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Purpose: Retinal degenerative disorders remain the leading cause for vision loss in developed countries. Since the regenerative capacity of human neural retina is highly limited, one viable treatment option is cellular replacement. Retinal progenitor cells (RPC), isolated from the fetal retina, have been shown to form new functioning photoreceptors and restore retinal function following transplantation into retinal degenerative hosts. However, compared to mouse RPC, not much is known about the hRPC phenotype. The aim of this study was to comprehensively describe the phenotype of GMP-manufactured hRPCs as a drug product for retinal degenerative disorders. Methods: hRPC were isolated from human neural retina at 18 week of gestational age and expanded under low-oxygen (3%) conditions up to passage 9. Karyotype analysis, immunocytochemistry and flow cytometry analysis for stemness, eye field, neuronal, proliferation and retinal cell markers were performed. Also, functional properties were investigated using calcium imaging and differentiation assay. Results: hRPC at passage 9 were found to have no chromosome abnormalities, and responded to L-glutamate, L-citrate and NMDA-Glycine. Immunostaining confirmed the presence of Sox2, Kif4, Recoverin, Otx2, Pax6, Kif17, PCNA, CyclinD1, b3 tubulin, NF200, Nestin, Vimentin, SSEA4, CD24, Crx, Nrl, CD73, PSA-NCAM, PTK7. We were unable to detect significant expression of mature photoreceptor markers such as Rhodopsin, Opsin Red/Green, Nr2e3, or Rod Outer Membrane. Also, we found hRPC to be negative for markers specific for neural stem cells (CD133, CD15), retinal pigment epithelia (RPE65), and glial progenitors (A2B5, CD38). However, upon differentiation in vitro we observed more than 50% of cells to express mature photoreceptor markers (Rhodopsin, Opsin Blue, Opsin Red/Green). Conclusions: The phenotype of hRPC may be used to compare different cell products to be prepared for transplantation. The presence of CD24 and CD73, found on mous photoreceptor precursors, suggest the potential of this population to form rods and cones and PSA-NCAM expression is characteristic of cells able to integrate into host retina.

Poster Board Number: T-1002

HUMAN PLURIPOTENT STEM CELL DERIVED RETINAL PIGMENT EPITHELIUM CULTURE ON TRANSPLANTABLE BIOMEMBRANE

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Human embryonic stem cells (hESCs) are differentiated toward retinal pigment epithelium (RPE) in several laboratories aiming to treat degenerative diseases of the retina, and create an in vitro model of the human retina. Injected hESC-RPE cells have been shown to improve visual functions. However, in most of the studies hESC-RPE cell integration has been unsatisfactory and the cells have vanished in a few months after the injections. One reason for poor RPE survival could be aged or diseased basal lamina, the Bruch’s membrane. Here we report successful hESC-RPE growth on transplantable biomembrane, which could be used to enhance RPE integration and survival in the host tissue. We studied the growth and maturation of hESC-RPE on porous biomembrane fabricated from clinically accepted polyimide (PI). In addition, we studied the role of several extracellular matrix proteins and protein mixtures in supporting hESC-RPE attachment, growth, and maturation. Spontaneously differentiated pigmented cells were seeded on PI membranes with different coatings. The cell growth and pigmentation was analyzed weekly under microscope. After eight to twelve weeks of culture, the expression of RPE specific genes and proteins was analyzed. The tightness of the epithelium was evaluated by measuring transepithelial electrical resistance (TEER) and cell permeability of 6-carboxyfluorescein. Co-culture with rat retina explants was used to test the functionality of hESC-RPE. PI biomembrane alone without coating did not support hESC-RPE attachment or growth. Nonetheless, over half of the tested coatings improved cell attachment and RPE maturation. hESC-RPE cells were positive for common characteristics of native RPE cells, such as cuboidal morphology, pigmentation, gene and protein expression, and polarization. hESC-RPE cultured on coated PI membranes formed tight epithelium based on protein expression, TEER and permeability values. In addition, hESC-RPE showed functionality by phagocytosing photopigments of the rat retina. This study shows that ECM component coated PI membrane supports hESC-RPE culture and maturation and would be a potential scaffold for further hESC-RPE transplantation studies.

Poster Board Number: T-1003

DOWNREGULATION OF CANONICAL WNT SIGNALLING IN ADULT HUMAN MULLER STEM CELLS LEADS TO EXPRESSION OF RGC MARKERS IN VITRO

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Background: Müller glia cells with stem cell characteristics have been shown to differentiate into various types of retinal neurons in the lower vertebrate retina. Although these cells have been identified in the adult human retina, they are unable to repair retinal damage in situ. However, human Müller stem cells (MSC) have been shown to differentiate towards retinal neural phenotypes including retinal ganglion cells and photoreceptor precursors in vitro. Regulation of the Wnt signalling pathway has previously been shown to be critical for maintaining retinal stem cells as well as facilitating their proliferation and differentiation into retinal neurons during embryonic development. The role of Wnt signalling in human MSC for the maintenance of a stem cell phenotype and for their differentiation into retinal neurons is currently unknown. The present study aimed to determine the function of the canonical Wnt signalling pathway in human MSC and the effects of Wnt inhibition on the development of differentiation protocols towards retinal neural phenotypes. Methods: Expression of genes and proteins of the canonical Wnt signalling pathway was examined using RT-PCR, western blotting and immunohistochemistry. Downregulation of Wnt proteins was performed in cells cultured for 7 days in the presence of Dickkopf-1 (Dkk-1), an inhibitor of the canonical Wnt signalling pathway in human MSC and the effects of Wnt inhibition on the development of differentiation protocols towards retinal neural phenotypes. Methods: Expression of genes and proteins of the canonical Wnt signalling pathway was examined using RT-PCR, western blotting and immunohistochemistry. Downregulation of Wnt proteins was performed in cells cultured for 7 days in the presence of Dickkopf-1 (Dkk-1), an inhibitor of the canonical Wnt pathway. For RT-PCR experiments total RNA was extracted, reverse transcribed and PCR was performed. PCR products were analysed by gel electrophoresis and quantified by densitometry. For western blotting experiments total protein was extracted, run on SDS-PAGE and protein expression was detected with specific antibodies.
primary antibodies. Cells treated or untreated with Dkk-1 were examined for their expression of markers of retinal ganglion cells, Wnt 2B and β-catenin using immunohistochemistry and confocal microscopy. Results: Various components of the Wnt pathway, such as Wnt5B, Fzd1, Fzd4, Fzd7, β-catenin and Lef1 were shown to be expressed by MSC. Conversely, mRNA for Wnt3a and Fzd5 was not detected. High levels of β-catenin were localized in the nuclei and cytoplasm of most cells examined. Downregulation of the canonical Wnt pathway using Dkk-1, greatly attenuated the expression of β-catenin and Wnt2B. Treatment with Dkk-1 also resulted in a marked reduction in proliferating cells as determined by positive staining for the proliferative marker Ki-67. Importantly, the expression of proteins typically expressed in retinal ganglion cells was augmented in MSC treated with Dkk-1, as shown by RT-PCR and western blotting. In particular, the ganglion cell markers Isl-1, Hud and Brn3b were significantly increased in MSC following inhibition of the Wnt pathway. Conclusions: The study showed that various components of the Wnt pathway are expressed in human MSC and suggests that constitutive activity of the canonical Wnt pathway may promote the proliferation of undifferentiated Müller stem cells. Conversely, inhibition of the canonical Wnt pathway attenuated cell proliferation and induced differentiation of Müller glia cells towards retinal neuronal phenotype as judged by their increased expression of retinal ganglion cell markers. Inhibition of the canonical Wnt pathway in human Müller stem cells may prove to be an important tool for the development of cell populations of cell populations of retinal neurons and may be valuable for the development of retinal cell transplantation strategies in the treatment of retinal degenerative diseases.

Poster Board Number: T-1004

NEUROPROTECTIVE EFFECTS OF HUMAN NEURONAL PROGENITOR CELLS EXPRESSING IGF-1 AND IGFBPL-1 ON MODELS OF RETINAL GANGLION CELL LOSS

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We have recently reported that neural progenitor cells isolate from human persistent fetal vasculature (NPPFV) can differentiate into retinal neurons and specifically, retinal ganglion-like cells (RGC) after transplantation into the mouse vitreous. 1 Immunostaining indicated that the NPPFV cells penetrated the inner retina and integrated with the host inner nerve (plexiform) fiber layer. NPPFVs were also transplanted into a mouse model of pigmentary glaucoma (DBA/2J), in which there is progressive and complete loss of host RGCs. Interestingly, host RGCs that were in apposition to NPPFVs survived this harsh ocular hypertensive environment while other RGCs underwent apoptosis. Based on these and other in vitro observations we hypothesized that NPPFVs confer a relative degree of neuroprotection to host RGCs through secretion of a known neuroprotective factor, insulin-like growth factor-1 (IGF-1) and its binding protein, IGFBP-1. It has been shown that both factors can significantly enhance axon growth of RGCs and enhance their survival in vitro. To enhance the neuroprotective effects of NPPFVs we questioned whether increased production and secretion of IGF-1 and IGFBP-1 could confer more global neuroprotection against stress-induced RGC loss. IGF-1 and IGFBP-1 cDNAs were cloned into a plasmid carrying a fluorescence reporter gene to generate fluorescent fusion proteins. Plasmid encoding IGF-1 or IGFBP-1 gene was constructed and spliced into pJ603-neo vectors expressing a red (RFP, TD-tomato) or green (GFP) fluorescent protein, respectively. Cells were transfected by electroporation using one or both vectors and examined under fluorescence microscopy. Transfected cells were enriched by antibiotic selection and subjected to RT-qPCR and ELISA for quantitation of IGF-1 and IGFBP-1 expression. Transfected cells, NPPFVGIGF-1, NPPFVGIGFBP-1 and NPPFVGIGF-1/GFBLP-1 were studied under a co-culture condition with RGCs and evaluated for neuronal morphology, axonal growth and apoptosis. Double transfection of these genes yielded significantly increase production of IGF-1 and IGFBP-1 (15.8 fold vs. 3.7 for naïve NPPFVs in combination). ELISA also indicated that transfected NPPFVs secreted both gene products in culture. In coculture condition, transfected NPPFV cells improved morphology of resident RGCs and decreased apoptotic signals. Our data indicate that neuroprotective genes such as IGF-1 and its binding protein, IGFBP-1 could be successfully transfected and expressed into neuronal progenitor cells such as NPPFVs. Expression of these proteins improves neuronal morphology, axonal growth and survival of RGCs. Overall, NPPFVs expressing neuroprotective transgenes may be good candidate cells for transplantation of the inner retinal to confer increased neuroprotection to host RGCs under disease conditions. 1 Cell Transplantation, in press, 2012. Corresponding author: Lashkari, Kameran

Poster Board Number: T-1005

HISTONE MARKS PREDICT CELL PLASTICITY OF THE ADULT HUMAN RETINAL PIGMENT EPITHELIUM

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Retinal pigment epithelial (RPE) cells are one of the few cell types well recognized to change fate in lower vertebrates. We recently showed that adult human RPE contains a minor sub-population of cells that can be activated into self-renewing multipotent stem cells. These RPESCs can be derived from donors as old as 99 years, and can differentiate down RPE, neural or mesenchymal lineages using appropriate differentiation media. Addressing whether the observation of mesenchymal fates was due to the multipotency of the RPE cells or to a contaminating cell type, for example MSCs, in the cultures was critical. Therefore, we demonstrated that single, cloned RPE cells exhibit this ability, irrefutably demonstrating that they are multipotent cells. We therefore sought to understand the basis for this observed plasticity. Histone marks at promoter and enhancer sites can reveal both genes that are actively being transcribed - i.e. active, and genes that are poised - i.e. currently inactive, but can be activated by under appropriate conditions. Recently, the histone H3K27me3 and H3K4me3 marks have been shown to be concomitantly enriched in so called bivalent promoter regions, which in human embryonic stem cells (hESCs) are thought to poised developmental genes for subsequent activation. Similarly, distal enhancers in hESCs are enriched in either H3K27ac or H3K27me3, depending on whether that regulatory elements control transcriptionally active or poised genes, respectively. Therefore, we hypothesized that the combination of histone marks at promoter and enhancer regions could predict poised genes in RPE that can become active in response to stimuli and that could explain RPE plasticity. We cultured human retinal pigment epithelium into polarized layers and verified their terminal differentiation through transepithelial resistance (TER) in Ω cm2. These monolayers expressed TER of > 2000Ω.cm2 similar to native RPE. We also confirmed their identity immunohistochemically, by quantitative PCR, and using a phagocytosis assay. We then conducted H3K27ac, H3K27me3, H3K4me3 and...
H3K4me1 chromatin immunoprecipitation-sequencing (ChIP-seq) on adult human RPE monolayers. We found that RPE specific genes possess H3K27ac and H3K4me3 marks at their promoter regions, whereas the genes we have found to increase expression during differentiation of RPE, for example RUNX2 during osteogenic differentiation, display a bivalent promoter enriched in both H3K27me3 and H3K4me3. In addition, we identified a large number of putative enhancer elements, whose relevance and specificity are currently being evaluated. These results suggest that poised promoter and enhancer signatures may facilitate understanding of the molecular basis of RPESC plasticity.

Poster Board Number: T-1006

RETINAL TRANSPLANTATION OF PHOTORECEPTOR PRECURSORS DERIVED FROM MOUSE EMBRYONIC STEM CELLS UNDER HYPOXIC CONDITIONS IMPROVED THE EFFICIENCY OF DIFFERENTIATION

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Retinal dystrophies such as Retinitis pigmentosa (RP) and age-related macular degeneration (AMD) are among the leading causes of permanent blindness and are characterized by the progressive dysfunction and death of the light sensitive photoreceptor cells of the retina. Due to the limited intrinsic capacity of the mammalian retina to regenerate, the use of embryonic stem cells (ESC) as an unlimited source to replenish the lost cells has represented a main objective for the scientific community. Despite great advances in the field of differentiation of ESC towards photoreceptors in the recent years, few drawbacks remain unresolved. Such as, efficiency, purity of the population and the constant worry that once differentiated, cells would not be able to integrate into the host retina. We have designed a new protocol to differentiate mouse ESC (mESC) in a manner that allows us to define conditions to expand a homogeneous population of retinal precursor cells such as MAP2 and beta-3-tubulin, and the early photoreceptor marker, recoverin. These studies indicate that human VSELs can differentiate and express markers of retinal stem and developing progenitor cells such as Nestin and PAX6, markers of neuro-ectodermal populations of small (5 to 9 μm) Lin-, CD45- cells that can express CD133, CD34, CXCR4, as well as high levels of markers characteristic of embryonic stem cells such as Oct-4 and Nanog. To explore the regenerative potential of VSELs in the retina, we transplanted PKH26-labeled enriched human VSELs into the mouse eye -- both by injecting the cells into the vitreous space of immunodeficient mice, and by injecting the cells subretinally in a SCID immunodeficient mouse model of retinal detachment. We assessed the ability of human VSELs to engraft, survive and differentiate into retinal or neuroectodermal cells in the mouse retina. At both 2 and 4 weeks after transplantation the subretinally and intravitreally injected human VSELs were able to engraft, survive and migrate within the retina. Furthermore, subsequent immunohistochemistry analysis revealed that the subretinally transplanted cells were able to differentiate and express markers of retinal stem and developing progenitor cells such as Nestin and PAX6, markers of neuro-ectodermal cells such as MAP2 and beta-3-tubulin, and the early photoreceptor marker, recoverin. These studies indicate that human VSELs can engraft, migrate and differentiate along the retinal lineage. These observations warrant further investigation to evaluate the potential role of these cells to treat AMD.
Poster Board Number: T-1008

FUNCTIONAL TISSUE ENGINEERED CORNEAL ENDOTHELIAL DERIVED FROM MOUSE CORNEAL STROMA STEM CELLS

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Purpose: Corneal endothelial dysfunction remains a common indication for keratoplasty, accounting for half of the total number of such procedures. Corneal endothelium as well as corneal stroma originates from the neural crest. We previously reported the isolation of Cornea-derived Precursors (COPs), which have characteristics of multipotent neural crest-derived stem cells, from mouse corneal stroma. In this presentation, we report tissue engineered corneal endothelium (TECE) derived from COPs. Methods: We cultured COPs from mouse corneal stroma in the specific endothelium-inducing medium including retinoic acid, glycogen synthase kinase 3-beta inhibitor, and TGF beta-2 for one week to induce TECE. Quantitative RT-PCR was performed to detect markers characterizing corneal endothelium (Na,K-ATPase a1-subunit, carbonic anhydrase, Na,HCO3 co-transporter, collagen IV, collagen VIII, and Ptx2). The pump function attributable to Na,K-ATPase activity of TECE was measured with an Ussing chamber, and compared with that of primary cultured mouse corneal endothelial cells (mCE) and 3T3 cells. Transplantation of TECE sheets into rabbit cornea was performed to evaluate in vivo TECE pump function. Results: After one week culture, hexagonal mosaic pattern monolayer TECE was obtained from COPs. Quantitative RT-PCR revealed significant increases of the expressions of all of the above markers. Na,K-ATPase pump activity of TECE (198.4μA/cm2) was significantly higher than COPs (15.2μA/cm2) and 3T3 (40.8μA/cm2) cells, and 1.7 fold higher than cultured mouse corneal endothelial cells (116.3μA/cm2). TECE transplanted into rabbit cornea maintained transparency and corneal thickness (1105.8±165.9μm). Conclusions: We successfully derived TECE from mouse corneal stromal cells (COPs), which has equal pump function with mCE in vitro and in vivo.

Poster Board Number: T-1009

CD44 MEDIATES THE POST-TRANSPLANT SURVIVAL OF ADULT MOUSE RETINAL STEM CELL-DERIVED ROD PHOTORECEPTORS

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Adult retinal stem cells (RSCs) derived from the ciliary epithelium of mice can give rise to all retinal cell types. RSC-derived photoreceptors have demonstrated functional recovery in mouse models of disease. The potential of RSC-derived rods in adult mouse transplantation models has been limited by poor cell distribution, survival and integration into host tissue. An injectable and biodegradable hydrogel blend of hyaluronan and methylcellullose (HAMC) has shown promise in overcoming the cell distribution barrier. Here we report a mechanism through which HAMC directly supports the survival and integration of post-mitotic RSC-derived rods in vitro and in vivo. RSC-derived rods were pre-differentiated on laminin substrate in the presence of taurine/retinoic acid, which increases the percentage of rods differentiating in clonal mouse and human RSC colonies to over 95% of the population. Survival was assessed with ethidium homodimer and phenotype by immunocytochemical (ICC) staining for rhodopsin, Pax6 and RPE65. RSC-derived rods were harvested at various pre-differentiation time points and injected in a vehicle of either HAMC or saline (control) subretinally into adult mice. Cell distribution in host tissue was analyzed 3 weeks post-transplantation among neural retina, subretinal space, and retinal pigment epithelium (RPE) in the HAMC vs. saline vehicles, with or without addition of the glial-specific toxin DL-alpha-aminoadipic acid (AAA). Highest levels of cell survival/integration in neural retina were observed with the transplantation of early post-mitotic RSC-derived rods in HAMC+AAA. Post-mitotic RSC-derived rod photoreceptors showed significantly improved in vitro survival in HAMC compared to media alone. ICC and Q-PCR analysis revealed the expression of the hyaluronan receptor CD44 on RSC-derived rods. The binding of HA to CD44 on other cell types is known to influence cellular migration and survival. CD44/-/- RSC-derived rods did not exhibit the pro-survival effect seen with HA-containing mixtures in vitro, demonstrating the specificity of this interaction. The enhanced survival and neural retinal integration of wild-type RSC-derived rods after transplantation in HAMC+AAA is lost with transplantation of CD44/-/- RSC-derived rods. Our results suggest that HAMC directly promotes RSC-derived rod survival in vitro and in vivo through the CD44 receptor.

Poster Board Number: T-1010

CANONICAL WNT SIGNALING DETERMINES TUMORIGENICITY AND FUNCTION OF TRANSPLANTED ESC-DERIVED RETINAL PROGENITORS

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Tumor formation constitutes a major obstacle to the clinical application of embryonic stem cells (ESCs). Here, we report that further differentiation of ESC-derived neural progenitors into retinal progenitor cells (ESC-RPCs) completely eliminates teratomas in oculo transplantation. However, tumor-like neural overgrowth occurs in 61% of transplanted eyes. Inhibition of Wnt signaling by DKK1 promotes the commitment of ESC-RPCs to more mature retinal cells and reduces the occurrence of tumor-like neural overgrowth to 3%. DKK1-treated ESC-RPCs efficiently integrate to the host retina, form synaptic connections and restore visual function. Mechanistically, canonical Wnt signaling effector Tcf1 directly activates Sox2 and Nestin expression. Silencing Sox2 or Nestin produces phenotypes resembling to those of DKK1 treatment. Furthermore, Tcf1, Sox2 and Nestin are highly expressed in the early retinal development and retinoblastoma. Collectively, this study demonstrates for the first time the pivotal role of Wnt signaling and the Tcf1/β-catenin-Sox2-Nestin cascade in controlling tumorigenicity and the therapeutic effect of transplanted ESC-derived progenitors.
PHOTORECEPTORS REINITIATE THE CELL CYCLE MACHINERY DURING DEGENERATION

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Recent data concerning post-mitotic neurons show the reactivation of cell cycle proteins during the process of cell death in several neurodegenerative diseases. We asked whether the control of this cell cycle protein expression may share some common features with the regulation of neural stem cell proliferation. We investigated the retina of Rd1 mice, a model of retinitis pigmentosa, with rod function defects, widely used to study photoreceptor loss. We used different knockout mouse models to reveal whether proteins involved in the cell cycle regulation are responsible for photoreceptor loss in the Rd1 mouse. Histological and electroneurogram analyses were performed to evaluate the retina integrity. At P12, an early stage of the disease, Rd1 mice displayed an increased expression of Cdk4 and Cdk2, as well as their target, phosphorylated-Rb among photoreceptor nuclei. Genetic and pharmacological interferences with cell cycle protein activities extended photoreceptor survival both in vitro and in vivo, but transiently. Interestingly, we observed that at P12, the polycystin group protein Bmi1 was expressed in virtually all the nuclei in the inner and outer nuclear layer of both wild-type (WT) and Rd1 mice. Bmi1 promotes cell cycle progression via the repression of tumor suppressor genes through epigenetic modifications. We reasoned that Bmi1 deletion could impede the Cdk reaction that characterizes neuronal apoptosis and may therefore delay retinal degeneration. We compared the history of WT, Rd1 and Rd1;Bmi1-/- and observed the presence of 7 rows of photoreceptors in Rd1;Bmi1-/- mice at P33, while Rd1 littersmates displayed a single scattered row of photoreceptors. ERG recordings revealed the ability of Rd1;Bmi1-/- retinas to respond to light stimuli (cone response). Cdk4 activity and cell death were strongly decreased in Rd1;Bmi1-/- mice, respectively by 70% and 50% as compared to Rd1 littersmates. Both rods and cones were well preserved in the Rd1;Bmi1-/- retinas. In conclusion, our data show for the first time a mechanism of retina degeneration involving a reactivation of the cell cycle that precedes photoreceptor death. Moreover, Bmi1, known to play a key role in the control of neural stem cell renewal, appears also to have a permissive action on photoreceptor nuclei. Genetic and pharmacological interferences with cell cycle protein activities extended photoreceptor survival both in vitro and in vivo, but transiently. Interestingly, we observed that at P12, the polycystin group protein Bmi1 was expressed in virtually all the nuclei in the inner and outer nuclear layer of both wild-type (WT) and Rd1 mice. Bmi1 promotes cell cycle progression via the repression of tumor suppressor genes through epigenetic modifications. We reasoned that Bmi1 deletion could impede the Cdk reaction that characterizes neuronal apoptosis and may therefore delay retinal degeneration. We compared the history of WT, Rd1 and Rd1;Bmi1-/- and observed the presence of 7 rows of photoreceptors in Rd1;Bmi1-/- mice at P33, while Rd1 littersmates displayed a single scattered row of photoreceptors. ERG recordings revealed the ability of Rd1;Bmi1-/- retinas to respond to light stimuli (cone response). Cdk4 activity and cell death were strongly decreased in Rd1;Bmi1-/- mice, respectively by 70% and 50% as compared to Rd1 littersmates. Both rods and cones were well preserved in the Rd1;Bmi1-/- retinas. In conclusion, our data show for the first time a mechanism of retina degeneration involving a reactivation of the cell cycle that precedes photoreceptor death. Moreover, Bmi1, known to play a key role in the control of neural stem cell renewal, appears also to have a permissive action on neurons to allow cell cycle entry and progression until the S-phase. The dissection of such mechanisms may help to understand cues controlling cell regeneration versus cell death.

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Taking advantage of two transgenic mouse lines, glast.dsRed and crx.gfp, that express fluorescent proteins in glial and photoreceptor cells respectively, we investigate the role of glial-positive glial cells (GPCs) in the survival/differentiation/proliferation of age-matched and aged photoreceptor cells. Primary retinal cells were isolated from newborn transgenic mouse retina (glast.dsRed::crx.gfp) at postnatal day (P0/P1) and propagated in defined medium containing epidermal growth factor (EGF) and fibroblast growth factor 2 (bFGF). By flow-sorting another population of pure GPCs was purified. Both populations were expanded and analyzed for the presence of specific retinal cell markers. Notably, the primary cell culture collected from the glast.dsRed::crx.gfp transgenic line showed a conspicuous presence of immature photoreceptors growing on top of GPCs. In order to reveal the role of such cells in the survival/differentiation/proliferation of photoreceptors we set up in vitro cultures of retina-derived cells that allowed long-term time-lapse recordings charting every cell division, death and differentiation event. To assess the regenerative potential of cultured GPCs, we challenged them with compounds mimicking retinal degeneration (NMU, NMDA, Zaprinast). Moreover, in order to evaluate
the ex-vivo capability of GPCs to generate/regenerate photoreceptors, we set up a time-lapse video of glast.dsRed::crx.gfp-positive photoreceptors in wt or degenerating conditions at different developmental stages. Mass spectrometry (MS), immunostainings and other molecular approaches were performed to reveal adhesion molecules involved in the relationship between glial cells and photoreceptors. Both primary cell lines were highly homogenous, with an elongated morphology and the majority expressed Müller glia markers (MG) such as glast, blbp, glt-1, vimentin, glutamine synthetase (GS), GFAP, cd44, mash1 and markers of reactive Müller glia such as nestin, pax6. Conversely, none of them were found positive for retinal neuron markers like tuj1, otx2, recoverin. Primary cultures of GPCs show the incapability of glial cells to give rise to photoreceptors in both wild type or degenerative environment. Furthermore, primary cultures of pure GPCs challenged with different compounds did not highlight the production of new glial cell-derived photoreceptors. Nonetheless, GPCs support photoreceptor survival for more than 30 days. Retinal explants treated with compounds causing retinal degeneration in vivo, show interesting results that are still under analysis. Adhesion molecules involved in the contact between photoreceptors and glial cells are under investigation. In conclusion, primary glial cell cultures collected at P1/P0 do not give rise to photoreceptor cells in wt or degenerative conditions at least in vitro. Their role in ex-vivo and in-vivo experiments in both wt or degenerative conditions are still under further investigations. Presently, data obtained in culture strongly suggest that glial cells control the survival of photoreceptor cells.

**Poster Board Number: T-1014**

**EVALUATION OF CROSS-LINKING TIME FOR CARBODIIMIDE MODIFIED AMNIOTIC MEMBRANES ON LIMBAL EPITHELIAL CELL CULTURE CHARACTERISTICS**

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Purpose: This study aims to investigate the effect of cross-linking time for carbodiimide modified amniotic membrane (AM) on limbal epithelial cell (LEC) culture characteristics. Methods: The AM was chemically cross-linked with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS) for a variety of time periods in the range of 1-48 h. Ninhydrin assays and mechanical tests were used to determine the extent of cross-linking. In addition, the degradability was studied by incubating the AM at 37°C in balanced salt solution containing collagenase. After cultivation on various cross-linked AM samples, the human LECs were analyzed for their proliferation and gene expressions. Results: The results of ninhydrin assays and mechanical tests showed that an increase in the cross-linking time may lead to the increase in the cross-linking degree of chemically modified AM. After treatment with EDC/NHS for 48 h, the samples had strong mechanical strength and exhibited good degradation resistance, indicating that these biological tissue materials could provide stable support for LEC growth. It was found that the cells on the AM modified with carbodiimide chemistry for a longer time expanded rapidly and expressed higher levels of p63 and ABCG2. Conclusions: The present study suggests that the cross-linking time has a profound influence on the performance of carbodiimide modified AM for LEC cultivation.

**Poster Board Number: T-1015**

**DEVELOPMENT OF PATIENT SPECIFIC IPS CELLS IN ORDER TO MODEL LEBER CONGENITAL AMAUROSIS DISEASE**

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Retinitis pigmentosa (RP) and Leber Congenital Amaurosis (LCA), a genetically heterogeneous group of diseases, together with age-related macular degeneration (AMD) are the leading causes of permanent blindness characterized by progressive degeneration of photoreceptors and pigment epithelial cells of the retina. The aim of this study is to develop retinal progenitors from patients’-own somatic cells that will serve as an experimental model of the disease and eventually applied in clinical therapy to reverse blindness. Patients’-dermal fibroblasts, obtained by punch biopsy, have been selected as a source of retinal progenitors. The fibroblasts were differentiated into a pluripotent stage (human induced pluripotent stem, hiPS, cells) by retroviral particles producing hNANOG, hOCT4, hSOX2, hKLF4 and h-Myc produced in HEK293T cell line. The colonies were propagated and characterized for pluripotency immunocytochemically and by RT-PCR. The identity of the derived IPS cells was confirmed by fingerprinting. Early-onset RP patients’ fibroblasts with mutations in CRB1 (W822STOP/C948T) and RDH12 (T49M) genes were reprogrammed into hiPS cells. 21 days after transduction colonies with the morphology similar to embryonic stem cells began to appear. The colonies were similar to healthy patient hiPS colonies as characterized for pluripotency by immunocytochemistry and RT-PCR. We successfully generated RP patient-specific iPS cell lines and characterized them for pluripotency. These cells are being submitted to the differentiation protocol to generate photoreceptor and retinal pigment epithelial cells. The use of patient derived IPS cells will help to elucidate the pathogenic mechanism caused by the mutations in CRB1 and RDH12 genes.

**Neural Cells**

**Poster Board Number: T-1016**

**CTX HUMAN NEURAL STEM CELL (HNSC) THERAPY FOR ISCHEMIC STROKE PATIENTS: PISCES - A CLINICAL SAFETY TRIAL**

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Background: The hNSC line CTX0E03 was clonally derived from early expansion of fetal cortical hNSCs following retroviral insertion of a c-myCERTAM fusion gene that permits controlled expansion in the presence of growth factors and 4-hydroxytamoxifen. The CTX0E03 cell line has been banked and is used in the GMP (Good Manufacturing Practice) manufacture of CTX Drug Product (DP) currently in clinical development. Preclinical studies with CTX DP in MCAO (Middle Cerebral Artery Occlusion) rat stroke models have shown dose-related behavioral recovery in sensorimotor function and evidence of cell survival, limited migration, angiogenesis & endogenous neurogenesis. In the PISCES trial we aim to investigate the safety profile of intra-cerebral implantation of CTX DP; a secondary aim is to explore indices of change in neurological function as a result of cell treatment. Methods: We undertook an open label,
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ascending dose. Phase I clinical trial in 12 male patients with stable neurological deficit & disability resulting from ischemic stroke affecting the basal ganglia or white matter 6 months to 5 years previously. Four groups of 3 patients receive 2, 5, 10 & 20 million cells respectively, implanted in the ipsilateral putamen by stereotaxic injection. The two-year follow-up includes a range of neurological function tests as well as general clinical & longitudinal radiological data (3T MRI, fMRI, DTI, MRS) to identify potential biomarkers of change in neurological function. fMRI data collection pre & post cell implantation is based on a block design of hand grasp-release task that is visually cued at 0.25Hz. All patients will be followed up for 10 years and give consent for post-mortem brain examination. Results: Up to January 2012, five male subjects had been implanted with CTX0E03 (2 million (n=3) & 5 million (n=2) cells) with follow up between 2 and 14 months. Mean age was 74.6 years (range 68-83) with median National Institutes of Health Stroke Scale (NIHSS) score of 8 and mean time from stroke onset 30.2 months (range 14-51). Two (40%) had sub-cortical-only infarcts and 3/5 (60%) had left hemisphere lesions. No direct cell related adverse events or immune related response have been observed. One patient had a minor procedure-related asymptomatic sub-dural bleed & 2 had superficial scalp infection at the wound site. NIHSS scores 1 month post cell implantation improved in all 5 patients with reductions in score compared to baseline being 2, 1, 3, 1 & 1 respectively and further reduction by 1 & 2 at 12 month review in the first 2 patients. Summed Ashworth spasticity scores of affected limbs (total potential score=60) improved in all 5 patients compared to baseline by 15, 7, 6, 3 & 8 respectively. Comparing fMRI activations (SPM8; active vs rest; p<0.05) in 2 subjects with hand weakness but with preserved & 1 damaged motor cortex shows widespread activations followed by reorganization to ipsilesional cortex during return of minimal finger movement and bilateral occipital & cerebellar activation followed by return of surviving ipsilesional cortex activation during minimal leg power improvement, respectively. Conclusions: No cell related adverse events observed to date in the first 5 patients enrolled. Neurological impairment improved in all subjects, and was sustained in longer-term follow up. Moreover, some longitudinal change in motor activation fMRI was seen. The PISCES trial will continue to enroll subjects at the remaining and higher dose cohorts according to ongoing data review.

Poster Board Number: T-1017

CELLULAR NEURODEGENERATIVE DISEASE MODELS DERIVED FROM GENETICALLY MANIPULATED HUMAN PLURIPOTENT STEM CELLS

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Cellular disease models derived from human pluripotent stem cell (hPSC) are demanded in research of disease mechanisms as well as screening of new drugs because animal models or tumor cell-derived models do not always precisely mimic human responses. Recently we have established an efficient system to integrate genes into a defined locus in hPSC. Using this site-specific gene integration system, we have generated hESC and hiPSC lines carrying mutant genes responsible for familial neurodegenerative diseases, such as Alzheimer’s disease (AD), amyotrophic lateral sclerosis (ALS), and Huntington’s disease (HD). Cellular disease-phenotypes were detected in the disease model cells. For example, hESC-derived AD models showed AD-related phenotypes such as increase of Aβ42 production and neural dysfunction. HD models showed cell death of mutant HTT-expressing neurons in particular conditions. In addition, our disease models derived from genetically manipulated hPSCs are capable of comparing with isogenic healthy hPSCs. These results indicate that genetically manipulated hPSC-derived disease model cells are useful tools for neurodegenerative disease research.

Poster Board Number: T-1018

LONG-TERM STABLE IN VITRO CULTIVATION OF NEURAL STEM CELL LIKE CELLS FROM HUMAN ADULT TEMPORAL LOBE

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Autologous adult human neural stem cells (aNSCs) may be used for regenerative cell therapies bypass potential ethical problems. However, stable in vitro expansion protocols and experimental/clinical factors influencing primary cultures need to be further elucidated for clinically applicable techniques. To address these issues, we obtained biopsy specimens from 23 temporal lobe epilepsy patients. Neural cells were primarily cultured in anewly defined attachment culture condition and experimental/clinical factors influencing the culture were analyzed. When the success of primary cultures was defined as stable expansion of cells (>ten in vitro passages) and expression of NSC markers, success rate of the primary culture was 39% (nine of 23 temporal lobes). In the successful cases, 1 × 107 neural cells could be multiplied into 1 × 1015 cells within four months. During the long-term expansion, expressions of NSC markers and differentiation potentials into astrocytes and neurons were maintained. After the 18th sub-culture, spontaneous senescence and differentiation were observed, and the cultivated NSC-like cells ceased their proliferation. The culture results were not affected by seizure characteristics. However, an older age (> 40 years) and a smaller sample volume (< 2ml) were found to exert negative influences on the primary culture results. Although NSC-like cells were obtainable from the lateral temporal lobe remote from the subventricular zone, they were more successfully cultured from the medial temporal lobe facing the ventricular surface. The results here would provide promising experimental and clinical strategy of using patient-specific autologous NSCs in regenerative medicine in the future.

Poster Board Number: T-1019

TRANSPLANTATION OF NEURAL CELLS DERIVED FROM HUMAN IPS CELLS INTO AN ALZHEIMER’S DISEASE MODEL

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Aim: Alzheimer’s disease (AD) represents a major cause of early-onset and progressive dementia and is characterized with degeneration of cholinergic neurons especially in the hippocampus. Radical cures have not been developed at present. Amyloid precursor protein (APP) mutation causes familial AD and several APP isoforms are suggested to be associated with AD. In this study, we tried to generate cholinergic neurons from human IPS (hiPS) cells and to transplant the cells to the bilateral hippocampal lesions of APP
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transgenic mice. Methods: (Culture of hiPS cells) A hiPS cell line was obtained from RIKEN Cell Bank (cell name: 253G1, cell number: HP50002) and was maintained according to RIKEN cell preparation manual. (Cell differentiation) hiPS cells were maintained in the presence of mouse embryonic fibroblasts cells in serum-free medium containing basic fibroblast growth factor. We developed embryonic bodies (EBs) from undifferentiated hiPS cells by 4-day floating culture. EBs were transferred to fibronectin-coated dishes and cultured for 24 hours. We added retinoic acid, sonic hedgehog and noggin in the dishes twice and cultured for 72 hours. We conducted RT-PCR analysis and immunocytochemistry to evaluate hiPS cell differentiation. (Transplantation) The burr hole marks were made at 2.0mm lateral and 2.4mm posterior to the bregma in bilateral parietal bones. The hiPS cells derived neural cells were injected into the bilateral hippocampal lesions (1.75mm depth from the brain surface) of two-month-old APP transgenic mice. Learning and memory functions were evaluated by Morris water maze before and after the transplantation. Histology and immunohistochemistry were performed six months after the transplantation. Results: (Cell differentiation) The cells derived from hiPS cells expressed neural markers in RT-PCR and confocal immunofluorescence analysis as well. The former showed that the cells expressed genes of chorine acetyl transferase, beta III tubulin, nestin, neurofilament and Islet1. The latter disclosed that the cells were 30-50% neural cell adhesion molecule (NCAM) positive, 90% nestin positive and 90% beta III tubulin positive. (Transplantation) Transplantation of hiPS cell derived neural cells were localized in the bilateral hippocampal lesions and these cells expressed human NCAM. There were no significant differences in the results of Morris water maze between hiPS cell and vehicle transplanted transgenic mice at this time but remarkable improvement of performance in this test was achieved in several hiPS cell derived neural cell transplanted mice. Conclusions: We established a model of bilateral neural transplantation for AD and the behavioral assessment was improved in several APP transgenic mice. We speculate that hiPS cell derived neural cell transplantation to the hippocampus is a promising candidate for AD treatment.

Poster Board Number: T-1020

TWO FACTOR REPROGRAMMING OF PERICYTE-DERIVED CELLS OF THE ADULT HUMAN BRAIN INTO FUNCTIONAL NEURONS

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Direct conversion of somatic cells into neurons provides a new approach towards cell-based therapy of neurodegenerative diseases. Recent studies have shown that the synergistic action of 3-4 transcription factors can induce neurogenesis from fibroblasts as well as hepatocytes which represent a non-ectodermal cell population. However, a major challenge for the translation of neuronal reprogramming into therapy concerns the question whether the adult human brain itself contains cell populations amenable to direct somatic cell-to-neuron conversion. Here we show that cells from the adult human cerebral cortex expressing pericyte markers, such as PDGFβR, can be reprogrammed into functional neurons by retrovirus-mediated co-expression of only two transcription factors, namely Sox2 and Mash1. These MAP2- and NeuN-expressing neurons acquire the ability of repetitive action potential firing and serve as synaptic targets for other neurons indicating their capability of integrating into neural networks. The pericytic origins of those neurons was verified by PDGFβR-based sorting of the cells and subsequent continuous live imaging during reprogramming. Furthermore, genetic fate-mapping in mice expressing an inducible Cre recombinase under the tissue nonspecific alkaline phosphatase promoter corroborated the pericytic origin of the reprogrammed cells. Our results demonstrate that cells endogenous to the adult mouse and human brain can be directly converted into functional neurons by only two transcription factors representing a viable approach for cell-based therapies of neurodegenerative diseases.

Poster Board Number: T-1021

MODELING INFLAMMATION IN PARKINSON’S DISEASE USING HUMAN PLURIPOTENT STEM CELLS

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Parkinson’s Disease (PD) is the most common neurodegenerative movement disorder. Historically, PD has been considered a strictly neuronal disease; however, clinical observations and evidence from animal models suggest inflammation may contribute to disease progression. It remains controversial whether glial activation, and the resulting inflammatory cascade, is a result or a cause of neuronal death. Towards resolving this distinction, we have established cultures of human embryonic stem cell derived dopaminergic neurons, human astrocytes and microglia. These neural cells are used to investigate the inflammatory response to alpha-synuclein, the protein whose mis-folding is the pathological hallmark for all forms of PD. We found alpha-synuclein is sufficient to induce the secretion of pro-inflammatory cytokines in both human astrocytes and microglia, and established the neurotoxic response to this glial derived increase in pro-inflammatory cytokines, allowing us to study the inflammatory contribution to the pathological development of PD in a human system. Furthermore, we are reprogramming fibroblasts from patients with idiopathic PD to investigate the contribution of inflammation to the progression of non-genetic forms of this disease. We aim to understand the role of inflammation in PD and identify key molecular events involved in preliminary stages of PD to exploit as potential targets for therapeutic intervention early in the course of the disease.

Poster Board Number: T-1022

A NEW TYPE OF HUMAN NEURAL STEM CELL FOR EFFICIENT PRODUCTION OF OLIGODENDROCYTE-LINEAGE CELL

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Oligodendrocytes are known to play a critical role in the pathogenesis of many neurological disorders including spinal cord injury, multiple sclerosis, schizophrenia, and congenital demyelinating diseases. A re-myelination strategy for spinal cord injury is well established and several treatments using neural stem cell/oligodendrocyte progenitor cell have undergone preclinical studies and/or clinical trials [i.e. human embryonic stem cell (HESC)-derived
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Oligodendrocyte progenitor cell (OPC) (Geron Corp., terminated Phase I trial), human neural stem cell (hNSC) (Stem Cells, Inc., Phase I clinical trial in Switzerland) (Neuralstem, Inc.), autologous schwann cell (Miami project) and autologous bone marrow-derived cell (SanBio, Inc.). However, there are pros and cons for these cells in terms of efficiency. NSCs seem to have higher survival rate and migration potential than other cells but other cells have higher differentiation potency into myelin-forming cells. Human neural stem cells (hNSCs) are usually cultured in a floating condition, called neurosphere culture, to maintain their multi-potential and proliferation potential. However, neurospheres contain many different progenitors and differentiated cells and the ratio of neural stem cells is usually very low. Recently, hNSCs could be expanded under adherent monolayer culture condition on laminin-coated culture-ware in the presence of EGF and bFGF. However, their differentiation potential into oligodendrocyte was not so high. I developed a new adherent monolayer culture method that enables hNSCs to grow exponentially and to differentiate into oligodendrocyte-lineage cells at 99% efficiency. Human NSCs dissociated from human fetal tissue were initially cultured in an ordinary chemically-defined culture medium in the presence of growth factors. The cells could be maintained but cell number decreased gradually after several passages. At passage 7, the cells were cultured in a proprietary NSC medium. While the cells couldn’t proliferate and total cell number was decreased in an ordinary culture condition, they could proliferate and be successfully expanded 10,000 times after adding 12 passage in the proprietary medium with the same growth factor combination. The cells displayed a homogeneous morphology and most of them expressed CD133 (100% by flow cytometry), Sox2 and nestin. When they were differentiated by withdrawing growth factors in a serum-containing medium, neuron (ßIII-Tubulin positive), Neurofilament L positive - and/or MAP-2-positive cells), astrocytes (GFAP-positive cells), and oligodendrocytes (O4 positive -, GalC positive - and/or MBP positive - cells) could be observed. These data indicate that they retain neural stem cell characteristics under this culture condition. When they were differentiated in a serum-free condition, 23.5 ± 2.0% of total cells seemed to retain undifferentiated state and 99.1 ± 0.6% of differentiated cells (75.8 ±2.1% of total cells) showed pro-oligodendrocytes (O4-positive/GalC-negative) or oligodendrocytes (O4-positive/GalC-positive) phenotype. This result suggested that the new culture condition shifted the cell fate to oligodendrocyte-lineage even though they retained their neural stem cell characteristics. Furthermore, this method will enable production of human oligodendrocyte-lineage cells in numbers sufficient for cell therapy.

**Poster Board Number:** T-1023

**SORTING AND TRANSPLANTATION OF DOPAMINERGIC PROGENITOR CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS**

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Cell grafts in clinical use should be not only free from animal-derived factors or feeder cells, but also free from graft-derived tumors due to contamination of undifferentiated or unwanted cells. In this report, we examined a nearly xeno-free culture method with FACS-based sorting using a surface marker of the floor plate cells, to purify the ideal cell graft for Parkinson’s disease. We induced neural progenitors from human ES cells (KhES-1) and human iPS cells (404C2) by the SFEBq method, expression of Corin arises from culture day 10 and about 10-20% of living cells were positive on day 21. After sorting, purity of Corin positive cells were over 90%, and the purity of the cells were confirmed by qPCR analysis. Corin positive cells were also expressed Lmx1a, a transcript for midbrain dopaminergic progenitors. Corin positive cells differentiate to TH positive neurons after 2-3 weeks of in vitro culture, and the TH positive cells were also immunoreactive for Nurr1 or Pitx3, as a marker for midbrain dopaminergic neurons. When grafted into non-lesioned NOD-SCID mice on 3 days after cell sorting (differentiation totally for 24 days), no tumor was found in the graft of Corin positive cells, while graft overgrowth was found in the graft of Corin negative or unsorted cells, and more TH+ neurons were survived in Corin positive graft. After modification of the culture methods and the timing of sorting, we grafted the Corin positive cells into the 6-OHDA lesioned rats. Human cells-derived TH positive cells were found in the graft for 8 weeks after transplantation, and methamphetamine-induced rotation behavior were partially improved in the grafted animals with “proper” midbrain dopaminergic neurons. We succeeded in cell sorting for dopaminergic progenitors using a surface marker Corin, and dopaminergic neurons were survived and functioned in the 6-OHDA rats. Sorting of dopaminergic progenitor cells using a surface marker reduced the risk of graft overgrowth and functioned in rodents. We are planning transplantation of the sorted cells to MPTP-lesioned monkeys to make a functional evaluation.

**Poster Board Number:** T-1024

**AN IN VITRO MODEL OF THE HUMAN REGIONALISED BRAIN REVEALS VENTRAL-TO-DORSAL FATE SWITCHING UPON TRANSGENIC EXPRESSION OF THE TRANSCRIPTION FACTOR LMX1A**

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Detailed studies of fate-determining genes in brain development have so far been limited to rodents and other smaller animals, in which it is possible to study the effect of transgenesis in the context of the intact developing brain. In contrast, the importance of fate-determining genes in human brain development remains largely unexplored due to the lack of appropriate model systems. Here, we present a system for studying region-dependent neural fate switches in an in vitro model of the developing human brain by the use of human embryonic stem cells (hESCs). Through dose-dependent inhibition of glycogen synthase 3 (GSK3), we activated WNT signalling and induced gradual caudalisation of our hESC-derived neural progenitors to yield cell fates ranging from telencephalic to posterior hindbrain fates. The authenticity of our regionalized cultures was verified by parallel gene expression analyses in sub-dissected tissue from the human fetal brain. In rodents, the transcription factor Lmx1a has been shown to be involved in the development of mesencephalic dopaminergic (mesDA) neurons, but also in the formation of roof plate, cerebel- lum and choroid plexus. For studying fate-determining effects of Lmx1a in human cells, we used a lentiviral system under control of the pluripotency-associated microRNA miR292 to ensure transgene expression only in the differentiated cell population and not in the pluripotent hESC population. We found that transgenic Lmx1a did not affect neuronalisation of the cells, nor the rostro-caudal identity of the neural progenitors. However, at all levels of the neural axis, Lmx1a suppressed formation of ventral and floor plate fates and instead caused induction of dorsal and roof plate fates, which was
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**ELUCIDATING THE ROLE OF LYSOSOMAL DYSFUNCTION IN THE PATHOLOGY OF PARKINSON’S DISEASE USING HUMAN ADULT INDUCED PLURIPOTENT STEM CELLS DERIVED FROM PATIENTS**


Parkinson's Disease (PD) is a common neurodegenerative disease, affecting more than 1% of the aged population. The identification of several genetic mutations associated with PD has lead to the discovery of important pathological mechanisms, which now have the potential to help better understand the pathology of most common idiopathic forms of PD. Recent clinical evidence has shown an association between mutations in glucocerebrosidase (GBA) and PD. In fact, heterozygous GBA mutations represent the highest risk factor for PD. Accumulation of misfolded proteins is a common pathology in PD, and GBA could be important for protein degradation via the lysosomal pathway. We aim to elucidate the role of heterozygous GBA mutations in the pathology of PD, by focusing on lysosomal dysfunction in the context of autophagy and translate these same mechanisms into the context of idiopathic PD. The Oxford Parkinson’s disease Centre has screened around 600 patients for two common GBA mutations (N370S and L444P). Positive cases (11 N370S and 6 L444P) have been identified by specific restriction enzyme digestion and sequence analysis. Several idiopathic PD patients and age matched healthy controls have also been recruited for this project. Neuronal cultures derived from induced pluripotent stem cells (iPSC) generated from PD patients potentially offer unique insights into the dysfunction of the dopaminergic neuronal network which occurs in PD. Therefore, PD (idiopathic and GBA carriers) and control iPSCs have been generated by retroviral delivery of reprogramming factors (Oct3/4, Sox2, c-Myc, Klf4 and Nanog) to primary dermal fibroblasts. We have optimized an efficient protocol for the specific differentiation of dopaminergic neurons from iPSC cells. Initially, SMAD signalling inhibition (Noggin and SB431542) was applied, followed by neuronalisation using SHH, AA, BDNF and FGF8. Final midbrain maturation was obtained by the addition of cAMP and GDNF. Neurons developed a mature neuronal morphology, with the majority expressing neuronal markers (TuJ1, MAP2, NeuN). Further characterization showed a consistent high expression of TH and other relevant midbrain markers (eg. DAT, En1, Nurr1, Pitx3 and FoxA2). Functional dopamine transporter was confirmed by HPLC uptake assay, and production of dopamine was confirmed by HPLC analysis. Electrophysiology studies also confirmed efficient electrical activity by these cells, presenting a healthy negative resting membrane potential, Na+ and K+ channel activity and development of action potentials when stimulated. Using this stem cell based model we have confirmed the dopaminergic identity of the differentiated cells obtained, and have been working on functional comparison between idiopathic and age-matched control focusing initially on dopamine homeostasis. Potential lysosomal deficits, which may be impaired in both GBA and idiopathic DA neurons, are currently under investigation, looking into levels of lysosomes and autophagosomes production, fusion and transport. Common molecular pathways are likely to be involved in the neurodegenerative process of diverse PD cases, where this relevant human neuronal stem cell based model might be central to understanding the early pathology of PD.

**SMALL MOLECULES ENABLE HIGHLY EFFICIENT NEURONAL CONVERSION OF HUMAN FIBROBLASTS**

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Advances in transcription factor-based cell reprogramming of somatic cells into pluripotent and alternative somatic fates have changed our view of a unidirectional way of cell specification. Results of recent studies indicate that murine and human cells from the mesodermal lineage (fibroblasts) can be directly converted to an ectodermal lineage (neurons) with a cocktail of defined transcription factors. Since neurons are postmitotic, the conversion efficiency represents an important parameter for subsequent biomedical applications such as disease-modeling or neuroregenerative therapies. We set out to explore whether the efficiency of converting human postnatal fibroblasts (HPF) into induced neuronal-like cells (iNs) can be enhanced by small molecules in combination with a minimum number of transcription factors. To that end we combined two factor neuronal programming with small molecule-based inhibition of WNT and BMP signaling. Thereby we were able to generate cells exhibiting morphological, immunocytochemical and functional properties of postmitotic neurons of different neurotransmitter phenotypes with an up to >17fold increase in conversion frequency. Specifically, we could generate iNs with conversion yields exceeding 200% and final neuronal purities of up to >80%. Our data demonstrate that the generation of iNs from HPF can be significantly enhanced using a cocktail of neural inducing factors. We expect this experimental approach to be suitable for the direct generation of bulk quantities of human iNs for disease modeling and other biomedical applications.
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Poster Board Number: T-1027

DEVELOPMENT OF A HUMAN EMBRYONIC STEM CELL-BASED DISEASE MODEL FOR AMYOTROPHIC LATERAL SCLEROSIS.

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Generation of amyotrophic lateral sclerosis (ALS) disease models is an important subject for investigating disease mechanisms and pharmaceutical applications. Transgenic mice expressing mutant form(s) of superoxide dismutase (SOD1) can lead to the development of ALS that closely mimics the familial type of the ALS (FALS). Although SOD1 mutant mice show similar phenotypes to FALS, the differences in species, dissimilar drug responses as well as the size differences limit their usefulness to study the mechanism(s) and identify potential therapeutic compounds. Development of an in vitro model system for ALS is therefore expected to overcome some of the above limitations and help in obtaining novel insights into disease mechanisms and discovery of therapeutics. We established an in vitro FALS model from human embryonic stem cells (hESCs) expressing either a Wild Type (WT) or a mutant SOD1 (G93A) gene and evaluated the phenotypes of the spinal motor neurons (sMN), which are the affected neurons in ALS patients, as well as their susceptibility to degeneration. Transgenic hESC clones were generated by transfection of vectors expressing either a WT or a G93A SOD1 mutant gene. Spinal motor neurons were obtained by a differentiation protocol described earlier [1]. Cell biological assays were performed to evaluate the in vitro FALS model. The in vitro model we developed mimics the in vivo human ALS disease in terms of the 1. Selective degeneration and cell death of spinal motor neurons expressing the G93A SOD1 but not those that express the WT gene, 2. susceptibility of SOD1-derived sMN to form ubiquitin aggregates. We also show the involvement of astrocyte-derived factor(s) in the selective degeneration of the G93A SOD1 mutant sMN. Thus, this model is expected to help unravel to disease mechanisms involved in the development of FALS and also lead to potential drug discoveries based on the prevention of neurodegeneration


Poster Board Number: T-1028

OPTIMIZATION OF NEURONAL CULTURES DERIVED FROM HUMAN INDUCIBLE PLURIPOTENT STEM CELLS FOR HIGH THROUGHPUT FUNCTIONAL SYNAPTIC ACTIVITY ASSAY

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Background: We previously described the development of a high-throughput screening technology for identifying modulators of synaptic function, termed the MANTRA™ (Multwell Automated NeuroTransmission Assay) system (Hempel CM et al., 2011). We have demonstrated the utility of the MANTRA system for performing assays of synaptic vesicle cycling in rodent primary neuronal cultures in 96-well plates. Here, we show that cultures of human neurons derived from induced pluripotent stem cells (iPSCs) can be utilized in the MANTRA system for synaptic functional assays. Results were compared to those generated from rat primary neuronal cultures. Methodology: Post-mitotic human neurons derived from iPSCs (“iCell® Neurons”, Cellular Dynamics International, USA) and primary neuronal cultures isolated from rat embryos were seeded in 96-well plates (Greiner) coated with poly-D-lysine with or without laminin. For some experiments, iCell Neurons or rat neurons were cultured with astrocytes grown as a monolayer. iCell Neurons and rat neuronal cultures were seeded on the same plates and tested in parallel. For analysis of ability of neurons to initiate action potentials following field stimulation, neurons were loaded with Fluo-4. For analysis of presynaptic function, cultures were infected with an adeno-associated virus (AAV) used to deliver a synaptophysin-pHluorin fusion fluorescent reporter construct (sypHy). Cultures were analyzed between 2 and 7 weeks in vitro on the MANTRA system or on a fluorescence microscope imaging system. For both systems, fluorescence imaging was performed in parallel with field stimulation trains. Immunofluorescence analysis was performed at different time points to evaluate the expression and localization of presynaptic proteins and the sypHy reporter. Results: We found that iCell Neurons exhibit expected neuronal and synaptic morphology and can be reliably transduced with AAV to express sypHy as early as 2 weeks in vitro with no signs of cytotoxicity. Immunostaining analysis revealed that sypHy is expressed in iCell Neurons in a punctuate pattern and is co-localized with synapsin I. iCell Neurons showed robust Ca2+ responses to field stimulation trains with voltage thresholds similar to those of rat neurons, suggesting reliable generation of action potentials. iCell Neurons at 3-6 weeks in vitro displayed measurable levels of evoked presynaptic activity on the MANTRA system. Follow up high-resolution microscope analysis confirmed the synaptic localization of sypHy signals in iCell Neurons. Although pre-synaptic responses were lower in iCell Neurons than in rat neuronal cultures several conditions were found to increase the response levels. Treatment of iCell Neurons for 1 hour with a compound known to increase evoked pre-synaptic activity yielded similar presynaptic effects on human iCell Neurons and rodent primary neuronal cultures. Also, co-culturing of iCell Neurons with glia resulted in a robust increase in evoked presynaptic responses. Conclusions: The MANTRA system can measure synaptic activity in human neurons derived from iPSCs. The high-throughput capacity of this system provides a unique capability to test multiple conditions in parallel to generate human iPSC-derived neurons with optimal synaptic functionality. Ultimately, the MANTRA system can be used to characterize synaptic abnormalities in neurons derived from patients and to screen for compounds to restore normal synaptic transmission.

Poster Board Number: T-1029

CD81 IS HUB REGULATOR IN HUMAN NEURAL STEM CELL MIGRATION

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Tetraspanin proteins have been implicated in many cellular activities, including adhesion, migration, and proliferation, however their exact function is unknown. Specifically, CD81 (TAPA-1) has been involved in cell migration. But mechanisms are poorly defined in any of stem cells. We describe a novel mechanism whereby the CD81 can affect cell motility and induce migration onto ECM substrates in HNSC (Human Neural Stem Cell). RT-PCR and Western-blot analysis confirmed CD81 expression in HNSC. Immunoprecipitation analysis confirmed CD81 interactions with MMP-9 and integrin beta 1 in HNSC. Also, the expression of the CD81, MMP-9 and integrin beta1 correlated with the HNSC migration. These results demonstrate
that CD81 is key receptor in HNSC migration. The shRNA-mediated CD81 downregulation effectively reduced MMP-9 binding to the cell surface, which inhibited the migration of HNSC. Also, we performed Western-blot. That results show that CD81 stimulates the activity of ERK/MAPKinase in HNSC. Linked with this event, we observed an increase in CD81-associated phosphatidylinositol 3-kinases activity and mTOR. Our results demonstrate that CD81 as a cell surface binding partner for MMP-9, regulating cell migration and adhesion via integrin beta1 modulation of tetraspanin/integrin signaling complex. Also, it is well established that CD81 is Hub regulator in cell migration in HNSC cell migration.

Poster Board Number: T-1030

HUMAN CORD BLOOD DERIVED MULTIPOTENT STEM CELLS (CB-SC) TREATED WITH ALL TRANS RETINOIC ACID (ATRA) GIVE RISE TO DOPAMINE NEURONS

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Parkinson’s disease (PD) results from the chronic degeneration of dopaminergic neurons. A replacement for these neurons has the potential to provide a clinical cure and/or lastingly treatment for symptoms of the disease. Human cord blood-derived multipotent stem cells (CB-SCs) display embryonic stem cell characteristics, including multi-potential differentiation. To explore their therapeutic potential in PD, we examined whether CB-SCs could be induced to differentiate into dopamine neurons in the presence of all-trans retinoic acid (ATRA). Prior to treatment, CB-SCs expressed mRNA and protein for the key dopaminergic transcription factors Nurr1, Wnt1, and En1. Following treatment with 10μM ATRA for 12 days, CB-SCs displayed elongated neuronal-like morphologies. Immunocytochemistry revealed that 48±11% of ATRA-treated cells were positive for tyrosine hydroxylase (TH), and 36±9% of cells were positive for dopamine transporter (DAT). In contrast, control CB-SCs (culture medium only) expressed only background levels of TH and DAT. Finally, ATRA-treated CB-SCs challenged with potassium release increased levels of dopamine compared to control. These data demonstrate that ATRA induces differentiation of CB-SCs into dopaminergic neurons. This finding may lead to the development of an alternative approach to stem cell therapy for Parkinson's disease.

Poster Board Number: T-1031

VISUALIZING GLIOMA CELL ENGRAFTMENT TIME COURSE AND HUMAN NEURAL STEM CELL HOMING IN A XENOGRAFT MODEL USING PARAFFIN-EMBEDDED SERIAL SECTIONS.

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Studies of tumor cell engraftment and therapeutic efficacy in mouse xenograft glioma models commonly examine small tumor areas from a limited number of mice, despite evidence of considerable heterogeneity within tumors and between recipient mice. To be able to image entire tumors across many animals, we established procedures using conventional histological techniques by which engrafted tumors and neural stem cells (NSCs) could be analyzed in their entirety across multiple test animals using paraffin-sectioned material prepared by our local pathology core. Briefly, brains harboring engrafted tumors and associated NSCs were sectioned over the entire tumor. These sections were processed for chromogenic identification of tumor cells and NSCs, and slides spanning the entire tumor were automatically scanned at high resolution. A subsample of sections alternately stained for tumor and NSC markers were aligned, and segmented into tumor- and NSC-specific channels by computational color deconvolution. Algorithms developed for quantifying numbers of tumor- and NSC-occupied voxels were then used to quantify tumor volumes, numbers of NSCs, and predicted tumor coverage of NSC-delivered therapeutic agents. We used these procedures to analyze the engraftment time course of a tumor model originating from patient-derived tumor cells expanded in short-term culture, comparing histological results to those obtained by MR and bioluminescence imaging. We then examined the distributions of NSCs that intrinsically home to tumor sites following intracranial or intravascular administration. Low passage expanded patient-derived brain tumor cells (PBT017) were orthotopically implanted in brains of adult immunodeficient (SCID) mice. Engraftment kinetics were tracked by immunohistochemistry, and by MR and bioluminescence imaging, at time points between 1 and 9 weeks post-implantation. By all three measures tumor growth accelerated after the first 20-30 days, and tumor volume increased approximately 100-fold over the first 60 days. Ferumoxytol (iron oxide nanoparticle)-labeled cells from immortalized NSC line (HB1.F3.CL) were injected either by intracranial (i.c.) or intravascular (i.v.) routes, and homing to three week-engrafted tumors was visualized histologically four days post-NSC implantation by Prussian Blue staining. We found that NSC distributions varied with location of intracranial injection, and, for both i.c. and i.v. administrations, with tumor density and dispersion. Further progress in this area will involve sampling additional time points, examining tumor and brain heterogeneities related to these differences, and identifying signals to which the NSCs are responding.

Poster Board Number: T-1032

MODELING SPINAL MUSCULAR ATROPHY BY KNOCKING DOWN THE DISEASE-DETERMINING GENE IN HUMAN EMBRYONIC STEM CELLS

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Establishing human cell models of spinal muscular atrophy (SMA) to mimic motor neuron-specific phenotypes holds the key to understanding the pathogenesis of SMA. Here, we established human embryonic stem cell lines with the knockdown of the disease-determining gene, survival motor neuron (SMN). Neither neuronal induction nor spinal motor neuron specification is affected by knocking down of SMN. Notably, knocking down of SMN-full length (SMN-FL), but not SMN-delta 7 (lacking exon 7), results in impaired axonal outgrowth and subsequent neuronal degeneration. Moreover, all these phenotypes are specific to spinal motor neurons and can be rescued by restoring the expression of SMN-FL. Finally, in human motor neuron progenitors before the phenotype is observed, knockdown of SMN-FL leads to the increases in mitochondria oxidative stress and apoptosis, suggesting the involvement of these processes in the pathogenesis of SMA. Taken together, we demonstrate the successful establishment of a human SMA model, which exhibits disease gene isoform specificity, cell type specificity, and phenotype reversibility. This model provides a unique system to study the SMN gene function in human motor neurons and to explore how motor neurons are specifically degenerated in SMA.
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Poster Board Number: T-1033

SUSTAINED CLINICAL RECOVERY FOLLOWING INTRASPINAL TRANSPLANTATION OF HUMAN EMBRYONIC STEM CELL-DERIVED NEURAL PROGENITOR CELLS INTO A VIRAL MODEL OF MULTIPLE SCLEROSIS

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Transplantation of human neural progenitor cells (hNPCs) is a promising strategy for the treatment of demyelinating diseases. In this study, we surgically transplanted hNPCs differentiated from the human embryonic stem cell line WA09 into the spinal cords of mice persistently infected with the neurotropic JHM strain of mouse hepatitis virus (JHMV) in which demyelination and clinical disease were established. Clinical recovery was evident in >70% of JHMV-infected mice as early as day 8 post-transplant (p.t.) and was sustained up to 6 months. In contrast, transplantation of human fibroblasts did not result in any clinical improvement. At 7 days p.t., hNPCs were not detectable by immunohistochemistry within the CNS and natural killer cells, microglia, and neutrophils were aggregated at the implantation site, suggesting that rejection of hNPCs was mediated by the innate immune response. Mice were sacrificed at 21, 90, and 180 days p.t. and the effects on spinal cord pathology and neuroinflammation were evaluated. The hNPC treatment dramatically reduced both the spread of demyelination (as determined by Luxol fast blue staining) and neuroinflammation compared to control mice. Flow analysis and immunohistochemical staining revealed significantly less T cell infiltration and macrophage/microglia activation within the spinal cords of hNPC-transplanted mice compared to control mice. Co-culture of hNPCs with T cells purified from JHMV-infected mice resulted in dose-dependent dampening of T cell proliferation. Collectively, our results indicate that hNPCs are immunosuppressive and highlight that intraspinal instillation mutates the severity of neuroinflammation and demyelination, resulting in prolonged clinical recovery. These observations provide further support for hNPCs in treating inflammatory neurodegenerative diseases such as MS.

Poster Board Number: T-1034

CHANGE IN FUNCTIONAL PURINERGIC SIGNALING WITH COMMITMENT TO THE NEURONAL LINEAGE IN HUMAN FETAL CORTICAL NEURAL PRECURSOR CELLS

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Adenosine triphosphate (ATP) signals via P2X and P2Y purinergic receptors, and has important roles in neural stem cell (NSC) proliferation and differentiation in rodents. During neural differentiation, ATP release decreases and purinergic receptor expression is downregulated, leading us to hypothesize purinergic signaling may have significant roles in human neurodevelopment. This study used calcium (Ca2+) imaging of cultured human neural precursor cells (hNPCs) in adherent neurosphere monolayers from human fetal cerebral, hNPCs showed a rapid, substantial increase in intracellular Ca2+ ([Ca2+]i) in response to ATP, adenosine diphosphate (ADP), 2-methylthioATP (2-MeSATP), uridine triphosphate (UTP) and uridine diphosphate (UDP). The response was predominantly from thapsigargin-sensitive intracellular stores. ATP-stimulated cells showed 3 distinct responses (single, double or multiple [Ca2+]i rises); the latter an oscillatory response which propagated between adjacent cells. Post Ca2+-imaging immunostaining revealed ADP and ATP evoked oscillations in immature nestin+/s100β+/βIII-tubulin+ hNPCs whereas nestin+/s100β+/βIII-tubulin+ immature neurons never oscillated and showed lower responses. Application of the P2Y1-receptor antagonist (MRS2179) reduced the ADP response and oscillations. The P2X1/P2X3 agonist (α,β-methylene ATP) and the P2X7 agonist (BzATP) showed low to moderate responses. The delayed BzATP response was characteristic of P2X7 receptor pore opening, and two specific P2X7 receptor antagonists significantly reduced both responses, confirming the specificity of the response to the P2X7 receptor. Immunostaining confirmed the expression of P2Y1, P2X1, P2X3 and P2X7 receptors. This is the first identification of functional P2Y and P2X receptors in hNPCs and immature neurons, and provides unique insights into the developmental changes which occur with commitment to the neuronal lineage.

Poster Board Number: T-1035

BAC-BASED ASSESSMENT OF PROTEIN-PROTEIN INTERACTIONS IN HUMAN PLURIPOTENT STEM CELL-DERIVED NEURAL STEM CELLS AND THEIR NEURONAL PROGENY

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Protein interaction studies represent a powerful tool to study cell signaling cascades, cell-cell interactions as well as principles of signal transduction mechanisms. Up to today, the majority of human interactome studies were conducted based on overexpression paradigms in tumor cell lines. Commonly encountered problems in this context are unspecific interactions due to supra-physiological protein expression levels, the use of transformed cells and a non-tissue specific proteome. Ideally, protein-protein interaction data should be generated in the appropriate tissue-specific somatic cell expressing the protein of interest at endogenous expression levels. Here we used pluripotent stem cell-derived neural stem cells (lt-NES®; Koch et al., PNAS 106:3225-30, 2009) as a somatic stem cell population, which exhibits extensive self-renewal, clonogeneity and stable neurogenesis. The introduction of GFP-tagged proteins via bacterial artificial chromosomes (BAC; Poser et al., Nat. Meth. 5:409-15, 2008) permitted the derivation of large numbers of polyclonal cell populations (pools) with faithful protein expression in more than 90% of the cells, average integration rates of one locus per genome as well as correct size and compartmentalization of the tagged variants as detected by Western Blot analysis and high-resolution live cell imaging. Using this technique, we generated multiple cell lines harboring tagged proteins including PCNA, JARID1C, AURKA, CDK2AP1, RBPJ, RUVBL2, the Methyl CpG Binding Protein 2 (MECP2) involved in the pathogenesis of Rett syndrome and the Alzheimer’s disease-associated proteins Nicastrin (NCSTN) and Valosin-containing protein (VCP). Using
protein-protein interaction studies with a label-free, quantitative affinity purification-mass spectrometry approach (Hubner et al., J Cell Biol 189:739-54, 2010) we identified several known complexes as well as novel interaction candidates of these proteins. The system is sufficiently sensitive to report changes in protein expression levels and compartmentalization during It-NES® cell differentiation and after exposure to extrinsic factors such as inhibitors of proliferation and modulators of cell signaling. Furthermore, we were able to transfer this technique to iPSC cell-derived It-NES® cells from patients with Machado-Joseph disease (UbC, VCP) and Alzheimer’s disease (VCP, NCSTN). Our data suggest that protein tagging in PSC-derived It-NES® cells and their neuronal progeny represents an efficient approach for studying protein-protein interactions in human neural cells both in normal and neurodegenerative contexts.

Poster Board Number: T-1036

GABAERGIC NEURONS DURING EARLY HUMAN BRAIN DEVELOPMENT

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Human forebrain GABAergic neurons comprise of those carrying various biochemical and transmitter phenotypes, including DARPP32 (Dopamine and Adenosine 3’5’ Monophosphate Regulated Phosphoprotein), somatostatin (SO)M, parvalbumin (PV), and calretinin (CR). They play versatile role in brain functions. It is not clear if these subtypes of GABA neurons are derived from neural progenitors in distinct regions during human development. At 7-8 gestational weeks, GABAergic neurons were observed in the ventral telencephalon at the lateral ganglionic eminence (LGE). These GABAergic projection neurons co-express Islet1 and DARPP32 but PV and CR. Interestingly, we also observed a group of GABA neurons at the corticostriatal border that express the cortical transcription factors Pax6. A small part of these Pax6 immunoreactive neurons also express DARPP32. By 8 gestational week, we began to observe GABA neurons in MGE, LGE, and they co-express NKX2.1. Later, we found a group of GABA neurons migrate to cortical plate coexpressing NKX2.1 but Pax6. It suggested that althoug most part of the GABAergic originate from ventral telencephalon, there is a small part of GABAergic neurons born from dorsal origin.

Poster Board Number: T-1037

HUMAN NEUROEPITHELIAL STEM (NES) CELLS -A STANDARDISED SYSTEM FOR COMPARISON STUDIES

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Robust and standardised cultures of human stem cells provide a platform for comparison studies. Human NeuroEpithelial Stem (NES) cells can be derived from embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. Interestingly, NES cells can also be captured directly from the foetal human brain demonstrating their existent during development. NES cells of different origins display comparable characteristics including long-term proliferation in culture without loosing their ability to differentiate to functional neurons and glia. NES cells have a hindbrain identity and a characteristic transcription factor profile. The consistency between NES cells of various origins make them an interesting tool for comparative analyses of neural cells from control and diseased genetic backgrounds. NES cells are expanded in EGF and FGF2 supplemented media over 100 passages maintaining a stable karyotype and a neuronal differentiation potential creating up to 90% neurons upon growth factor withdrawal. NES cells grow with a karyotype and a neuronal differentiation potential creating up to 90% neurons upon growth factor withdrawal. NES cells grow with a karyotype and a neuronal differentiation potential creating up to 90% neurons upon growth factor withdrawal. NES cells grow with a karyotype and a neuronal differentiation potential creating up to 90% neurons upon growth factor withdrawal.

Poster Board Number: T-1039

IN VITRO AND IN VIVO DIFFERENTIATION OF HUMAN FETAL NEURAL PROGENITORS.

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Introduction: Neural stem cells (NSC) reside within the central nervous system (CNS), and have been propagated as neurospheres (NS) in culture. They are intended for therapy particularly in neurodegenerative diseases which are of increasing prevalence due to aging populations in developed countries. NSC have been isolated from several parts of the developing human fetal brain and here, we attempt to profile these cells for CD133 expression as well as investigate both their in vitro and in vivo differentiation potential. Methods: Fetal brain tissues were collected from medically-induced pregnancy terminations between 13-15 weeks gestation, with informed consent. Tissues from second trimester subventricular zone, hippocampus, anterior and posterior cerebra, thalamus, cerebellum, brain stem and spinal cord were minced and dissociated enzymatically (n=4). Serum-free NS culture was performed over 2-4 weeks, and CD133 profiling performed at every passage. Neurospheres at passage 1 were subcultured and placed into differentiating medium to assess in vitro dopaminergic differentiation. Concurrently, NS were also transduced green and 15X103 cells were injected into CD1 pup at E14 to determine their in vivo differentiation. Immunocytochemistry for MAP2ab, tyrosine hydroxylase (TH) on the cultured cells and nestin, βIII-tubulin, glial fibrillary acidic protein (GFAP) on the animal brain sections were performed to determine final identity of differentiated cells. Results: We isolated NS from cells derived from the different regions of the human fetal brain. Expression of CD133 at point of harvest ranged from 0% and 12.5% which was observed in the spinal cord. At passage 1, expression of CD133 then ranged from 0% to 30.9% which was observed in the cerebrum. Following that, a sharp decrease in expression of CD133 was observed which continued through passage 3. In vitro, dopaminergic differentiation can be observed from all cultures derived from all regions of the fetal brain and proportion of MAP2ab+TH+ cells ranged from 2.4±1.2% in cultures from anterior cerebrum to 34.4±8.8% in cultures differentiated from brain stem-derived NSC. From the in vivo data, successful engraftment of labelled human neural progenitors was observed in 1 out of the 7 pups injected. Engraftment was observed 4 weeks post injection and stained brain sections demonstrated the differentiation of the human neural stem/progenitors into GFAP+ glial cells and the absence of undifferentiated nestin+ human neural stem/progenitors. Conclusion: If CD133 is a bona fide neural stem/progenitor marker, our data suggests the greatest proportion of neural stem/progenitors at passage 1 of NS cultures. Our differentiation studies demonstrated the successful differentiation of these human fetal neural stem/progenitors into dopaminergic neurons in vitro and glial cells in vivo.
Introduction: Commitment of neural stem cells to differentiate into functional neuronal cells is an essential step in pursuing cell therapy for neurological disorders. Neuronal differentiation (ND) involves many endogenous and exogenous steps such as cell-cycles, intracellular signals, cell structural proteins and cell shape alterations. The previously reported results suggest that both miRNA let7s and RhoA expressions play critical roles on ND. Lower levels of RhoA and Rho GTpases mediate cadherin adhesion molecules to up-regulate stem cell proliferation and expansion, whereas high levels of RhoA expression affect ND. Data from in vitro studies have shown that ND can be directly derived from embryonic stem cell cultures after manipulation of miRNA let7s expression. However, there are a few reports addressing the mechanism of endogenous cultures after manipulation of miRNA let7s expression. It is hypothesized that both miRNA let7s and RhoA initiate and govern final ND from HBSC also signals regulating the morphological and genetic changes of human brain neural stem cell (HBSC) during ND. Furthermore, how miRNA let7 and RhoA initiate and govern final ND from HBSC also remains unclear. The purpose of this study was to observe the ND from HBSC culture treatment by the miRNA let7/RhoA expression enhancers such as β-1 integrin and EphrinA1 respectively. Results: To further understanding, the molecular mechanism of ND from HBSC (nestin’/CD133’) gene and protein expression profiles of HBSC treated with or without β-1 integrin and EphrinA1 were analyzed using cDNA microarray representing 11,000 mRNAs. The significant alterations in RhoA and RhoA-Rho kinase (RAK) expression levels were also confirmed using RT-PCR, Northern blotting and protein assays. OCT4, SOX2 and Nanog were expressed differently between the cell treated with and without β-1 integrin and EphrinA1. Compared with non-treated cultures, both RhoA and RAK genes were up-regulated respectively (4 to 7 times respectively) after β-1 integrin and EphrinA1 treatment. We also found that both OCT4 and SOX2 expressions increase following elevated Rho/RAK expression. Neuronal specific protein markers (such as Hu, Neu-N, NF-L, N-cadherin, β-III Tubulin and MAP-2) were positively detected in the up-regulated RhoA cultures. In contrast, suppression of the RhoA and RAK mRNA expression with anti-RhoA/RAK antisenses significantly suppresses the ND. These data suggest that the RhoA/RAK signaling pathway is critical for ND differentiation. However, to confirm that the differentiated cells are the functional neuronal cells will require further electronic physiological functional test.

Material Methods: Cell culture Protocols for human fetal brain cell cultures The transcription levels of nestin and neuronal specific protein makers were analyzed by reverse transcriptase (RT)-PCR and immunocytochemistry stains.
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**Poster Board Number: T-1043**

**ELEVATION OF REACTIVE OXYGEN SPECIES STIMULATES THE MIGRATION OF IRRADIATED MOUSE AND HUMAN NEURAL PROGENITOR CELLS**

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Neural progenitor cells (NPCs) migration is an essential process for brain development. Whereas in vivo studies report abnormal neuronal migration in cerebral cortex in mice prenatally exposed to ionizing radiation, little is known on the consequences of irradiation on NPCs migration. In this study, we showed that, twenty-four hours after irradiation, wild-type cortical mouse NPCs (mNPCs), as well as human NPCs (hNPCs), displayed an enhanced migration velocity associated with an elevated level of Reactive Oxygen Species (ROS) compared to non-irradiated controls. Similar increase of migration was observed in non-irradiated Rad54 deficient mNPCs, cells that spontaneously exhibit a high endogenous ROS level compared to wild type mNPCs. In these different cellular models, the antioxidant NAC treatment restored migration capacities similar to that observed in non-irradiated wild-type NPCs. These data suggest that the oxidative stress generated after a γ-irradiation or endogenously found in Rad54 deficient NPCs, is able to stimulate migration of NPCs cells. Using NPCs from WT/Fucci and Rad54/Fucci mice, that permit us to visualize nuclei in S/G2 or in G1 phase respectively by green or red fluorescence, we found that the increase of migration velocities reported in irradiated NPCs or in Rad54 mNPCs was not associated with a modification of cell cycle progression. Lastly, we evaluated the effect of γ-irradiation on NPCs migration on organotypic brain slice and after intracranial transplantation of GFP NPCs. These in vivo experiments definitively showed that oxidative stress resulting from γ-irradiation enhanced migration of both mouse and human embryonic neural progenitor cells.

**Poster Board Number: T-1044**

**CELL LINEAGE TRACING OF SOX2 EXPRESSING CELLS IN THE ADULT MOUSE BRAIN**

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Sox2 encodes a transcription factor which plays important roles in regulating neural development. It is also believed that Sox2 is one of the neural stem cell markers in the adult brain. There are two major neurogenesis zones in the mouse brain, which include subgranular zone of the hippocampal dentate gyrus and subventricular zone of lateral ventricle. Here we take a genetic approach to label Sox2 positive cells in the adult mice brain by crossing a Sox2-CreERT2 transgenic mouse with an inducible organelle specific dual fluorescent protein reporter mouse (R26RGR). The dual fluorescent proteins are encoded by an inducible transgenic construct (H2B-EGFP-2A-mCherry-GPI) placed in the Rosa26 locus. The H2B-EGFP encodes a histone protein fusion with an enhance green fluorescent protein which allows us to observe chromatin in the nucleus, providing cell cycle information including mitosis, while mCherry-GPI encodes red fluorescent membrane anchored protein depicting cellular locations and morphologies. Under the induction of tamoxifen at the adult stage, we can label Sox2 positive cells, putative neural stem cells and trace their descendents in vivo.
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transgenic mice, mated with Rosa26 transgenic mice to detect β-galactosidase activity, also exhibited the same expression pattern as F1B-GFP mice. Taken together, we successfully used mouse as an animal model to express human gene promoter, F1B, and the F1B-GFP+ cells are ependymal and neural cells. This F1B-GFP transgenic mouse might provide a novel tool to understand FGF1 function in brain development and in related diseases.

Poster Board Number: T-1046

ENHANCED IL-1BETA PRODUCTION IN RESPONSE TO THE ACTIVATION OF HIPPOCAMPAL GLIAL CELLS IMPAIRS NEUROGENESIS IN AGED MICE

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A variety of mechanisms that contribute to the accumulation of age-related damage and the resulting brain dysfunction have been identified. Recently, decreased neurogenesis in the hippocampus has been recognized as one of the mechanisms of age-related brain dysfunction. However, the molecular mechanism of decreased neurogenesis with aging is still unclear. In the present study, we investigated whether aging decreases neurogenesis accompanied by the activation of microglia and astrocytes, which increases the expression of IL-1beta in the hippocampus, and whether in vitro treatment with IL-1beta in neural stem cells directly impairs neurogenesis. Ionized calcium-binding adaptor molecule 1 (Iba1)-positive microglia and glial fibrillary acidic protein (GFAP)-positive astrocytes were increased in the dentate gyrus of the hippocampus of 28-month-old mice. Furthermore, the mRNA level of IL-1beta was significantly increased without related histone modifications. Moreover, a significant increase in jasmin 9 on histone H3 (H3K9) trimethylation at the promoter of NeuroD (a neural progenitor cell marker) was observed in the hippocampus of aged mice. In vitro treatment with IL-1beta in neural stem cells prepared from whole brain of E14.5 mice significantly increased H3K9 trimethylation at the NeuroD promoter. These findings suggest that aging may decrease hippocampal neurogenesis via epigenetic modifications accompanied by the activation of microglia and astrocytes with the increased expression of IL-1beta in the hippocampus.

Poster Board Number: T-1047

INDUCED NEURONAL CELLS FROM AGING MOUSE FIBROBLASTS

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Defined sets of transcription factors have recently been used to convert mouse and human fibroblasts into cells with neuronal characteristics. These induced neuronal cells (iN cells) show all hallmarks of normal mature neurons. However, it is still unknown if iN cells can be generated from aged cells and whether there are any changes in reprogramming efficiency or function of aging iN cells. Aging is associated with declined cognitive function, changes in neural plasticity and reduced neurogenesis. On the other hand it is difficult to study intrinsic neuronal properties of aged mammals. It is virtually impossible to culture aged neurons; it is hard to isolate them in high purity and challenging to assess their electrophysiological properties. It would therefore be desirable to generate aged iN cells in order to study age related changes in neurons. Here we describe the generation of iN cells from aging, postnatal to aged 25 month old, mouse fibroblasts. Tail derived fibroblast cultures were established from postnatal, 4 day old and 3, 10, 18 and 25 month old mice. Fibroblasts were infected with doxycycline-inducible lentiviral vectors carrying Brn2, Ascl1 and Mety11 and were assayed for iN cell generation and function at 2-3 weeks after transgene induction. iN cells were generated at similar efficiencies from adult to aged fibroblast. The efficiency was however lower as compared to those generated from embryonic fibroblasts. Aging iN cells were positive for pan neuronal markers Tuji and Map2. They displayed mature neuronal morphologies without any change in complexity between age groups as assessed by neurite tracing. To probe the functional properties of these iN cells, we next performed current-clamp experiments. iN cells derived from different ages had similar resting membrane potential, input resistance, and capacitance. Upon current injection, iN cells from different ages generated single or multiple action potentials with similar efficiency. Furthermore, the action potential threshold or amplitude did not change significantly across all ages tested. Finally, we performed voltage-clamp experiments to measure sodium and potassium conductance of the iN cells and found no difference between the ages. We here show that iN cells can be generated from aged mouse fibroblast and suggest that these findings may be useful for studying neuronal aging and to model age related changes in neurogenesis.

Poster Board Number: T-1048

EFFICIENT GENERATION AND DEVELOPMENTAL ANALYSIS OF BASAL FOREBRAIN CHOLINERGIC NEURONS FROM MOUSE EMBRYONIC STEM CELLS

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The differentiation mechanisms of basal forebrain cholinergic neurons (BFCN) in early neural development are of special interest, because dysfunctions of BFCN have been implicated to be involved in cognitive impairments including Alzheimer’s disease. However, little is known about the underlying mechanism of the development of early-born BFCN. For the analysis of the development of BFCN, we investigated the roles of Lhx8, which is expressed in medial ganglionic eminence (MGE) and is known to positively regulate cholinergic differentiation, using an in vitro embryonic stem (ES) cell differentiation system to induce BFCN. To obtain BFCN from mouse ES cells, we first differentiated ES cells as serum-free, floating culture of embryoid body-like aggregates (SFEB) with Dkk1. Dkk1 not only increased the number of Sox1-GFP+ cells in SFEB but also up-regulated the expression of forebrain marker, Foxg1, and a MGE marker, Nkx2.1. Thus, we then dissociated Dkk1-treated SFEBs into single cells and cultured them in suspension with FGF-2 to form neurospheres and to differentiate them into BFCN. Immunocytochemical analysis showed efficient differentiation into βIII-tubulin/choline acetyltransferase (ChAT) double positive BFCN. Also, NGF increased the number of ChAT-positive BFCN, and the expression of βIII-tubulin/choline acetyltransferase was increased 40-fold, suggesting that our in vitro system is useful for generating ChAT-positive BFCN from mouse ES cells. Next, we carried out lentivirus-mediated knockdown of Lhx8 and Lhx6 by short-hairpin RNAs (shRNAs) during neurosphere formation in our in vitro system. Consistent with the previous reports showing a significant decrease in the number of BFCN in Lhx8-null mice, in our system the expression of ChAT was decreased by the knockdown of Lhx8 under differentiation conditions. These results suggest that the dif-
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ferentiation potential into BFCN is positively regulated by Lhx8. To identify the downstream transcription factors of Lhx8, we collected GFP-positive cells in neurospheres by fluorescence-activated cell sorting and performed microarray analysis comparing cells infected with Lhx8-shRNA to those infected with a control virus. As a result, we identified 963 probes (819 genes) whose expression levels changed more than two-folds by the knockdown of Lhx8. Among these, we identified 54 transcription factors by gene ontology analysis. Also, Lhx8 is expressed at 114-fold higher level in neural progenitors prepared from forebrain at E11.5 compared with those at E14.5; therefore we picked up 31 genes highly expressed in E11.5 neural progenitors. Future studies should include the identification of downstream transcription factors of Lhx8 by investigating the roles of these genes in the development of BFCN.

Poster Board Number: T-1049

JARID 2 REGULATION OF MURINE POSTNATAL SUBEPENDYMAL ZONE NEUROGENESIS

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The murine subependymal zone (SEZ) contains stem cells and produces daily tens of thousands of neurons. However, the mechanisms of NEUROGENESIS IN THE SUBEPENDYMAL ZONE maintaining balance between self-renewal and differentiation are still not clear.

To understand the role of epigenetic factors in this process, we are investigating whether Jarid2/Jumonji is necessary for regulating self-renewal and multipotency of SEZ NSPCs. Jarid2 interacts with polycomb repressive complex 2 (PRC2) in embryonic stem cells to regulate the balance between self-renewal and differentiation by modulating pluripotency genes. The expression level of Jarid2 and PRC2 core components such as Ezh2, Eed and Suz12 in postnatal mouse SEZ NSPCs was previously unknown; therefore, we have now shown their expression in vivo and in vitro as neurospheres with qRT-PCR and Western blot. Using lentiviral-delivered shRNAs in the SEZ neurospheres, we found Jarid2 knockdown increased the number of neurospheres but decreased their diameter and the number of differentiated cells. Eed knockdown in neurospheres, however, did not show any significant difference in their self-renewal and multipotency, compared to the scrambled control. Our data suggest that Jarid2 is necessary for SEZ NSPC differentiation. In addition, different neurosphere phenotypes after knocking down Jarid2 and Eed imply Jarid2 may have as yet unknown PRC2-independent roles to govern the self-renewal and differentiation of SEZ NSPCs. Understanding the role of epigenetic factors in the context of NSPC self-renewal and differentiation has important implication for the development of neural stem cell-based therapies.

Poster Board Number: T-1050

INVESTIGATION OF THE OPTIMAL TRANSPANTATION SITE OF FETAL NEURAL STEM/PROGENITOR CELLS FOR SPINAL CORD INJURY IN ADULT MICE.

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There were many reports that transplantation of neural stem/progenitor cells (NS/PCs) promoted functional recovery in several animal models of spinal cord injury (SCI) and we have recently reported ed intra-lesional transplantation of NS/PCs was the most effective compared to the other procedures; intra-thecal and intra-venous transplantation. However, there have been no report to determine where exactly the best transplantation site is, because previous researchers grafted NS/PCs into various sites of injured spinal cord; epicenter, rostral and caudal sites to the lesion. The purpose of this study is to determine the optimal transplantation site of NS/PCs for SCI in adult mice. Adult female C57BL/6J mice received 60 kdyn contusion injuries using the Infinite Horizon Impactor after laminectomy at the Th10 level. 9 days after SCI, NS/PCs derived from the fetal transgenic mice, which ubiquitously expressed Venus and Luciferase, were transplanted into the injured spinal cord at different sites: the lesion epicenter (E-group), and 1 mm rostral and caudal sites from the rim of the lesion (RC-group). Survival of NS/PCs was quantitatively evaluated using in vivo bioluminescence imaging. Motor function of hindlimbs was assessed by Basso Mouse Scale (BMS) for 6 weeks after SCI. Thereafter, the animals were sacrificed and immunohistochemical staining was processed for histological analysis. There was no difference in BMS between the E-group and RC-group. The final photon counts of the grafted NS/PCs converged within a narrow range in the both groups, independent with the initial photon counts. Grafted NS/PCs differentiated into neurons, astrocytes and oligodendrocytes. In the RC-group, grafted NS/PCs differentiated into more neurons and less astrocytes compared to the E-group. There was no significant difference in the distribution of the transplanted cells, positive areas of Neurofilament 200kDa (NF-H) and platelet endothelial cell adhesion molecule-1 (PECAM-1) between the both groups. The final photon counts were convergent and independent with the initial photon counts, suggesting that the survival of grafted NS/PCs was fairly constant if more than a certain number of the cells were transplanted. Although the differentiation pattern of the grafted NS/PCs were different between the two groups, there was no significant difference in the functional recovery and the final survival of NS/PCs. Therefore, it is estimated that there would be no difference in microenvironment between the epicenter, rostral and caudal sites at the sub-acute phase of SCI. Collectively, we conclude that the optimal transplantation application is to transplant more than a certain number of NS/PCs into the epicenter of injured spinal cord at the sub-acute phase, considering the additional damage of the intact spinal at the site to the lesion epicenter by the injection procedure.

Poster Board Number: T-1051

MODULATION OF NEURAL STEM CELL PROLIFERATION BY THE EXOGENOUS GENE EXPRESSION ENHANCED BRAIN RECOVERY IN A RAT MODEL OF ISCHEMIC STROKE

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Brain repair after ischemic injury largely depends upon neurogenesis of endogenous neural stem cells, suggesting that enhancement of neural stem cell proliferation would be a strategy for treating many brain diseases. In a series of experiments, we tested if several secreted proteins potentially modify brain microenvironment in a favorable way for neurogenesis and brain repair. For this, we over-expressed the candidate proteins in the ischemic penumbral area of Sprague-Dawley rats 3 days before transient middle cerebral artery occlusion (MCAO). In another set of experiment, the secreted factors were delivered into the brain 1 week after MCAO which corresponded to the subacute stage of stroke. Behavioral recovery was assessed for 5 weeks with a battery of behavioral tests including modified neurologic severity score (mNSS), accelerated rotarod and foot fault tests. We found that, among the factors, SDF1 demon-
stated a significant improvement of neurologic deficits resulting from ischemic brain injury. BrdU-labeling experiments demonstrated new-born (BrdU+) cells in the SVZ and striatum were dramatically increased by the exogenous SDF1 and most of the BrdU+ cells were co-stained with doublecortin and PSA-NCAM. Convincingly, the number of BrdU+ cells was highly correlated with the results of behavioral tests. We also found that the number of newly formed neurons and glial cells in the peri-infarct area was increased by the overexpressed SDF1. In summary, we observed the therapeutic efficacy of SDF1 regardless of delivery time post-stroke, indicating the therapeutic potential of this protein in treating stroke patients of various stages of stroke. (This research was supported by Stem Cell Research Program (2010-0020407) funded by the Ministry of Education, Science and Technology of Republic of Korea).

Poster Board Number: T-1052

PERLECAN IS NECESSARY FOR THE MAINTENANCE OF CD133 EXPRESSING NEURAL STEM CELLS IN THE ADULT MOUSE SUBVENTRICULAR ZONE.

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In the subventricular zone (SVZ) of the adult brain, GFAP+ neural stem cells (NSCs) are tightly associated with fractones and blood vessels basement membranes. Perlecan, a major heparan sulfate proteoglycan (HSPG) is present in the basement membranes of the SVZ blood vessels and in fractones. However, the role of perlecan in the neurogenic niche has remained largely unknown. Perlecan-null mice die at birth because of premature cartilage development. To restore cartilage abnormalities, we created lethality-rescued mice, which expressed recombinant perlecan in cartilage, using a cartilage-specific Col2a1 promoter/enhancer. In this study, using this mouse model deficient in perlecan in the adult brain, we assessed the content of extracellular matrix in the neurogenic niche and investigated the behavior of NSCs in the absence of perlecan. We observed no obvious change in the constitution and integrity of the blood vessel basement membranes and in the fractones of the SVZ despite the absence of perlecan. To discriminate GFAP+ NSCs from GFAP+ SVZ astrocytes, we used the stem cell marker CD133. We observed a decrease in the number of CD133+GFAP+ NSCs in the SVZ of perlecan null mice. Finally, the number of new neuroblasts (expressing the immature neuronal marker doublecortin) in the SVZ and the integration of new neurons (expressing the mature neuronal marker NeuN) in the olfactory bulb were also decreased in perlecan null mice. These results suggest that perlecan is necessary for the maintenance of CD133+GFAP+ NSCs population and for neurogenesis. This study brings the first evidence of a key role of perlecan in the adult neural stem cell niche.

Poster Board Number: T-1053

HISTAMINE PROMOTES NEURONAL DIFFERENTIATION OF CULTURED RAT MIDBRAIN NEURAL PRECURSOR CELLS BUT DIMINISHES DOPAMINE NEURON NUMBERS IN VITRO AND IN VIVO

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Histamine (HA) is a neuroactive molecule that is involved in the regulation of diverse physiological functions in adult organisms, such as wakefulness and sleep, motor activity, as well as thermic and endocrine modulation. During central nervous system (CNS) development, histamine (HA) is one of the first neurotransmitters to appear, presenting higher concentrations than those observed in the adult brain. Particularly, in the region of ventral midbrain (VM), HA increases its concentration up to 5-fold at embryonic days 14-16 and then its levels are decreased close to birth, reaching the low concentrations that are present in the adult brain. Interestingly, neuronal differentiation in the VM correlates temporally with the increase in the concentration of HA in this region. The role of HA during brain development has been elusive. In this work, we aimed to study the effects of HA on neural precursor cells (NPC) of rat VM in vitro and in vivo. We analyzed the effect of several HA concentrations on proliferation, differentiation and cell death of cultured VM NPC. We found that VM cells express HA receptors, and that HA was able to significantly increase neuronal differentiation from 21% to 37%. Pharmacological assays revealed that this HA-induced neuronal differentiation is due to activation of H2 receptors. We observed that HA increased 2-fold apoptotic cell death compared to control conditions, evaluated by TUNEL assay. An interesting finding was that the proportion of dopaminergic neurons was selectively and significantly decreased after HA treatment of VM NPC. We also evaluated the role of HA in vivo through intraperitoneal injections in the developing brain, and found that HA administration decreased the number of dopaminergic neurons in the VM, without affecting gabaergic neurons. Taken together, these results suggest that HA might be acting as a transient signal that modulates neurogenesis and dopamine neuron differentiation/survival in the midbrain during CNS development.

Poster Board Number: T-1054

REGULATION OF ADULT MOUSE NEURAL STEM CELLS BY TLR9 MEDIATED SIGNALING.

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Adult tissue-specific stem cells possess the ability to self-renew and generate functionally differentiated cells that replenish lost cells throughout an organism’s lifetime. Stem cell functions are controlled by extracellular cues from the niche and by intrinsic genetic programs within the cells. Recent studies have shown that the adult mammalian brain retains neural stem cells (NSCs) in the subventricular zone (SVZ) of the lateral ventricle and subgranular zone (SGZ) of the hippocampal dentate gyrus. NSCs generate three major cell types in the central nervous system (CNS), i.e., neurons, astrocytes and oligodendrocytes. It has been shown that their differentiation is strongly influenced by local environment and extracellular cues from such as Wnt produced by surrounding astrocytes. Microglia is a bone marrow-derived macrophage-like cell that plays an important role in brain immunity. We have previously found that microglia are localized adjacent to NSCs in the SGZ of the hippocampal dentate gyrus. This finding implies that, similarly to astrocytes, microglia can also affect the behavior of NSCs as a niche component. In epileptic conditions, activation of microglia occurs concomitantly with the increase in NSC proliferation, tempting us to hypothesize that NSC proliferation is increased by as yet unknown factors derived from microglia. To examine this hypothesis, we focus on Toll-like receptors (TLRs), since they have been known to activate microglia leading to the massive induction of gene expression of secreted factors. TLRs have been reported to be expressed in CNS cells, therefore we confirmed TLRs...
expression in the adult hippocampus by immunohistochemistry. We found that TLR9 is expressed in microglia, which reside in the SGZ. To reveal the role of TLR9 on NSC regulation in an epileptic condition, kainate was intraperitoneally administrated to wild type or tl9r−/− knockout mice. We observed that the lack of TLR9 resulted in promotion of kainate-induced cell proliferation. As a next step, we sought to identify the proliferating cells in tl9r deficient mice following seizure induction with kainite by analyzing the phenotype of BrdU-labeled dividing cells. We found that the majority of these proliferating cells were neuroblasts, which are committed to differentiate into neurons. In addition, the number of newly generated mature neuron in the granular cell layer was significantly increased in tl9r−/− knockout mice compared to that in wild type mice 4 weeks after kainite-induced seizure. This finding has prompted us to further examine whether behavior of NSCs are regulated by TLR9 expressed in microglia. In vitro studies indicated that microglia stimulated with TLR9 ligand released soluble factors that attenuated neuronal differentiation. Taken together, these data raise a possibility that TLR signaling in microglia regulates kainate-induced neurogenesis in the adult mouse hippocampus.

**Poster Board Number: T-1055**

**SUPPRESSED CYTOKINE EXPRESSION IN NEONATAL RAT BRAINS IMMEDIATELY FOLLOWING TRAUMATIC BRAIN INJURY INDICATES A RAPID ENDOGENOUS ANTI-INFLAMMATORY RESPONSE**

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The timing of therapeutic intervention in neurological disorders, especially neurotraumas such as traumatic brain injury (TBI) is critical (Glover et al., 2012 in press), with the acute phase already influenced by genetic and histologic perturbations including aberrant neurovascular inflammation and apoptosis (Borlongan and colleagues, 2009, 2010). Although immediate cell death cascades have become established in adult TBI, the immediate pathophysiology underlying neonatal TBI is poorly understood. The objective of the present study was to determine the role of cytokine regulation following TBI in neonatal rats. Seven-day old Sprague-Dawley rats were subjected to TBI using the controlled cortical impact (CCI) injury model. Age-matched littersmates that did not receive TBI served as the control for this study. Immediately following TBI (within 15 minutes), rats were euthanized and the brains were divided into the ipsilateral (left) and contralateral (right) hemispheres and flash frozen in liquid nitrogen. A BioRad 23-Plex panel was used to measure cytokine levels. Surprisingly, the data revealed that 18 of the 23 cytokines analyzed were significantly downregulated in the hemisphere contralateral to the TBI impacted hemisphere. Three cytokines, namely, IL-5, IL-6 and MIP-3α were identified as significantly suppressed in both ipsilateral and contralateral hemispheres of neonatal TBI rats compared to the control rats. The remaining 5 cytokines did not significantly differ between TBI and control rats, indicating an overwhelming downregulation of the entire panel of cytokines analyzed here. A parallel study processing the plasma of the same cohort of neonatal TBI and control rats revealed neither downregulation nor upregulation of the same cytokines analyzed in the brain tissue, suggesting a highly localized cytokine suppression in the brain during the early injury phase that was not reflected in the peripheral circulation. In stark contrast to the reported early pro-inflammatory response exhibited in adult TBI, the present neonatal TBI study demonstrated a reversed cytokine profile of downregulation. These results suggest a robust and immediate endogenous anti-inflammatory response is mounted by the contralateral hemisphere, a brain region remote from the site of injury. This study demonstrates that the highly plastic neonatal brain is equally capable of cytokine regulation following TBI, which may be a unique feature of the young brain’s regenerative capacity.

**Poster Board Number: T-1056**

**ADULT NEUROGENESIS AND NEURONAL REGENERATION IN THE ZEBAFISH BRAIN**

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The subventricular zone (SVZ) of the adult mammalian brain contains neural stem cells (NSCs) that generate neuronal progenitor cells (NPCs) to be supplied into the olfactory bulb (OB) in the physiological condition. However, brain insults such as ischemic stroke and traumatic brain injury stimulate NSCs in the SVZ to produce NPCs migrating to other brain regions for regeneration. The regenerative capability of such endogenous NSCs is insufficient to promote the functional recovery. To overcome this difficulty, we need to understand the mechanisms of adult neurogenesis and brain regeneration more precisely. The telencephalic ventricular zone (VZ) of adult zebrafish, in which powerful genetic approaches and in vivo imaging techniques are available, is an excellent comparative model to study the unsolved problems in neurobiology of the mammalian SVZ. We established a zebrafish model to study the cellular and molecular processes underlying adult neurogenesis and brain regeneration. Systematic immunohistochemical and ultrastructural studies of the adult zebrafish telencephalic VZ have revealed that its cytoarchitecture is similar to the VZ in the developing mammalian brain. Live imaging of fluorescently-labeled NPCs has shown that NPCs in the telencephalic VZ migrate along the blood vessels to the OB in adult zebrafish. Telencephalic injury induced coordinated cellular processes that underlie neuronal regeneration: the up-regulated proliferation of NPCs in the telencephalic VZ and the differentiated NPCs into mature neurons at the injury site. Eventually, the adult zebrafish brain appeared fully repaired within a month after the lesion. Moreover, we compared neurogenesis in the telencephalic VZ of young adult, mid-age, and elderly zebrafish. The number of NPCs migrating towards the OB was gradually decreased with age. The capacity for neuronal regeneration in the telencephalic VZ of young adult, mid-age, and elderly zebrafish. The number of NPCs migrating towards the OB was gradually decreased with age. The capacity for neuronal regeneration in the telencephalic VZ of young adult, mid-age, and elderly zebrafish. The number of NPCs migrating towards the OB was gradually decreased with age. The capacity for neuronal regeneration in the telencephalic VZ of young adult, mid-age, and elderly zebrafish. The number of NPCs migrating towards the OB was gradually decreased with age. The capacity for neuronal regeneration in the telencephalic VZ of young adult, mid-age, and elderly zebrafish. The number of NPCs migrating towards the OB was gradually decreased with age. The capacity for neuronal regeneration in the telencephalic VZ of young adult, mid-age, and elderly zebrafish. The number of NPCs migrating towards the OB was gradually decreased with age.
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the mRNA level but have not been characterized at a functional ing sites. The hSCNs used for this study were derived from the both ligand and voltage-gated ion channels in 384 parallel record-

neurons using an automated patch clamp system that measures enous ion channels in human embryonic stem cell (hESC)-derived patch-clamp electrophysiology studies, here we characterize the drug discovery. To evaluate the suitability of hSCNs for automated systems is currently limited to recording from large scale screening of compounds for effects on ion channel activity through direct electrophysiological measurements. This type of platform technology can accelerate the drug discovery and development process for ion channel targets. However, the application of automated systems is currently limited to recording from cell lines that overexpress the ion channel of interest in a non-na-
tive context. In contrast, human stem cell-derived neurons (hSCNs) have shown promise to be a more physiologically relevant tool for functional recovery after ischemic injury. Here, we examined the efficacy of in-
jection into the striatum or the SVZ of a lentivirus-expressing Wnt3a on neurogenesis and functional outcome following focal striatal ischemic injury induced by local administration of the vasoconstric-
tor endothelin-1. Results: Treatment with Wnt3a in the striatum significantly enhanced functional recovery after ischemic injury on the 28th day and increased the number of BrdU-positive cells differ-

teinated into mature neurons in the ischemic striatum. Treatment with Wnt3a in the SVZ significantly enhanced the functional recover-
y from the second day after injury and increased the number of immature neurons in the striatum and the SVZ on the second day after injury. In addition, we found reduced disseminated neuronal injury in the group treated with Wnt3a in the SVZ. Conclusions: Our data suggests that gene therapy using Wnt3a contributes to func-
tional recovery after ischemic injury, through increasing neurogen-

esis or neuronal survival in the ischemic striatum.

CHARACTERIZATION OF EMBRYONIC STEM CELL-

derived neurons on a high-throughput automated patch clamp system
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The advent of automated patch clamp systems has enabled the large scale screening of compounds for effects on ion channel activity through direct electrophysiological measurements. This type of technology can accelerate the drug discovery and development process for ion channel targets. However, the application of automated systems is currently limited to recording from cell lines that overexpress the ion channel of interest in a non-na-
tive context. In contrast, human stem cell-derived neurons (hSCNs) have shown promise to be a more physiologically relevant tool for drug discovery. Similar to primary neurons, hSCNs endogenously express ion channels in a more native-like context, enabling better pharmacological characterization of candidate compounds during drug discovery. To evaluate the suitability of hSCNs for automated patch-clamp electrophysiology studies, here we characterize the electrophysiological and pharmacological properties of endog-

enous ion channels in human embryonic stem cell (hESC)-derived neurons using an automated patch clamp system that measures both ligand and voltage-gated ion channels in 384 parallel record-
ing sites. The hSCNs used for this study were derived from the WA09 (H9) hESC line and express a broad array of ion channels at the mRNA level but have not been characterized at a functional level. To date, we have demonstrated the functional expression of multiple voltage-gated ion channels in these hSCNs; a full biophys-
cal and pharmacological characterization of these and other ion channel types expressed at the transcript level is underway. This study demonstrates that the combination of human stem cell-
derived neurons with a high-throughput automated patch clamp system potentially offers a powerful assay for ion channel drug discovery in a more biologically relevant system.

VOLUNTARY EXERCISE RESTORES THE SOCIAL RECOGNITION IMPAIRMENT IN DOMINANT NEGATIVE DISC1 MICE: IMPLICATION FOR SCHIZOPHRENIA
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Background: Schizophrenia (SCZ) is a severe neuropsychiatric disor-
der affecting about one percent of the population. Current pharma-
cological treatment options have insufficient effect on negative symptoms, such as social withdrawal, and cognitive decline. Clinical studies indicate that physical exercise can attenuate negative symptoms and improve cognitive deficits, possibly by enhancing adult neurogenesis. The aim of the present study was to determine whether voluntary wheel running can improve the SCZ-associated phenomenotypes of dominant-negative DISC1 transgenic mice (DN-DISC1). Results: DN-DISC1 and wild-type mice were placed for 14 days in an active cage with free access to a running wheel . Thereafter, mice underwent behavioral tests and brains were pro-
cessed for tissue analysis. We could not observe differences in social interaction between the wild type and DN-DISC1 transgenic mice. Nonetheless, a significant deficit in social recognition was detected in the DN-DISC1 mutant mice which was markedly reversed by voluntary exercise in both females and males. We also found that female mice stayed in the active cage, restored the impairment ob-
served in Y maze performance which is attributed to hippocampal dysfunction. ELISA analysis showed two fold increase in BDNF levels in frontal cortex of WT mice after physical exercise. In contrast, in the DN-DISC1 mice, physical exercise didn’t induce the BDNF levels.

Discussion: Our preliminary results suggest that physical exercise can attenuate the impaired social and cognitive phenomenotypes exhib-
ited by the DN-DISC1 mice. In parallel, we observed no alteration in BDNF levels in the DN-DISC1 mice after physical activity which may indicate for their dysfunction in neurogenesis processes. Further tissue analysis of the brains will be conducted in order to reveal a possible mechanism underlying this behavioral data.

LMX1A DEFINES MIDBRAIN DOPAMINERGIC NEURONS: FACS AND FICTION
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The derivation of specific neuronal subtypes from human pluripo-
tent stem cells (hPSCs) at high purity is currently hampered by the inherent heterogeneity of existing differentiation protocols. Thus cell grafts may be contaminated with unwanted cell types, resulting in tumor formation and/or lack of functional integration. Isolating committed progenitors for use in both in vitro and in vivo applica-
tions is highly attractive since their reduced structural fragility when compared to post mitotic neurons, and proliferative potential allows for expansion prior to use. Previous work in this laboratory
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has demonstrated the isolation of committed mouse dopaminergic (DA) progenitors using a LIM homeobox transcription factor 1 alpha (Lmx1a) homologous recombinant reporter cell line. Depending on the culture method, the isolated Lmx1a+ progenitors give rise to enriched DA neurons in terminal cultures. In this study we investigate the potential of Lmx1a as a marker for the enrichment of DA neurons from hPSCs. We report that correct regionalisation of differentiating hPSCs during neural induction is required for the FACS isolation of committed floorplate (midbrain) DA progenitors using a human Lmx1a reporter cell line. These progenitors, when expanded as neurospheres and replated, give rise to enriched populations of functional bonafide midbrain DA neurons. Our findings show that it is possible to define and isolate cells suitable for either cell replacement therapies or the development of in vitro models of neurotoxicity.

Poster Board Number: T-1061

CULTURE, CHARACTERIZATION AND DIFFERENTIATION OF NEURAL STEM CELLS FROM GUINEA PIGS (CAVIA PORCELLUS)

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Neurogenesis in adulthood of most mammals persists in two main brain areas: the hippocampus dentate gyrus (DG) and the subventricular zone of the lateral ventricle (SVZ). This study aimed to cultivate, characterize and differentiate neural precursors of fetal guinea pigs (Cavia porcellus). Culture of cell suspensions obtained from fetal SVZ were plated on Poly-Hema (Poly [2-hydroxyethyl methacrylate]) treated flasks and maintained in EGF and FGF-2-supplemented high glucose medium. After 7 to 10 days of culture, rounded, solid cellular aggregates with irregular surface and variable size were observed in the supernatant. These neurospheres (NSFs) were submitted to passages with or without enzymatic dissociation. Non-dissociated NSFs increased in size and number after passage whereas dissociated cells retained their proliferative capacity and eventually generated new NSFs. Dissociated and non-dissociated NSFs were frozen and thawed and the viability pre- and post-freezing, and after 1 to 15 days were maintained. The colorimetric method MTT revealed no differences in viability for thawed cells, examined soon after thawing or after 1 week or 1 month of thawing. In NSF cultures submitted to differentiation in EGF/FGF-2 deprived medium, a significant number of morphologically different, adherent cells were observed after 7 days. These cells were positive by immunostaining for markers of neural precursors (Nestin), neurons (β-Ill-tubulina), oligodendrocytes (mGalC) and astrocytes (GFAP). Thus, these results demonstrated the obtainance of NSFs with progenitor potential from SVZ of guinea pig fetuses.

Poster Board Number: T-1062

ESTABLISHMENT OF INDUSED PLURIPOTENT STEM CELL LINES FROM PARKINSON DISEASE PATIENTS FOR MOLECULAR MECHANISMS STUDY


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Parkinson’s disease (PD) is the second most common neurodegenerative disorder (after Alzheimer’s disease). Parkinson’s disease is a gradually progressive, degenerative neurologic disorder. Although typically a sporadic disease, mutations in some genes have been identified as a cause of late-onset, autosomal dominant familial PD that is clinically and neurochemically indistinguishable from sporadic PD. Mutations in the leucine-rich repeat kinase 2 (LRRK2) are the most common cause of familial and sporadic PD identified to date. Mutation G2019S causes from 3% (Asia) to 41% (Northern Africa) of familial PD cases. Although some progress on LRRK2 involvement in the disease progression has emerged during last years absence of adequate model complicates investigation of human diseases and development of novel therapies. Recent advances in cell reprogramming technologies facilitate the development of human cell models that allows precise mechanisms disease investigation. Using skin biopsies from PD patients with LRRK2 gene mutation (G2019S) we reprogrammed dermal fibroblasts using lentiviral constructs carrying Yamanaka’s factors (Oct4, Sox2, KIf4, c-Myc). Obtained iPSC clones were morphologically indistinguishable from human embryonic stem cells colonies. We analyzed PD patient-specific iPS lines for the expression of main markers of pluripotency i.e. Oct4, Sox2, Hesx1, Sall1, TRA-1-60, TRA-1-81, SSEA4, etc. Established cell lines were also analyzed for the ability to differentiate into the cells belonging to all three germ layers. We developed an effective protocol of iPSCs differentiation into tyrosine hydroxylase - positive dopaminergic neurons. Generation of “diseased” iPSC lines from different patients carrying common genetic alterations allows use of phosphoproteomic approaches to identify possible molecular pathways of disease development and to find electrophysiological differences in neuronal network formation.

Poster Board Number: T-1063

NEURAL STEM CELLS DIRECTLY DIFFERENTIATED FROM PARTIALLY REPROGRAMMED FIBROBLASTS RAPIDLY ACQUIRE GLIOGENIC COMPETENCY

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Neural stem cells (NSCs) were directly induced from mouse fibroblasts using four reprogramming factors (Oct4, Sox2, KIf4, and cMyc) without the clonal isolation of iPSCs. These NSCs gave rise to both neurons and glial cells even at early passages, while early NSCs derived from clonal ESCs/iPSCs differentiated mainly into neurons. EGF-dependent neural sphere cultivation efficiently propagated these gliogenic NSCs and eliminated residual pluripotent cells that could form teratomas in vivo. We concluded that these directly induced NSCs were derived from partially reprogrammed
cells, because dissociated ESCs/iPSCs did not form neurospheres in this culture condition. These NSCs differentiated into both neurons and glial cells in vivo after being transplanted intracranially into mouse striatum. NSCs could also be directly induced from adult human fibroblasts. The direct differentiation of partially reprogrammed cells may be useful for rapidly preparing NSCs with glioegenic competency.

**Poster Board Number: T-1064**

**PHENOTYPIC SCREENING FOR NEUROGENESIS: FROM IN VITRO TO IN VIVO**

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One of the most remarkable forms of structural plasticity in the adult brain is the generation of new functional neurons from adult neural precursors cells (NPCs). There is accumulating evidence that neurogenesis in the adult hippocampus contributes to brain physiology and disease, but its precise physiological role remains elusive. Conceptually, this process can be divided into four steps: (i) proliferation; (ii) neuronal fate determination; (iii) survival and neuronal/synaptic maturation of new neurons; and (iv) functional integration of new neurons into the pre-existing neuronal network. Here we describe the development of phenotypic in vitro screening assays using human embryonic stem cell derived NPCs as a cellular model to investigate neurogenesis. Using expression profiling of differentiating cells and exposure of NPCs to bioactive small molecules, we can demonstrate that neurogenesis relevant signaling pathways are active in this in vitro cell model. These experiments demonstrate that this human NPC model represents a neuronal relevant transcriptional and biological state, which can unleash the identification of a plethora of targets spreading the druggable genome. We identify through a screening cascade of High-Throughput Screening, image based High Content Analysis (neurite outgrowth, synaptic maturation) in human NPCs and subsequently in mouse model of adult hippocampal neurogenesis, new active CNS compounds/targets. These compounds may help in identifying novel regenerative medicines, and may ultimately elucidate novel mechanisms modulating adult neurogenesis.

**Poster Board Number: T-1065**

**IN VIVO FATE AND FUNCTION OF NEURAL STEM CELL GRAFTS AND OF THE SUBSEQUENT ENDODER GIOUS BRAIN IMMUNE RESPONSES**

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In order to understand the physiological mechanisms needed for transplanted neural stem cells (NSC) to regenerate the injured central nervous system (CNS), we have aimed over the past years to characterize eGFP/Luciferase-expressing adherently growing cultures of murine embryonic (E14) brain-derived NSC, which were characterized by the expression of specific marker proteins and by their in vitro tri-lineage differentiation potential. Next, the therapeutic potential for intravenous administration of NSC was investigated in the experimental autoimmune encephalomyelitis mouse model for multiple sclerosis. The absence of therapeutic benefit was due to cell retention in lung capillaries, as demonstrated by real time in vivo bioluminescence imaging (BLI). Finally, we also investigated the in vivo remyelination capacity of NSC upon direct grafting into the demyelinated CNS of cuprizone-treated mice. At two weeks post-grafting, proper differentiation and integration of grafted NSC was hindered by strong microglial and astrocyte responses in both healthy and demyelinated CNS tissue, as demonstrated by quantitative in vivo BLI and histological analysis. Therefore, we hypothesize that the therapeutic potential of NSC grafting, as observed by other studies, cannot be accounted for by adequate cell graft migration, survival and/or integration, but may potentially be explained by induced inflammatory responses following cell grafting.

**Poster Board Number: T-1066**

**A POTENTIAL APPLICATION OF OLFACTORY BULB-DERIVED ADULT NEURAL STEM CELLS**

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Neural stem cells (NSCs), which are responsible for continuous neurogenesis during the adult stage, are present in human adults. Typical neurogenic regions are the hippocampus and the subventricular zone; recent studies have revealed that NSCs also exist in the olfactory bulb. Olfactory bulb-derived neural stem cells (OB NSCs) have the potential to be used in therapeutic applications and can be easily harvested without harm to the patient. Through the combined influence of extrinsic cues and innate programming, adult neurogenesis is a finely regulated process occurring in a specialized cellular environment, a niche. Understanding the regulatory mechanisms of adult NSCs and their cellular niche is not only important to understand the physiological roles of neurogenesis in adulthood, but also to provide the knowledge necessary for developing new therapeutic applications using adult NSCs in other organs with similar regulatory environments. We will present our recent progress of the study of adult NSCs regarding the potential of regenerative medicine, primarily in the area of diabetes therapy. The utility of NSCs derived from the olfactory bulb is presented along with the rationale for why this might be an acceptable treatment modality. The potential use of stem cells for the testing and recommendation of specific therapeutics for mental disorders is also presented.

**Poster Board Number: T-1067**

**NESTIN-POSITIVE STEM/PROGENITOR CELLS WITH NEURAL DIFFERENTIATION POTENTIAL ARE PRESENT IN THE MENINGEAL NICHE DURING DEVELOPMENT UP TO ADULTHOOD.**

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Meninges, which include arachnoid and pia mater, cover the entire CNS and are filled with cerebrospinal fluid produced by choroid plexi. Recently, we found that meninges host a population of immature precursors with neural differentiation potential that can be activated by injury. In this work, we asked whether these precursors in meninges were developmentally conserved from embryo to adult. Therefore, in samples from embryo (E14, E20), perinatal (P0, P15) and adult rats, we: i) quantify the distribution, the number and
the proliferation rate of nestin-positive cells in meninges; ii) characterize the overall meningeal cell composition and its changes during the developmental stages; iii) assessed the stem/progenitor cells potential in vitro. The cellular and extracellular composition of meninges across development were analysed and the expression of markers of undifferentiated cell (Nestin and Vimentin), neural crest (p75), neural progenitors (Sox2, DCX, Tuj1), astrocytes (GFAP), pericytes (NG2), endothelial cells (CD31) and ECM components such as fibronectin, heparan sulphate and laminin, assessed by confocal microscopy. Cells expressing the stem cell marker nestin were found in meninges as early as E14. The number and density of these cells decreased with the animal age and represent the 13.3±4.4% of the adult rat meninges cells. Proliferating K667-nestin positive cells were found in all developmental stages even that their number significantly decreases (E20 16.5%, P0 10.9%, P1 8.7%±6 and in adult 4.8%; p<0.0001). Moreover, Oct4- and DCX- positive cells were also found at all ages. Meninges nestin-positive cells were distinct from NG2-, GFAP- and CD31-positive cells. To further characterize meninges as stem cell niche, we analysed the gene expression of stem cell-related markers including nestin, pou5f1, vimentin, cspg, dcx, pax6 and sox2 by laser capture microscopy. Quantification of the developmental changes of microdissected meningeal tissue confirm the persistence of stem cells-related gene during all the stages. Finally, we analysed the in vitro stem cell properties of meninges nestin-positive cells during development up to adulthood. As expected from the ex-vivo observation, the number of colony forming unit (CFU) and cell growth rate, decreased with rat age. Under differentiating conditions, nestin-positive cells expanded from all rat ages underwent neural differentiation. These data suggest that the meninges are a putative new stem cell niche capable of housing and maintaining up to adulthood a population of stem/progenitor cells with neural differentiation potential. Further investigation will elucidate any functional role of the meningial stem cell niche and of its nestin-positive stem/progenitor cells in brain development and in adulthood.

Poster Board Number: T-1068

EVIDENCE FOR NEUROGENESIS BY LATENT PARENCHYMAL PROGENITORS AFTER STROKE

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In the adult mouse brain, neurogenesis is limited to two small regions, one of which is the subventricular zone (SVZ) of the lateral ventricles. In response to stroke, neural stem cells in the SVZ increase their proliferation, and neuroblasts ectopically migrate toward the lesion site. Despite this well-described response, it is not known whether all stroke-induced ectopic neuroblasts are derived from the SVZ. It has been shown that following a cortical stab injury, parenchymal astrocytes, which are normally non-neurogenic, can be isolated and propagated as multipotent neurospheres in vitro. It has remained unknown whether parenchymal astrocytes can also display such neurogenic potential in vivo. To test this, we used Connnexin-30-CreER transgenic mice or a Cre-expressing adenoovirus to inherently label parenchymal astrocytes, and subsequently subjected animals to experimental ischemic stroke through transient occlusion of the middle cerebral artery. We will present data providing evidence for stroke-induced local neurogenesis from parenchymal astrocytes in vivo. Our findings highlight a previously unknown level of plasticity in the adult brain and suggest a possible new target for therapeutic interventions that aim to repair the brain after ischemic injury.

Poster Board Number: T-1069

UNIQUE GENE EXPRESSION PROFILES OF NEURAL STEM CELLS AND THEIR PROGENY IN THE ADULT BRAIN

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Neural stem cells (NSCs) generate new neurons throughout life in two regions of the mammalian brain: the subventricular zone (SVZ) lining the lateral ventricles, and the hippocampal dentate gyrus (DG). Adult neurogenesis has been implicated in tissue homeostasis, physiologic brain function, and has been also associated with a number of neuro-psychiatric diseases. To characterize the molecular signature of NSCs and immature neurons we isolated NSCs and their progeny from the adult DG and SVZ using transgenic reporter mice expressing either a GFP reporter under the control of the Sox2 promoter (labeling NSCs), or a DsRed reporter under the control of the doublecortin (Dcx) promoter (labeling immature neurons). Transcriptome analyses revealed distinct gene expression profiles between NSCs and immature neurons. One example is the growth factor insulin-like growth factor 2 (IGF2), which was highly expressed in DG NSCs in contrast to SVZ NSCs and immature neurons. We show that IGF2 controls proliferation of DG NSCs in vitro and in vivo via the AKT pathway. Gene expression profiling of NSCs and newborn neurons may reveal novel genes/pathways that control distinct steps in the course of adult neurogenesis.

Poster Board Number: T-1070

NEURAL STEM/PROGENITOR CELLS DAMAGED BY REACTIVE OXYGEN SPECIES EVOLVED BY PHOTOSENSITIZING REACTION

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We want to know how the growth of neural stem/progenitor cells and their differentiation are affected by reactive oxygen species evolved in photosensitizing reaction, because of the similarity between the stem cells and the tumor cells in central nervous system. We investigated the effects of two photosensitizers (rhodamine 123 and hematoporphyrin) on the mouse neural stem/progenitor cells cultured in vitro under the illumination. ABC transporters were expressed in the cells, and could pump rhodamine 123 and hematoporphyrin out of the cells. Under the illumination of strong actinic light with those photosensitizers, reactive oxygen species was evolved to injure the cells. Number of viable cells decreased under illumination through apoptosis and necrosis. Those cell-killing activities were not clearly dependent on the presence of inhibitors for ABC transporters. Immunocytochemical staining with showed that immature cells with markers of neural stem/progenitor cells (Sox2, CD133, nestin) were more sensitive to the reactive oxygen species than the differentiated cells.
METABOLIC CONTROL OF ADULT NEURAL STEM CELL ACTIVITY

Braun, Simon M G1, Knobloch, Marlen1, Zurkirchen, Luise1, von Schoultz, Carolin1, Zamboni, Nicolas2, ArauzoBravo, Marcos1, Kovacs, Werner1, Karalay, Oszlem1, Suter, Ueli1, Roccio, Marta1, Lutolf, Matthias1, Semenikovich, Clay1, Jessberger, Sebastian1

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Neurogenesis relies on an intricate balance between quiescence and proliferation of neural stem cells (NSCs) to ensure the lifelong generation of newborn neurons in the adult brain. The proliferative activity of NSCs is governed by highly regulated molecular mechanisms, many of which are yet to be identified. Using metabolic profiling of adult NSCs we observed that these cells are in a distinct metabolic state when compared to their differentiated progeny or other dividing neural cells. In particular, NSCs display high levels of de novo lipogenesis, a metabolic pathway upregulated in many cancer cells. Here we show the role key lipogenic enzymes play in maintaining NSC proliferation using pharmacological inhibition and conditional genetic deletion of these genes. Furthermore, we found that this pathway is tightly regulated in the hippocampal neurogenic niche through the specific expression of proteins which can inhibit the activity of these lipogenic enzymes within the quiescent pool of NSCs. Our findings underline the essential role of lipid metabolism in regulating the proliferative activity of adult NSCs.

NEURONAL INDUCTION IN GRAFTS OF NEURONAL PROGENITORS TO SITES OF SPINAL CORD INJURY

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Keywords: spinal cord injury, neural stem cells pinal cord injury (SCI) causes loss of neurons and axons at the lesion site, frequently resulting in permanent loss of function. One repair strategy is to use cell transplantation as a means of replacing injured neurons, providing a potential mechanism for forming poly-synaptic relays across sites of injury. The aim of the present study is to compare the fate of freshly isolated embryonic neural cells versus cultured, pre-differentiated neural cells that are transplanted into the injured adult spinal cord. Neural stem cells that express green fluorescent protein (GFP) were harvested from embryonic day 14 (E14) rat spinal cord and grafted into a C3 dorsal column wire lesion site in wild-type adult rats, 2 weeks after SCI. The grafts filled the injury site and matured into both neurons and glia; neurons extended axonal processes over long distances into the host spinal cord both caudally and rostrally. Fetal NPCs were generated from neurospheres derived from E14 spinal cord. When grafted into the adult injured spinal cord, these cells differentiated into glia. To increase neuronal differentiation/maturation from neurospheres, the cells were pre-differentiated into neuronal progenitors as monolayer cultures for 3, 5, 7 days using retinoic acid and sonic hedgehog. The cells expressed the early neuronal progenitor markers, β III tubulin and Nestin. When grafting these pre-differentiated neuronal progenitors/early neurons into adult SCI lesion sites, almost no mature spinal cord neurons were detected in the cell graft 4 weeks after transplantation. Instead the graft consisted mainly of glial cells. Overall graft survival was good. These findings confirm the pro-glial environment of the adult spinal cord, indicating that even pre-differentiation into neuronal phenotypes are insufficient to lead to a neuronal fate in vivo. Further differentiation steps are required to promote retention of the neuronal phenotype in grafted neural progenitors. Such work is ongoing. ACKNOWLEDGEMENTS: We thank the Rat Resource and Research Center, University of Missouri, Columbia, Missouri, for providing GFP rats. This work was supported by the Veterans Administration, NIH (NS09881), CIRM, Wings for Life, Canadian Spinal Research Organization, the Swiss Institute for Research into Paraplegia, and the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation.

MODELING PARKINSON’S DISEASE IN A PETRI DISH: EFFECT OF A-SYNUCLEIN ON DOPAMINERGIC NEURONS DERIVED FROM MOUSE INDUCED PLURIPOTENT STEM CELLS

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Parkinson’s disease (PD) affects 1% of the population over the age of 60 and by the year 2030 the number of cases is estimated to double. PD is the second most prevalent neurodegenerative disorder. Lewy bodies are the hallmarks of PD pathology and accumulate the protein α-synuclein. α-synuclein overexpression is a causative factor for neurodegeneration as shown in Mendelian forms of PD. α-synuclein upregulation has also been consistently reported in toxicological animal models of PD. Several recent publications propose that α-synuclein can have a prion-like effect, where the disease state protein conformation can be transmitted between neurons. The disease relatively specifically targets A9 dopaminergic (DA) neurons in the midbrain and spares adjacent A10 DA neurons. In comparing these two populations in vitro we will explore the differences in susceptibility for PD. We hypothesize that A9 DA derived neurons will be more vulnerable to extracellular α-synuclein compared to A10 DA neurons in a new induced pluripotent stem cell (iPSC) model. We have generated iPSC lines by taking mouse tail fibroblasts and transfecting them with the four factors; specifically, Oct4, Sox2, Klf4 and l-Myc in a retroviral vector. The mouse tail fibroblasts are reprogrammed and iPSC colonies are picked after 21-30 days. Following characterization for pluripotency, the iPSCs are guided to a neuronal fate by using a novel four-stage protocol employing a feeder-free culturing method. Embryoid body formation is carried out in mESC media with the SMAD inhibitors Dorsomorphin (1 μM), SB431542 (2 μM) and the MEK inhibitor PD0325901 (1μM). After plating, the cells are cultured in N2/B27 media plus growth factors for 16 days and yield a significant percentage of beta III tubulin positive cells and tyrosine hydroxylase (TH) positive cells. Within the TH-positive population, A9 and A10 neurons can be distinguished using FoxA2 and calbindin markers, respectively. Challenging our iPSC-derived neurons with α-synuclein protein at different concentrations for 24 hours and subsequent flow cytometry and immunocytochemistry will illuminate the viability of A9 dopaminergic neurons versus adjacent A10 neurons. Comparing relative survival percentages of DA neurons can shed light on the inherent differences between A9 and A10 neurons. These differences are important when trying to understand why A9 neurons are more susceptible in PD. In addition, we believe that mouse dopaminergic neurons derived from induced pluripotent stem cells can be a valuable model of PD for 1) examining the characteristics of DA neuronal subtypes, 2) using these neurons for neurotoxicity
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testing, e.g. challenge with MPP+ and paraquat, and 3) establishing drug screening approaches. If deemed successful, our iPSC-derived neuronal model will be a valuable tool to study Parkinson’s disease in a petri-dish.

**Poster Board Number: T-1074**

**RFX TRANSCRIPTION FACTORS AND FGF1 SUSTAIN NEURAL STEM AND PROGENITOR CELLS THROUGH AURORA-A KINASE ACTIVATION**

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Fibroblast growth factor 1 (FGF1) has been suggested as an important growth factor for many biological processes, such as neurogenesis. FGF1 and FGF-1B promoter-driven green fluorescent protein (F1BGFP) have been used to isolate neural stem/progenitor cells (NSPCs) with self-renewal and multipotency. However, the mechanisms that regulate the maintenance of F1BGFP(+) NSPCs are still unknown. In this study, we provide several lines of evidence to show the regulatory mechanisms for F1BGFP expression and the maintenance of F1BGFP(+) NSPCs: (i) RFX2 and RFX3 transcription factors could directly bind the 18-bp cis-element (-484 to -467) of F18 promoter, and are crucial for F1BGFP expression; (ii) F1BGFP(+) cells have significantly higher levels of AurA activation, neurosphere formation than GFP(-) cells. Protein kinase inhibitor, staurosporine, RFX2-siRNA and RFX3-siRNA could reduce FGF-1B promoter activity, AurA activation and neurosphere formation. These inhibitory effects could be rescued by FGF1 treatment. (iii) AurA kinase specific inhibitor, VX-680, could significantly reduce the AurA activation and neurosphere formation of F1BGFP(+) NSPCs. However, these reductions could not be rescued by additional FGF1 treatment. (iv) Autocrine/paracrine activation of AurA of F1BGFP(+) cells could be inhibited by FGF1-neutralizing antibody and FGFR (SU5402). Furthermore, AurA activation in F1BGFP(+) cells could also be inhibited by PI3K/AKT inhibitor, suggesting FGF receptors and AKT signaling pathways play important roles in the activation of AurA kinase and the maintenance of F1BGFP(+) NSPCs. (v) As a proof of principle, F1BGFP(+) cells are identified as a subset of B1 NSPCs with single primary cilium which will absorb through AurA activation in the lateral wall of the adult mouse brain. In conclusion, our results suggest that RFX2/3 transcription factors are crucial for F1BGFP expression levels. F1BGFP(+) cells have sustained AurA activation through FGF1-FGFR and PI3K/AKT pathways.

**Poster Board Number: T-1075**

**PS7 CONTROLS QUIESCENCE OF ADULT HIPPOCAMPAL NEURAL STEM CELLS**

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Neural stem cells (NSCs) in the adult mammalian hippocampus constitute a relatively quiescent cell population, but the molecules responsible for their quiescence have remained unknown. In this study, we found that the cyclin-dependent kinase inhibitor Cdkn1c (ps7) regulates the quiescence of NSCs in the adult mouse hippocampus. Selective deletion of the ps7 gene in NSCs initially resulted in their recruitment into the cell cycle and increased neurogenesis in young adult mice and even in aged mice. In contrast, long-term deletion of the ps7 gene through adulthood resulted in NSC exhaustion and a reduced level of neurogenesis in aged mice. Furthermore, our results support the notion that dynamic changes in ps7 levels underlie the reversal of NSC quiescence in response to neurogenic stimuli such as epileptic seizures and running. The regulation of NSC quiescence by ps7 might thus have important implications for lifelong neurogenesis and neural plasticity in response to external stimuli.

**Poster Board Number: T-1076**

**MICRORNA-124 IS A NEURONAL FATE DETERMINANT IN THE SUBVENTRICULAR ZONE**

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MicroRNAs are small, non-coding endogenous RNA that bind to and regulate mRNA targets, making them central players of gene regulation. miR-124 is one of the most abundant miRNA in the brain and several studies have shown that it plays a role in neural stem cell (NSC) regulation. To study the role of miR-124 in the postnatal subventricular zone (SVZ), we have generated a transgenic reporter mouse that allows visualisation of miR-124 activity in the brain in vivo. We found that miR-124 is not present in NSC, but its activity is initiated in transient amplifying progenitors and it remains expressed in differentiated neuronal progeny. When we stably suppressed miR-124 activity in vivo in the SVZ, adult neurogenesis was blocked and ectopic astrocytes, derived from the SVZ, were found in the olfactory bulb. On the contrary, when we over-expressed miR-124 in the SVZ, it reduced expression levels of miR-124 in vivo and positions miR-124 as a neuronal fate determinant in the postnatal SVZ.

**Poster Board Number: T-1077**

**SYNTHETIC POLYMER-BASED NEURAL STEM CELL NICHE IDENTIFICATION**

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FGF2 and Wnts are known to be secreted from microenvironments surrounding neural stem/progenitor cells (NSCs/NPCs) and act as mitogens and contribute to the maintenance of NSCs/NPCs. We have reported that these growth factors promote NSCs/NPCs proliferation via the common signaling pathway, i.e., GSK3β inactivation, β-catenin accumulation, and cyclin D1 expression. We have also shown that this β-catenin accumulation results in potentiation of Notch signaling which leads to inhibition of neuronal differentiation (Shimizu et al., Mol. Cell. Biol., 2008). Furthermore, we recently found that cyclin D1, a downstream factor of FGF2 and Wnts, inhibited astrocyte differentiation through disturbing LIF/STAT3-mediated astrocyte-specific gene expression in a manner independent of cell cycle progression (Bizen et al., unpublished data.). Thus, the molecular mechanisms for NSC/NPC maintenance partly involve FGF2, Wnt, and Notch signaling pathways. NSCs are thought to require a specific niche for the maintenance of multipotency throughout a lifetime. However, despite the above mentioned findings, its molecular basis is poorly understood. It seems difficult to analyze the roles of the interaction between NSCs/NPCs and niche molecules, because of complexity of input stimuli from many niche components (such as ligands, cytokines, and ECM molecules) and NSC/NPC surface molecules. Although conventional approaches such as gene expression profiling and mass spectrom-
Abstract: Poly (lactic-co-glycolic acid) (PLGA) has been widely applied to tissue engineering as a good biocompatible material and useful biomaterials in a range of applications. We fabricated silk fibroin hybrid film and confirmed the influence of adhesion and proliferation on OECs according to content of silk fibroin. Olfactory ensheathing cells (OECs) were seeded on PLGA/silk /synthetic hybrid films using 0, 10, 20, 40 and 80 wt% of silk fibroin. Among them, one acrylate-based polymer called “PA518” maintained a high level of nestin expression of NSCs/NPCs, in spite of the differentiation-inducing condition without FGF2. Furthermore, we found that the neurosphere-forming ability and multipotency of NSCs/NPCs plated on PAS18-coated dishes was approximately 5-fold higher than those of NSCs/NPCs cultured on conventional poly-L-ornithine/fibronectin-coated dishes. Taken together, we here suggest that PAS18 is a niche-mimic molecule for NSC/NPC maintenance. Concurrently, we also screened glioma stem cell (GSC) niche mimicry and identified several hit polymers that supported the self-renewing proliferation of GSCs. These achievements may contribute not only to the understanding of NSC/GSC regulation but also to the development of novel therapeutic strategy for the regeneration medicine and cancer treatment in view of stem cells.

Poster Board Number: T-1078
EFFECT OF PLGA/SILK FIBROIN HYBRID FILM ON PROLIFERATION AND ATTACHMENT OF OLFACTORY ENSHEATHING CELLS
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EFFECT OF POLY(LACTIC-CO-GLYCOLIC ACID) DESIGNATION ON OLFACTORY ENSHEATHING CELLS: PHASE II MAIL OUTLINE

Introduction
1. Background
    a. Olfactory ensheathing cells (OECs) are critical in the development and regeneration of the olfactory system.
    b. PLGA is a well-known biodegradable polymer used in various biomedical applications.

2. Objective
    a. To investigate the effect of PLGA on the proliferation and attachment of OECs.

3. Materials and Methods
    a. OECs were cultured on PLGA films with different concentrations of silk fibroin.
    b. Proliferation and attachment were assessed using MTT and FACS analysis.

4. Results
    a. PLGA with 40% and 80% silk fibroin showed increased proliferation and attachment.

5. Conclusion
    a. PLGA/silk fibroin hybrid films are promising for OECs application.

Keywords: PLGA, OECs, proliferation, attachment.
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Placenta-derived multipotent cells (PDMCs) isolated from the human placenta are a population of multipotent cells that are able to differentiate into multiple cell types. It is well known that 1-methyl-3-isobutylxanthine (IBMX) induces the differentiation of PDMCs to repair injured white matter remain unclear. Here, using cell-type- and region-specific genetic labeling methods in a mouse HI model, we characterized the Olig2-expressing OPCs. We found that after HI, OPCs increased in the posterior part of the subventricular zone (pSVZ) and migrated into the injured white matter. However, their oligodendrocytic differentiation efficiency was severely compromised compared with the OPCs in normal tissue, indicating the need for an intervention to promote their differentiation. Erythropoietin (EPO) treatment is a promising candidate, but it has detrimental effects that preclude its clinical use for brain injury. We found that long-term post-injury treatment with a non-erythropoietic derivative of erythropoietin, asialo-erythropoietin (AEPO), promoted the maturation of pSVZ-derived OPCs and the recovery of neurological function, without affecting hematopoiesis. These results demonstrate the limitation and potential of endogenous OPCs in the pSVZ as a therapeutic target for treating neonatal white-matter injury.

**Poster Board Number: T-1081**

**UNRAVELING THE FUNCTION OF A NOVEL SPLICE VARIANT OF NURR1 AND ITS IMPLICATIONS IN TAILORING MIDBRAIN DOPAMINERGIC NEURONS**

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Recent work in the field of neuronal differentiation has given critical importance to generate midbrain dopaminergic (mDA) neurons from pluripotent stem cells and fibroblasts due to its physiological and clinical implications. Expression of the orphan nuclear receptor Nur1 gene has been shown to be critical for the generation, survival and maintenance of mDA neurons and a key requirement for inducing mDA phenotype. Here, we report a splice variant of Nur1 isolated from the mouse brain which lacks a part of its ligand binding domain (LBD), leading to in-frame deletion of 37 amino acids. Expression analysis of the splice variant named as Nur1d, during embryonic (mouse) midbrain development revealed its differential temporal expression pattern. Interestingly, we found the mRNA expression of Nur1d to be spatially overlapping with the expression of the wild type Nur1 only during the later differentiation stages of mDA neurons. Transient over expression studies on embryonic mouse ventral mesencephalon (VM) neural precursor’s revealed that Nur1 significantly enhances the number of tyrosine hydroxylase (Th) positive neurons compared to Nur1d. Intriguingly, the Th positive neurons obtained from Nur1d transfected cultures demonstrated a more matured neuronal phenotype with significant increase in neurite length and expression of late mDA markers. To dissect possible distinct roles of Nur1 and Nur1d during mDA development, we performed luciferase reporter assays using the Th and BDNF promoters on embryonic mouse VM primary cultures and secondary cell lines transfected with Nur1 or Nur1d. Here, we observed Nur1 strongly potentiates the transcriptional activity of the Th promoter while in contrast Nur1d significantly enhances the transactivation of the BDNF promoters. Furthermore, we investigated the neuroprotective ability of Nur1 and Nur1d transfected embryonic mouse VM cultures against the cytotoxic effects of 6-hydroxydopamine (6-OHDA). We found that the viability of mDA neurons upon 6-OHDA challenge was significantly enhanced in cultures transfected with Nur1d compared to Nur1, demonstrating the robust neuroprotective potential of Nur1d. In order to investigate the functional switch of the Nur1d splicing variant on a structural level we are presently conducting differential nuclear magnetic resonance (NMR) analysis of Nur1 and Nur1d. Taken together our data indicate that Nur1d attributing mDA neurons with enhanced maturation and survival could be utilized in cellular differentiation paradigms aiming to obtain more mature and robust mDA neuron phenotype.

**Poster Board Number: T-1082**

**IDENTIFICATION OF A NEURAL INDUCTION DOMAIN IN BC-BOX PROTEINS AND NEURAL INDUCTION OF SOMATIC STEM CELLS BY TRANSFER OF ITS DOMAIN PEPTIDE**

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Pluripotent somatic stem cells have potential to differentiate to neurons, and they are hopeful to be used as drafted donor cells for neuronal regenerative therapy. However, the grafted cells little survive and differentiate to functional neurons in recipient neural tissue. To overcome the problem, neural induction using neurotrophic factors or gene transfer has been employed before grafting, but neurotrophic factors do not occur specific neural induction, while gene transfer has risk of vector. If a neural induction domain for somatic stem cells exists in proteins to induce neural differentiation, its identification can contribute to neuronal regenerative therapy through neural induction of somatic stem cells using the neural induction domain. We previously demonstrated that von Hippel-Lindau tumor suppressor (VHL) protein has a function of neural induction in neural stem cells (NSCs) without any neurotrophic factors. Then, we hypothesized that a neural induction domain potentially exists in the VHL protein. Here we identify a neural induction domain for somatic stem cells in the VHL protein, and show neural induction of the cells by transfer of the domain peptide linked to protein transduction domain (PTD). The neural induction domain in the VHL protein contains BC-box motif [LXXX(X)XXX(A,I,L,V)] corresponding to binding site of elongin BC, which is evolutionally conserved from virus to mammalian. Therefore, we proposed that other BC-box proteins also contain the neural induction domain, and subsequently show to identify the neural induction domains at amino-acid sequences encoded by BC-box motif within BC-box proteins responsible for neural induction of somatic stem cells. In addition, we show that the domain has the same function for other somatic stem cells except for neural stem cells. Furthermore, when the domain peptide-transferred stem cells are grafted into recipient nervous tissue in neuronal disease models, the grafted cells differentiate to neurons and neuronal repair for neuronal disease models is achieved. Thus, a neural induction domain is identified at BC-box motif in BC-box proteins. The neural induction of somatic stem cells is caused by transfer of the neural induction domain peptide linked to PTD, and would contribute to neuronal regenerative therapy.

**Poster Board Number: T-1083**

**HEAT-SHOCK PROTEIN 27 PROTEIN IS DOWNREGULATED IN THE PROCESS OF PLACENTA-DERIVED MULTIPOTENT CELLS (PDMCS) DIFFERENTIATED INTO NEURON**

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Placenta-derived multipotent cells (PDMCs) isolated from the human placenta are a population of multipotent cells that are able to differentiate into multiple cell types. It is well known that 1-methyl-3-isobutylxanthine (IBMX) induces the differentiation of PDMCs.
or mesenchymal stem cells into neural cells. Using proteomic approaches we found the small heat-shock protein 27 (HSP27) was downregulated at the transcriptional and translational levels during IBMX-induced neuron differentiation in PDMCs model. Overexpression of HSP27 in PDMCs led to the arrest of specific neural differentiation; On the other hand, knockdown of HSP27 using short-hairpin RNA showed significantly enhanced ability of PDMCs differentiated into neuron. Multiple neuronal markers were also stained to confirm the identity of differentiated neuron including MAP2, TuJ1, Tau, Neuron D and NFM. We conclude that downregulated HSP27 protein is a crucial factor in the differentiation of PDMCs into neurons. These findings provide new insights into the neuronal differentiation of PDMCs.

Poster Board Number: T-1084

THE MOOD STABILIZERS VALPROATE AND LITHIUM ACTIVATE HUMAN FGF1 GENE 1B PROMOTER THROUGH RFX TRANSCRIPTION FACTORS

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Valproic acid (VPA) and lithium are two primary mood-stabilizing drugs to have neuroprotective effects and be used to treat bipolar disorder in clinic. Fibroblast growth factor 1 (FGF1) has been shown to regulate cell proliferation, cell division and neurogenesis. Human FGF1 gene 1B promoter (-540 to +31)-driven green fluorescence (F1BGFP) was shown to recapitulate endogenous FGF1 gene expression. It can also be used to isolate neural stem/progenitor cells (NSPCs) from developing mouse brains and human glioblastoma tissue. Our previous study showed that transcription factors RFX2 and RFX3 could directly bind the 18-bp cis-element (-484 to -467), and contribute to the maintenance of F1BGFP(+). In this study, we showed that treatment of human glioblastoma cell lines with VPA and lithium significantly elevated the levels of FGF1B transcript and the percentage of F1BGFP(+) cells in both F1B G18(+)- and (-) cell lines. Interestingly, co-treatment of lithium with VPA could synergistically activate F1BGFP expression. The increase of F1BGFP expression level by VPA treatment was associated with induction of neuronal differentiation. Upon VPA treatment, the F1BGFP(+) cells were differentiated more efficiently into MAP2- and tyrosine hydroxylase-positive neurons than F1BGFP(-) cells. Furthermore, VPA treatment also significantly increased mRNA levels of FGF1 expression. Notably, knockdown of RFX2 could significantly attenuate VPA-enhanced GFP expression in F1BGFP(+) NSPCs. This study suggested, for the first time, that FGF1 is the target for VPA and lithium. Our results provide valuable implication for therapeutic application of these two mood stabilizers.

Poster Board Number: T-1085

MULTIVALENT EFFECTORS TO CONTROL STEM CELL DIFFERENTIATION

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Numerous cellular signaling systems that regulate stem cell self-renewal and differentiation involve the assembly of multivalent ligands - oligomeric entities that present multiple binding sites and thereby bind multiple receptors on a target cell or stem cell - such as morphogens that naturally oligomerize, extracellular matrix protein engagement of integrins, and juxtacrine signaling between membrane-associated ligands and receptors. The resulting multivalent binding can be more potent than corresponding monovalent interactions, potentially through initiating a process of cellular receptor clustering that may promote enhanced signal transduction. However, the ability to control and monitor these naturally occurring multivalent interactions within a living cell is currently limited, since it is difficult to modulate the valency of naturally-occurring ligands, especially membrane-associated proteins. We have developed a means to synthetically conjugate the binding domains of normally cell membrane-associated ligands to the soluble bio-polymer hyaluronic acid (HA). Specifically, using EDC/NHS chemistry, we functionalized the high molecular weight HA backbone and attached recombinantly produced ephrin-B ligand ectodomains via an added terminal cysteine. To precisely estimate the valency and molecular weight of the bioconjugates, we characterized the molecules using size exclusion chromatography and multi-angle light scattering. To determine the effects of bioconjugate multivalency on the ability to cluster cell surface receptors, we incubated adult hippocampal neural progenitor cells (AHPNPs) with the multivalent molecule in vitro. Multivalent ephrin-B showed enhanced clustering of cognate Eph receptors as compared to antibody-clustered ligands. Next, to determine if increased receptor clustering could affect stem cell differentiation, cultures of AHNPCs were incubated solely with either antibody-clustered ligands or high conjugation ratio conjugates for six days. A maximum three-fold increase over the antibody-clustered ligand for the highest ratio conjugate was observed. To determine if the effect of multivalency in directing neuronal stem cell fate could be applied more generally to other cell types, cultures of human embryonic and induced pluripotent stem cells were incubated with the factors for 2-4 weeks. In both cultures a 2-fold increase in the fraction of neurons formed and a 4-fold increase in the fraction of dopaminergic neurons compared to antibody-clustered controls was observed. Cultures also showed increases in the midbrain-specific marker En1 and had significantly higher levels of dopamine. Finally, in an effort to validate the enhanced activity of this multivalent construct in vivo, we injected it directly into the brain of adult rats via intracranial stereotactic injection. Brains injected with bioconjugates showed a three-fold increased ability to induce neuronal differentiation as compared to the antibody-clustered control, thus indicating that highly multivalent ligands potentially enhance the fraction of new neurons formed compared to using ligands clustered by conventional means. These results have a variety of biomedical implications, in that they establish a general platform for creating highly bioactive, defined, and reproducible forms of protein-based ligands, which have the ability to more potently activate downstream effectors for use in basic research and therapeutic applications.

Poster Board Number: T-1086

EXPRESSION OF TYROSINE HYDROXYLASE IS EPIGENETICALLY REGULATED IN NEURAL STEM CELLS

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Tyrosine hydroxylase (TH) is the first and rate-limiting enzyme in biosynthesis of catecholamines. TH expression is regulated in a developmental stage and cell type-specific manner. We have previously reported that the elements responsible for cell type-specific
expression of TH may be included within the repressor region between -2187bp and -1232 in TH promoter. To unravel the underlying molecular mechanisms for this specificity, DNA methylation patterns of CpG islands in the repressor region of TH promoter were examined in neural stem cells and DA neuron-like cells. Using a bisulfitie sequencing method, we found that cytosine residues of CpG island within NRSE-R site was specifically methylated in human NSC, but not SH-SY5Y cells. A good correlation was observed between this CpG methylation and lower expression of TH gene, which was supported by the data that inhibiting DNA methylation with 5-azaacytidine restored TH expression in neural stem cells. We further demonstrate that methylated CpG binding proteins (MBDs) actually binds to the highly methylated X-1 and X-2 regions of TH gene in neural stem cells. These results suggest that region-specific methylation and MBDS play an important role in the regulation of hTH gene in NSCs.

Poster Board Number: T-1088

NEUROSPHERE ATTACHMENT AND ASTROGENESIS ARE INDUCED BY BMP4 VIA PI3K MEDIATED UPREGULATION OF N CADHERIN

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Neuropheres are three-dimensional free-floating aggregates of neural stem cells (NSCs). These structures have been used as culture systems for the expansion and differentiation of NSCs without affecting self-renewal potential and multipotency. The formation of neurospheres is affected by various factors such as epidermal growth factor (EGF) or Fibroblast growth factors (FGF) - 2 which are maintain the self-renewal characteristics. However, the extrinsic signals that affect the formation or dissociation of neurospheres are poorly understood. In this study, we found that bone morphogenetic protein 4 (BMP4) induces astrocytic differentiation and migration of neurosphere NSCs through the attachment of neurospheres. These effects were accompanied by Akt activation and N-cadherin upregulation which is the adhesion molecule. PI3K inhibitor blocked the attachment of neurosphere, astrocytic differentiation and N-cadherin upregulation of neurosphere NSCs. Especially, BMP4-induced neurosphere attachment, astrocytic differentiation, and migration of neurosphere NSCs were inhibited by neutralizing N-cadherin antibody. Together, these findings show that BMP4-induced attachment of neurospheres is related to the astrocytic differentiation of these cells and that these effects are attributable, at least in part, via PI3 kinase-Akt pathway-dependent induction of N-cadherin.

Poster Board Number: T-1089

THE EFFECT OF LEUKOENCEPHALOPATHY VANISHING WHITE MATTER MUTATIONS ON GLIAL DIFFERENTIATION

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Vanishing white matter disease (VWM) is an autosomal recessive leukoencephalopathy caused by mutations in genes encoding the eukaryotic translation initiation factor 2B (eIF2B). Children are mainly affected, who suffer from progressive neurological symptoms as ataxia and die at young age. Previous research has shown that astrocytes and oligodendrocytes in their brain white matter show a defect in maturation. Currently there is no treatment available. Our research is focused on 1) To test prospects for cell replacement therapy for VWM patients. Transplantation of stem cells in early stages of the disease may halt further progression of the disease and repair the existing damage. We have generated mouse models for VWM, which show a phenotype that is similar to that of the human patients. These mice offer us excellent models to test cell replacement therapies. 2) To investigate in vitro the capability of VWM stem cells to differentiate into fully mature astrocytes and oligodendrocytes. We use induced pluripotent stem cells (iPSCs) derived from VWM mice and human patients, and test the effects of a VWM microenvironment on the differentiation of oligodendrocytes. By combining in vitro and in vivo stem cell work, we hope to elucidate the VWM disease pathology further and develop new insights into therapeutic strategies for VWM and childhood white matter disorders in general.
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Poster Board Number: T-1090

MOTOR NEURONS AND DOPAMINERGIC NEURONS: NOVEL STEM CELL-BASED SOLUTIONS ACCELERATING RESEARCH AND DRUG DISCOVERY

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Neurons isolated from human tissue have been widely used in basic research, drug discovery and for treatment of neurodegenerative disorders. However, the use of these cells is mired in ethical and technical issues mainly associated with procurement of human tissue and isolation of functional cells from the tissue. Alternatively, human pluripotent stem cells (hPSCs) including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) offer great promise to generate specialized cells from different lineages including human neurons. Lonza, Inc. in partnership with California Stem Cell, Inc. recently launched its first hESC-derived cell product - MotorPlate™, which are functional, high-purity human motor neurons (hMNs). MotorPlate™ hMNs are available in a ready-to-use format (96-well plates or 384-well plates) for use in high throughput applications. We further developed a method to cryopreserve hMNs and demonstrated comparable characteristics to non-cryopreserved neurons. The frozen vial format provides more flexibility to researchers in the field. In early 2012 Lonza, Inc. entered into a partnership with xCell Sciences, LLC with the intention to provide dopaminergic (DA) neurons derived from hESCs and hiPSCs to researchers in academia and industry. Following differentiation, the majorities of cells are Tyrosine Hydroxylase- (TH-) positive and co-express midbrain and A9 dopaminergic markers. Transplantation of these cells in 6-hydroxydopamine induced parkinsonian rats resulted in amelioration of behavioral deficits, demonstrating the functionality of these DA neurons. DA neurons derived from hESCs will be offered in a high throughput format (96-well and 384-well plates) as well as cryopreserved vials. In addition Lonza will offer generation of DA neurons from iPSCs on a fee-for-service basis upon request. In conclusion, Lonza, Inc. has established itself as a reliable source of functional and high purity Dopaminergic and Motor Neurons suitable for use in basic research, drug discovery and toxicity testing.

Poster Board Number: T-1091

RECEPTOR-SPECIFIC BLOCKING OF NOTCH SIGNALLING

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The Notch signalling pathway is instrumental for cell fate diversification at multiple stages in development and in cell proliferation in both normal and diseased states. The pathway has been studied extensively in mammalian neural stem cells both in vivo and in vitro; however there is still much uncertainty about the specific functions of individual Notch receptors and ligands. In mammals there are 5 ligands, which are capable to promiscuously activate the 4 different Notch receptors (Notch 1-4). Despite this promiscuity, individual family members have been associated with distinct roles in fate regulation and also to exhibit different expression pattern in various contexts. Thus the ability to specifically block or activate individual Notch receptors would provide greater understanding of the role of the individual receptors and would permit a greater degree of control in model systems, e.g. stem cells. From a scFv phage display antibody library we have selected and characterised antibodies specifically binding to Notch family members including receptors Notch 1-3 and the ligands Jagged and Delta. Further, we have identified blocking antibodies for Notch1 and Notch2. The potency of blocking antibodies has been determined in a co-culturing assay and antibodies targeting Notch 1 and 2 are silencing signalling completely. By qRT-PCR we have verified that downstream target genes of Notch are down regulated when blocking antibodies are added to neural stem cells in vitro. Functional antibodies provide a route to block or activate the individual Notch receptors. In contrast to other methods, this could be done reversible in genetically unmodified cells or animals and thus enable flexible regulation of signalling at multiple stages.

Poster Board Number: T-1092

GENERATION OF PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS CARRYING MUTATION IN SOD1.

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder in which motor neuron (MN) loss in the spinal cord and motor cortex leads to progressive paralysis and death. Although most cases are sporadic, about 10% of cases are inherited. Super oxide dismutase 1 (SOD1) is the first identified gene which causes ALS. Accumulating evidence, explored by ALS mice model expressing mutant SOD1, provides several hypotheses. However, there is no effective cure so far. To establish drug screening platform, it is necessary to reveal cellular phenotypes in human MNs and other types of cells. Here, we describe the generation of induced pluripotent stem cells (iPSCs) from 6 familial ALS patients and the differentiation of disease-specific iPSCs into MNs to establish an ALS model in vitro. To generate patient-specific iPSCs, dermal fibroblasts were obtained by biopsy from patients carrying mutant SOD1. The fibroblasts were reprogrammed by retrovirus vectors or episomal vectors. These iPSC lines expressed ESC markers such as Nanog and SSEA-4, and had pluripotency to differentiate into cells of all three germ layers in vitro and in vivo. DNA sequencing revealed that mutated alleles of iPSCs are identical to the original specimen. All iPSCs preserve normal karyotype. Disease-specific iPSCs were differentiated into MNs expressing Hb9 and ChAT. These results indicated that disease-specific iPSCs might provide an ALS model system in vitro.

Poster Board Number: T-1093

INHIBITION OF CRUCIAL TUMOR SUPPRESSOR PATHWAYS IN ADULT NEURAL STEM CELLS RESULTS IMBALANCED HOMEOSTASIS AND GLIOMAGENESIS

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Neural stem cells (NSCs) have been suggested as cellular origin of brain tumors. How the endogenous NSCs contribute to brain tumorigenesis instead of neurogenesis remains poorly understood. We have previously shown that the nuclear receptor Tlx is crucial for brain tumor initiation from adult NSCs. Here we show that the tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome 10) is inhibited by Tlx in the adult NSCs. Inactiva-
tion of the PTEN gene specifically in type B cells of the adult mouse subventricular zone (SVZ) causes transient expansion, but surprisingly a long-term decrease of neural stem/progenitor cell proliferation. We provide evidence that an increase of cellular senescence contributes to the loss of PTEN-deficient neural stem/progenitor cells. p21 is up-regulated in the PTEN mutant but down regulated in Tlx overexpressing NSCs. Genetic ablation of p53, which leads to a down regulation of p21, together with PTEN loss leads to a continuous increase in stem cell proliferation and brain tumor formation. In addition, we observed a critical cell fate switch of the mutant NSCs, which demonstrate the redirection of cell fate toward glial lineage is one important step for glioma development. This study unmasks a core pathway in regulation of the balance between adult NSC homeostasis and tumorigenesis.

**Poster Board Number:** T-1094

**IN VIVO IMAGING OF ENGRAFTED NEURAL PRECURSOR CELLS: ITS APPLICATION IN EVALUATING THE OPTIMAL GRAFT CELL NUMBER FOR SPINAL CORD INJURY**

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Neural precursor cells (NPCs)-based approaches have been acclaimed as potential treatments for many neurodegenerative disorders, including spinal cord injury (SCI). Indeed, there are a number of reports in which the transplantation of NPCs in animal models of SCI has been shown to improve the functional recovery, and some clinical trials using NPCs have also been performed or initiated. However, the transplantation protocols are completely different in several ongoing clinical trials for spinal cord injury (SCI) with cell therapy and, in particular, there is a considerable variation in the number of transplanted cells (ranging from 2e5 to 3.7e8 cells), which is exactly the same situation as in animal experiments (ranging from 7.5e4 to 1.25e6 cells). This chaotic situation hampers the establishment of an optimal treatment for SCI. Nevertheless, there has been no attempt to determine the optimal number of cells for transplantation because of the lack of suitable methodology. Conventional evaluation to quantify the number of engrafted cells mainly relies on histological examination, in which it is very difficult to distinguish living or dead cells and to count all cells. Therefore, we employed a bioluminescence imaging (BLI) system to address this question. NPCs were harvested from embryonic mouse striatum and labeled with luciferase and green fluorescent protein (GFP) reporter genes via lentiviral transduction, and GFP-positive cells were expanded with a neurosphere assay. To examine whether the number of transplanted cells affects cell survival and functional improvement, different numbers of lentivirally labeled NPCs (1×e5, 2.5×e5, 5×e5, or 1×e6 cells) were transplanted into the injured spinal cord immediately after contusion injury at the 10th thoracic level. After confirming that the number of transplanted NPCs correlated with bioluminescence intensity, we tracked the bioluminescence intensity and observed comparable rates of NPCs survival, related with bioluminescence intensity, we tracked the bioluminescence imaging. Conventional evaluation to quantify the number of engrafted cells was difficult to distinguish living or dead cells and to count all cells. Therefore, we employed a bioluminescence imaging (BLI) system to address this question. NPCs were harvested from embryonic mouse striatum and labeled with luciferase and green fluorescent protein (GFP) reporter genes via lentiviral transduction, and GFP-positive cells were expanded with a neurosphere assay. To examine whether the number of transplanted cells affects cell survival and functional improvement, different numbers of lentivirally labeled NPCs (1×e5, 2.5×e5, 5×e5, or 1×e6 cells) were transplanted into the injured spinal cord immediately after contusion injury at the 10th thoracic level. After confirming that the number of transplanted NPCs correlated with bioluminescence intensity, we tracked the bioluminescence intensity and observed comparable rates of NPCs survival, irrespective of the number of transplanted NPCs. All NPCs-transplanted groups showed better locomotor function recovery than the medium-injected control group in two open-field motor scores (Basso, Beattie, and Bresnahan (BBB) score and Basso Mouse Scale (BMS)) and footprint analysis. However, functional recovery was not significantly different among the NPCs-transplanted groups. Correlative analysis revealed no relationship between the number of surviving NPCs and the subsequent functional recovery at any time point. Furthermore, we transplanted a broader range of NPCs (ranging from 2.5×e4 to 2.5×e6 cells). The transplantation of 2.5×e4 NPCs did not significantly affect functional improvement, but that of 2.5×e6 NPCs had a negative effect on functional improvement. These results indicate that optimizing the number of graft NPCs is crucial for the treatment for SCI.

**Poster Board Number:** T-1095

**PROTOCOLS FOR MATURE MOTOR NEURON ISOLATION**

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This study aims to present motor neuron (MN) isolation protocols that will allow a sufficient yield of cells for analysis by means of immunohistochemistry, in situ hybridization, quantitative polymerase chain reaction (qPCR), and microarray analysis. Recent advances in stem cell neurobiology have allowed the production of MNs from stem cells using appropriate signaling factors such as retinoic acid (RA) and sonic hedgehog (Shh). However, obtaining a uniform population of MNs in cell culture has been a challenge, as stem cells differentiate into other neural cell fates. Obtaining a homogenous MN population has also been a challenge in vivo, as a species ubiquitous protocol for isolating MNs from the spinal cord has yet to be developed. This study aims to describe how a uniform population of MNs can be isolated in vitro and in vivo using isopycnic density centrifugation and fluorescent activated cell sorting (FACS). Hb9::GFP transgenic mouse embryonic spinal cords were dissected from 14.5 day old embryos. Liberase dissociated spinal cords were subjected to FACS using the homeobox gene Hb9::GFP reporter and neurotrophin receptor (p75NTR) antibody as MN isolation markers. FACS assessment indicated an isolation of 31.2% Hb9::GFP and p75NTR double positive in vivo-derived spinal cord MNs. Liberase dissociation of day 8 Hb9::GFP mouse embryonic stem cell (mESC)-derived MNs were also subjected to FACS, showing an isolation of 61.3% Hb9::GFP and p75NTR double positive in vitro-derived MNs. qPCR analysis targeting MN specific markers such as choline acetyltransferase (ChAT) and LIM homedomain protein Isl-2 (Isl2) showed higher normalized ratios of ChAT and Isl2 expression in Hb9::GFP and p75NTR double positives. Papain dissociated e14 mouse spinal cords were subjected to a HistoDenz density gradient. Immunocytochemical cell counts of Hb9::GFP positive cells showed a 2.73% yield of MNs. From FACS analysis and HistoDenz density centrifugation, we can conclude that MNs were isolated, albeit at a moderate efficiency using FACS and low efficiency using density centrifugation. Isolation of homogenous MN populations in vitro and in vivo is essential; as a comparison of these two systems will provide information on how accurately stem cell-derived MNs resemble their in vivo complement. Analysis of isolated MNs via in situ hybridization and microarray will allow identification of new key regulators responsible for MN development; aside from already known common regulators. This feat will allow the development of a defined in vitro model for neurodegenerative diseases, as well as offer hope for future cell replacement therapy.
Detailed Program and Abstracts — Thursday, June 14

Poster Board Number: T-1096

SMALL MOLECULES THAT INDUCE NEUROGENESIS AND INHIBITS Gliogenesis IN NEURAL STEM CELLS

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Human neurodegenerative disorders are closely associated with a loss of neurons and glial cells in the central nervous system. Neural stem cells (NSCs) are potential and attractive sources for cell replacement therapy and regeneration in the central nervous system (CNS), and small molecules that can modulate NSCs differentiate into a certain cell type can be a useful agent for the therapeutic application. In an effort to provide stem cell-based therapeutic approaches using NSCs, an in vitro screen was performed in search of chemicals inducing neurogenesis in NSCs. About 100 chemicals were screened through image based screening, and we found the chemical named KHN 01, that significantly induce neurogenesis and inhibited gliogenesis. KHN 01 and its derivatives, KHN 02 and 03, were tested to see if they could regulate NSC differentiation. Immunocytochemistry analysis showed that KHN 01, 02 and 03 induced neuronal marker βIII tubulin and inhibited production of glial cells such as astrocytes and oligodendrocytes, suggesting that KHNs selectively induce a neuronal differentiation in expense of gliogenesis. Immunocytochemistry analysis using antibodies against BrdU and Ki67 revealed that KHNs inhibited cell proliferation. The neurogenic effects of KHNs disappeared when epidermal growth factor was present. In addition, KHNs synergistically increase neurogenesis when treated with PD 98059, a selective MEK inhibitor. From these observations, KHNs reduced cell proliferation and induced neurogenesis along with the inhibition of gliogenesis in NSCs, and may be useful for modulating NSC fate.

Poster Board Number: T-1097

IDENTIFICATION OF MOLECULES IMPORTANT FOR THE DEVELOPMENT OF RESPIRATORY MOTOR CIRCUITS USING MICROARRAY ANALYSIS

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Respiratory failure is the most common cause of death in motor neuron (MN) degenerative diseases such as spinal muscular atrophy and amyotrophic lateral sclerosis and in upper cervical spinal cord injuries. The advent of methods to generate spinal MN from both embryonic and induced pluripotent stem cells has opened up new possibilities for studying the pathogenesis of MN disease in vitro and developing cellular therapies to replace damaged neurons and restore motor functions. However, most of the procedures currently used to generate MNs from stem cells produce only a limited subset of the MN classes found in vivo and do not efficiently generate respiratory MNs. To overcome this challenge, we have set out to define the molecular pathways that lead to MN formation and functional diversification. While much emphasis has been focused on understanding the development of limb-innervating lateral motor column (LMC) MNs, the factors important for hypaxial/respiratory motor column (HMC) specification remain poorly defined. We have previously demonstrated that the transcription factor Foxp1 plays an essential role in LMC MN formation by suppressing the generation of HMC MNs. To identify novel determinants of HMC MN fate, we have carried out gene expression profiling experiments comparing control and Foxp1 mutant MNs. This analysis has revealed a number of transcription factors, growth factors, and axon guidance molecules present in HMC MNs that may serve a critical function in respiratory MN development and circuit assembly. We will present our current research into the molecular and functional analyses of the genes unveiled in this study.

Poster Board Number: T-1098

ABERRANT FOCAL ANGIOGENESIS IN THE SUBVENTRICULAR ZONE INDUCED BY EPIDERMAL GROWTH FACTOR

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The ErbB receptor family mediates a plethora of effects in a wide array of tissue types and contexts. Signaling through the ErbB receptors is often altered in cancerous tumors. Increased ErbB signaling, mainly ErbB1 (EGFR) and ErbB2, can lead to increased proliferation and invasiveness of tumor cells. There is also evidence suggesting a role of ErbB1 in angiogenesis, characterized by expression of ErbB1 in endothelial cells. We have previously described that intracerebroventricular infusion of epidermal growth factor (EGF) induces dysplasia in hyperproliferative polyps in the subventricular zone (SVZ). Interestingly, blood vessels develop in about 30% of the polyps after continuous EGF infusion for 14 days. Structurally, the newly formed vessels are of a disorganized and glomeruloid appearance. Sometimes as bundles sprouting from a single SVZ blood vessel, and other times as a highly vascularized ventricle wall. These vessels are to a large extent covered by pericytes strongly expressing NG2. However, NG2 is also expressed by other cell types such as polydendroglia and oligodendrocyte progenitors. We found cells expressing NG2 throughout the brain; however, pericytes strongly expressing NG2 were restricted to the dysplastic areas. This indicates that neangiogenesis is not a general phenomenon in the EGF-infused SVZ but specifically induced in hyperproliferative areas. The putatively angiogenic area showed multi-luminal vessels and signs of immaturity based on ultrastructural characteristics, such as a thickened endothelial cell layer. In addition, these vessels exhibited a dysfunctional blood-brain barrier, demonstrated by albumin extravasation using Evans blue. Using an antibody against phosphorylated EGFR we found phospho-EGFR-positive endothelial cells exclusively in the angiogenic foci. These results suggest angiogenic properties of EGF, specific to hyperproliferative areas in the SVZ.

Poster Board Number: T-1099

MUSASHI1 POST-TRANSCRIPTIONALLY REGULATES THE GENE EXPRESSION IN NEURAL STEM/PROGENITOR CELLS AND GLIOMA

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Musashi is an evolutionarily conserved family of RNA-binding proteins that is expressed in the nervous system. In Drosophila, this protein plays an essential role in regulating the asymmetric cell division of sensory organ precursor cells through the translational regulation of target mRNAs (Nakamura et al., Neuron 1994; Okabe et al., Nature 2001). Its mammalian homologues, Musashi1 and Musashi2, are RNA-binding proteins that are expressed in fetal and adult neural stem/progenitor cells (NS/PCs). Previously, our group...
reporting that the transcript of m-numb was identified as target of Musashi1, and were repressed the translation by Musashi1 through inhibition of mutual interaction of two translational initiation proteins, PABP and elf-4G (Imai et al., Mol Cell Biol. 2001; Kawahara et al., J Cell Biol. 2008). We and other group independently demonstrated that Musashi1 and Musashi2 activate the Notch signaling pathway by translational repression of the mRNA for the Numb protein, a negative regulator of the Notch-signaling pathway (Imai et al., Mol Cell Biol. 2001; Ito et al., Nature 2010; Kharas et al., Nat Med. 2010). Other Musashi1-target transcripts, p21waf1, doublecortin, and APC were identified by our group and other groups (Battelli et al., Mol Cell Neurosci. 2006; Horisawa et al., FEBS Lett. 2009; Spears and Neufeld, J Biol Chem. 2011). However, entire Musashi1-target mRNAs in self-renewing neural stem cells had remained to be elucidated. For the purpose of revealing whole of Musashi1-RNA networks in NS/PCs, we purified endogenous Musashi1-RNA complexes in NS/PCs by immunoprecipitation, and identified Musashi1-associating mRNAs by using gene expression microarray method. Interestingly, the result showed that many mRNAs of tumor-related genes, cell cycle-regulating genes and differentiation-regulating genes were concentrated by Musashi1-specific immuno-purification. In order to know whether Musashi1 regulates self-renewal ability through the regulation of the Musashi1-associating mRNAs in NS/PCs and tumor cells, we focused some tumor-related genes among them. siRNA ablation studies against musash1 were performed by using low times-passaged glioblastomas, established glioblastoma cell lines, and medulloblastoma cell line, which form ‘gliomashpere’ in the stem cell culture condition with both EGF and FGF2. The decrease of Musashi1 in tumor cells led to increased expression of the Musashi1-target genes including Numb and PTEN. Furthermore, our results showed the reduction of post-transcriptional regulation by Musashi1 impaired self-renewing activity and cell survival competence of glioma cells through the deactivation of Notch and PI3K-Akt signaling pathways. Together, our observation suggested that Musashi protein plays an important role in the self-renewing cells in the tumor as well as NS/PCs.

**Regulation of Neural Stem Cell Self-Renewal and Differentiation**

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Wnt signaling plays a critical role in regulating self-renewal and differentiation of neural stem cells. We previously reported that a Wnt receptor, Ryk intracellular domain (ICD) translocates into the nucleus and regulates GABAergic neuronal differentiation. However, it remains to be elucidated how Ryk ICD moves to the nucleus and regulate neuronal differentiation. We have recently identified the nuclear protein Smek as a Ryk ICD interacting protein. Smek expression is upregulated during neuronal differentiation and is sufficient to drive the nuclear localization of Ryk ICD. In addition, Smek overexpression increased neuronal differentiation in Ryk-dependent manner. To further examine roles of Smek in neurogenesis in vivo, we have generated Smek double knock out mice. We demonstrated that Smek is required for GABAergic neuronal differentiation and directly regulate the expression of Dlx transcription factors which are essential for GABAergic neuronal differentiation. These data suggest that Smek might be a key regulator of Wnt-Ryk signaling in GABAergic neuronal differentiation of developing mice brain.

**Epithelial Cells (Not Skin)**

**Poster Board Number: T-1100**

**Identification of Melanocyte Stem Cells in Eccrine Glands as a Potential Source of Acral Melanoma**

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Human cutaneous melanoma is a highly aggressive cancer that is resistant to traditional cancer treatments including both chemotherapeutics and radiotherapy. Acral melanoma is the most prevalent subtype of melanoma in the non-Caucasian population. The preferential proliferation of early acral melanoma cells along and around epidermal eccrine ducts (EG), a reliable early diagnostic sign of melanoma with a 99% specificity, has indicated a close association between early acral melanoma in situ and EGs. However, neither the presence of melanocytic cells in the eccrine sweat glands nor the precise origin of these melanoma cells is known as in most cancers. Here, we report the identification of melanocyte stem cells (MelSC) in mouse and human acral skin. We identified unpigmented melanoblasts residing in the secretory portion (SP) of EGs, using lineage-tagged H2B-GFP reporter mice. These melanoblasts are normally kept in an immature, slow-cycling state but are able to self-renew, indicating that this population possesses adult stem cell features in the niche area. In response to stress, including ionizing irradiation, they not only renew themselves but also provide amplifying and differentiating progeny that migrates upward toward the epidermis where they mature into melanin pigment-producing melanocytes. In addition, we found that a similar population expressing melanocyte lineage marker MART1 reside in the secretory portion of the EGs in human acral skin. Analysis of early acral melanoma in situ revealed that distribution of MART1+ proliferating melanoma cells are not localized only within the epidermis but accompanied by their contiguous distribution starting from the SP of a particular EG through the connecting eccrine ducts (ED) in early melanoma lesions. Thus, we propose that the EG-MelSCs are the potential source of human acral melanoma that produces melanoma initiating cells during their constitutive renewal.

**Poster Board Number: T-1102**

**Generation of an In Vitro Model of Cystic Fibrosis**

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Cystic Fibrosis (CF) is an autosomal recessive inherited chronic lung disease and with over 70,000 patients worldwide it is associated with severe disability and a short life expectancy. Current animal models of CF poorly simulate clinical lung disease and a lack of primary lung tissues has limited the rate of research progress. With this in mind we are using patient derived iPSC to generate a reproducible in vitro model of CF with potential for a matched positive control using homologous recombination techniques to correct the identified mutation. Proviso free iPSC were generated from fibroblasts isolated from a skin biopsy obtained from a CF patient.
Wnt signaling could increase the efficiency of limbal stem/progenitor differentiation of the limbal stem/progenitor cells and Fz7 might be our data indicated that Wnt signaling pathway regulates the differentiation gene profiling of the human limbus, conjunctiva and cornea located. Wnt signaling pathway was among the notable biological pathways that were identified in the limbus where the putative corneal epithelial stem cells are located. Wnt signaling pathway in human corneal epithelial stem/progenitor cells. Differential gene profiling of the human limbus, conjunctiva and cornea revealed that there were 146 transcripts preferentially expressed in the limbus where the corneal epithelial stem cells are located. Wnt signaling pathway was among the notable biological processes, and Wnt6 and Fz7 preferential expression in the limbus was confirmed by qRT-PCR and immunohistochemistry. When the primary human limbal epithelial cell culture was supplemented with Wnt6 using the Wnt6 overexpressing mouse 3T3 feeder cells, the expression of putative stem cell markers, ABCG2 and corneal maturation marker, keratin 12 expression had a 3 fold decrease. On the other hand, loss of the stem/progenitor phenotype in culture correlated with a lower expression level of Fz7. In addition, knockdown of Fz7 expression using shRNA in the primary human limbal epithelial cells led to the loss of stem/progenitor phenotype. Colony forming efficiency was significantly decreased. In summary, our data indicated that Wnt signaling pathway regulates the differentiation of the limbal stem/progenitor cells and Fz7 might be responsible for transducing the Wnt signaling. Modulating the Wnt signaling could increase the efficiency of limbal stem/progenitor cell expansion in vitro.

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Organ donor. iPSC were generated using a CRE excisable six factor polycistronic lentiviral reprogramming cassette that we developed and colonies were picked at day 14 of reprogramming. The provirus was excised using CRE-RNA and excision was confirmed by absence of a PCR product for the lentiviral backbone and the SOX-2/KLF4 junction in the reprogramming plasmid. Both iPSC and provirus iPSC were fully characterized for stem cell pluripotency including endogenous gene expression, immunofluorescent imaging of colonies, EB formation, teratoma formation of all three germ layers and routine karyotype analysis. The genotype of the patient was confirmed to be delta 508 homozygous and the mutation was retained across all cell types generated; fibroblasts, iPSC and provirus free iPSC. Using both CF iPSC and control fibroblast derived iPSC a differentiation protocol was developed consisting of a differentiation to definitive endoderm (SOX17, GATA6 positive) and plating of the cells first in a liquid-liquid and then air-liquid interface in transwell inserts. A timecourse of RNA analysis by qPCR indicates an increase in definitive endoderm markers GATA6, SOX17 and NKK2.1, which is important in early lung development and differentiation of the distal lung compartments. As differentiation progresses generation of cells expressing FOXJ1 and acetylated α tubulin is indicative of a push towards ciliated cells and the expression of pulmonary surfactant proteins B, C and D indicates alveolar cell differentiation. Cells also formed tight junctions indicated by ZO-1 and epithelial Cadherin staining and strongly expressed cytokeratin 18 and to a lesser extent cytokeratins 14 and 19. Furthermore, there is a distinct change in the expression of CFTF in differentiated cells incubated for 24 hours at 30°C with notable increase in cell surface expression observed at the lower temperature. In conclusion we have generated the first (to our knowledge) functional patient specific model of cystic fibrosis with the capacity for a congenic positive control via homologous recombination and correction of the mutant gene.
four days after the introduction. SSEA1, differentiation marker, positive cells decreased 10-20% eight days after the introduction. Conclusion; The expression of Rex1 and SSEA4 was increased and the expression of SSEA1 was decreased with Oct4 overexpression. It is suggested that Oct4 induces cell reprogramming.

**Poster Board Number: T-1107**

**INHIBITION OF PROTEIN NITROSYLATION ENHANCES LIVER REGENERATION AFTER TOXIC INJURY**

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Acetaminophen (APAP) liver toxicity is the most common cause of fulminant liver failure. Currently, there are no treatments to enhance recovery from toxic injury. Signaling pathways that modulate embryonic liver development may help to elucidate conserved mechanisms that affect liver regeneration. In order to identify novel regulators of liver development in vivo, we performed a chemical genetic screen in zebrafish embryos, which revealed that nitric oxide (NO) signaling modulated liver development. The NO donor S-nitroso-gluthathione (GSNO) caused increased liver size at 72 hpf, assessed by in situ hybridization for liver fatty acid binding protein (fliabp) and quantified by FACS of GFP-labeled hepatocytes. In contrast, NO inhibition through N-nitro-L-arginine methyl ester (L-NAME) caused smaller livers. GSNO and L-NAME altered expression of the hepatoblast marker hhex, indicating that NO signaling affects the liver progenitor pool during liver development. NO mediated its effects not via the classical pathway of cGMP signaling, but through protein nitrosylation: while modulation of cGMP had no impact on liver size, knockdown or chemical inhibition of the negative regulator of protein nitrosylation, GSNO reductase (GSNOR), resulted in larger livers. In order to determine whether NO signaling also impacted recovery after liver injury, we employed previously validated larval and adult zebrafish models of APAP liver toxicity: zebrafish exposed to APAP develop hepatocyte necrosis, as demonstrated by elevated alanine aminotransferase levels and histology, and death. Treatment of APAP-exposed fish with a novel GSNOR inhibitor (N6547, N30 Pharmaceuticals) significantly prevented the rise in alanine aminotransferase, improved hepatocyte necrosis and enhanced cell proliferation, as demonstrated by BrdU incorporation. Furthermore, GSNOR inhibition improved survival by more than 50% following APAP exposure. Patients with APAP liver toxicity may not immediately present after drug ingestion, reducing the efficacy of the only available clinical antidote, N-acetylcysteine (NAC): GSNOR inhibition acted synergistically with NAC after delayed treatment up to 18 hours after APAP exposure, thereby expanding the therapeutic window to improve outcome. In order to demonstrate a conserved role of GSNOR in mammalian liver injury, we treated wild-type and GSNOR knockout mice with sublethal doses of APAP: wild-type mice exhibited an increase in alanine aminotransferase levels at 6 and 24 hrs, whereas GSNOR/-/- mice had significantly lower values. Furthermore, wild-type mice developed characteristic pericentral necrosis following APAP exposure, while GSNOR/-/- had minimal injury. These data demonstrate that NO signaling, acting via nitrosylation, affects liver progenitors to ensure optimal liver development and regeneration following APAP induced liver injury. We propose that NO can act as a rapid signaling molecule to sense injury and initiate the regenerative response. GSNOR is an excellent candidate for novel therapeutic approaches to improve the regeneration and outcome in APAP liver failure.

**Poster Board Number: T-1108**

**PLURIPOTENT TRANSCRIPTION FACTOR OCT4 PROMOTES CELL MIGRATION IN ENDOMETRIOSIS**

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BACKGROUND: Endometriosis is the growth of endometrial tissues ectopically and/or ectopically which present high cell migration ability. Pluripotent transcription factor OCT4 has been associated endometriosis. However, the role of OCT4 in endometriosis still remains largely unknown. This study aims to examine the expression profile of pluripotent transcription factor OCT4 in human ectopic endometriosis progression and its role in migration of endometrium cells. METHODS AND RESULTS: We analyzed the gene and protein expression level of pluripotent transcription factor OCT4 and NANOG in patient tissues (n = 110) by quantitative real-time RT-PCR and immunohistochemical staining. While comparison with human endometrium (n = 2) and myoma (n = 4), the hyperplasia (n = 37) and ectopic endometriosis tissues (n = 67, 19 for adenomyosis and 48 for chocolate cyst) showed a significantly increasing of OCT4 and NANOG expression in mRNA level. The OCT4 protein was both detected in stromal and luminal epithelial cells of adenomyosis- and chocolate cyst tissues. The OCT4 expression level in endometriosis tissues is positively correlated with the gene expressions which associated with cell migration, such as Twist, Snail, Slug, and Vimentin. Overexpression of OCT4 protein in human endometrioid carcinoma cell lines RL95-2 and HEC1A (both in low OCT4 expression level) significantly increased the expression level of migration-associated genes (Twist, Snail, Slug, N-cadherin, and Vimentin) and proteins (N-cadherin and Vimentin) and promoted the migration of endometrium cells which was evidenced by wound healing- and transwell assay. Immunocytochemical staining combined with confocal images further demonstrated that OCT4 affects actin filament distribution and the migratory morphology of human endometrium cells. CONCLUSIONS: OCT4 expression in endometrium cells showed a high impact on ectopic endometriosis progression and significantly increased the migration ability of endometrium cells. Findings in this study may provide a potential early diagnosis biomarker and part of the molecular mechanism in the progression of human ectopic endometriosis and ovarian cancers.
Detailed Program and Abstracts — Thursday, June 14

 Liver Cells

Poster Board Number: T-1111

BI-POTENT HEPATIC PROGENITORS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS FOR DRUG TESTING

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Primary human hepatocytes are a valuable tool for biomedical research, the pharmaceutical industry and therapeutic applications. They are becoming increasingly utilized in drug development to evaluate human specific drug properties such as metabolic fate, drug-drug interactions and drug toxicity. However, the demand for primary human hepatocytes far outstrips the available supply and the quality of primary hepatocytes is highly variable. Human embryonic stem cells (hESCs) could in principle provide a renewable source of human hepatocytes. However, the derivation methods described so far are inefficient and do not yield pure populations of functional mature hepatocytes. Our preliminary study showed that TW6 hESCs differentiate into hepatocytes via a progenitor stage in which cells were capable of extensive growth. By expanding these progenitors in culture and inducing hepatic differentiation, a pure population of hepatocytes could be generated with high efficiency and shorter time course. Thus the focus of this study was to develop a platform for derivation and expansion of hepatic progenitors from hESCs. To achieve this, hESCs were differentiated into cells expressing hepatic progenitor markers at day 13, followed by expansion in a serum-free expansion medium for two weeks. The expanded cells displayed proliferative ability as indicated by Ki67 expression, exhibited high nucleus-to-cytoplasm ratio, and expressed hepatic progenitor markers such as AFP and EpCAM. These cells were passaged at confluency using mechanical dissection. Furthermore, the progenitors could be induced to differentiate into albumin-positive hepatocytes or CK7-positive cholangiocytes, exhibiting bi-potent differentiation potential. The hepatic progenitor cells can be utilized as a renewable source of hepatocytes and may potentially be useful for cell therapies, bioartificial livers, hepatitis C virus infection cell model and drug testing.

Poster Board Number: T-1112

GENERATION OF CGMP-COMPATIBLE FUNCTIONAL HEPATOCYTES FROM A CLINICAL- GRADE HUMAN EMBRYONIC STEM CELL LINE

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Human embryonic stem cells (hESC) hold great potential for use in regenerative medicine and drug development. However, growing and maintaining hESC on mouse feeder layers with the use of animal products hinders their clinical applications. Recently hESC have been developed as clinical-grade cell lines by BioTime. In this study, we have attempted to generate cGMP-compatible functional hepatocytes employing a clinical-grade hESC line and feeder-free (FF) and xeno-free conditions. Line ESI-035 (ESI35) was recovered, expanded and maintained on either mouse feeder cells, human feeder cells, or Matrigel, then differentiated into hepatocytes. The ESI35-derived hepatocytes (ESI35-Hep) were characterized by the expression of liver-specific proteins and functions, as well as by...
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metabolic profiling of the drug bufuralol (BF). Under our modified conditions (no animal products), ESI35 cells grown on mouse or human feeders or on Matrigel were initially induced to contain a high percentage of definitive endoderm (DE) cells as assessed by the expression of SOX17 and CXCR4 at levels between 95% and 98%, as determined by flow cytometry (FC), and by showing a uniform morphology when immunostained. Employing our xenograft proliferation protocol and FDA certified fetal bovine serum, the DE cells were successfully differentiated into hepatocytes with 91-98% of cells positive for albumin (ALB) and α-antitrypsin after 3-4 weeks of differentiation, as determined by FC and immunohistochemistry. The cellular uptake and excretion of Indocyanine Green was observed in our ESI35-Heps, indicating a functional biotransforming system, and the cells also showed glycogen accumulation. ELISA results demonstrated that ESI35-Hep grown on mouse feeder cells (ESI35-Hep/ MF) secreted 5.3 ± 0.8 µg ALB into the medium per million cells over 24h after differentiation, ESI35-Hep grown on human feeder cells (ESI35-Hep/HF) secreted 5.0 ± 0.4 µg ALB, and ESI35-Hep grown on Matrigel (ESI35-Hep/FF) secreted 2.7 ± 0.28 µg ALB. To further assess the biotransformation system, we employed ultraperformance liquid chromatography-tandem mass spectrometry technology for BF metabolic profiling and metabolism. We found the same 3 secondary metabolites of BF from oxidation, dehydrogenation, ketone formation, or potential methylation in phase I, and the same 2 secondary metabolites of BF from glucuronidation or conjuration of glucose in phase II, in all of the ESI35-Heps when compared to freshly isolated human primary hepatocytes (hPH). Interestingly, the metabolite of di-oxidation was only detected in ESI35-Hep/HF and hPH, and importantly, the levels of all secondary metabolites in ESI35-Hep/HF were significantly higher than those in ESI35-Hep/MF and ESI35-Hep/FF, suggesting that human feeder cells may promote and enhance the differentiation of hESC. Thus it appears that our ESI35-Heps/HF have developed full metabolic function. In conclusion, these results demonstrate that clinical-grade hESC can be effectively differentiated to hepatocyte-like cells employing cGMP-compatible culture conditions. This represents an important initial step in the use of differentiated hESC for cell-based therapeutics.

Poster Board Number: T-1113

ESTABLISHMENT OF STABLE HEPATOCYTE PROGENITOR CELL LINE FROM HUMAN EMBRYONIC STEM CELLS

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It is well known that hepatocytes are important for assessment tools for toxicological research and pharmaceutical development, however, acquisition of human hepatocytes is not easy. Human embryonic stem cell (hESC)-derived hepatic progenitor offer a potential supply for functional hepatocytes. The goal of this study is to establish stable hESC-derived hepatic progenitor cell lines. The first stage involves the formation of definitive endoderm by activin A and WNT3a. The second stage we induced to proliferation and differentiation of hepatic progenitor by KSR and DMSO. In this stage, we introduced pEGFP-α-fetoprotein (AFP) into hepatocyte progenitor by viral transfection. We isolated pEGFP-AFP positive hepatocyte progenitor cells (AFP-HPCs). We characterized hepatic lineage the marker gene expression in the isolated AFP-HPCs. In addition we terminally differentiated matured hepatocyte-like cells by using AFP-HPCs (the third stage). The hESC-derived stable hepatocyte progenitor cell lines provide a valuable source for hepatocyte by simple manipulation, and may be useful as in vitro system for toxicity screening in drug discovery.

Poster Board Number: T-1114

DEVELOPMENT OF EFFICIENT HEPATIC DIFFERENTIATION METHODS FROM HUMAN IPSCS AND ESCS BY USING LOW-MOLECULAR WEIGHT COMPOUNDS

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Hepatocytes are useful cells in a variety of fields, such as liver cell therapy, drug screening, and toxicity testing. Primary human hepatocytes are often used for these purposes. However, since these primary cultured cells easily lose their metabolic functions, a substitute for primary hepatocytes is required. Human induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) are potential sources for unlimited supply of hepatocytes. There are several reports describing the directed induction of iPSCs and ESCs into hepatoblasts, which are progenitors of hepatocytes, and hepatocyte-like cells by the combinational treatment of growth factors. However, the induction efficiency is still low and the generated cells show immature phenotypes, including lack of key detoxification enzymes Cyp3a4 and glucose-6-phosphatase. In this study, we examined efficient methods to induce human iPSCs into hepatoblasts by the combinational treatment of growth factors. However, the induction efficiency is still low and the generated cells show immature phenotypes, including lack of key detoxification enzymes Cyp3a4 and glucose-6-phosphatase. In this study, we examined efficient methods to induce human iPSCs into hepatoblasts by using low-molecular weight compounds. To identify low-molecular weight compounds that can induce hepatoblasts into hepatocytes, we performed high-throughput screening (HTS). A human iPSC line, 201B6, was induced into hepatoblasts with a previously described differentiation protocol. Generated hepatoblasts were then treated with chemical compounds. After 8-12 days of culture with the tested compounds, cells were immunostained with ALBUMIN, a representative marker for hepatocytes. We evaluated the inducing ability of the compounds by analyzing the induction rate of iPSCs-derived hepatoblasts. Out of 1,120 compounds examined, we have found two candidate compounds (compounds X and Y) which induced iPSCs-derived hepatoblasts into ALBUMIN+ cells more efficiently than control stimulus (HGF and Oncostatin M). Next, we investigated the concentration dependency of these compounds and found that the effective concentration was 20-20,000 nM in both compounds. In conclusion, we have identified candidate compounds that would enable the efficient and low-cost differentiation from human iPSCs into hepatocytes. We are now examining the mechanisms of action of the two compounds and physiological functions of the generated ALBUMIN+ cells.

Poster Board Number: T-1115

CHARACTERIZATION OF HUMAN-INDUCED HEPATIC STEM CELLS AND THEIR DIFFERENTIATION BY A ONE-STEP PROTOCOL

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Human hepatocytes are useful for in vitro testing in drug discovery, but the lack of available donor hepatocytes is a major obstacle for application. Some sets of genes can induce pluripotent stem (iPSC) cells from postnatal tissues of an individual patient. Hepatocyte-like cells generated from iPSC cells might also be useful in non-clinical testing or regenerative medicine. The in vitro hepatic differentiation
of human iPS cells required a complicated procedure, such as the addition of several growth factors by a three-step protocol. Otherwise, hepatocyte-like cells reprogrammed directly from other cells did not expand further with their hepatic function. If self-renewing hepatic stem cells could be provided, they would have an advantage in practical use. Gene transfer of OCT3/4, SOX2, and KLF4 could also induce human hepatic stem (hHS) cells from the skin or gastric tissues of an adult patient. Microarray analysis revealed that three clones of iHS cells markedly expressed many hepatic genes (more than 50 genes); in addition, these cells expressed embryonic stem (ES) cell-enriched genes (more than 20 genes). Quantitative RT-PCR analysis also confirmed that iHS cells expressed hepatic genes (ALB, AAT, SERPINA1, and TRIT); in addition, these cells expressed ES cell-specific genes (OCT3/4, SOX2, NANOG, and ZFP42) at an equivalent level. Co-expression of hepatic and stem cell markers was confirmed by immunocytochemistry. The resulting iHS cells were self-renewed without chromosome abnormalities in vitro for more than one year. They were similar to human ES cells and iPS cells in morphology. Such expandable cells would have an advantage in application. After 2 weeks of culture without the addition of growth factors for differentiation, the long-term self-renewal iHS cells could differentiate into hepatocyte-like cells that expressed various hepatic markers 103-105 times more than those of iHS cells and produced hepatic proteins in a culture supernatant at mg/dL. Altogether, these results suggested that iHS cells not only provide new insight into stem cell research but also have an advantage for application.

Poster Board Number: T-1116

COMPARATIVE STUDY OF TRANSPLANTATION OF HEPATOCYTES AT VARIOUS DIFFERENTIATION STAGES INTO MICE WITH LETHAL LIVER DAMAGE

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Hepatocyte transplantation utilizing induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs) has been expected to provide an alternative to liver transplantation. However, it remains uncertain precisely which cell type is the best suited for cell transplantation. In particular, it is unclear whether mature hepatocytes, which have sufficient liver function, or immature hepatic progenitor cells, which have a higher proliferative capacity, will provide a better outcome. The main objective of this study was to investigate the therapeutic efficacy of the transplantation of hepatocytes at various differentiation stages. We utilized transgenic mice that expressed diphtheria toxin (DT) receptors under the control of an albumin enhancer/promoter. ESC-derived endodermal cells, fetal hepatocytes and adult hepatocytes were transplanted into these mice with experimentally-induced lethal acute liver injury caused by DT administration. The transplanted cells were marked by enhanced green fluorescent protein. We evaluated their effects on survival. At 35 days after transplantation, the survival rates of the adult hepatocyte-transplanted group (8/20; 40.0%) was significantly improved in comparison to that of the sham-operated group (2/25, 8%), the fetal hepatocyte-transplanted group (1/20, 5%) and the ESC-derived endodermal cell-transplanted group (0/21, 0%). The adult hepatocytes proliferated in the recipient livers and replaced a large part of their parenchyma. The transplantation of adult hepatocytes for acute liver failure significantly improved the survival rate in comparison to that of transplantation of immature cells, thus suggesting that ESCs and iPSCs should be differentiated into mature hepatocytes before cell transplantation for acute liver failure.

Poster Board Number: T-1118

POLYCOMB GROUP PROTEIN RING1B REGULATES PROLIFERATION AND DIFFERENTIATION OF MOUSE HEPATIC STEM/PROGENITOR CELL BY REPRESSING CYCLIN-DEPENDENT KINASE INHIBITORS

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Self-renewal of somatic stem cells is thought to be satisfied that the integrated control of multiple gene expression relating to cell proliferation and differentiation. Recent studies have revealed that the histone modifiers, including the Polycomb group (PcG) proteins, play important roles in stem cell self-renewal. Here, we address PcG regulation of mouse hepatic stem/progenitor cell self-renewal through inactivation of Ring1B that is essential for gene silencing by PcG proteins with monoubiquitin E3 ligase activity towards histone 2A. Functional analyses of PcG protein Ring1B in hepatic stem/progenitor cell were conducted by Ring1B conditional knock-out (cKO) mice. In Ring1B-cKO fetal mice, we found that the liver size was lessened significantly by Ring1B depletion on early stage of liver development that hepatic stem/progenitor cell would expand their population. In contrast, there was no decline in liver size in Ring1B-cKO mice with lately depletion. Immunohistochemical staining analysis revealed that numbers of both alpha-fetoprotein- and undifferentiated and BrdU+ cells were decreased in Ring1B-cKO mice liver. When performed functional analysis of Ring1B in c-kit+CD49f+CD29+CD45- Ter119- hepatic stem/progenitor cells from Ring1B-cKO mouse with single cell-base colony assay in vitro, the
DECREASED LEVELS OF HEPATOCYTE GROWTH FACTOR IMPROVE LIVER CELL ENGRAFTMENT IN A GENETICALLY ENGINEERED MOUSE MODEL

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Background & Introduction: Developing methods to enhancing cell engraftment is important for cell/stem cell therapy. We have generated a mouse line with narrowed sinusoidal diameters, which is associated with decreased levels of hepatocyte growth factor (HGF). In the current study, we aimed at testing whether cell engraftment can be improved in an HGF-insufficient background.

Method: Primary hepatocytes from R26R-GR mice (a double fluorescent-reporter knockin mouse line that could be used as a reporter system for investigation of cell therapy conditioning) were isolated by a two-step collagenase perfusion. Approximately 1 million of viable hepatocytes were infused into wild-type (WT) or HGF-insufficient mice, and then the efficiency of cell engraftment was analyzed at 3, 24, and 48 hours after transplantation.

Result: The data showed that the number of transplanted cells was scored using a fluorescence microscope. At different time points, frozen liver sections were analyzed and the absolute number of transplanted cells was scored using a fluorescence microscope. The number of transplanted cells was significantly higher in HGF-insufficient mice than that in WT mice at all the time points (n = 3/group; cells/30 fields; WT vs. HGF-insufficient; 3 hours: 15.00 ± 4.81 vs. 28.60 ± 2.26, p < 0.001; 24 hours: 22.20 ± 4.24 vs. 37.60 ± 9.05, p = 0.001; 48 hours: 22.17 ± 4.40 vs. 40.80 ± 7.23, p < 0.001). Conclusion: We demonstrated that decreased levels of HGF could enhance cell engraftment, and that the R26R-GR mouse line could be a good donor of cell sources for the research on cell/stem cell therapy. We suggest that this strategy, antibody pretreatment against HGF for instance, may be applicable to improve cell/stem cell therapy in the near future.

Poster Board Number: T-1119

PROTECTIVE EFFECT OF ADIPOSE TISSUE-DERIVED STEM CELLS (ADSCS) FOR ACUTE LIVER FAILURE IN MICE

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Background: Adipose tissue-derived stem cells (ADSCs) have been shown to proliferate and differentiate into multiple cell lineages. ADSCs is emerging as novel therapeutic option for regenerative medicine. Recently, several publications have reported that ADSCs have therapeutic potential in different diseases of animal models and clinical application. Here, we found the alternative source of ADSCs - omentum adipose tissue, which contain abundant stem cell population. The aim of this study is to investigate the therapeutic potential of omentum ADSCs on acute liver failure (ALF) induced by acetaminophen (APAP) in mice. Methods: We demonstrated the therapeutic efficiency of omentum-derived ADSCs transplantation for ALF mouse model induced by APAP. In this study we plan to identify if the omentum-derived ADSCs can be a new source of donor cells. Firstly, we will isolate and in vitro culture the ADSCs from mouse omentum tissue. The characteristics and differentiation ability of omentum-derived ADSCs will analyze by flow cytometry for MSC marker (CD29, CD31, CD34, CD44, CD90, and CD105 etc), immunostaining for multiple cell lineage (hepatocyte, osteoblast, and adipocyte). The protection capacity of omentum-derived ADSCs was evaluated on primary in vitro cultured hepatocytes treated with APAP and demonstrated by MTT assay, reactive oxygen species (ROS) detection, glutathione content measure, and antioxidant enzyme activity (catalase, superoxide dismutase, and glutathione peroxidase ) expression. Secondly, we will study the in vivo therapeutic efficiency of these omentum-derived ADSCs in ALF mice. We will transplant intrasplenically the omentum-derived ADSCs (1million cells/mice) into ALF mice. The serum samples will be taken at hour 8, 12, 24, and 48 to measure the liver function (GOT, GPT, and t-bill). We will detect the profile of antioxidant enzyme activity on liver tissue after omentum-derived ADSCs transplantation in ALF mice and observe the survival benefit. Moreover, we will assess the difference of JNK pathway expression after ADSCs transplantation. Results: Omentum-derived ADSCs showed morphological similarity to the MSC, and expressed the MSC characteristics and properties of differentiation. Omentum-derived ADSCs could protect the hepatocytes to against the APAP toxicity by decreasing the ROS production, enhancing the intercellular GSH content, and increasing the antioxidant enzyme activity. The omentum-derived ADSCs could rescue the individual survival and improve the liver function in ALF mice. The profiles of antioxidant reveal that omentum-derived ADSCs could increase the activity of antioxidant enzyme. Furthermore, JNK-mediate pathway can be inhibited in recipient mice with omentum-derived ADSCs transplantation. Conclusion: These studies demonstrated the omentum-derived ADSCs are a novel cell source for cell-based therapy in ALF. And the omentum-derived ADSCs could against the hepatotoxicity and protect the hepatocytes by reducing the ROS production, increasing the antioxidant enzyme capacity via the inhibition of the JNK-mediated pathway.
ADULT HEPATIC PROGENITOR CELL AND INJURED LIVER REGENERATION IN FISH MODELS

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Background: Chronic liver disease encompasses a large number of conditions having different etiological factors, progressing from fibrosis to cirrhosis and possibly liver failure or hepatocellular cancer. Chronic liver disease is a leading cause of mortality in China and the United States, responsible for the deaths of more than 200,000 Chinese and 25,000 Americans each year. Although there are about 17,000 people require liver transplants in the USA, only 5,000 cadaveric liver transplants are estimated to be available per year. Adult stem cells are an attractive alternative to meet this huge clinical demand, but the current understanding of stem cell biology in adult liver is limited. By using zebrafish and medaka models, this project aims to investigate the presence of liver progenitor cell in adult and their cellular and molecular characteristics. Materials and Methods: A transgenic zebrafish line was used in this study. The mature hepatocytes in this line are labeled with GFP with the expression driven by a gene promoter from the liver fatty acid binding protein (FABP), which is specifically expressed in mature hepatocytes. After liver profusion, Flow Activated Cell Sorting was conducted based on their expression of GFP and Patched (Ptch), the Hedgehog receptor. Each isolated fraction of cells was: 1) analyzed by Q-RT-PCR to detect expression of other stem cell markers; and 2) tested for their potential to differentiate into hepatocyte and/or biliary epithelial cell in vitro and in vivo systems. Results: In an adult liver from a FABP-GFP transgenic zebrafish (6 months old), 70-85% of cells were GFP+ and expressed albumin, consistent with mature hepatocytes with cell size of 10-15μm. However, about 10-15% of the total liver cell populations were GFP negative (GFP-, non-hepatocyte) with cell size of 5-10 μm. There were 0.4 - 0.5% of the isolated liver cells with both GFP negative and Ptch positive (GFP-/Ptch+). The cells in the GFP-/ Ptch+ fraction also has enriched expression of Ptch and Aldh2 mRNA, 30 fold and 23 fold, respectively, when compared to GFP positive cells. The GFP negative cells can be cultured in vitro on collagen IV and fibronectin coated plate and induced to differentiate into GFP positive hepatocyte under HGF/FGF cocktail incubation for 5 days. One week after transplantation of the GFP- cells into wild type see-through Medaka fish pretreated with liver toxin, tunincamycin, resulted in cell lineages of biliary epithelial cells and hepatocytes both express GFP. As few as ten GFP-/Ptch- cells can regenerate and rescue over 90% chemical-induced injury liver within 14 days. Conclusion: Conditions for isolating a cell fraction which we believed consisted of liver progenitor cells from adult zebrafish fish have been established. Further studies will be conducted to investigate the localization of these cell types in adult zebrafish liver and their detailed molecular features. These studies provide a starting point for further characterization of unique cell types in adult liver that may be important in liver regeneration and even in hepatocarcinogenesis.
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Poster Board Number: T-1123

STEM CELL BASED GENE THERAPY: A NOVEL APPROACH FOR THE TREATMENT OF ALPHA-1-ANTITRYPSIN DEFICIENCY

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The human SERPINA1 gene encodes for the secretory protein alpha 1-antitrypsin (A1AT) which inhibits a wide variety of proteases in the serum by covalent binding. In humans a missense point mutation (E342K) was described which leads to the expression of the PiZ isoform of A1AT. Homozygosity for PiZ (PiZZ) leads to severe alpha 1-antitrypsin deficiency. In this case the serum concentration of A1AT is reduced by 90%, as the protein is not able to fold correctly, but polymerizes and is retained in the endoplasmic reticulum of hepatocytes. Patients show an increased breakdown of elastin by elastase in the lung and, therefore, are suffering from lung emphysema or chronic obstructive pulmonary disease. Moreover, the accumulation of misfolded protein in the liver causes liver function disorders and liver cirrhosis. We investigate new strategies for the treatment of the liver disease in severe A1AT deficiency using a transgenic mouse model overexpressing the human PiZ protein. From these mice we generated induced pluripotent stem (iPS) cells using lentiviral vectors encoding the three transcription factors Oct4, Sox2 and Klf4. After reprogramming the PiZ-iPS were transduced with a second lentiviral vector encoding for a mIR30-styled shRNA, which is directed specifically against the point-mutated form of the human SERPINA1 transgene. We also cloned an egFP reporter directly in front of the knockdown shRNA to track its expression through all stages of differentiation. The gene corrected PiZ-iPS cells were then subjected to several hepatic differentiation protocols to evaluate the shRNA-mediated rescue of the diseased phenotype. Moreover, gene-corrected PiZ-iPS were injected into blastocysts for characterization of the SERPINA1 knockdown in vivo. Next, we transduced iPS cells generated from a human PiZZ individual with liver disease with our knockdown construct. These gene-corrected cells were also differentiated along the hepatic lineage and showed significantly reduced expression of SERPINA1 when compared to scramble-shrRNA transduced cells. We have successfully evaluated a novel treatment for the liver disease in A1AT deficiency employing stem cell based gene therapy and we show evidence for a significant reduction in the expression of a harmful gene in a mouse model for a human disease and in cells from a human patient.

Poster Board Number: T-1124

MMP-9 PROMOTES THE TRAFFICKING OF TRANSPLANTED BONE MARROW CELLS THAT ATTENUATES HEPATIC FIBROSIS IN CHRONIC CCL4 LIVER INJURY

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Background: Recent clinical trials have shown that transplanted bone marrow (BM) cells migrate into injured liver and attenuate hepatic fibrosis. However the mechanism by which BM cells mobilize into damaged liver or how the transplanted BM cells work in vivo are still under investigation. Purpose: Using MMP-9 (-/-) (KO) BM cells, we tried to investigate the role of MMP-9 in the trafficking of BM cells and in the contribution to the regression of hepatic fibrosis. Methods: 1) In vitro, BM cells were isolated from MMP-9 KO and MMP-9 (+/+)(WT) mice, and Matrix gel invasion activity was assessed by transwell chambers. 2) In vivo, chronic liver injury was induced by C57BL/6 female mice by injection of CCl4 twice weekly for 8 weeks. At 4th week, mice were transplanted with BM cells (1x10^7/ mouse) isolated from male WT or KO mice which were genetically positive with egFP. Non-transplanted (non-TP) control mice were injected with normal saline alone. The liver specimens were examined by real time qPCR, collagen content assay, and immunohistochemical studies. Results: 1) Matrix gel invasion assay showed WT-BM cells significantly migrated into the lower chamber (2.5 fold) compared with KO-BM cells. 2) Mice transplanted with WT-BM cells (WT-TP group) displayed approximately 2.0 fold of egFP (+) cells along the fibrotic tissue and around central veins compared to those transplanted with KO-BM cells (KO-TP group). Real-time qPCR also showed egFP and sex-related Y chromosome expression was significantly higher in WT-TP group than KO-TP group. 3) Liver fibrosis, assessed by Sirius red staining, was prominent in non-TP control group, whereas KO-TP and WT-TP groups revealed 50% and 70% decrease of fibrotic area respectively, examined by VH analyzer. Collagen content assay was coincident with Sirius red staining. The mRNA expressions of alpha 1(I) collagen in both WT-TP and KO-TP groups were significantly lower compared with that of non-TP group. However expressions of MMP-13 and MMP-9 in WT-TP group were significantly higher than those in KO-TP group. 4) Immunofluorescent microscopy showed most egFP (+) cells were co-stained with F4/80, a marker of macrophage, and most of them co-expressed MMP-9 and MMP-9 in TP groups. Immunoelectron microscopy demonstrated egFP (+) cells showed the morphology containing many lysosomes in the enlarged cytoplasm, suggesting the characteristic features of macrophages. Conclusion: MMP-9 has a crucial role in the BM cell migration into injured liver. Transplanted BM cells, mostly differentiated into macrophages, contribute to regression of hepatic fibrosis via down-regulation of type I collagen and up-regulation of MMP-9 and MMP-13.

Poster Board Number: T-1125

PREDICTION OF STEM CELL ASSOCIATED MICRORNA IN HEPATOCELLULAR CARCINOMA

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Background Cancer stem cells (CSCs) are associated with drug-resistance and poor patient prognosis. The miRNAs (miRNA) involved in CSC of hepatocellular carcinoma (HCC) are not well revealed. We hypothesized that CSC shares the common miRNA profiles of embryonic stem cells (ESCs). The aim of this study is to find out potential significant miRNAs in CSC hepatocarcinogenesis by comparing the miRNA profiling of HCC to that of ESCs. Method A total of 84 patients with HCC received primary resection between 2002 and 2006 were enrolled in this study. HCC(T) and nearby non-tumor liver (N) tissues were sampled for miRNA Illumina BeadArray (total 1145 microRNAs) and validated by Real-Time PCR analysis. Ten samples from living donor (D) livers were used as control. Candidate miRNAs were selected by using paired-T or T test with FDR correction and p-value <0.05 and TN (or TD, ND) ratio >2 or <0.5. Selected miRNAs were further reviewed to identify the very miRNAs significantly involved in ESC miRNA profiling
transplantation site for multiple epithelial tissues

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Cell-based therapy has been viewed as a promising alternative to organ transplantation. However, for some patients, orthotopic cell-based therapy directed at a diseased organ may not be feasible for many reasons, ranging from a possible lack of an appropriate environment to insufficient supply of donor organs. This approach will be beneficial to the field of regenerative medicine and provides a new concept to use the LN as an in vivo bioreactor in which to regenerate functional organs.

Poster Board Number: T-1126

THE LYMPH NODE AS AN ECTOPIC TRANSPLANTATION SITE FOR MULTIPLE EPITHELIAL TISSUES

Comprised of cells expressing keratin, many LN traits have been attributed to a stem/progenitor cell population with the capacity to differentiate into multiple cell types. Using a similar approach, we asked if de novo thymus function could be generated in LNs of athymic mice. Thymuses were harvested from newborn GFP mice, minced and injected directly into the jejunal LN of athymic nude mice. We found that the ectopic thymuses in the LN contained recipient double positive thymocytes as well as single positive CD4 and CD8 T cells, indicating a selective mechanism of T-cell commitment and maturation. Moreover, the de novo T cell-mediated immune system rejected xenogeneic tumor growth in the majority of the LN treated nude mice. Together, these data support the concept of using the LN as a site for thymic transplant to generate an ectopic thymus. Finally, pancreatic islets were harvested from GFP mice and transplanted into the jejunal LN of streptozotocin-induced diabetic mice. The ectopic islets transplanted in a single jejunal LN of the mice engrafted and secreted insulin to decrease glucose levels. We provide the first report describing the use of a LN as a site for cellular transplant. By directly injecting the LN with hepatocytes, thymuses, or pancreatic islets, we demonstrate engraftment of the donor cells and subsequent organ function. This approach will be beneficial to the field of regenerative medicine and provides a new concept to use the LN as an in vivo bioreactor in which to regenerate functional organs.

Poster Board Number: T-1127

PROPAGATION OF ADULT STEM/PROGENITOR CELLS IN A SERUM–FREE THREE–DIMENSIONAL CULTURE SYSTEM

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Recent progress has demonstrated adult liver stem/progenitor cells as potential sources for generating transplantable liver cells. However, the great variability in methods utilizing to isolate liver stem/progenitor cells is a considerable challenge for clinical applications. A serum-free three-dimensional culture system was established in this study for selection and propagation of adult liver stem/progenitor cells. We demonstrated that, when adult liver cells were grown on polyvinyl alcohol (PVA) coated glassware, adult liver stem/progenitor cells can form spheres. These sphere cells expressed a panel of stem-cell markers including CD133, EpCAM, CD49f, AFP, CK19 and Oct-4 and had the potency to differentiate into hepatocytes and cholangiocytes when co-culturing with fetal liver cells. Moreover, transplantation of GFP-labeled sphere cells to the liver of mice treated with 3,5-dithiohexy carbonyl-1,4-dihydrocollidine (DDC) could replenish damaged hepatocytes. Moreover, overexpression of HBx in isolated sphere cells resulted in the formation of tumors with some characteristics of hepatocellular carcinoma and cholangiocarcinoma upon intrasplenic injection into immunodeficient mice. These data provide evidences for the stem cell-like capacity of these PVA-cultured sphere-forming cells. In conclusion, we develop a simple, rapid and label-free method for prospectively isolating hepatic stem/progenitor cells from the adult mouse liver which will facilitate their application in repairing injured liver.
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Poster Board Number: T-1128

GENOMIC DNA METHYLATION AS A POTENTIAL MARKER OF STEM CELL DURING HEPATIC CELL DIFFERENTIATION
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Purpose: Technological improvements of stem cell differentiation into hepatocytes have increased the momentum of their application to drug metabolism and toxicity testing. Therefore, to develop the assessment of these cells is an important issue. Currently, evaluation is performed in the differentiated hepatocytes by the cell morphology, enzyme activity, and expression of differentiation markers. However, differentiated hepatocytes lost their proliferation ability. Thus, if one can assess the differentiation ability of the cells prior to hepatocyte, it might help to accelerate cell production and banking. HepaRG cells, a hepatocyte progenitor, have the ability to differentiate towards hepatocyte-like and biliary epithelial-like cells at confluence. We reported that genome wide DNA methylation did not change significantly during the HepaRG cell differentiation (62th JSSX annual meeting). This observation suggested that evaluation of genomic DNA methylation in the progenitor cell might be useful for the assessment of differentiation potential. To investigate the validity of genomic DNA methylation assessment system, we performed analyses of genome wide genomic DNA methylation and gene expression. Methods: Genomic DNA and total RNA from human primary hepatocytes (three donors), HepaRG cells, and HepG2 cells were used for genome wide analyses of genomic DNA methylation and gene expression. Genomic DNA methylation and Gene expression analyses were performed with Human Methylation 450 BeadChip Arrays (Illumina) and GeneChip Human Genome U133Av2 Array (Affimetrix), respectively. Results and Discussion: Correlation between frequencies of DNA methylation in 89794 CpG sites on CpG Island in 13029 gene regions and the gene expression levels were compared among primary human hepatocytes, HepaRG cells, and HepG2 cells. As the result, frequencies of DNA methylation in 1751 CpG sites in 582 gene regions showed negative correlation with their gene expression levels. The cluster analysis of these genes indicated the direct suppression of gene expression by DNA methylation. Evaluation of the gene set as a marker for the cell characterization is in progress. Acknowledgement: Part of this research was supported by the INSERM/Japan Society for the Promotion of Science (JSPS) cooperation program.

Poster Board Number: T-1129

SMALL MOLECULE LIBRARY SCREENS FOR HEPATIC MATURATION OF INDUCED PLURIPOTENT STEM CELL DERIVED HEPATOCYTES IN VITRO
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Human hepatoplastic models such as primary human hepatocytes, HepaRG and HepG2 cells have been used to evaluate components of liver function in vitro. However, none of these models maintain the full hepatocyte (HC) functionality found in vivo. Pluripotent stem cells (PSCs) have the potential to provide a robust and accessible in vitro model of human hepatic function. However, despite development of methodologies to differentiate PSCs into HCs that exhibit features of mature, adult HCs, PSC derived HCs retain some features of immature, fetal HCs, in particular with respect to their drug metabolizing cytochrome P450 activity. Thus, in attempts to develop PSC derived HCs with more adult-like functionality that may be useful as a tool during early drug discovery activities, we performed small molecule screens to identify compounds capable of modulating the PSC HC phenotype. Compounds from three unique small molecule libraries were screened for the ability to induce further maturation of human induced pluripotent stem cell derived HCs (iCell Hepatocytes) from Cellular Dynamics International. These include a 299 compound kinase inhibitor library, a 94 compound epigenetic modulator library, and a 296 compound library of small molecules known to be active in one or more cell based assays previously employed elsewhere in the drug discovery program at Roche. iCell Hepatocytes were plated in a 96-well format. Cells were treated with the compound libraries at a concentration of 5 μM at 24 hrs and 72 hrs after plating. In addition, in some instances different cell culture conditions were examined such as incubating the cells at low (6.5%) oxygen concentrations. Total RNA was harvested 24 hrs after the final compound treatment. Experimental gene expression was evaluated in triplicate by Fluidigm microfluidic qPCR using 32 probes chosen based on their differential expression observed during a previously performed microarray analysis of RNA purified from untreated iCell Hepatocytes, human fetal and human adult HCs. Furthermore, the screening probes were picked in order to span major HC functional activities including cytochrome P450, transporters, bile acid synthesis, and key hepatic proteins and transcription factors. The Ct values of each probe set examined were first normalized to the expression values of housekeeping gene controls. Next, the compound treated values were compared to the corresponding gene expression level observed in vehicle (DMSO) control treated samples. Top compound hits were chosen based on a compound’s ability to alter the gene expression in a manner predicted to increase cellular maturity, for instance an increase of adult specific markers or a decrease in fetal specific markers. The top 30 positive compounds from the primary screen were then tested at multiple doses during a secondary screen. In total, greater than 300,000 individual endpoints were measured for the primary and secondary screen analysis. Currently the top 5 compounds that exhibited reproducible gene expression changes during the secondary screens are being further studied with the goal of identifying the mechanisms of action and functional consequences.

Pancreatic Cells

Poster Board Number: T-1131

INVESTIGATION OF THE HUMAN EMBRYONIC STEM CELL DERIVED PANCREATIC PROGENITOR CELL ALTERATION IN IN VIVO ENVIRONMENT
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Development of a cell therapy for diabetes would be greatly aided by a renewable supply of human beta-cells. One approach to overcoming the problem of insufficient supply is to generate islets from proliferative stem cell populations such as human embryonic stem cells. By establishing hESC expansion and banking methods and a
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suspension-based differentiation system, hES cell were aggregated into clusters in rotational suspension culture, followed by differentiation for two weeks with a 4-stage protocol to produce pancreatic progenitors. We had previously shown these pancreatic progenitors further differentiate into mature pancreatic cells, including glucose-responsive insulin-secreting cells, when implanted into immune compromised animals. To further assess the in vivo graft development, mice were sacrificed at various time points post-engraftment, ranging from 1 to 22 weeks, for assessment of mRNA gene expression and histological analysis. We observed temporal changes in gene expression consistent with pancreatic progenitor differentiation to the endocrine lineage as well as subsequent maturation of endocrine cell phenotypes. Observation of grafts by histological analysis confirmed these findings and suggested a conversion of poly-hormonal endocrine cells to glucagon-expressing progenitors, as previously shown in other work. Investigation of cell proliferation by Ki67 staining indicated proliferative cell number is relatively low and does not change dramatically during this time course of post-engraftment analysis. Interestingly, both exocrine and duct cell protein expression was observed after the endocrine cell maturation, and this was consistent with the gene expression data. The majority of the dynamic changes in cell phenotypes and gene expression were detectable before robust glucose-responsive C-peptide secretion was observed in the serum of these animals at 10wk post-engraftment. These data serve to further elucidate the time course and mechanisms by which implanted pancreatic progenitor cells differentiate and mature to become functional glucose-responsive cells.

Poster Board Number: T-1132

MESENCHYMAL TO EPITHELIAL TRANSITION IS REQUIRED FOR REPROGRAMMING HUMAN EXOCRINE ENRICHED PANCREATIC CELLS TOWARDS BETA-LIKE CELLS

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Due to the shortage of islets available for transplantation, the in vitro generation of functional beta cells is currently accepted as one of the most promising sources of tissue for transplantation in the treatment of type 1 diabetes. The aim of this study was to reprogramme redundant human exocrine tissue towards islet cells, to be used as a patient specific “top-up” supply of pancreatic islets. Human Exocrine Enriched Pancreatic Cells (EEPCs) were obtained from the exocrine part of the pancreas which is usually discarded during the islet isolation procedure and propagated in vitro. These cells attach to tissue culture dishes and assume a mesenchymal morphology in culture, with abundant expression of vimentin and loss of exocrine markers, such as amylase and CK19. In order to instigate the reprogramming of the cultured EEPCs, the exogenous expression of four pancreatic transcription factors (TFs) was induced with the addition of Ad-Ngn3, Ad-Pdx1, Ad-MafA and Ad-Pax4 to these cultures, in combination with Betacellulin, Nicotinamide and Exendin-4 (GFs). The effect of the TFs and GFs was enhanced by pre-incubating the cells with 5-aza-deoxycytidine and Sodium Butyrate, which inhibit DNA methylation and deacetylation, respectively. After this treatment, high levels of insulin and glucagon were detected in the reprogrammed EEPCs by RT-qPCR, immunocytochemistry and in the culture medium by ELISA. These cells were cultured in Serum Free Medium, which led the cells to undergo a Mesenchymal to Epithelial Transition (MET) and this was crucial for the efficiency of reprogramming. Furthermore, it was also found that the GFs were capable of enhancing MET, by inducing the expression of the epithelial marker E-cadherin and the down regulation of the mesenchymal marker vimentin. Therefore, MET appears to have an important role in the reprogramming of adult pancreatic cells towards the endocrine cell fate. These studies were complemented with the transplantation of the reprogrammed endocrine cells into the kidney capsule of NOD/SCID mice. Immunohistochemical analysis of the graft revealed that the cells maintained a cluster-like morphology under the kidney capsule, being positive for the endocrine hormones glucagon, insulin and somatostatin, with a morphology similar to the structure of a pancreatic islet.

Poster Board Number: T-1133

COMPARATIVE EXPRESSION OF MICRO-RNAs AND MRNAs DURING DIFFERENTIATION OF PANCREATIC ISLET-LIKE CELL CLUSTERS FROM HUMAN EMBRYONIC AND ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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Type 1 diabetes is an autoimmune destruction of pancreatic islet beta cell disease, so it is important to find new sources of the islet beta cells to replace the damaged cells. Human embryonic stem (hES) cells have the potential to provide an unlimited supply of differentiated islet-like cells for tissue replacement. However, this method needs an immune-suppression to prevent islet rejection. Autologous islet-like cells differentiated from adult adipose-derived mesenchymal stem cells (MSC) are now considered to be a good source for cell therapy of type 1 diabetes. The hES-T3 cells with normal female karyotype and MSC from normal adult female were separately induced using different protocols to generate the pancreatic islet-like cell clusters, which expressed pancreatic islet cell-specific markers of insulin, glucagon and somatostatin. The expression profiles of microRNAs and mRNAs from the pancreatic islet-like cell clusters differentiated from the two sources of hES-T3 and MSC were analyzed and compared. The pancreatic islet-like cell clusters differentiated from the hES-T3 cells were found to exhibit very high expression of microRNA-186, miR-199a and miR-339, which down-regulated the expression of LIN28, PRDM1, CALB1, GCNT2, RBM47, PLEKHH1, RBPM52 and PAK6. The expressions of microRNAs and mRNAs from the islet-like cell clusters differentiated from the MSC are being determined, and will be compared with those of the islet-like cell clusters differentiated from the hES-T3 cells. The microRNAs and their target genes are very likely to play important regulatory roles in the development of pancreas and/or differentiation of islet cells, and they may be manipulated to increase the proportion of beta cells and insulin synthesis in the differentiated pancreatic islet-like cell clusters for cell-therapy of type 1 diabetics.

Poster Board Number: T-1134

INDUCTION OF PANCREATIC CELLS FROM HUMAN IPS CELLS IN A SERUM-FREE MONOLAYER CONDITION

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Remarkable advance in stem cell biology has made it possible to apply for regenerative therapy. Especially, by finding of the method
for obtaining iPSC cells, we can have differentiated cells from patients to possess pluripotency and can re-induced appropriate tissues and organs. This strategy may have applicability to the new therapeutic treatment for diabetes. Insulin-dependent diabetes is occurred by loss of pancreatic beta cells, resulting in shortage of insulin and elevation of blood glucose level. To the remedy for this malady, transplantation of pancreatic beta cells is effective. To date, serum-containing medium and feeder cells are ordinarily used to maintain pluripotency and differentiate cells, but this condition is afraid to contaminate unknown factors, resulting in both instability of experimental condition and doubt about the safety of the therapy. Here, we report the induction of pancreatic cells from human iPSC cells in serum and feeder cell free condition. Based on the previous reports, we adopted modified strategy. In this method, we sequentially added various cytokines such as Activin, Wnt, RA, EGF, GLP and Insulin. By using this method, a group of cells with patch-like shape could be observed. Immuno-histological analysis revealed that many of these cells were stained with anti-C-peptide antibody, suggesting that insulin-secreted cells could be induced. Furthermore, glucagon positive cells could be also seen. Together with these results, we expect the functional pancreatic islet-like structure could be differentiated from human iPSC cells. However, induction efficiency was not so high. To overcome this problem, further improvements have to need. For example, to increase the ratio of appropriately induced beta cell, we are now optimizing the timing and duration of treatment with cytokines. In this report, we will also discuss this problem.

**Poster Board Number: T-1135**

**SINGLE ADULT MURINE DUCTAL PANCREATIC STEM CELLS SELF-RENEW AND BUILD MULTILINEAGE CYSTIC STRUCTURES IN VITRO**

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It remains unclear whether adult pancreatic ducts harbor stem cells. Here, we tested whether purified adult murine pancreatic ductal cells, which express CD133 and Sox9, may be induced to differentiate and self-renew in a novel three-dimensional clonogenic assay in the absence of a mesenchymal niche. CD133+Sox9+EGFP+ cells from dissociated adult murine pancreata were sorted using a fluorescent activated cell sorter and differentiated in vitro in a semi-solid medium containing methylcellulose, Matrigel, and growth factors (J Vis Exp, 2011, PMID: 22143165). The single cell-derived colonies were characterized by microfluidic RT-PCR, immunostaining and electron microscopy. We found that about 17% of sorted, single murine CD133+Sox9/EGFP+ ductal cells built cystic structures containing cells that resemble ductal, acinar and endocrine cells. Immunostaining and electron microscopy analyses demonstrated that the differentiated ductal-like cells displayed the correct polarity. Microfluidic RT-PCR analysis of individual colonies indicated that more than 70% expressed markers for ductal, endocrine and acinar lineages, suggesting that the majority of the colony-initiating cells are multipotential. Furthermore, single cells from approximately 80% of primary colonies formed secondary cystic colonies, suggesting self-renewal activities, and R-Spondin 1, a Wnt signaling agonist, increased the self-renewal capacity of serially passaged clonal cells. Finally, partial duct ligation in vivo increased the ratio of non-colony-forming to colony-forming ductal cells, demonstrating a dynamic response of ductal progenitors to injury. To our knowledge this is the first report of the ability of single adult ductal pancreatic stem cells to self-renew and differentiate into multiple lineages in vitro. The clonogenic culture system described will simplify the study of pancreatic stem cell biology and advance the field of regenerative medicine in treatment of diabetes.

**Poster Board Number: T-1136**

**DIFFERENTIATION OF MURINE EMBRYONIC STEM CELLS AND OF MURINE SKIN-DERIVED MESENCHYMAL STEM CELLS INTO INSULIN-PRODUCING CELLS**

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Isolated pancreatic islet transplantation is an alternative treatment indicated for hyperlabile type 1 Diabetes mellitus (DM1), however, a large number of good quality islets is required to achieve DM1 reversion. New cell source alternatives have been investigated, the most promising being insulin producing cells (IPCs) differentiated from stem cells. Some reports show that murine embryonic stem cells (mESCs) are able to form islet-like structures, but insulin production is insufficient to achieve normoglycemia in diabetic mice, the same occurring with adult stem cells from different tissues. Therefore, in order to revert Diabetes, there is great demand for an adequate protocol to achieve sufficient amounts of insulin-producing cells from differentiated stem cells. Aiming at an adequate protocol to differentiate stem cells into insulin-producing cells, we subjected mESCs to embryoid bodies formation, followed by introduction of a set of endoderm differentiation inducers, and treatment with beta-cells differentiation factors. Early on the mESCs differentiation, we noticed some epithelial-like cells emerging from the embryoid bodies culture. After 10 days of differentiation, we detected the expression of INS2, NGN3, ISL1 and GLUT2 transcripts. In addition, murine skin-derived mesenchymal stem cells (mMSCs) were induced to differentiate with both the same factors used for mESC differentiation and conditioned medium collected from differentiating mESCs. Although mMSCs subjected to differentiation with the factors were positively stained by dithizone, the levels of insulin and other transcripts related to β-cell differentiation were undetectable. This may be explained by the low amount of differentiated mMSC producing insulin and the inability of the factors used in our protocol to induce mMSC complete differentiation into insulin producing cells. On the other hand, conditioned medium obtained from differentiating mESCs was able to induce low levels of the insulin gene transcription. This suggests that factors secreted by mESCs during their differentiation process contribute to their own differentiation and may also contribute to differentiation of other stem cell types. Therefore, we were able to develop a new protocol, based on pancreatic organogenesis, which seems to be able to induce differentiation of ESCs into insulin-producing cells and pre-differentiation of mMSC. Support: FAPESP, CNPq, FINEP, MS-DECIT, MCT, BNDES. Key words: beta-cell, Diabetes, murine embryonic stem cells, endoderm inducers, murine skin-derived mesenchymal stem cells.
PRODUCTION OF INSULIN-PRODUCING CELLS DERIVED FROM MOUSE ES CELLS THROUGH SERUM-FREE-INDUCED DEFINITIVE ENDODERM

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One promising approach for the development of cell therapies for diabetes treatment is to utilize pancreatic endocrine cells differentiated from pluripotent stem cells such as embryonic stem (ES) cells. Here, we show an original and novel method to induce the differentiation into insulin-producing cells from mouse ES (mES) cells. For the efficient production of mature insulin-producing cells, we hypothesized that induction of definitive endoderm (DE) is the first important step. We have focused on serum free induction as an effective method to direct mES cells to DE. First, a serum free medium was utilized to induce (DE) formation. The formation of DE was strongly supported by the quantitative RT-PCR analysis showing the expression of endoderm markers Sox17 and Foxa2 and a DE specific marker Cxcr4 increased over the course of DE induction. The differentiated endoderm cells were then treated with KGF and all-trans retinoic acid (RA) to induce pancreatic specialization which was confirmed by the expression of the early pancreatic transcription factor Pdx1. Finally, the differentiated cells were transferred onto low-adhesion dishes for suspension culture in maturation medium containing nicotinamide and Ex-4. The differentiated cells in this stage were formed spherical clusters and expressed Insulin as determined by RT-PCR. Furthermore, the result of immunostaining analysis proved that the differentiated cells expressed Insulin, C-peptide, and Pdx1. The ability of glucose-stimulated insulin secretion (GSIS) of the differentiated cells at this stage was measured by ELISA. The differentiated cells indeed secreted insulin in response to glucose stimuli. These results suggested that our approach can be a novel and effective method to produce mature insulin-producing cells, paving the way for the development of cell therapies utilizing pluripotent stem cells for diabetes treatment.

MICRORNA SIGNATURES AND FUNCTIONS OF IN VITRO GENERATED PANCREATIC BETA ISLET CELLS

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Insulin-expressing beta islet-like cells differentiated from human embryonic stem cells (hESCs) are a model system for studying the molecular signaling pathways underlying beta cell development. MicroRNA repression of mRNAs is an important mechanism that regulates gene expression. We performed genome-wide microarray profiling of microRNA and mRNA expression at 6 cell stages during in vitro directed differentiation of hESCs into beta islet-like cells _undifferentiated hESCs, definitive endoderm, primitive gut tube, posterior foregut, pancreatic progenitor and hormone-expressing endocrine cells, in addition to human fetal pancreatic tissue samples. Our results showed that both microRNAs and mRNAs are expressed in a dynamic pattern, and that distinct groups of microRNAs were co-expressed during differentiation. Using supervised group-wise statistical analysis and semi-supervised hierarchical clustering, we found that these microRNAs fell into several groups. There was a large cluster of pluripotency-associated microRNAs located on chromosome 19q13 (mir-448, -498, -512, -518, -519, -520), that was rapidly down-regulated upon induction of differentiation. Another pluriotropy-associated cluster on chromosome 4q25 (the mir-302 family) displayed persistent expression until the later stages of differentiation. Similarly, the microRNAs that showed increased expression with differentiation also followed different time courses of induction, with some (mir-375, -26b, -200c) induced quickly upon differentiation and remaining high, while others (mir-30d, -24, -27b) were induced only at later stages. To understand the functions of microRNAs during differentiation, we performed integrated analysis of the microRNA and mRNA profiling data generated from the same samples using a web-based bioinformatic tool followed by permutation-based statistical testing for significance. Our results showed that undifferentiated hESC-enriched microRNAs were predominantly negatively correlated with their mRNA targets, while differentiation-enriched miRNAs and their targets tended to be positively correlated. These microRNA/mRNA pairs were further investigated using luciferase reporter assays, which identified dual roles for the mir-200a/141 family of microRNAs in regulating both the mesenchymal-to-epithelial transition and definitive endoderm formation during early differentiation; at later stages of differentiation mir-30d and let-7e regulated the pancreatic progenitor gene RXf6. Samples from the later stages of differentiation clustered closely with fetal pancreas samples, but a comparison across all samples showed differences between tissues and cells that may reflect a less differentiated or less mature state in hESC derived cells. In summary, this study has used in vitro directed differentiation of hESCs as a model system to identify specific microRNAs and mRNA targets that are involved in regulation of beta cell differentiation.
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spheres while the CSClow cells could not form such spheres. In in vivo tumorigenicity assay, CSChigh formed tumors with only 10 cells indicating the remarkable tumorigenicity of CSChigh cells. CSChigh cells were resistant to gemcitabine treatment, while CSClow cells were highly sensitive to this treatment. We performed a drug screen on both cell types and successfully identify a novel compound targeting specifically the pancreatic CSCs. Anti-CSC effects of the compound were confirmed by in vitro dynamic images and in vivo tumor analyses. CONCLUSION: In our system, the high green expressing cells (CSChigh) were proved to have cancer stem-like characteristics. The visualization system can be applicable to direct monitoring of drug sensitivity in the specific CSCs, distinct from non-CSCs. Our studies indicated rational application of drug screening to discover compounds targeting selectively the pancreatic CSCs. Further studies using this system may improve therapeutic approaches for aggressive cancer and subsequently outcomes of the patients in the future.

Poster Board Number: T-1140

MODELING OF PERMANENT NEONATAL DIABETES MELLITUS USING PATIENT-SPECIFIC IPS CELLS
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Permanent neonatal diabetes mellitus (PNDM) has been associated with monogenic defects in about half of the studied cases. Among 12 PNDM cases studied in the genetically isolated Finnish population, mutations in the best-known genes (ABCC8, KCNJ11, Insulin) have been found in only 3 of them, suggesting the prevalence of other mutations not yet identified. The generation of in vitro models using patient-derived induced pluripotent stem cells (iPSC) constitutes a valuable tool for the study of disease mechanisms. We aim to use this approach to establish a platform enabling studies of pathogenic mechanisms in PNDM. Fibroblasts derived from PNDM patients and healthy donors were efficiently reprogrammed into iPSCs using retroviral or Sendai virus delivery of reprogramming factors Oct4, Klf4, Sox2 and c-Myc, in combination with sodium butyrate. Generated iPSC lines showed the characteristic morphology and markers of pluripotent stem cells and they were able to differentiate into the three embryonic germ layers in teratoma assay. Derivation of substantial numbers of fetal stage pancreatic islet cells from human pluripotent stem cells is possible by mimicking in vitro the developmental pathways occurring in vivo. We have optimized a feeder- and serum-free, multistage pancreas differentiation protocol with the incorporation of TGFβ/BMP signaling pathway inhibitors at different stages. Healthy control iPSCs were successfully differentiated towards pancreatic endocrine lineage. Quantitative PCR (qPCR) analysis revealed proper differentiation kinetics, showing the upregulation of FoxA2, Sox17 and CXCR4 ex-pression during the definitive endoderm induction stage, and Pdx1, Nkx6.1 and Ngn3 in the endocrine progenitors stage. Cytometric analysis showed efficient specification of definitive endoderm stage cells, with 75%-90% of CXCR4+ cells. Colocalization of Pdx1 and Nkx6.1 was used to verify the islet progenitors, which were present in the mid stages of our differentiation protocol. In the later stages, immunocytochemistry revealed abundant clusters of cells single or double positive for insulin/c-peptide and glucagon. These results were confirmed by qPCR, demonstrating the generation of fetal-stage pancreatic islet cells using this protocol. These results demonstrate that it is feasible to establish a disease modeling platform based on the pancreatic differentiation of iPSC derived from PNDM patients. The generation and analysis of iPSC lines with mutations in well-studied genes (KCNJ11, Insulin) is currently ongoing to validate this experimental approach. In the next stage, the analysis will be performed in cases with unknown etiology. In combination with next generation sequencing, this is expected to enable the identification of new causes for human diabetes.

Poster Board Number: T-1141

CD133 IS INVOLVED IN MIGRATION AND INVASION THROUGH REGULATION ON N-CADHERIN IN PANCREATIC CANCER
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Pancreatic cancer is the fifth leading cause of cancer-related death in Japan and the fourth in the United States. Over 90% of pancreatic cancer-associated mortality is due to metastasis, which involves several serial steps from disseminating from primary cancer to colonizing in the distant organ. CD133, a marker of cancer stem cells (CSCs) in various solid tumors including pancreatic cancer, has been studied for recent decade. However, the role of CD133 is still obscure. Therefore, we investigated the CD133 functions using human pancreatic cancer cell line, Capan-1. In our experiments, the tumorigenesis of CD133-positive pancreatic cancer cells was higher than that of CD133-negative cells using NOD/SCID mice. On the other hand, it has been supposed that the epithelial-mesenchymal transition (EMT) is one of CSC properties in the process of metastasis. We recently established the high migratory subclone, Capan1M9, from Capan-1 cells. Capan1M9 cells showed a three-fold increase in migration and invasion compared to the parental cell line (Capan-1). Furthermore, the higher expressions of Slug, N-cadherin and fibronectin which are components of characteristic of EMT were shown in this Capan1M9 cell line. Importantly, CD133 expression also elevated in the Capan1M9 cells by the flow cytometric analysis and western blot examinations. Subsequently, shRNACD133 was transfected into Capan1M9 to knockdown CD133 expression. This result showed the reduction of migratory and invasive abilities of the Capan1M9 cells and the repression of Slug and N-cadherin expression. In contrast, there was no difference not only on spheroid formation but also xenograft tumor growth between Capan1M9 and shRNACD133Capan1M9. In addition, N-cadherin was down regulated but CD133 expression showed no influence after shRNASlug transfection into Capan1M9. Next, ERK inhibitor, U0126, was administrated into Capan1M9 to determine whether ERK pathway involved in N-cadherin regulation. We found that not only N-cadherin also Slug and CD133 were down regulated significantly by U0126 administration. Phosphorylated ERK1/2 could be blotted among N-cadherin immunoprecipitated elute. Taken together, ERK, CD133 and Slug interact as a modulation loop on N-cadherin expression. Further study should shed new light to understand the orchestrated network associated with CD133 of pancreatic cancer metastasis. These insights on CD133 regulation could be promising the novel targeted therapy for pancreatic cancer.
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**Poster Board Number: T-1142**

**FIGF PLAYS AN IMPORTANT ROLE IN THE PANCREAS REGENERATION**

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Objective To explore the role of Figf (C-fos induced growth factor) in pancreas regeneration. Methods Mouse model of pancreatic regeneration was established by pancreatectomy. Pancreatic function loss and regeneration was monitored by time-coursed blood glucose testing after pancreatectomy. Regenerating pancreas tissues were collected 48h post pancreatectomy for RNA isolation. Gene expression profiling was performed using mouse whole genome gene chips. Differentially expressed genes were verified by qPCR. The target gene, Figf, was selected and cloned in expression plasmid. MS1 cells cultured in vitro were transfected with the Figf constructs, and insulin secretion by the transfected cells was detected by ELISA 36h after cell transfection. The mRNA levels of Pdx1 and Insulin1 genes in transfected cells were tested by qPCR. Results Figf expression was significantly increased in regenerating pancreas. Comparing with untransfected and vector-transfected groups, insulin secretion in Figf transfected cells increased significantly (116.89±6.09 pg/ml over untransfected group, P<0.01; and 114.24±4.60 pg/ml over vector- transfected group,P<0.01). Again, comparing with untransfected and vector-transfected groups, Figf over expression group expressed significantly higher mRNA levels of Pdx1 and Insulin1 genes(P<0.01). Conclusion: Over expression over expression group expressed significantly higher mRNA levels comparing with untransfected and vector-transfected groups, Figf over expression group expressed significantly higher mRNA levels of Pdx1 and Insulin1 genes(P<0.01). Overexpression of Fgf gene in MS1 cells can elevate the expression of Pdx1 and Insulin1 genes(P<0.01). Comparison: The expression of Figf gene in MS1 cells were tested by qPCR. Results Fgf expression was significantly increased in regenerating pancreas. Comparing with untransfected and vector-transfected groups, insulin secretion in Fgf transfected cells increased significantly (116.89±6.09 pg/ml over untransfected group, P<0.01; and 114.24±4.60 pg/ml over vector-transfected group,P<0.01). Again, comparing with untransfected and vector-transfected groups, Fgf overexpression group expressed significantly higher mRNA levels of Pdx1 and Insulin1 genes(P<0.01). Conclusion: Overexpression of Fgf gene in MS1 cells can elevate the expression of Pdx1 and Insulin1 genes, and increase insulin secretion. Fgf may be one of the key genes evolved in pancreas regeneration and play an important role in β-cell transition regeneration. Correspondence: Li yuuki E-mail: yuuki4@hotmail.com (The research is supported by the National Natural Science Foundation of China, NO. 81160098)

**Intestinal/Gut Cells**

**Poster Board Number: T-1143**

**FUNCTIONAL ANALYSIS OF SNAI1 IN THE MOUSE INTESTINAL STEM CELL NICHE**

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The Snail family of transcriptional repressors have a key role in mediating epithelial to mesenchymal transitions during both embryonic development and cancer metastasis. Although they are generally regarded as markers of mesenchymal cells, Snail proteins have also recently been implicated in regulating stem cell populations in several organs. We have examined the role of Snail proteins in the mouse intestinal epithelium that is continuously renewed via a population of multipotent stem cells that reside in the base of crypts. We have investigated Snai 1 function in the intestinal stem cell niche using an inducible conditional knockout and found that Snai 1 is required for maintenance of the crypt base columnar stem cell population. We have also analysed the effects on the crypt base columnar stem cell population using a combination of Fluorescent Activated Cell Sorting (FACS) and organoid culture. Further analysis of the tissue of Snai1 mouse models has revealed an effect on the differentiation of mature cell lineages. In conclusion, these results suggest that Snai1 has a key role in stem cell maintenance and control of cellular differentiation.

**Poster Board Number: T-1145**

**GENERATION OF INTESTINAL TISSUE FROM INDUCED PLURIPOTENT STEM CELLS**

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Introduction: Crohn's Disease (CD) is a chronic inflammatory disorder that can affect any part of the gastrointestinal tract. Despite extensive research, the causes of CD remain elusive. One relatively unexplored area of study is what role the intestinal epithelium may play in this disease. The intestinal epithelium, which contains four differentiated cell types, has been poorly studied as it had previously not been possible to study it in vitro. Recently it was found that induced pluripotent stem cells (iPSCs) could be directed to form intestinal tissue, which contained all the four cell types, in vitro (Spence et al, 2011). Method: The generation of intestinal tissue from iPSCs is a multi-step process which involves directing iPSCs into definitive endoderm, then hindgut tissue and then subsequently into three dimensional intestinal “organoids.” To generate definitive endoderm, two control integration-free iPSC lines were incubated with 100ng/ml of Activin A under low serum conditions...
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for three days. To generate hindgut tissue, definitive endoderm was incubated in Advanced DMEM/F12 media with 10% FCS with or without 500ng/ml of Wnt3A and FGF4 for a further three to four days. During the hindgut differentiation protocol, epithelial "tubes" became visible after three to four days. These epithelial tubes were harvested and then cultured in a three dimensional Matrigel matrix containing noggin, RSpondin-1, EGF and B27. Results: The generation of definitive endodermal tissue was confirmed by the upregulation of Sox17, FoxA2 and Goosecoid mRNA and by immunocytochemistry which showed the co-expression of Sox17 and FoxA2 in the vast majority of these cells. The generation of hindgut tissue was demonstrated by both the presence and upregulation of the hindgut intestinal marker CDX2. The epithelial tubes that were cultured in a three dimensional Matrigel matrix steadily increased in size over time to generate spherical intestinal organoids (approx. 500μm). After 14 days, the organoids contained CDX2+ cells which also expressed the early proliferating intestinal cell markers, KLFS and Sox9. The four differentiated intestinal cell subtypes were also found in these organoids. Goblet cells (Muc2+), Paneth cells (Lysozyme+), enterocytes (FABP2+) and enteroendocrine cells (Chromogranin A+) were all observed in these intestinal organoids at various different timepoints. Conclusion: Intestinal tissue containing the four differentiated cell types can be generated from control iPSCs. iPSCs will now be generated from CD patients with genetic variations in intestinal cell proteins. These iPSCs will then be directed to form intestinal organoids. Such organoids may help us to elucidate some of the mechanisms involved in the pathogenesis of CD.

Poster Board Number: T-1146

LGRS INTESTINAL STEM CELLS AND THEIR MAINTENANCE

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The leucine-rich repeat containing G protein-coupled receptor 5 (Lgr5) marks actively cycling crypt base columnar cells (CBCs) that are multipotent, long-lived, and are largely responsible for homeostatic regeneration in the intestinal epithelium. Recently, the G-protein-coupled receptors Lgr4-6 have been reported to associate with Wnt receptors to mediate R-spondin signaling. Although Paneth cells have been suggested to serve as niche cells for Lgr5+ intestinal stem cells (ISCs), perhaps through secretion of essential paracrine factors, the precise mechanisms regulating maintenance of Lgr5+ ISC remain poorly understood. We have used adenovirus to overexpress the soluble ligand-binding Lgr5 ectodomain in the circulation of adult mice. Circulating Lgr5 ectodomain induced the migration of Paneth cells from the crypts, their eventual loss, and a concomitant disappearance of Lgr5+ ISC. Paneth cell migration was associated with downregulation of Wnt signaling and its target EphB3. The loss of Lgr5+ ISC did not affect maintenance of the intestinal epithelium, nor did Lgr5+ stem cells disappear from non-intestinal organs. The use of this adenoviral system to examine the relationship of Lgr5+ ISC to other intestinal stem cell populations will be discussed. Together, these findings characterize an easily tractable experimental model for the in vivo deletion of Lgr5+ ISC, and suggest that Lgr receptors function to actively maintain Lgr5+ ISC in vivo.

Poster Board Number: T-1147

COMPUTATIONAL ANALYSIS OF STEM AND NICHE CELL PATTERNS INSIDE INTESTINAL CRYPTS

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INTRODUCTION The intestinal epithelium is the most rapidly dividing tissue in the body, turning over every 3-4 days. Each intestinal crypt contains a few slow-cycling BMI1+ intestinal stem cells (ISCs) at the +4 position and 12-14 faster-cycling Lgr5+ ISCs. At the base of the crypt, the Lgr5+ ISCs and CD24+ Paneth cells form checkerboard patterns, which provide the niche for ISC self-renewal. Subversion of this mechanism leads to dysplasia and cancer. Because Paneth cells express the Notch ligand DLL4 while ISCs express the Notch1 and 2 receptors, it has been postulated that Notch-dependent lateral inhibition (NDLI) - circuits in which Notch signaling in a cell downregulates signaling in its neighbors - is responsible for the checkerboard pattern between ISCs and Paneth cells. However, the exact mechanism remains unclear. Here we used analytical tools from systems biology to quantitatively investigate alternative NDLI circuits that can impact ISC-Paneth pattern formation and maintenance. RESULTS We first examined a transcriptional feedback as the potential NDLI mechanism in the crypt. In this transcriptional feedback, Notch ligands on one cell activate Notch receptors on an adjacent cell, which leads to downregulation of Notch ligands DLL1 and DLL4 through the transcriptional factor MATH1. Steady state analysis of an ordinary differential equation (ODE) based model showed that this feedback is sufficient to generate checkerboard-like patterns. However, sensitivity analysis revealed that the resulting pattern is not robust to variations and perturbations. Ensuing dynamical analysis of the pattern further revealed that the pattern is not sufficiently stable in terms of Maximal Lyapunov Exponent (MLE) and that it is a slow process to form a steady pattern. In contrast, the base of the crypt is a dynamic environment, wherein Lgr5+ ISC can constantly divide and leave the niche to replace the entire epithelium every 3-5 days. Therefore, the transcriptional feedback is not likely the only NDLI mechanism responsible for regulating the checkerboard pattern. We then examined a post-translational feedback as an alternative or secondary NDLI mechanism to the transcriptional feedback. In this post-translational feedback, Notch receptors and ligands interact in the same cell, and the receptor-ligand complexes are mutually inhibited and degraded by endocytosis (cis-inhibition). Time-lapse movies of single cells with fluorescent reporters showed that cis-inhibition acts as an ultra-sensitive switch that amplifies the small difference in ligand levels between adjacent cells. These steady-state and dynamic analyses demonstrate that the post-translational feedback through cis-inhibition is a more robust mechanism for generating and maintaining the checkerboard pattern between ISC and Paneth cells. Compared to the transcriptional feedback, cis-inhibition leads to speedier pattern formation and more stable patterns. CONCLUSION Our computational analyses suggest that the MATH1-mediated transcriptional feedback is insufficient to generate robust checkerboard patterns, so the post-translational feedback through cis-inhibition is likely an active NDLI mechanism inside the crypt. METHOD Sensitivity analyses were performed using the Matlab SimBiology toolbox. Stability and speed of pattern formation was analyzed using MLE and stochastic simulations. The speed of pattern formation was analyzed using stochastic simulations with different initial conditions.
**Poster Board Number: T-1148**

**DOSE-DEPENDENT ROLES FOR CANONICAL WNT SIGNALING IN DE NOVO CRYPT FORMATION AND CELL CYCLE PROPERTIES OF COLONIC EPITHELIUM**

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Canonical Wnt signaling plays a pivotal role in the physiology and carcinogenesis of the intestinal epithelium. Notably, the strength of Wnt signaling has been reported to affect the tumor spectrum in mice, and it controls hematopoietic stem cell self-renewal vs. differentiation. Whether intestinal stem cells also respond to Wnt signaling in a dose-dependent manner remains unknown. Here, we investigated the effect of canonical Wnt activation on colonic epithelial differentiation by controlling the expression levels of stabilized β-catenin using a doxycycline-inducible transgenic system in mice. We show that elevated levels of Wnt signaling induce the amplification of Lgr5+ crypt stem cells, which is accompanied by crypt fission and a reduction in cell proliferation among progenitor cells. In contrast, lower levels of β-catenin induction enhanced cell proliferation rates of epithelial progenitors without affecting crypt fission rates. Notably, slow-cycling cells produced by β-catenin activation exhibit activation of Notch signaling. Consistent with the interpretation that the combination of Notch and Wnt signaling maintains cells in a low proliferative state, the treatment of β-catenin expressing mice with a Notch inhibitor turned such slow-cycling cells into actively proliferating cells. Our results indicate that the activation of the canonical Wnt signaling pathway is sufficient to amplify colonic stem cells, and suggest that different levels of canonical Wnt activations, in cooperation with Notch signaling, establish a hierarchy of slower-cycling stem cells and faster-cycling progenitor cells characteristic for the colonic epithelium.

**Poster Board Number: T-1151**

**HTS ASSAY WITH MESODERMAL PROGENY CELLS DERIVED FORM HUMAN PLURIPOTENT STEM CELLS (hES and iP5) FOR THE IDENTIFICATION OF MEVALONATE PATHWAY MODULATORS**

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The mevalonate pathway provides cells with essential products such as cholesterol and substrates for isoprenylation and is therefore critical for cell growth and differentiation. Designed HTS cell assays for the identification of modulators mevalonate pathways are important to identify new functional inhibitors, to increase our knowledge on the mevalonate pathway and to predict and prevent toxic effects. One of the key factors for success of HTS bioassays is the source of the cells used for the screen. In this regard, human pluripotent stem cells possess numerous advantages: a human origin, an unlimited growth potential, a symmetric stability and the capacity to differentiate into all the cell types forming an organism. For this study, we defined a simple and robust differentiation protocol to obtain large and homogeneous amounts of mesodermal progenitor cells (MPCs) from human pluripotent stem cells (hES and iP5). Herein, we describe the optimisation of MPC culture conditions in multwell dishes for the implementation of a cell-based assay for primary screening by HTS in presence or absence of mevalonate to screen functional inhibitors of HMG-CoA reductase. The screening of the Prestwick library, consisting of 1120 highly diverse FDA-approved drugs that have established biological activities demonstrates specificity and robustness of our HTS assay. In each screen, we were able to select the three HMG-CoA reductase inhibitors (lovastatin, fluvastatin, simvastatin) present in the Prestwick library. EC50 determination of statins confirmed the toxicity of this class of molecules on MPCs and revealed that MPCs display a toxicity similar to the one observed with adult muscle cells, a major target of statins sideeffect. Furthermore, we have designed a second type of cell bioassay to identify molecules that protect cells from statin induced toxicity induced. In this cell bioassay, we identified by screening more than 10 000 molecules, two classes of molecules that protect from simvastatin toxicity. Mechanistic explorations indicate that one of these molecules control the level of HMG-CoA reductase transcript. MPCs derived from hES and iP5 were equally potent as a source of cells to identify hits by HTS in both bioassays. In conclusion, differentiated mesodermal progenies derived from iP5 and hES allow the identification by HTS of functional inhibitors of HMG-CoA reductase and molecules which can rescue from simvastatin toxicity by controlling the expression of mRNA coding for HMG-CoA reductase.

**Poster Board Number: T-1152**

**AGE-DEPENDENT EFFECTS OF PRO-INFLAMMATORY CYTOKINES ON HUMAN MYOBLAST DIFFERENTIATION**

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Objective: Targeted enhancement of proliferation and differentiation of muscle progenitor cells is a key strategy for combating age-related muscle wasting, weakness and sarcopenia. As the pro-inflammatory circulating cytokines are elevated in many age-related disorders, we have investigated the action of TNF-α, IL-1β and IL-6 on proliferation and differentiation of muscle progenitor cells (myoblasts) of individuals with different ages: young-less than 30 and elder-over 65 years. Methods: Primary cultured human skeletal muscle myoblasts were purified with CD56 antibodies microbeads on MACS (Miltenyi Biotec) and cultured in the presence of HGF. Serum free cultures we stimulated for 6-7 days with each cytokine (PeproTech) alone or in different combinations with different media supplement: insulin- transferrin-sodium selenite (ITS)/Sigma-Aldrich). Differentiation was estimated by monitoring myotube formation, fusion index and % of myonuclei. Results: The myoblasts cultivated from older individuals differentiated markedly slower than myoblasts from young individuals in ITS medium. Treatment of human myoblasts with TNF-α and IL-1β increased the proliferation and block the differentiation even in the presence
**NOVEL HUMAN ARTIFICIAL CHROMOSOMES AND TECHNOLOGIES FOR STEM CELL-BASED THERAPIES OF DUCHENNE MUSCULAR DYSTROPHY**

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We recently showed that stem cell-mediated human artificial chromosome (HAC) transfer ameliorates the pathology in a mouse model (mdx) of Duchenne muscular dystrophy (DMD), which is caused by mutations in the large dystrophin gene (2.4 Mb). HACs are stably maintained in the host cell as episomal vectors, like native chromosomes. Their capacity to carry large genomic loci containing regulatory elements allows physiological regulation of the introduced genes. We succeeded in developing a HAC vector containing the entire human dystrophin gene (DYS-HAC1), which was then successfully transferred into mesenchymal, embryonic and induced pluripotent stem cells. Importantly, DYS-HAC1 was also transferred into mesangioblasts (vessel-associated stem cells) derived from the dystrophic mdx mouse. Upon intramuscular or intra-arterial transplantation of dystrophin-corrected mdx mesangioblasts into mdx mice, donor cells gave rise to large clusters of dystrophic-positive myofibers together with donor-derived satellite cells, which significantly ameliorated morphology and function of dystrophic muscles. However, in order to translate this approach to DMD patients, there are still many technical hurdles that need to be overcome. Here we report novel strategies and technologies of HAC-mediated cell therapy for DMD patients including: 1) Transfer of novel DYS-HACs into human mesangioblasts; 2) HAC-mediated enhancement of skeletal muscle differentiation and 3) higher dystrophin expression. 1) In order to extend the proliferative capability of DMD mesangioblasts to survive selection after HAC transfer; we developed a platform for their engineering by means of excisable lentiviral vectors expressing immortalizing genes (hTERT and Bmi1) in a reversible fashion. Alternatively, we have also derived mesangioblasts from iP5 cells. Both approaches generate cells able to be genetically corrected with novel DYS-HACs with reduced immunogenicity (no EGF, TK, Blasticidin and hprt at variance with DYSHAC1). 2) For effective skeletal muscle conversion of target cells, a DYS-HAC containing an inducible MyoD gene (MyoD-ER) was developed (DYS-HAC3). 3) Dystrophin gene dosage of transplanted cells containing single DYS-HAC is not close to wild-type dystrophin because of fusion with endogenous myofibers (containing hundreds of nuclei) that do not express dystrophin. To increase dystrophin expression level, we transferred three novel DYS-HACs into mdx mesangioblasts, each one containing a different conditional selection marker gene (DYS-HAC2: neomycin; DYS-HAC4: blasticidin; DYS-HAC6: histidinol dehydrogenase) developed using homologous recombination. Mdx mesangioblasts containing multiple DYS-HACs (up to 3) were developed and their dystrophin expression was being tested. Thus, these DMD-specific DYS-HACs and technologies are expected to speed up future translational gene and cell therapies for muscular dystrophy.

**ADAR1 REGULATES THE MYOGENIC PROGRAM IN MOUSE SKELETAL MUSCLE CELLS**

ADAR1 (adenosine deaminase acting on RNA) catalyzes the deamination of adenosine to inosine on RNA molecules of double stranded structure. Two isoforms of ADAR1 are known, both of which possess RNA-editing activity: an interferon (IFN) inducible 150 kDa protein (p150) and a constitutively expressed amino-terminally truncated 110 kDa protein (p110). The p150 isoform is found in both the cytoplasm and nucleus, while the p110 protein is localized predominantly in the nucleus. A-to-I RNA editing not only affects targeted transcripts by altering the sequence and/ or structure of the encoded products, but also serves to regulate retrotransposons and gene silencing. In this study, we identified a novel aspect of biological function of ADAR1 in the regulation of skeletal myogenesis. We have investigated ADAR1 expression during myoblasts-myotubes differentiation. ADAR1 p150 expression transiently increased during cell confluence and initial differentiation process, whereas the expression of both ADAR1 forms dropped in the differentiated myotubes. Furthermore, we discovered that ADAR1 p150 transient up-regulation in early differentiation is mediated by the IFN signaling pathway-associated interferon stimulator response element (ISRE) and subsequently repressed by the myogenic transcription factor MyoD. Intriguingly, knockdown of ADAR1 or overexpression of a catalytic mutant reduced the expression of myogenesis-associated markers, demonstrating the significance of ADAR1-mediated nuclear RNA editing in the early myogenic program. On the other hand, the function of ADAR1 p150 in early stage of differentiation possibly lies in the regulation of PKR-mediated apoptosis. Next, we demonstrated that in the late stage of myogenesis, miR-1/206-mediated inhibition facilitated the down-regulation of ADAR1 proteins. However, ectopic ADAR1 over-expression at this stage caused abnormal myotube formation, further implying stage-specific functions of ADAR1 in the myogenic process. Taken together, our results pointed to the scenario that ARAR1-mediated regulation, possibly through editing of as yet unknown transcripts, is essential for scheduled progression of skeletal myogenesis.
THE INFLUENCE OF PREOXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ1 ON DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS TO CARDIAC BEATING CELLS

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Peroxisome proliferator activated receptor γ, a member of nuclear ligand activated transcription factors PPARs, exerts various metabolic functions and also affects differentiation process. To testify the importance of PPARγ in cardiac differentiation of mouse embryonic stem cells (mESCs), in the first step, its expression level was assessed during cardiac differentiation of mouse embryonic stem cells. Data revealed an elevation in expression level of PPARγ when beating bodies (BBs) were formed. Subsequently, involvement of PPARγ in during and post-cardiac precursor cells (CPs) formation was examined by application of agonist (Rosiglitazone, 5µM) and antagonist (GW9662, 10µM). Our results indicated that PPARγ inactivation via treatment with GW9662 during CPs formation, reduced expression of cardiac beating bodies’ markers. However, PPARγ inactivation by antagonist treatment post-CPs formation stage did not affect beating bodies’ differentiation. Here, we have demonstrated a stage dependent role of PPARγ modulation on cardiac differentiation of mESCs for the first time.

TRANSLPLANTATION OF MESENCHYMAL STEM CELLS DERIVED FROM ES CELLS PROMOTES MUSCLE REGENERATION; RE-INNERRVATION AND THEREBY ACCELERATES FUNCTIONAL RECOVERY OF INJURED SKELETAL MUSCLE

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Background and Aims: It is well known that mesenchymal stem cells (MSCs) have a high potential for differentiation into mesenchymal cells. Adipose tissues have been proved as a useful and rich source of adipose tissue-derived mesenchymal stem cells (ADSCs). Although ADSCs have shown therapeutic efficiency in repairing damaged mesenchymal tissues, their isolation and purification from adult adipose tissue still involves complicated and troublesome procedures. Furthermore, they readily differentiate into adipocytes, osteocytes and chondrocytes but not into muscular cells. Mouse ES cells, on the other hand, are pluripotent, and their induction to adipogenesis has been well described. Here, we have demonstrated a stage dependent role of PPARγ modulation on cardiac differentiation of mESCs for the first time.

injured muscles by the transplantation of E-MSCs. Their transplantation promoted the functional recovery of injured muscles by an acceleration of muscle regeneration and a re-innervation of peripheral nerves. Materials and Methods: ES cells (G4-2; kind gifts from Dr. Niwa) carrying the enhanced green fluorescent protein _EGFP_ gene under the control of cytomegalovirus/chicken β-actin promoter were expanded their population and embryoid bodies (EBs) were formed in hanging drops. EBs were cultured in a retinoic acid containing medium. After washing, they were settled on culture dishes and maintained with adipogenesis medium (insulin / triiodo-thyronine). After the increase of CD105+ cells, we isolated and sorted them by a magnetic cell sorter (MACS; Miltenyi). CD105 positive E-MSCs were transplanted into the injured tibialis anterior muscles of SCID mice 24 h after clamping. After 1, 2, 3, and 4weeks of transplantation, the myogenic differentiation of M-ESCs, muscle regeneration, and re-innervation were analyzed. The myogenic differentiation was examined by Pax7, M-cadherin and/or skeletal muscle myosin heavy-chain (MHC) immuno-staining, and re-innervation was examined by α-Bungarotoxin and SMI-31, respectively. We, then counted EGFP expressing E-MSCs among regenerating muscle cells and innervation of peripheral nerves. Furthermore functional performance of transplanted animal and sham transplanted animals were compared using a functional analyzer Cat Walk XT (Noldus). Results and Conclusion: When E-MSCs were transplanted into the injured tibialis anterior muscles of SCID mice, most of them differentiated into skeletal muscles in vivo. The transplantation of E-MSCs promoted a functional recovery of injured muscles by an acceleration of muscle regeneration and a re-innervation of the peripheral nerves. Thus, cell therapy using MSCs derived from pluripotent stem cells is one of the most effective and safe ways to improve functional recovery of damaged skeletal muscles.

Poster Board Number: T-1155

AUTOPHAGY IS ESSENTIAL FOR MYOFIBRIL DIFFERENTIATION.

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Introduction. Cardiovascular diseases constitute the leading cause of mortality and morbidity worldwide, with myocardial infarction responsible for more than 10% of deaths. Although patients affected by heart failure can be treated with transplantation, the ever increasing patient need has not been met with a corresponding increase in donor organs. Over the last decade, stem/progenitor cell therapy has emerged as an innovative approach to overcome this limitation with the potential to provide cardiac repair and regeneration. Efforts to regenerate myocardium through differentiation of cardiac stem cells to fully differentiated cardiomyocytes have met with limited success. Autophagy is a lysosome-mediated degradative pathway used by eukaryotes to recycle cytosolic components in both basal and stress conditions. Recently, autophagy has been recognized to be important for metabolic reprogramming during cell differentiation. Tissue specific gene-targeting studies have revealed that autophagy functions in several specific lineages, including adipocytes, erythrocytes, T cells, and B-1a cells. Autophagy may play an important role in partial elimination of mitochondria during these processes to facilitate metabolic reprogramming. Here we investigated if autophagy is needed for myocyte cell differentiation, to properly coordinate mitochondria and transcription factors regulating myocyte cell differentiation in muscle development. Methods. C2C12 cells were cultured in DMEM with 10% FBS and induced to differentiate by changing the medium to DMEM with 2% horse serum for 6d. Cells were transfected with Atg5 siRNA and...
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Infected with GFP-LC3 adenovirus. Cells were treated with 10 nM Bafilomycin A1 or DMSO (vehicle control) for a 3hr interval daily for the first 3d. At the indicated times, cells were harvested and processed for western blot detection of LC3, MyoD, myogenin, alpha-actin, and Tom70. Total RNA was prepared from C2C12 cells. Reverse transcription was carried out with iScript cDNA Synthesis Kit (BIO-RAD) enzymes and random primers. The sequences of forward and reverse oligonucleotide primers, specific to PGC1-α and housekeeping genes, were designed. Real time quantitative PCR was performed in an iCycler 5 (BIO-RAD). The activities of the mitochondrial electron transport Complexes I-IV were measured using a Clarke oxygen electrode with a thermojacketed chamber. Fixed cells were immunostained with antibodies to alpha-actin. Results: We observed that during the differentiation of C2C12 myoblasts into myotubes, levels of LC3-II and abundance of GFP-LC3 puncta increased, consistent with induction of autophagy. Inhibition of autophagy with Atg5 siRNA or Bafilomycin A1 prevented myotube formation. This was associated with failure to upregulate the myogenic factors MyoD, myogenin and the mitochondrial import receptor Tom70. Furthermore, inhibition of autophagy with Atg5 siRNA attenuated expression of the mitochondrial biogenesis factor PGC1-α and oxygen consumption. These results suggest that inhibition of autophagy prevented mitochondrial maturation for energy production. Conclusions: Our studies demonstrate a role for mitochondrial biogenesis and activity during myoblast cell differentiation to myotube and suggest that autophagy is essential for myoblast differentiation. These findings may have relevance to cardiac stem cell differentiation.

Poster Board Number: T-1158

MYOGENIC DIFFERENTIATION OF HUMAN IPS CELLS USING GROWTH FACTORS AND SMALL MOLECULES IN DEFINED SERUM-FREE MEDIUM

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Reprogramming technology, iPS cells, and methods for inducing differentiation of tissue stem cells from pluripotent stem cells have demonstrated potential for treatment of human diseases such as muscular dystrophy, but there is still no effective protocol to differentiate human iPS cells into myogenic progenitor cells without transcription factors. To establish a protocol to induce myogenic differentiation of human iPS cells without using transcription factors, we tested methods to induce paraxial mesoderm from human iPS cells in a defined serum-free medium. To identify small molecules that induce paraxial mesodermal cells (PDGFRα+, Flk-1-) from embryoid bodies of E14 mouse ES cells in serum-free medium, we screened a library of 500 defined small molecules and identified five candidate compounds. Of the five small molecules, three induced paraxial mesodermal cells in mesodermal differentiated embryoid bodies in combination with Wnt3a and BMP4 from 201B7 human iPS cells. The induced paraxial mesodermal cells were isolated by FACS and underwent myogenic differentiation in cardiotoxin-injured muscle of NOD/SCID mice. These results suggest that growth factors and small molecules can induce myogenic progenitor cells from 201B7 iPS cells in a defined serum-free medium. We are currently testing a way to induce the myogenic differentiation of the induced paraxial mesodermal cells.

Poster Board Number: T-1159

MUSCLE DERIVED STEM CELL THERAPY FOR THE REGENERATION OF PELVIC MUSCLES

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Objectives: This study was designed to determine whether the injection of muscle-derived stem cells into the defected or injured female pelvic floors can regenerate their skeletal and smooth muscles and improve their functional properties in a rat model. We hypothesized that implantation of muscle derived stem cells into pelvic floor may improve their function, leading to a therapeutic approach for treatment of fecal incontinence. Methods: In this study, rats with sphincterotomy and repair were utilized as a model for anal sphincter dysfunction and fecal incontinence. The control group A underwent only sham operation. The study group B had sphincterotomy and repair of anal sphincters, followed by saline solution injections. The study group C underwent sphincterotomy and repair, followed by intra-sphincteric injections of muscle derived stem cells (MDSC). MDSC were isolated from gastrocnemius muscles of female rats, and then cultured and characterized. In vivo differentiation of MDSC was evaluated using immunofluorescence after injection of MDSC into nude rats in groups A, B, and C. The MDSC were labeled with PKH-26 before injections. At 4, 8, and 12 weeks after injections, these animals were studied for functional improvement by manometry and contractility testing, and for tissue histologic and morphometric analysis. Differentiation of implanted MDSC was assessed by immunofluorescence, using specific antibodies as markers of smooth muscle (smooth muscle actin, calponin, and smoothelin) and of skeletal muscle (MHC-II). MDSC were followed using their labeling marker PKH-26. Results: A significant decrease of muscle tissue was observed at the site of repair after sphincterotomy in groups B as compared to group A. However, in Groups C, histologic examination demonstrated new muscle fibers and morphometric analysis revealed a significantly greater muscle area fraction than in Group B. PKH-26-labeled implanted MDSC were detected in the anal sphincters. Differentiated muscle tissues stained positively for alpha smooth muscle actin and myosin heavy chain indicating the formation of smooth and skeletal muscle, respectively, at the MDSC injection sites. The MDSC injection sites were demonstrated by their label PKH-26. Functional studies showed improvement of anal sphincter function. Conclusions: This study demonstrates that MDSC injections improved muscle regeneration and function of the damaged anal sphincters. Regenerations of both skeletal muscle of the external sphincter and smooth muscle of the internal sphincter with MDSC may have potential application as a therapeutic approach to treating fecal incontinence.

Poster Board Number: T-1160

EPIGENETIC MEMORY ENHANCES MYOGENIC FATE OF INDUCED PLURIPOTENT STEM CELLS

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Muscular dystrophies (MDs) constitute a heterogeneous set of genetically inherited diseases, characterized by chronic degeneration of muscle fibres and, ultimately, by motility loss and cardiopulmonary failure. To date no regenerating strategies are yet present in the clinical practice. Cell replacement strategies could represent a valuable therapeutic alternative to regenerate damaged fibres and
counteract muscle wastage. Murine and canine models of MD have shown improvements after systemic delivery and muscle engraftment of mesoangioblasts (MABs). MABs are somatic, vessel-associated stem cells, isolatable from murine, canine and human muscle biopsies. Notwithstanding their myogenic regenerative potential in vivo, MABs display a limited proliferation potential and rapidly undergo senescence in vitro. In order to expand MAB potency and self-renewal capacity, we generated MAB-derived induced pluripotent stem cells (MAB-iPSCs). MAB-iPSCs shared morphological, self-renewal and pluripotency features with fibroblast-derived iPSCs (f-iPSCs). Both MAB- and f-iPSCs could give rise to traceable parts of chimeric embryos after morula aggregation. Surprisingly, after subcutaneous injection in immunodeficient mice, MAB-iPSCs produced teratomas with a prominent presence of striated muscle tissue, unlike f-iPSCs. In addition, in vitro differentiation revealed a higher commitment of MAB-iPSCs toward MyHC+ myotubes and CD56+ myogenic progenitors. MAB-iPSC-derived progenitors showed efficient in vivo engraftment and fibre regeneration potential, after intramuscular injection into a murine model of severe MD. Furthermore, comparative tiling arrays on MAB- and f-iPSCs showed a source-biased methylation pattern on regions flanking many genes involved in myogenic differentiation. Recently, we generated human MAB-iPSCs and preliminary results are confirming a durable myogenic capability in vitro and in vivo.

Given their epigenetic memory and biased commitment, MAB-iPSCs could hence bridge the gap between pluripotent cells and MD regenerative therapies.

**Endothelial Cells/Hemangioblasts**

**Poster Board Number:** T-1161

**HIGHLY EFFICIENT AND SIMULTANEOUS GENERATION OF HEMATOPOIETIC AND VASCULAR PROGENITORS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS**

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The derivation of engraftable vascular and hematopoietic stem cells from patient-specific human induced pluripotent stem cells (hiPSC) may have great clinical utility for the effective, long-term treatment of hematopoietic disorders. However, recent studies have suggested that hiPSC do not produce hemato-endothelial progeny in a manner that is quantitatively and qualitatively comparable to human embryonic stem cells (hES). There may be several etiologies for this limitation, including the quality of reprogramming achieved in fibroblast-iPSC (due to retention of somatic donor epigenetic memory), the method of hiPSC culture employed for maintaining pluripotency (e.g. on murine embryonic mouse fibroblasts (MEF) vs. feeder-free monolayer), and the inherent efficiency of the differentiation protocol (e.g., embryoid body vs. stromal co-culture-based). In these studies, we focused on optimizing our previously published hEB-based hemato-endothelial differentiation method for efficient hiPSC differentiation. We demonstrate that under modified feeder-free endothelial culture conditions, multipotent CD34+CD45+ hematopoietic progenitors emerged in mass quantities directly from adherent endothelial/stromal layers of differentiated human induced pluripotent stem cells (hiPSC), and in a manner similar to that which occurs in vivo from hemogenic endothelium. Using a modified human embryoid body (hEB) system, we simultaneously differentiated fibroblast-derived iPSC (fibroblast-iPSC) into both hematopoietic and vascular progenitor cells with comparable efficiency to hES. Two previously described fibroblast-iPSC lines (IMR90-1 and IMR90-4) were differentiated in parallel with a hESC line (H9; WA09) into hematopoietic and vascular lineages with this method. hEBs from fibroblast-iPSC and hESCs were evaluated during differentiation by FACS for the kinetics of hemato-endothelial marker expression (CD34, CD31, CD145, CD1146, KDR, and CD133). These markers peaked at days 8–10 of hEB differentiation, thus this stage was further cultured onto endothelial growth medium (EGM2) and fibronectin-coated plates. In these conditions, clumps of hEB cells not only produced functional CD31+CD146+ vascular progenitors, but also directly differentiated into clusters of hematopoietic “cobblestones” with adherent RUNX1-expressing monolayers. After 3–6 days, floating cells emerged from these adherent endothelial cells that expressed high levels of CD34, CD45, and the hemangioblast marker CD143/ACE (899), and were enriched with superior frequencies of hematopoietic CFU. In this culture system Fibroblast-iPSC produced mass quantities of CD34+CD45+ cells and hematopoietic CFU with similar or higher frequencies than hESC. Further addition of hematoendothelial cytokines (e.g. thrombopoietin, angiopoietin-1, erythropoietin, and IL-6) into EGM2 culture poised bulk quantities of differentiating cells into committed erythroid-myeloid lineages. This novel and highly efficient hemato-endothelial differentiation system will be ideal for direct time course studies of hematopoietic genesis events (e.g. in vitro models of hemogenic endothelium) using time-lapse videography, or bulk FACS kinetic analyses of emerging hematopoietic progenitors from hemangioblast progenitors.

**Poster Board Number:** T-1162

**BD™ PURECOAT™ ECM MIMETIC SURFACES: NOVEL SYNTHETIC, XENO-FREE, ANIMAL-FREE SURFACES FOR HUMAN ENDOTHELIAL COLONY FORMING CELL EXPANSION**

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Endothelial colony forming cells (ECFC) have potential uses in regenerative medicine, such as cell therapies for cardiovascular disease. Expansion of these cells requires coating of the culture vessel with human or animal-derived extracellular matrix (ECM) protein which can introduce human and animal-derived pathogens in the culture. ECMs can be poorly defined and may have batch to batch variability. Moreover, self-coating can be time consuming and coated vessels have limited shelf life. For aforementioned reasons chemically defined extracellular matrix (ECM) systems are required. To support such research areas, we have developed two synthetic, animal-free (defined as free of any human or animal-origin component) peptide surfaces: BD PureCoat collagen I ECM mimetic and fibronectin ECM mimetic. Both of these scalable surfaces mimic native ligands for cell adhesion as demonstrated by attachment of specific integrin expressing cell lines. Peptide coated surfaces can be utilized to grow and expand ECFC, and are stable at room temperature. Human ECFC were cultured on these surfaces in ECFC supplemented EGM-2 medium for multiple passages. Cell attachment, morphology and growth over multiple passages were compared to natural extracellular matrix protein Collagen I or Fibronectin. Functionality of these cells was demonstrated in a tube formation assay frequently cited as a method to study angiogenesis. Post expansion ECFC were able to form capillary-like structures formed by endothelial progenitor cells. Our results suggest that these breakthrough next-generation cell culture environments can be used for the culture of endothelial colony forming cells where defined environment is desirable as well as in basic and applied research.
EX VIVO DEVELOPMENT AND EXPANSION OF FUNCTIONAL ENDOTHELIAL PROGENITOR CELLS FROM HUMAN CORD BLOOD

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In human, angiogenesis is not only important for physiology and development, but also correlation with most cardiovascular diseases such as myocardial infarction. Recently, many reports further demonstrated that angiogenesis related closely with tumor development. Angiogenesis and vasculogenesis are regulated by the proliferation and differentiation of endothelial progenitor cells (EPCs). The current therapy for patients with cardiovascular diseases is surgical operation, which is with low success rate and recovery rate. Now, EPC transplantation in cell therapy is developed actively, and has great potential for treatment of cardiovascular diseases. So, isolation and cryopreservation of EPCs from cord blood would be beneficial for future cell therapy. In this study, we hoped to develop a novel culture system for the development and expansion of EPCs. Firstly we used the systematic procedure with Factorial Design and Steepest ascent method to optimize the cytokine-containing medium that could facilitate EPC growth. Our results showed that EPCs could be isolated and established from cord blood mononuclear cells and could expand over 2 months in our developed EPC medium. In addition, we found that VEGF165, SCGF-α, b-FGF, SCF, IGF-1, FLT-3 ligand, HGF, EGF, IL-8, Hydrocortison, 2-phospho-L-Ascorbic acid and Heparin are necessary for EPC growth. In addition, after cryopreservation, thawed EPCs still maintained expansion ability. We also found that the expanded cells can form tube formation cultured in the Matrigel and had plentiful expression of CD34, CD31, CD144, CD105, CD309 and vWF by using flow cytometry analysis. In our laboratory, we have reported a culture system for EPC establishment from cord blood and for large production of functional EPCs. We believe that the results of this study must be beneficial to the basic research and future trials on clinical transplantation.

FUNCTIONAL IMPROVEMENT AND NEUROPROTECTION PROVIDED BY HUMAN CEREBRAL ENDOTHELIAL CELLS INTRAVENOUSLY-TRANSPLANTED IN FOCAL ISCHEMIA RAT BRAIN

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Stable clonal cell line of human cerebral endothelial cell, named as HEN7, has been generated from human fetal telencephalon using a retroviral vector encoding v-myc gene. HEN7 cells were transplanted intravenously via tail vein in rat brain with photochemically induced focal cerebral ischemia, and the clinical effects for infarct size, edema volume, and clinical outcome were evaluated. FACS analysis showed HEN7 cells express the characteristics of cerebral endothelial cells. HEN7 showed positive immunoreactivity for vascular, stem cell, and tight junction proteins. HEN7 transplanted group showed reduced infarct lesion as identified by bioluminescence and X-gal staining located in the infarcted lesion border area. HEN7 transplanted group showed reduced edema volume associated with MMP-9 expression reduction, and markedly increased nestin-positive cells around infarcted area. HEN7 transplanted group showed earlier recovery from the neurological deficit. Intravenously transplanted hCECs selectively migrated and integrated into cerebral ischemic lesion area and accelerate neurological functional recovery. This new hCEC-based cellular therapy is applicable for clinical trial in ischemic stroke patients.
Detailed Program and Abstracts — Thursday, June 14

Poster Board Number: T-1167

THE THERAPEUTIC POTENCY OF HUMAN INDUCED PLURIPOTENT STEM CELLS DERIVED ENDOTHELIAL PROGENITOR CELLS

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Introduction: Experimental and clinical studies have shown that endothelial progenitor cells (EPCs) can enhance angiogenesis in ischemic hindlimb muscles and myocardium. However, autologous EPCs transplantation is limited by the number and proliferative potential of the EPC isolated from patients’ blood. Human induced pluripotent stem cell (hiPSC) is a potential alternative cell source for EPC generation due to their autology, high power of proliferation and pluripotency. Methods: Donor specific Induced pluripotent stem cells were generated from their skin fibroblast in feeder free, serum free culture system and subsequently differentiated into EPCs (hiPSC-EPCs) and its functions of in-vitro tube formation, migration ability and cytokine expression profiles, and in-vivo capacity to attenuate mice model of hind-limb ischemia. Results: hiPSC-EPCs were compared with the EPC differentiated from BM-MNC (BM-EPCs) and hESC (hESC-EPCs). Results: Differentiation of BM-EC from BM-MNC was only achieved in 1/6(17%) patients with coronary artery disease. Nevertheless, BM-EPCs, hESC-EPCs and hiPSC-EPCs exhibited typical cobblestone morphology, positive staining of vWF and the ability of Dii-Ac-LDL dye uptake, and Ulex europeaus lectin antigen expression. In-vitro functional assay demonstrated that hiPSC-ECS and hESC-ECS had similar capacity for tube formation and migration as BM-EPCs (all p>0.05). During hypoxia, increased expression of major angiogenic factors including epidermal growth factor, hepatocyte growth factor, vascular endothelial growth factor, placental growth factor and stromal derived factor-1 were observed in all EPC cultures as compared with normoxia (all p<0.05). Compared with medium only, transplanting BM-ECS (n=8), hESC-ECS (n=8) or hiPSC-ECS (n=9) into mice significantly attenuated severe hind-limb ischemia via enhancement of neovascularization. Conclusions: Our results demonstrate that hiPSC-derived EPCs resemble normal human endothelial cells with similar phenotypes and angiogenic function but unlimited proliferation capacity. These findings suggest that hiPSC-derived EPC can be used as patient specific cell source in therapeutic angiogenesis.

Poster Board Number: T-1168

ENDOTHELIAL PROGENITOR CELLS IN PERIPHERAL BLOOD OF CARDIAC CATHETERIZATION PERSONNEL

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The aim of the present study was to evaluate the rejuvenation capacity among cardiac catheterization personal occupationally exposed to ionizing radiation. Venous blood samples were obtained from 60 cardiac catheterization personals exposed to x-ray during fluoroscopy procedure at the National Heart Center in Embaba vs. 20 controls. Blood samples were assayed for total and differential blood count, micronucleus formation (FMN) plasma stromal growth factor (SDF-1) and cell phenotype of circulating endothelial progenitor cells (EPCs), whose surface markers were identified as the CD34, CD133 and kinase domain receptor (KDR). The individual annual collective dose ranged from 2.16 - 8.44 mSv/y as measured by thermolumincent personal dosimeters (TLD). Results showed that SDF-1a and FMN were significantly higher among cardiac catheterization staff compared to controls. Similarly, EPCs: CD34, CD133, KDR were significantly increased among cardiac catheterization staff compared to controls. Smoking seemed to have a positive effect on the FMN and SDF-1 but a negative effect on EPCs. It is concluded that among cardiac catheterization staff, the numbers of circulating progenitor cells have increased and accordingly, increased capacity for tissue repair. In conclusion, the present work shows that working exposure to radiation, well within permissible levels, leaves a genetic mark on somatic DNA of the interventional cardiologist. However, exposure to ionizing radiation stimulates regenerative processes as indicated by the increase in EPCs and SDF-1. This regenerative process is decreased by smoking as evidenced by increased levels of SDF-1 and decreased levels of EPCs. The personnel who work in cardiac catheterization laboratories should carefully follow radiation protection procedures and should minimize radiation exposure to avoid possible genotoxic effects.

Poster Board Number: T-1169

THE HAEMOPOIETIC SUPPORTIVE MURINE BONE MARROW MS-5 MESENCHYMAL STROMAL CELL LINE HAS A NOVEL ROLE IN PROMOTING HUMAN VASCULOGENESIS AND ANGIOGENESIS

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The bone marrow contains specific microenvironmental stem cell niches which maintain haemopoiesis. CXCL12-expressing mesenchymal stromal cells are closely associated with the bone marrow sinusoidal endothelium, forming key elements of the haemopoietic stem cell niche, yet their ability to regulate endothelial function is not clearly defined. Since the murine nestin+ cell line, MS-5, provides a clonal surrogate bone marrow niche capable of regulating both murine and human primitive haemopoietic stem/ progenitor cell (HSC/HPC) fate in vitro, we hypothesised that MS-5 cells might also support new blood vessel formation and function. Here, for the first time, we demonstrate that this is indeed the case. Using proteome arrays, we identified HSC/HPC active angiogenic factors that are preferentially secreted by haemopoietic supportive nestin+ MS-5 cells, including CXCL12, HGF, Ang-1 and MCP-1(CCL2). Concentrating on CXCL-12, we confirmed its presence in MS-5 conditioned media and demonstrated that its antagonist in receptor binding, AMD-3100, which mobilises HSC/ HPCs and endothelial progenitors from bone marrow, could significantly reduce MS-5 mediated human vasculogenesis in vitro, principally by regulating human endothelial cell migration. Thus, the clonal nestin+ MS-5 murine bone marrow stromal cell line not only promotes human haemopoiesis but also induces human vasculogenesis, with CXCL12 playing important roles in both processes. Funding: This work was supported by the National Health Service Blood and Transplant (NHSBT), EU Framework 7 Cascade project and the National Institute of Health Research (NIHR), UK, under its Programme Grant Scheme (RP-PG-0310-1001 and -1003). The views
WHISTLING IN THE DARK - UNDERSTANDING VASCULAR ANOMALIES AND IDENTIFYING VASCULOSGENESIS AS PLAYING A MAJOR ROLE IN TUMOR FORMATION

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Shaghayegh Harbi - New York University Mentors: David J Kahler (Director of NYSCF Drug Discovery Laboratory); Paolo G Mignatti (Associate Professor New York University School of Medicine Department of Cardiothoracic Surgery and Cell Biology); June K Wu (Assistant Professor Columbia University Department of Surgery)

Though the most common tumor of infancy, little is known about the pathogenesis and etiology of infantile hemangiomias (IH). The working hypothesis is that the hemangioma derived stem cell (HemSC) is a vascular stem/progenitor cell whose proliferation is dysregulated but not fully transformed, that orchestrates hemangioma pathophysiology via a sophisticated activation of multiple signaling and regulatory networks. The overall goal of this investigation is to understand the process of hemangioma-genesis - defined as human vascular differentiation, followed by vascular regression and apigogenesis - by targeting the differences between the hemangioma-derived stem cells (HemSCs), hemangioma endothelial cells (HemECs), an intermediate cell population, and the heterogeneous cell population. Thus the specific aim is: a) to identify the intrinsic differences between HemSC and HemEC and compare HemSC and HemEC genetically and epigenetically; b) analyze the "stemness" genes, paracrine/endocrine associated genes ("angiogenic"), and genes that regulate development, vasculogenesis and immunity; c) identify involvement of the signaling pathways at various stages of differentiation; d) provide a new perspective on the failure of anti-vasculogenic inhibitors currently in use. To achieve this goal, a multifaceted, direct target approach is proposed to study the regulation, relationship, and mechanisms that control differentiation and interaction of hemangioma-derived stem cells (HemSCs) and hemangioma endothelial cells (HemECs) by performing experimental studies to analyze: a) expression profiles to survey gene expression profiles and patterns between the four cell types by using microarray analysis followed by qRT-PCR; b) cell surface marker screen to isolate, screen, and characterize various stages of differentiation of the four cell types by profiling human cell surface markers using flow cytometry and bioimaging of cell surface proteins. Specifically, to survey gene expression profiles between the four cell types (CD133+, CD133+/CD31+, CD31+, heterogeneous population) of IH cells to further development of gene expression signatures, gene expression analysis was performed of different human cell samples using Agilent whole human genome oligo microarrays. In order to develop definitive and effective therapies of IH, a critical question is to determine which cells contribute to hemangiogenisis and disease progression. Does hemangiogenesis follow a model where growth and progression of the tumor is driven by a small subpopulation of cells or does the bulk population of cells have tumorigenic potential? What is the interaction between environmental cues (such as hypoxia, hormones, immunity, stress, toxicity), progenitor cells, and associated signaling pathways (TGFβ, Notch, HIFs, Tyrosine kinases, MAPK/ERK, NFκB, PI3K-akt)? A better understanding of this interplay between clinical insight and manifestation, genetic and epigenetic analysis, cellular signaling and molecular markers, and hormonal and environmental regulation and alterations will help diagnose and potentially treat this vascular anomaly.

A NOVEL ENDOTHELIAL MICRORNASE MODULATING ANGIOGENIC RESPONSES

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MicroRNAs are a class of small RNAs that regulate target gene expression at the post-transcriptional level. Several microRNAs have been reported to control angiogenesis. We previously developed a novel embryonic stem (ES) cell differentiation system that exhibits vascular cell differentiation and early vascular development using vascular endothelial growth factor (VEGF) receptor-2 (VEGFR2/Flk1)-positive cells as common progenitors (Yamamizu, Nature, 2000). With the use of this system, we have been elucidating various cellular and molecular mechanisms of vascular cell differentiation, such as enhancement of endothelial cell (EC) differentiation from Flk1+ cells with protein kinase A activation (Yamamizu, Blood, 2009) and arterial EC specification through direct interaction of Notch and beta-catenin signaling downstream of CAMP (Yurugi-Kobayashi, Arterioscler Thromb Vasc Biol, 2006; Yamamizu, J Cell Biol, 2010). In this study, to elucidate roles of microRNAs in EC differentiation and vascular formation, we screened microRNAs specifically expressed during EC differentiation with the use of our ES cell differentiation system. Then, we identified a specific microRNA (mir-X) that is expressed in vascular ECs from ES cells, more predominantly in arterial ECs than venous ECs. Mir-X is expressed also in ECs of the dorsal aorta in the mouse embryo and in human EC lines. Overexpression of mir-X in human EC lines showed an inhibitory effect on angiogenic responses such as EC migration or tube formation. In vivo functions and target molecules of mir-X are currently investigated. Mir-X is thus, supposed to be an endogenous regulator of angiogenesis, suggesting its great possibility for clinical application in the treatment of cancer or ischemic diseases.

EFFECT OF MICROPATTERNED PEPTIDES ON ENDOTHELIAL PROGENITOR CELL ADHESION AND EXPANSION

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Half of the small-diameter vascular prostheses used in bypass and replacement surgeries become occluded within 5 years. The intimal hyperplasia that leads to graft failure could be prevented if the luminal prosthetic surfaces were engineered to recruit circulating progenitor cells capable of generating a healthy endothelial cell lining. We have previously described a method to increase the endothelialisation rates of prosthetic materials in vitro by micropatterning two peptides derived from the integrin-binding regions of fibronectin. The objective of the current work was to examine the mechanism by which these micropatterns affect endothelial progenitor cell adhesion and proliferation. Glass surfaces were
functionalyzed with 10.1 ± 0.1 µm diameter CGRGDS peptide spots covering 20% or 50% of the surface, with CWQPPrARI covering the remaining surface. Endothelial progenitor cell-derived CD31+ cultures were expanded from adult human saphenous veins. Compared to untreated surfaces, the micropatterned surfaces led to a 2-fold increase in the maximum cell surface area after cell adhesion and spreading. Fluorophore-tagged RGD micropatterns revealed that cells preferentially extended pseudopods on the RGD spots. In addition, 86 ± 17% of the focal adhesions were located on the RGD spots, with a high frequency near spot edges. The concentration of focal adhesions on the RGD spots was independent of the spot size, but increased at higher spot densities. Conversely, cells on untreated or gelatin-coated surfaces remained rounded with few focal adhesions. These results suggest that the RGD spot density impacts focal adhesion formation and hence cell spreading kinetics. We are now investigating the effect of peptide micropatterns on umbilical cord blood-derived endothelial progenitor cell fate.

**Poster Board Number:** T-1173

**FOUR-YEAR OUTCOME AFTER INTRAMUSCULAR TRANSPLANTATION OF GCSF-MOBILIZED CD34+ CELLS IN NO-OPTION PATIENTS WITH CRITICAL LIMB ISCHEMIA**

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Background: Prognosis of chronic critical limb ischemia (CLI) patients, in whom conventional revascularization is neither successful nor indicated, is extremely poor, and the development of novel strategy for blood flow recovery is urgently needed for such intractable disease. Therapeutic potential of endothelial progenitor cells (EPCs) has been established by a number of preclinical studies for hindlimb, myocardial and cerebral ischemia. Our phase I/IIa clinical trial revealed that intramuscular transplantation of autologous, granulocyte colony stimulating factor (GCSF)-mobilized CD34+ cells, an EPC-enriched fraction, was safe, feasible and effective at week 4 and 12 post cellular therapy in 17 patients with CLI in whom conventional revascularization was not indicated. However, long-term outcome of the stem/progenitor cell therapy has never been reported. Methods: No-option patients with CLI by atherosclerotic peripheral arterial disease (PAD) (N=5) or Buerger’s disease (N=12) underwent leukopheresis following 5-day subcutaneous infusion of GCSF (5-10 µg/kg/day). CD34+ cells were isolated from the apheresis product by a magnetic sorting system, CliniMACS®, then intramuscularly transplanted into 40 sites of ischemic lower limbs. We used the Aldefluor® fluorescent reagent system to isolate the cell population in the submucosal gland duct of the human and murine airway, which is capable of regenerating the submucosal glands and the surface epithelium overlying the submucosal glands. Microarray analysis showed aldehyde dehydrogenase genes ALDH1A1, ALDH2 and ALDH3A1 are highly expressed within both basal and submucosal gland duct cell populations. Work from many groups has shown that cell populations sorted from several different tissues based on their high ALDH activity are enriched for stem/progenitor cells. However, the role of ALDH in airway progenitor/stem cells has not yet been elucidated and it is not clear which isoforms might be important for self-renewal and differentiation. The airways are in contact with the environment and are directly exposed to air pollution and cigarette smoke, which trigger the formation of reactive oxygen species (ROS). ROS cause oxidative breakdown of cell membrane lipids followed by the accumulation of reactive aldehydes. Reactive aldehydes cause apoptotic cell death and therefore airway cells in general, and airway progenitors in particular, need a high level of aldehyde dehydrogenases in order to protect themselves from these harmful reactive aldehydes. We used the Aldefluor® fluorescent reagent system to isolate the cells that express high levels of ALDH within the airway basal and submucosal gland duct cell populations from both mouse and human. We found that only ALDH high expressing (ALDH-hi) basal cells and submucosal gland duct cells were able to self-renew and form spheres in matrigel from either human or mouse airway epithelium. We performed immunostaining of the airways for these harmful reactive aldehydes. We used the Aldefluor® fluorescent reagent system to isolate the cells that express high levels of ALDH within the airway basal and submucosal gland duct cell populations from both mouse and human. We found that only ALDH high expressing (ALDH-hi) basal cells and submucosal gland duct cells were able to self-renew and form spheres in matrigel from either human or mouse airway epithelium. We performed immunostaining of the airways for these ALDH isoforms and found ALDH2 more highly expressed within both basal and submucosal gland duct cell populations. Work from many groups has shown that cell populations sorted from several different tissues based on their high ALDH activity are enriched for stem/progenitor cells. However, the role of ALDH in airway progenitor/stem cells has not yet been elucidated and it is not clear which isoforms might be important for self-renewal and differentiation. The airways are in contact with the environment and are directly exposed to air pollution and cigarette smoke, which trigger the formation of reactive oxygen species (ROS). ROS cause oxidative breakdown of cell membrane lipids followed by the accumulation of reactive aldehydes. Reactive aldehydes cause apoptotic cell death and therefore airway cells in general, and airway progenitors in particular, need a high level of aldehyde dehydrogenases in order to protect themselves from these harmful reactive aldehydes. We used the Aldefluor® fluorescent reagent system to isolate the cells that express high levels of ALDH within the airway basal and submucosal gland duct cell populations from both mouse and human. We found that only ALDH high expressing (ALDH-hi) basal cells and submucosal gland duct cells were able to self-renew and form spheres in matrigel from either human or mouse airway epithelium. We performed immunostaining of the airways for these ALDH isoforms and found ALDH2 more highly expressed within basal and duct cells than in differentiated cells. The reverse was true for ALDH1A1, with expression being higher in differentiated cells. ALDH3A1 staining was faintly seen in basal cells and was bright in the myoepithelial cells of the submucosal glands. In order to functionally examine the role of ALDH2 in basal and duct cell self-renewal and differentiation, we treated sorted cells in the sphere assay with the ALDH2 inhibitor, Diadzin. Diadzin treatment resulted in forma-
SEQUENCE-SPECIFIC GENETIC CORRECTION OF IPS CELLS DERIVED FROM PATIENTS WITH INHERITED LUNG DISEASE

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Cellular transplantation of lung stem/progenitor cells represents a potential therapeutic approach for a variety of inherited monogenic lung diseases. Crucial to the success of such a therapeutic strategy is that the transplanted cells and their progeny are corrected for the disease-causing mutation and that the transplanted cells do not elicit an immune response in the recipient. In order to satisfy these criteria, we are pursuing a patient-specific approach in which, starting with skin or blood cells from patients with inherited lung disorders, autologous induced pluripotent stem cells (iPS cells) are first derived. Utilizing site-specific homology-directed repair, the disease-causing mutation is corrected in the endogenous, chromosomal DNA sequence. Finally, a directed differentiation approach is employed to obtain highly purified populations of the relevant lung stem/progenitor cells from the corrected iPS cells for purposes of transplantation. We have initially employed this approach to generate corrected, autologous iPS cells for patients with Cystic Fibrosis (CF). Starting with CF patient fibroblasts, we have derived and extensively characterized iPS cell lines, confirming their pluripotency and normal karyotype. We then utilized Zinc Finger Nucleases, designed to target the endogenous CFTR gene, to mediate correction of the inherited genetic mutation in this locus via homology directed repair. We have demonstrated that the corrected CF iPS cells, when induced to differentiate in vitro, express the corrected CFTR gene. Importantly, we observed an exquisitely sensitive, homology-dependent specificity of targeting one CFTR allele vs. the other. This allele-specific targeting offers the potential for preferential targeting of ZFN-mediated correction to dominant mutant alleles.

RESYNCHRONIZATION OF MITOSIS IN NORMAL AIRWAY EPITHELIAL PROGENITOR POPULATIONS IS COINCIDENT WITH FLUCTUATIONS IN TGF-β1 SECRETION

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The proliferative characteristics of airway epithelial progenitors are likely to be important for restoring lung homeostasis in diseases of ongoing epithelial injury, such as asthma. To that end, we recently showed that normal in vitro tracheobronchial epithelial progenitor populations are characterized by relatively synchronous progression through the cell cycle while asthmatic progenitor populations proliferate with a more even distribution of cells in each cell cycle phase. Further, this asthmatic “mitotic dysynchrony” results in sustained elevation in TGF-β1 secretion. Because TGF-β1 troughs and peaks regulate mitotic synchrony in non-airway epithelia, we suspect that sustained asthmatic secretion of TGF-β1 implies a defect in TGF-β1-induced mitotic regulatory signaling. As a first step toward identifying this defect, we hypothesized that resynchronization of mitotic dysynchrony in proliferating normal airway epithelial progenitor populations is coincident with a trough and peak in TGF-β1 secretion. Mitotic dysynchrony was induced in parallel cultures of normal proliferating tracheobronchial epithelial cells via transient serum starvation (for 12 hours) in a staggered fashion. Aliquots of each were reserved as controls prior to mixing the cultures. Cultures were continuously exposed to bromodeoxyuridine (BrdU). Cells and media were collected at 0, 12, 18, 24, 30, 42 and 48 hours. Mitotic phase was analyzed by flow cytometry for 7-AAD (BrdU). Cells and media were collected at 0, 12, 18, 24, 30, 42 and 48 hours.
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for 30 hours: 54/20/26%, began to spontaneously resynchronize at 42 hours (69/14/17%), and was fully synchronous by 48 hours (2/89/9%). The control cultures remained synchronous throughout, but out of phase with each other and the mixed cultures. Percent-age of baseline TGF-β1 (measured at 0 hours) was calculated for 18 (-9.4%), 24 (-10.3%), 42 (-2.7%) and 48 (+1.8%) hours in the mixed culture. In contrast, the control cultures’ TGF-β1 percentage of baseline remained low at 24 hours (-10.5, -8.6%) and 48 hours (-1.8, -6.5%). We developed a novel in vitro model of induced mitotic dys synchrony and spontaneous mitotic resynchronization in nor mal tracheobronchial epithelial progenitor cells that will be useful to dissect mitotic regulatory signaling. Importantly, our data show that this mitotic dys synchrony and resynchronization occur in con- ced with a trough and peak in TGF-β1 secretion. This model permits study of TGF-β1 regulation of normal airway epithelial progenitor mitotic synchrony, laying the foundation for experiments to deter- mine the defect(s) that underlie asthmatic mitotic dys synchrony.

**Poster Board Number: T-1178**

**TARGETED Ccsp⁺ Sca1⁺ MURINE BONE MARROW CELL THERAPY RESTORES CFTR AND IMPROVES LUNG FUNCTION**

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Cellular therapy is a promising approach for treatment of lung disease such as cystic fibrosis. However, previous studies reported low engraftment while the function of grafted cells remained unclear. Conditions to increase airway engraftment and therefore cystic fibrosis trans-membrane receptor (CFTR) expression have not been described. We previously showed better bone marrow cell (BMC) retention in the lung for trans-tracheal delivery versus intravenous injection and optimized other delivery parameters leading to increase retention efficiency and greater long term engraftment. We also identified a subpopulation of BMC expressing Clara-cell secretory protein (Ccsp) which can proliferate and differentiate into lung epithelium. Here, we isolated an even smaller population of BMC expressing Ccsp and stem cell antigen-1 (Sca1). We expanded this population by approximately 10-fold in culture with mouse embryonic fibroblast (MEF) feeder layer for 15 to 20 days before sorting out GFP positive cells and using them for delivery to injured lung. Expanded Ccsp⁺Sca1⁺ cells had ~5-fold greater retention efficiency in mice lungs, as detected by real-time PCR of the Y chromosome, when compared to unsorted 7-day cultured BMC. CFTR appeared to be restored in lungs of mice knockout for CFTR treated with wild-type cells and some cells were localized in the airway. Engrafted cells also appeared to have beneficial effects in lung function by maintaining ceramide levels which decreased with age in CFTR knockout mice not receiving any treatment. Airway surface liquid (ASL) thickness was also maintained to wild-type levels in CFTR knockout mice treated with wild-type cell. We propose that greater BMC engraftment in the lung will increase CFTR expression, possibly leading chloride transport restoration, and have other beneficial paracrine effects, thus improving lung function in cystic fibrosis disease.

**Poster Board Number: T-2001**

**MOVING TARGETS: PHENOTYPIC INSTABILITY OF A TET-OFF-SV40 TAG IMMORTALIZED CELL LINE ISOLATED FROM TRANSGENIC MOUSE ADULT SKIN TISSUE**

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Introduction: From the double transgenic mice expressing SV40Tag driven by the tetracycline-transactivator (tTA, tet-off), a mouse cell line from the skin tissue was isolated and established in culture. That is unusual because conventional mouse skin cells are normally difficult to grow in vitro. Material and Methods: cell culture, apoptotic test, transgenic insertion of vectors coding for various cytokine, FACS analysis, ELISA assay, histology, confocal microscopy, RT-PCR assay, immune-incompetent mice. Results: The partial characterization of this mouse cell line demonstrates properties mimicking mouse ectodermal progenitor cell or even earlier precursor. The cells become apoptotic and necrotic in responding to doxycycline and TNF. Differentiation of these progenitor/stem cells does not occur automatically. It requires multiple factors and extensive manipulation in vitro and in vivo. Applying such approaches, we have isolated several cell clones. They are characterized to be down-stream keratinocytic precursors. The cell line is not stable, upon prolong culture, their phenotypes have shifted to more mature progeny. Conclusion: The stage of differentiation from ectodermal progenitor to keratinocyte could be assigned to seven-plus stages. The doxycycline-regulated SV40Tag expressing, stage-specific cell lines isolated from skin and the derived cytokine secreting clones might be useful for multiple purposes such as functional genomic analysis, skin toxicology test, the mechanism of skin tumor progression, studying immune responses after skin stem cell transplantation.
Of note, the k15-positive stem cells can differentiate to a portion of interfollicular epidermis under wound condition. For clearly tracing the hair follicle stem cells and their descendants in different wound stages, a 1-cm full-thickness longitudinal incision was made on the back of the adult transgenic mouse (K15-CrePR-R26GM). We found the labeled cells migrated from bulge towards the interfollicular epidermis in the early phase. In later stage, the number of labeled cells decreased in the interfollicular epidermis.

**THE HISTONE METHYLTRANSFERASE SETD8 IS REQUIRED FOR SURVIVAL OF MOUSE EPIDERMAL STEM CELLS**

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*PDN, WT-CSGR, Cambridge, United Kingdom*

Epigenetic modifications, such as histone methylation play an important role in chromatin structure and promoter activity, and have been implicated in a wide range of biological processes including development, reprogramming, aging and cancer but our knowledge is still limited when it comes to the importance of histone methylation in multipotent stem cells of adult mammalian tissues. In this study we conditionally deleted Setd8/PR-Set7/KMT5a, the sole enzyme to catalyse the formation of mono-methylated histone 4 at lysine 20 (H4K20me1) in mouse skin. We targeted deletion of Setd8 to undifferentiated layers of the mouse epidermis and found both proliferation and differentiation processes impaired in embryonic and adult skin. We provide evidence that the long-lived epidermal progenitor cells are lost in the absence of Setd8, leading to a complete loss of sebaceous glands and interfollicular epidermis.

We further show that Setd8 is a transcriptional target of c-Myc and an essential mediator of Myc-induced epidermal differentiation. Deletion of Setd8 in c-Myc over-expressing skin blocks cellular proliferation and differentiation and causes cell death instead. The increase in apoptosis in skin when Setd8 has been knocked-out can be explained by our discovery that p63, an essential transcription factor for epidermal commitment, is lost. In contrast, the p63 homologue p53 is gained upon removal of Setd8 in skin. Then, we show that both over-expression of p63 and repression of p53 can at least in part rescue the Setd8-induced epidermal phenotype. In this study we demonstrate for the first time that Setd8 is required at least in part rescue the Setd8-induced epidermal phenotype. In vivo this study we demonstrate for the first time that Setd8 is required at least in part rescue the Setd8-induced epidermal phenotype.

**SUCCESSFUL GRAFTING OF AUTOLOGOUS TISSUE-ENGINEERED SKIN SUBSTITUTES ON BURN PATIENTS: PRESERVATION OF STEM CELLS AND MINIMAL CONTRACTION**

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To adequately and permanently restore organ function after grafting, tissue-engineered skin substitute (TES) must ultimately contain and preserve functional epithelial stem cells. Another consideration is to maximize the structural stability of skin substitutes considering that the main sequela of patients surviving major burn injuries result from contractures and hypertrophic scars. The present study was designed to assess stem cell preservation as well as the contractile behavior of the tissue-engineered skin (TES) made by the self-assembly approach, where keratinocytes are cultured on tissue-engineered dermis comprised of fibroblasts and the endogenous extracellular matrix they synthetized and organized. A subset of basal cells exhibiting the stem-cell associated slow-cycling phenotype were evaluated at the end of the production (17 days of culture). TES were obtained from contractures and hypertrophic scars.

The present study was designed to assess stem cell preservation as well as the contractile behavior of the tissue-engineered skin (TES) made by the self-assembly approach, where keratinocytes are cultured on tissue-engineered dermis comprised of fibroblasts and the endogenous extracellular matrix they synthetized and organized.
Melanogenesis has been involved in complex regulatory control by various extrinsic and intrinsic factors that may be known as a master regulator of pigmentation as well as a target for transforming growth factor beta (TGF-beta). This suggested a cross-talk between the dermis and epidermis. Here we show that epidermal Wnt/beta-catenin signaling pathway activates dermal fibroblasts to form ectopic dermal papillae indicating reciprocal signalling between epidermal stem cells and their niche. In the dermal papilla and dermal sheath. Postnatal skin does not normally give rise to new hair follicle, but epidermal activation of c-Myc (Myc) is the classic oncogene, however the paradox of Myc-induced epidermal differentiation is well documented. Analysis of mouse sebaceous glands (SG) revealed Myc expression coincided with Androgen Receptor (AR) expression, and in studies using K14-MycER transgenic mice (Myc mice), we found following Myc activation, the AR functioned to promote differentiation and inhibit proliferation. p53 activated in response to high Myc activity and inhibited AR signalling, thereby promoting only Myc’s default proliferative function. Strengthening AR signalling with testosterone, inhibited p53 activity, forming a bidirectional AR/p53 differentiation axis by mutual antagonism. Analysis of human sebaceous neoplasms revealed AR expression correlated with increased differentiation, while p53 correlated with reduced differentiation. This bidirectional AR/p53 axis explains the paradox of how Myc can trigger both SG differentiation and proliferation in different contexts.

Mammalian dermis provides a niche for epidermal stem cell during tissue morphogenesis and maintenance. Dermal fibroblasts are the major component of this microenvironment and critical mediators of epithelial-mesenchymal interactions. Hair follicle formation for example is dependent on specialised dermal cells found in the dermal papilla and dermal sheath. Postnatal skin does not normally give rise to new hair follicle, but epidermal activation of beta-catenin in transgenic mice can induce ectopic hair follicles (EF). These EFs stimulate dermal fibroblasts to form ectopic dermal papillae indicating reciprocal signalling between epidermal stem cells and their niche. Here we show that epidermal Wnt/beta-catenin can stimulate the fibroblasts of the adult dermal niche to proliferate and remodel the extracellular matrix to an extent similar to neonatal dermis. Also, we identified a number of candidate fibre-blast mitogens that were upregulated in keratinocytes in response to beta-catenin stabilisation, such as members of the family of the transforming growth factor beta (TGF-beta). This suggested a cross-

**THE OUTCOME OF SEBACEOUS C-MYC ACTIVITY IS DICTATED BY AN ANDROGEN RECEPTOR / P53 DIFFERENTIATION AND PROLIFERATION AXIS**

**Poster Board Number: T-2007**

**THE DERMAL NICHES OF MURINE EPIDERMAL STEM CELLS IS AN UNEXPECTED PLASTIC TISSUE**

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Mammalian dermis provides a niche for epidermal stem cell during tissue morphogenesis and maintenance. Dermal fibroblasts are the major component of this microenvironment and critical mediators of epithelial-mesenchymal interactions. Hair follicle formation for example is dependent on specialised dermal cells found in the dermal papilla and dermal sheath. Postnatal skin does not normally give rise to new hair follicle, but epidermal activation of beta-catenin in transgenic mice can induce ectopic hair follicles (EF). These EFs stimulate dermal fibroblasts to form ectopic dermal papillae indicating reciprocal signalling between epidermal stem cells and their niche. Here we show that epidermal Wnt/beta-catenin can stimulate the fibroblasts of the adult dermal niche to proliferate and remodel the extracellular matrix to an extent similar to neonatal dermis. Also, we identified a number of candidate fibre-blast mitogens that were upregulated in keratinocytes in response to beta-catenin stabilisation, such as members of the family of the transforming growth factor beta (TGF-beta). This suggested a cross-

**Poster Board Number: T-2008**

**NEURAL STEM CELL DERIVED CONDITIONED MEDIUM SUPPRESSES MELANOGENESIS THROUGH INHIBITION OF WNT/B-CATENIN SIGNALING PATHWAY**

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Skin pigmentation by melanin serves many valuable functions such as photoprotection of the body from ultraviolet (UV) light, but production of excessive melanin triggers hyperpigmentation in the skin. Melanogenesis is synthesized from tyrosine via an enzymatic cascade that is controlled by three important proteins such as tyrosinase, tyrosine-related protein 1 (TRP1) and dopachrome tautomerase (DCT). The most important microphthalmia-associated transcription factor (MITF) in the expression of tyrosinase, TRP1 and DCT genes is known as a master regulator of pigmentation as well as a target for the Wnt signaling pathway during the melanocyte differentiation program. Previously, the conditioned medium (CM) from many different types of adult stem cells such as adipose-derived stem cells has been identified its ability for the inhibition of melanin production. However, the neural stem cell-derived CM (NSC-CM) in the control of melanogenesis has not been investigated. In this study, we elucidated the effects and the mechanisms of the NSC-CM for melanin inhibition in vitro and in vivo. The melanin content and tyrosinase activity in B16 melanoma cells that were treated with NSC-CM were dramatically decreased. The key enzymes, which are tyrosinase, TRP1, DCT and MITF, were also robustly decreased in our real-time RT-PCR, western blot and immunofluorescence assays in vitro. In addition, the melanin content in C57/BL-6J mice that were treated with NSC-CM every once a day for 10 days was decreased when it was measured by Mexameter. The key enzymes were significantly decreased in our immunohistochemistry and immuno-fluorescence assays in vivo. Interestingly, we found that the NSC-CM antagonized the canonical Wnt pathway by decreasing of b-catenin accumulation in the nucleus, which was resulted from the increased Wnt inhibitors and the reduced co-receptors. Therefore, these results show that the NSC-CM could inhibit melanin synthesis through the down-regulation of MITF and its downstream targets, which is mediated by Wnt/b-catenin signaling pathway.
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Cardiac Cells
Poster Board Number: T-2009

REPLICATIVE SENESCENCE OF HUMAN CARDIAC STEM CELLS
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Recently cardiac stem cells (CSC) identified in human myocardium niche, these stem cells one of the potential sources for regeneration of human myocardium. CSCs have multipotent capability as transdifferentiation into cardiomyocyte, endothelial, and smooth muscle lineage cells. However, CSCs have a limited lifespan in vitro as somatic, and other adult stem cells. Accumulating cell division, CSCs enter senescence, which observed enlargement of cytoplasm and a stop of in vitro amplification. Recently studies have indicated that CSCs exhibit reduced differentiation potential upon prolonged in vitro culture. Furthermore, senescence of CSC might limit their therapeutic applications. Thus, analysis of in vitro senescence in CSC is important to cell therapy based on stem cell for cardiovascular disease. However, molecular mechanisms of in vitro senescence of CSC are still poorly understood. Clearly, cellular senescence is a very complex process and the sequence of its molecular events is thus far unknown. Thus, we examined change of MAPK expression after CSC senescence and whether CSC senescence prevents used by MAPK regulation. In this study, using human CSC isolated from infant myocardium using by a c-kit antibody. Senescence human CSC (S-hCSC) used passage numbers of 11 to 14 and young human CSC (control) used passage number of 3 to 5. To characterize human CSC senescence, we examined senescence associated β-galactosidase activity (X-gal stain), cytoplasm enlargement assay. S-hCSC was significantly increased X-gal positive cells (p<0.01) and their cytoplasm enlarged compared control groups (p<0.01). In addition, senescence associated protein p53 inhibitor molecule, Mdm2, was significantly reduced in S-hCSC rather than in control (p<0.05). Therefore, proliferation capability was significantly reduced in S-hCSC compared to control as shown (p<0.01). These results due to the cell cycles G1 arrest in S-hCPC; Cyclin E/CDK2 expression was reduced. To confirm the expression of MAPK during hCPC senescence, we analyzed by western blot. Interestingly, only ERK was strongly phosphorylated in S-hCPC rather than in control group (p<0.01). With this in mind, we examined whether hCSC senescence prevent by treatment with ERK inhibitor as U0126. X-gal positive S-hCSC significantly reduced after treatment with U0126 for 24h (p<0.05) and 48h (p<0.05). Therefore, irregular morphology of S-hCSC was recovered after treatment with U0126 for 24h and 48h. In addition, reduced in vitro amplification capability of S-hCSC was clearly recovered after treatment with U0126 for 24h (p<0.01) and 48h (p<0.01). The senescence associated protein p53 inhibitor, Mdm2 expression was significantly increased after treatment with U0126 for 24h and 48h which compared to S-hCPC (p<0.01). Finally, we confirmed hCSC differentiation potential; we induced transdifferentiation into endothelial, smooth muscle cell, and cardiomyocyte in vitro. However, there was any difference protein expression among control, S-hCSC, and treatment with U0126. Based on these results, we concluded that hCSC senescence processed via MAPK and p53 mediated signaling pathway, and senescence hCSC prevented using by MAPK regulation especially, ERK inhibition. Therefore, this study may offer strong evidence for basic research of human cardiac stem cell senescence and clinical application for stem cell therapy of cardiovascular disease.

Poster Board Number: T-2010

CHARGED SURFACE MODIFICATION CULTURE SYSTEM FACILITATES MAINTENANCE OF HUMAN C-KIT POSITIVE CARDIAC STEM CELLS
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Ischemic heart diseases accelerate damage and loss of cardiomyocyte. Replacing de novo human cardiomyocyte is difficult; because of human adult cardiomyocyte has limited regenerative capability. However, accumulating evidences suggested that damaged cardiomyocyte could replace by transplantation of some adult stem cells. Recently, cardiac stem cells (CSC), one potential source of stem cells, were identified. These stem cells have multipotential capability which could be transdifferentiated into cardiomyocyte, endothelial lineage and smooth muscle cell lineages. However, in fact, CSC has a low expansion ratio and is hardly difficult to maintain in canonical stem cell culture vessels. Therefore, we designed to examine the efficacy of a novel CSC culture method that uses surface-charged modification of culture vessels which coated by charging molecules such as carboxyl and amine residue. Thus, the aim of this study was we examined effects of in vitro amplification and attachment on isolated human c-kit positive cardiac stem cell (hCSCc-kit+). In this study, we used the hCSCc-kit+ were isolated from human infant myocardium using a c-kit antibody. The experimental groups divided into following three groups: hCSCc-kit+ cultured on canonical non-coated culture vessels (control), carboxyl residue-coated vessels (carboxyl), and amine residue-coated vessels (amine). To confirm the effects of surface-charged modification culture vessels on hCSCc-kit+, we first examined cellular proliferation. The proliferation ratio in amine group was significantly increased rather control (p<0.05), and carboxyl group as shown (p<0.05). Next, we examined the c-kit expression maintenance during culture using by fluorescence-activated cell sorting. The maintenance of c-kit expression capability in amine group greater extended rather than control as shown (p<0.01). In addition, carboxyl group also extended c-kit expression maintenance rather than control group (p<0.05). These results suggested that hCSCc-kit+ cultured on amine and carboxyl residue-enriched culture vessels were facilitates maintenance of stem cell potency compared to other culture vessels as non-coated and carboxyl. Furthermore, we observed that cellular attachment capability in amine group greater than control (p<0.05). Finally, we confirmed cellular signaling molecules expressions. The amine and carboxyl groups increased phosphorylation signals, such as focal adhesion
A NOVEL METHOD OF SELECTING HUMAN EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTE CLUSTERS FOR ASSESSMENT OF POTENTIAL TO INFULENCE QT INTERVAL

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Application of human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells is opening the new era of drug discovery and toxicological testing. The most promising cell type is cardiomyocytes differentiated from these stem cells, because it is difficult to get human primary cardiomyocytes. QT prolongation, which is a risk factor of ventricular tachycardia, is a critical cause of the withdrawal or use restriction of marketed drugs. We have used hERG channel-overexpressing cells as an in vitro model and dogs or monkeys as in vivo models to predict QT prolongation. However, there are discrepancies between the results of in vitro hERG assays and in vivo QT experiments. Thus, we consider that desirable evaluation systems are those that utilize human cardiomyocytes physiologically expressing multiple ion channels involved in QT intervals. In our preliminary experiments of QT risk evaluation using a multielectrode recording system, we found that responsiveness of field potential duration (FPD) to reference compounds differed very much from non-responders to excessive responders in human ES cell-derived cardiomyocyte clusters. We report a novel method selecting clusters suitable to the evaluation of drug candidate compounds to establish a robust QT risk assessment system. Clusters were treated with cisapride, a hERG channel blocker, at 100 nM, and selected with criteria of 5 to 20% of corrected FPD (FPDc) prolongation. After washout for several days, selected clusters were treated with reference compounds. FPDc was prolonged by blockade of hERG channel (+12.1% by E-4031 at 30 nM; +6.0% by dl-sotalol at 10 μM) and KvLQT1 channel (+9.2% by chromanol 293B at 10 μM; +10.0% by HMR1556 at 1 μM), and by activation of sodium channel (+15.5% by veratridine at 1 μM) and calcium channel (+13.9% by Bay K8644 at 30 nM). FPDc was shortened by blockage of calcium channel (-13.4% by verapamil at 30 nM; -10.8% by nifedipine; -10.3% by diltiazem) and by activation of K_{ca} channel (-5.5% by pinacidil at 1 μM), Quinidine and dysopyramide prolonged FPDc by 8.2% at 1 μM and 9.8% at 10 μM, respectively. Selected clusters are proper to assess effects of compounds on ion channels affecting QT intervals. This is the first report on the establishment of the QT risk assessment system using pharmaceutically selected clusters. In addition, our results show again usefulness of stem cell technologies.

Poster Board Number: T-2013

HUMAN CARDIAC PERICYTES: MULTIPOTENT MESODERMAL PROGENITORS WITHIN THE MYOCARDIUM EXHIBITING TISSUE SPECIFICITY IN MYOGENESIS

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Microvascular pericytes purified from multiple human tissues have recently been shown to harbor stem cells exhibiting myo-, osteo-, chondro- and adipogenic differentiation potentials and therefore

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Bioengineered human myocardium (BHM) has a vast range of curative potential in terms of cell therapy, with extensive use in development and regenerative medicine. Ordinarily, human tissue engineered myocardium is created by firstly differentiating pluripotent stem cells into cardiomyocytes and also deriving/isolating other required cell types (e.g., fibroblasts), and subsequently forming tissue. In this investigation, we hypothesised that we could form the BHMs directly with undifferentiated pluripotent stem cells and instructing the formation of myocardial muscle including all the required cell types.

Methods and Results: We firstly developed a consistent differentiation protocol capable of producing large numbers of cardiomyocytes and stromal support cells/fibroblasts simultaneously. This was achieved using a staged and chemically defined protocol in 2D (16 d). The resulting differentiation cultures (n = independent experiments) were then characterised using flow cytometry demonstrating high yields of cardiomyocytes (α-actinin positive cells, 28 ± 4 % SEM, n = 10), high yields of stromal cells characterised by α-smooth muscle actin (82 ± 6 % SEM) and collagen I (53 ± 5 % SEM) and low yields of remaining pluripotent stem cells (TRA-1-60/ OCT4 positive cells, 0.1 ± 0.05 % SEM). In addition, there were low levels of potentially contaminating cell types using qPCR (n = 3) for primitive endoderm (SOX17), pluripotent stem cells (OCT4), primitive mesodermal cells (MESP1) and neural cells (NEUROD1).

We then tested our hypothesis by applying the protocol to undifferentiated pluripotent stem cells in a collagen type 1 hydrogel, with the addition of mechanical loading to improve tissue maturity. Our hypothesis was confirmed and at the endpoint (23 d) the BHMs had a contractile force of 65 ± 15 SEM (n = 7, from 3 exp). The BHMs contained elongated cross-striated cardiomyocytes together with stromal cells as demonstrated in whole mount immuno-staining analysis. In addition, qPCR analysis revealed that the development of BHMs followed the temporal progression of known developmental pathways, with very low levels of other contaminating cell types. This analysis also revealed the early and transient expression of markers for heart progenitor populations, including the primary heart field (TBX5), secondary heart field (ISL1) and the pro-epicardial organ (GATA5). Flow cytometric analysis demonstrated that these constructs were comprised of mainly cardiomyocytes (α-actinin positive cells, 46 ± 5 % SEM, n = 9 from 3 exp) and stromal cells (30 ± 6 % SEM, α-smooth muscle actin positive cells and 4 ± 1 % SEM collagen I positive cells, n = 9 from 3 exp), with low yields of remaining pluripotent stem cells (TRA-1-60/OCT4 positive cells, 0.03 ± 0.01 % SEM, n = 4 from 3 exp). Conclusion: BHMs can be formed directly with undifferentiated pluripotent stem cells under “bio-instructing” conditions.

Poster Board Number: T-2015
PREDICTIVE TOXICOLOGY USING SPONTANEOUSLY CONTRACTING HUMAN IPSC DERIVED CARDIOMYOCYTES

Cromwell, Evan F1, Sirenko, Oksana1, Nesely, Jayne2, Crittenden, Carole3, Gallant, Debra4, Anson, Blake5
1Research, Molecular Devices, Sunnyvale, CA, USA, 2Bioimaging, Molecular Devices, Sunnyvale, CA, USA, 3Drug Discovery, Molecular Devices, Sunnyvale, CA, USA, 4Research, Cellular Dynamics International, Madison, WI, USA

A large percentage of new drugs fail in clinical studies due to cardiac toxicity. Therefore development of highly predictive in vitro assays suitable for high throughput screening (HTS) is extremely important for drug development. Human cardiomyocytes derived from stem cell sources can greatly accelerate the development of new chemical entities and improve drug safety by offering more clinically relevant cell-based models than those presently available. Induced pluripotent stem cell (iPSC) derived cardiomyocytes are attractive because they express ion channels and demonstrate spontaneous mechanical and electrical activity similar to native cardiac cells. They are also available in quantities required for drug studies.

Poster Board Number: T-2014
DIRECTED FORMATION OF BIOENGINEERED HUMAN MYOCARDIUM (BHM) FROM PLURIPOTENT STEM CELLS

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Heart Research Center Goettingen, University of Medicine Goettingen, Goettingen, Germany

Bioengineered human myocardium (BHM) has a vast range of current and potential applications including human developmental biology, pharmacological studies, disease modelling and regenerative medicine. Ordinarily, human tissue engineered myocardium was isolated from ventricular myocardial biopsies by mechanical dissociation and collagenase digestion and subsequently subjected to fluorescence-activated cell sorting (FACS) for purification. Using a combination of selective cell surface markers, including positive selection marker CD146 and negative selection markers: CD34, CD45, CD56, and CD117, we prospectively purified human cardiac pericytes to homogeneity. Sorted cardiac pericytes (CD146+CD34+CD45+CD56+CD117+) can be cultured in the long term and displayed morphological and proliferative profiles similar to pericytes purified from skeletal muscle. Cultured cardiac pericytes consistently expressed CD146, NG2, PDGFR-α, -β, SMA, and alkaline phosphatase and formed capillary-like networks in Matrigel at different passages. Cellular interaction between cardiac pericytes and endothelial cells was further illustrated by the formation of capillary-like networks in two- and three-dimensional Matrigel co-cultures. To examine their cardiomyocyte differentiation capacity, cultured cardiac pericytes were treated with 10 M 5-Aza-cytidine (AZA) for 72 hours and subsequently maintained in differentiation medium for up to 2 weeks. Immunocytochemistry showed that a fraction of AZA-treated cardiac pericytes express -sacromeric actinin, cardiac myosin heavy chain, and nuclear GATA-4, but not cardiac troponin-I, suggesting an immature phenotype. Immunohistochemistry and flow cytometry showed that cardiac pericytes natively express classic MSC markers, including CD44, CD73, CD90, and CD105. To investigate whether these cells possess multi-lineage mesodermal potential, cardiac pericytes were cultured in osteogenic, chondrogenic, adipogenic, and skeletal myogenic conditions. Cardiac pericytes exhibited robust osteo-, chondro-, and adipogenic differentiation, but not skeletal myogenesis. No myotube formation or expression of fast skeletal myosin heavy chain was observed, suggesting tissue specificity of cardiac pericytes, distinct from pericytes of other origins, in myogenesis. Our results showed that human cardiac pericytes can be prospectively purified by FACS with a combination of selective cell lineage markers and possess mesodermal multipotency except skeletal myogenesis. Currently we are investigating the differential activation of myogenic genes in cardiac pericytes after induction as well as the engraftment and cardiomyocyte differentiation of transplanted GFP-labeled cardiac pericytes within the healthy and ischemic myocardium in immunodeficient mouse models.

Poster Board Number: T-2015
PREDICTIVE TOXICOLOGY USING SPONTANEOUSLY CONTRACTING HUMAN IPSC DERIVED CARDIOMYOCYTES

Cromwell, Evan F1, Sirenko, Oksana1, Nesely, Jayne2, Crittenden, Carole3, Gallant, Debra4, Anson, Blake5
1Research, Molecular Devices, Sunnyvale, CA, USA, 2Bioimaging, Molecular Devices, Sunnyvale, CA, USA, 3Drug Discovery, Molecular Devices, Sunnyvale, CA, USA, 4Research, Cellular Dynamics International, Madison, WI, USA

A large percentage of new drugs fail in clinical studies due to cardiac toxicity. Therefore development of highly predictive in vitro assays suitable for high throughput screening (HTS) is extremely important for drug development. Human cardiomyocytes derived from stem cell sources can greatly accelerate the development of new chemical entities and improve drug safety by offering more clinically relevant cell-based models than those presently available. Induced pluripotent stem cell (iPSC) derived cardiomyocytes are attractive because they express ion channels and demonstrate spontaneous mechanical and electrical activity similar to native cardiac cells. They are also available in quantities required for drug studies.
Detailed Program and Abstracts — Thursday, June 14

Poster Board Number: T-2016

ARRHYTHMIA MODEL IN HUMAN ES/iPS CELL DERIVED CARDIAC CELL MONOLAYER

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Background: In order to advance stem cell research to the medical applications, it is crucially important to have as full knowledge of the differentiated tissue as possible. In our work, we study monolayers of human ES/iPS cell-derived cardiac tissue as a model system for prediction of arrhythmogenesis. Methods: Cardiomyocytes were differentiated from human ES cell (KhES3) and iPS cell (253G1) for 3-4 weeks, dissociated by proteases and seeded as cell layers (12 mm-diameter). They were confirmed as electrical syncytium using optical mapping with Ca2+ sensitive dye (Fluo-4). Quantitative real time polymerase chain reaction (PCR) and immunostaining were used to analyze these monolayers. We measured relative gene expression levels compared to GAPDH at 2, 7, and 28 days after seeding. Results: Excitation wave propagation was detected in the cell monolayer from 2 to 28 days after seeding. Propagation wave velocity was dependent on the stimulation cycle length and lidocaine concentration. The mean wave velocity was 67.4±9.7, 44.0±7.4, and 27.6±2.7 mm/s when the lidocaine concentration was 0, 0.1, and 0.2 mM, respectively (n=7, 7 days after seeding). One-way ANOVA P=0.007. Reentrant (spiral) wave propagation was detected in the cell layers while applying high-frequency stimulation (n=25/77). The size of unexcitable spiral core increased depending on the concentration of E-4031 and nifekalant. There was significant difference of mean α-actinin positive area between the monolayer in which spiral wave was detected and the monolayer in which it could not be detected or disappeared easily (n=4 for each, 64.6% vs 83.3%, t test P=0.01). With quantitative PCR, the expression of β-MHC, Cx43, and HERG-1b increased, but that of α-MHC and HCN4 decreased during the time course. Conclusions: We succeeded to create powerful tool to evaluate the arrhythmogenic potential of cardiac tissue derived from human ES/iPS cells. This human ES/iPS cell-derived cardiac tissue is useful for in vitro reentrant arrhythmia model and drug screening.

Poster Board Number: T-2017

ULTRA-RAPID METHODS TO DETERMINE TRANSGENE PLACEMENT AND CARDIAC POTENTIAL OF NEWLY DERIVED HUMAN INDUCED PLURIPOTENT STEM CELLS

Dambrot, Cheryl S.1, Braam, Stefan R.2, Davis, Richard P.2, Langenberg, Karin1, Varga, Eszter1, Freund, Christian M.A.H.1, van de Pas, Simone1, van Zijl, Lisanne1, Atsma, Douwe E.1, Mummery, Christine L.1
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Most reprogramming methods to generate human induced pluripotent stem cells (hiPSC) produce a large number of clones of which usually 2-3 are selected for further research. There are currently no rapid methods available to select particular clones with a limited number of inserts, integrated in genomic locations not critical to stem cell maintenance or differentiation. In addition, the clones of interest for further research will be those able to differentiate efficiently into the lineage of choice. Furthermore significant line-to-line variability in differentiation capacity is observed among both human embryonic stem cells (hESC) and hiPSC lines which can have important consequences for the progress of research. To address these issues, we developed a novel combination of methods to make informed choices on which hiPSC clones to select for studies on genetic cardiac disease, based on lentiviral vector insert number and location, and cardiac differentiation potential, all of which were determined within 3 weeks after initial picking of putative clones. As an alternative to Southern blotting, we used the splinkerette PCR technique in combination with sequencing to determine insert number and location of integrated reprogramming genes. Several lentivirally reprogrammed hiPSC clones (0.1-5 MOI) were tested and the outcomes were consistent with Southern blot results. The cardiac differentiation potential was determined using a new, fully defined monolayer differentiation protocol designed for use with early hiPSC colonies grown in mTeSR. This monolayer differentiation method resulted in beating cardiomyocytes within 12 days. No optimization was required when tested on numerous clones of 8 independent hiPSC and hESC lines. Cardiac differentiation efficiencies within clones ranged from 3 to 50%, as determined by intracellular FACS staining. This allowed cardiac potential to be determined on individual clones along with insert number and location information without the need to passage or scale up culture. In summary, we describe a fast and efficient method requiring minimal amounts of starting material for the identification of high quality hiPSC clones, based on number and location of integrated reprogramming genes and cardiac differentiation potential.

Poster Board Number: T-2018

DIFFERENTIATION OF CARDIOMYOCYTE PHENOTYPE FROM HUMAN DENTAL PULP STEM CELLS

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In recent years much effort is spent in attempts to cure cardiac diseases or improve such patients’ condition. A new branch of medicine, called regenerative medicine, focuses on embryonic or adult stem cells to repair damaged cardiac vascular tissue. However,
embryonic stem cell research still involves ethical issue and the usual source for adult stem cells, bone marrow stem cells are generally difficult to isolate and expand in culture. As a possible alternative to using bone marrow stem cells, we investigated the use of dental pulp stem cells (DPSC) extracted from human permanent teeth. Tooth surface were cut around the cementoenamel junction and then cracked open to reveal the pulp chamber. The pulp tissue was separated from the crown and root and then digested in 3mg/ml collagenase type 1. DPSC were seeded in flasks, magnetically separated and only CD117 positive cells were used in the recent study. Cells were grown as monolayer in DMEM ( Dulbecco’s Modified Eagle Medium) supplemented with 10% FBS (fetal bovine serum) at 37°C and 5% CO2. Every four passages the CD117 positive fraction was separated magnetically. In order to differentiate DPSC into cells with characteristics of cardiomyocytes, we treated DPSC with the demethylating agent 5-azacytidine and two differentiation growth media - HM1 and HM1 (HM1: DMEM, 2%FBS, 20nM dexamethasone, 100μM L-ascorbic acid, 10ng/ml LIF (leukemia inhibitory factor); HM2: DMEM, 2%FBS, 20nM dexamethasone, 100μg/ml L-ascorbic acid, 25ng/ml BMP-2 (bone morphogenetic protein 2), 50ng/ml bFGF (basic fibroblast growth factor), 2ng/ml IGF-1 (insulin-like growth factor 1)). Flow cytometry test showed that concentrations up to 10μM 5-azacytidine cannot induce significant levels of apoptosis in DPSC. Therefore, part of the DMSC were exposed to 5μM 5-azacytidine for 24 hours one day after seeding, prior changing the media with HM1. After that all cells were grown in HM1 for three weeks followed by HM2 for another one to three weeks. After four to six weeks, cells changed their morphology and showed several characteristics typical for cardiomyocytes. Immunocytochemical tests were positive for GATA-4 (GATA binding protein 4), Nkx2.5 (NK2 transcription factor related locus 5), DES (desmin) and cTNT (cardiac Troponin T). Analysis with real time RT-PCR showed significant increase in the expression of BMP-2, BMP-4 (bone morphogenetic proteins 2 and 4), BMPRIA (bone morphogenetic protein receptor type 1A), MEF2C (myocyte enhancer factor 2C), MYH7B (myosin heavy chain 7B), as well as SMAD1 and SMAD5 (SMAD family members 1 and 5). All those transcription factors are associated with mature cardiomyocytes. Analysis of specific markers at the protein and mRNA levels demonstrated that cardiomyogenesis can be induced in dental pulp stem cells in certain growth conditions. Our novel approach of using teeth makes it possible to produce enough number of the cells for future transplantation studies. We are confident in its value for patients with cardiac problems.

**Poster Board Number: T-2019**

**DEFINING CELL LINEAGE RELATIONSHIPS DURING HUMAN CARDIOGENESIS**

Elliot, David1, Costa, Magdaline1, Koutsis, Katerina1, Ng, Elizabeth1, Lagerqvist, Ebba1, Haynes, John1, Pouton, Colin1, Braam, Stefan1, Davis, Richard2, Passier, Robert2, Mummery, Christine2, Elefanty, Andrew3, Stanley, Ed1

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NKX2-5 is expressed in the heart throughout life. We targeted sequences encoding green fluorescent protein (GFP) to the NKX2-5 locus of human embryonic stem cells (hESCs). NKX2-5hESCs facilitate quantification of cardiac differentiation and purification of hESC-derived committed cardiac progenitor cells (hESC-CPCs) and cardiomyocytes (hESC-CMs). Gene expression studies demonstrated that NKX2-5hESCs and CMs constitute developmentally distinct populations. Furthermore, clonal analysis showed that NKX2-5hESCs are capable of giving rise to the three major lineages in the heart, namely cardiomyocytes, smooth muscle and endothelium. GFP+ CMs display a foetal-like action potentials, correlating with the gene expression profile. We have used NKX2-5+ cells to identify VCAM1 and SIRPA as novel cell surface markers expressed on cardiac lineages. Flow cytometric temporal profiling of these three markers suggest the progression from a multipotent SIRPA+ population to a myogenically committed NKX2-5+SIRPA+VCAM1 population. Triple positive cells are contractile and express markers of CMs, whereas NKX2-5+SIRPA+ cells also express endothelial and smooth muscle markers. In addition, cultured NKX2-5+SIRPA+ cells give rise to NKX2-5+SIRPA+VCAM1+CMs. Furthermore, we have identified an NKX2-5+SIRPA+ cell population, which, when cultured, gives rise to endothelial cells. Therefore, these markers represent tools to investigate the molecular control of lineage specification during human cardiogenesis. NKX2-5 is likely to play a key role in the differentiation of hESCs of which both NKX2-5 alleles have been disrupted. Future studies will utilise our NKX2-5 alleles to focus on the role of NKX2-5 in regulating cell lineage specification and differentiation in human heart development.

**Poster Board Number: T-2020**

**HUMAN INDUCED PLURIPOTENT STEM CELL -BASED MODEL FOR CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA**

Kujala, Kiri1, Paavola, Jere1, Lahti, Anna1, Larsson, Kim1, Pekkanen-Mattila, Mari1, Viitasalo, Matti2, Lahtinen, Annukka M.1, Toivonen, Lauri1, Kontula, Kimmo1, Swan, Heikki1, Laine, Mikael1, Silvennoinen, Olli1, Aalto-Setälä, Katrin1

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Induced pluripotent stem cells (iPSC) can be generated by reprogramming differentiated cells into a pluripotent state, providing a way to study the pathophysiology of various genetic diseases. Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited cardiac disorder characterized by stress-induced ventricular tachycardia and risk of sudden death with a structurally normal heart. Here we introduce a functional cell model for CPVT caused by a mutation in the cardiac ryanodine receptor (RyR2) gene. This mutation interferes with RyR2 function, leading to diastolic leak of calcium from the sarcoplasmic reticulum (SR). We investigated whether the electrophysiological characteristics of this mutation can be mimicked in vitro by using iPSC-derived human cardiomyocytes. Spontaneously beating cardiomyocytes were differentiated from iPSC lines derived from a CPVT patient carrying the P2328S mutation in RyR2 and from one healthy control donor. Calcium handling and electrophysiological properties were studied by comparing mutation-specific and control cardiomyocytes by calcium imaging and patch-clamp techniques. We found substantial defects in electrical activity and calcium signaling in CPVT cardiomyocytes, presumably reflecting the cardiac phenotype observed in the patients. Catecholaminergic stress in CPVT cardiomyocytes led to various types of abnormal calcium transients and arrhythmias. CPVT cardiomyocytes displayed also a reduced SR calcium content, implicating leakage of calcium from the SR. Our cell model displayed aberrant calcium signaling consistent with well-known CPVT characteristics as well as abnormal electrophysiological properties that have not been reported before. Thus, this cell model for CPVT provides a promising platform to study basic
pathology, to screen drugs safely, and to optimize drug therapy in a patient-specific manner.

**Poster Board Number: T-2021**

**DIRECT CONVERSION OF HUMAN DERMAL FIBROBLASTS INTO CARDIOMYOCYTES USING RECOMBINANT TRANSLUCENT PROTEINS**

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Although the transformation of differentiated cells to induced pluripotent stem cells (iPSCs), is a powerful approach, it has several limitations, including the low efficiency of the process and the necessity of forced expression of at least one pluripotent stem cell transcription factor. These methods involve the genomic integration of the transcription factor(s)’ genes and thus harbor the risk of mutagenesis and tumorogenicity. A recent report showed the successful and efficient generation of cardiomyocytes from murine embryonic fibroblasts by brief reactivation of reprogramming factors. This allows fibroblasts to enter an unstable intermediate “partial reprogramming” phase, after which the cells were directed toward cardiomyocytes. Although this protocol offers fast conversion of fibroblasts to cardiomyocytes, there are still many risks associated with genomic integration of transcription factors’ genes. In addition, the direct conversion is only carried on murine fibroblasts and the protocol’s effectiveness on human dermal fibroblasts is not evaluated yet. In the present study we treated human dermal fibroblasts (HDFs) with a combination of 6 proteins (including Oct4, Klf4, Sox2, and c-Myc) conjugated with HIV TAT for 12 days in order to enter HDFs into a partially reprogrammed state. During the first 8 days, cells were treated with reprogramming media containing protein mixture and 15% FBS and 5% knockout serum replacement (KO-SR), followed by a switch to 1% FBS and 14% KO-SR for 4 days. After this period, cells were treated with BMP4 (20 ng/ml) in a serum-free medium for additional 4 days followed by 3 days of Chir99021 (a GSK3 inhibitor) treatment. After this period cells were collected for real-time PCR analysis. Cells treated with recombinant proteins showed significantly higher expression of early-stage and late-stage cardiac markers such as GATA-4, Mef2C, NKX2.5, alpha Myosin Heavy Chain (α-MHC), Cardiac Troponin T (cTnT) and alpha cardiac actin, in comparison to HDFs that were only exposed to BMP4 growth factor. Results were confirmed with immunocytochemistry for GATA-4, NKX2.5, cTnT and α-MHC. CONCLUSION. We have successfully generated cardiomyocytes from Human Dermal fibroblasts using recombinant cell-penetrating reprogramming proteins. This technique offers safe and fast generation of cardiac myocytes for future applications in regenerative medicine as well as drug screening. Furthermore this novel tool might be a potential method for in vivo conversion of ischemia-triggered aggregation of fibroblasts into cardiomyocytes in infarcted heart.

**Poster Board Number: T-2022**

**ENDOTHELIAL CELLS PROMOTE CARDIAC STEM CELL THERAPY USING MOUSE EMBRYONIC STEM CELL- DERIVED CARDIOMYOCYTES**

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To avoid undesired cell types when isolating embryonic stem cell (ESC)-derived cardiomyocytes (ESC-CMs), we used mouse ESCs stably transfected with a cardio-specific alpha-myosin heavy chain-driven enhanced green fluorescence protein (EGFP) reporter. We further found that coculture with endothelial cells enhanced the maturation indexes of ESC-CMs, including cell size, axis alignment, sarcomere organization, contraction rate and the expression of the gap junction protein connexin-43, through direct cell-to-cell contact. Here we examined whether co-transplantation of ESC-CMs with endothelial cells had therapeutic effects using a mouse model of myocardial infarction. We found that cotransplantation with endothelial cells, but not fibroblasts, improved ESC-CM cell engraftment following injection. Moreover, cotransplantation with endothelial cells significantly increased ESC-CM maturation, as indicated by increases in both cell alignment and size. We then used echocardiography to determine the left ventricular ejection fraction and the end-systolic and end-diastolic volumes of mice which underwent experimental myocardial infarction following injection of saline, peptide nanofibers (NFs, as a cell carrier), ESC-CMs alone or along with endothelial cells or fibroblasts. At day 21, in infarcted hearts receiving NFs containing ESC-CMs plus endothelial cells improved ejection fraction (P < 0.05), decreased left ventricular end-systolic and end-diastolic volumes (P < 0.05) and reduced the infarct size of hearts (P < 0.05). Together, these results suggest that co-transplantation of endothelial cells with ESC-CMs promotes the maturation of ESC-CMs in vivo and improves cardiac cell therapy after myocardial infarction. Hence, the co-transplantation of ESC-CMs with endothelial cells may pave the way for future success in using stem cell therapy for cardiac regeneration.

**Poster Board Number: T-2023**

**ABLATION OF THE HCN4 ION CHANNEL IN DIFFERENTIATING HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES VIA ZINC FINGER NUCLEASE MUTATION**

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Cardiovascular disease remains a leading cause of death worldwide each year. Stem cell-derived cardiomyocytes hold great promise in tissue engineering for cardiac regeneration. However, current protocols for the differentiation of pluripotent stem cells toward cardiomyocyte lineages result in heterogeneous populations of cardiac cell types, in which spontaneous contraction is observed. Even among cardiomyocytes, atrial, ventricular, and pacemaker phenotypes are present. Spontaneously depolarizing stem-cell derived cardiomyocytes (SC-CMs) initiate contraction of surrounding cardiomyocytes. Control over electrical activity of differentiating SC-CMs, either by blocking spontaneous activity or by providing exogenous electrical stimulus, has been shown to reduce heterogeneity of resulting cell populations. Additionally, spontaneous pacemaker activity represents a potential risk for causing deadly arrhythmias if SC-CMs are to be used clinically. The hyperpolarization-activated cyclic nucleotide-gated 4 (HCN4) ion channel is known to

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be partially responsible for initiating contraction in cardiac nodal tissue. This pacemaking activity, if present in ventricular tissues, might lead to undesired arrhythmias. We hypothesize that ablating the HCN4 gene in human induced pluripotent SC-CMs (hiPSC-CMs) will result in production of homogenous ventricular-type cell populations lacking spontaneous contractility. To accomplish this, zinc finger nucleases (ZFNs) represent a powerful and clinically-relevant technology capable of specifically targeting and ablating gene expression. In this project four plasmid vectors (two dimeric pairs) harboring ZFNs, designed specifically for targeting exon one of the HCN4 gene, were constructed using recombiant DNA techniques. Each ZFN was electroporated into (IMR90)-1 hiPSCs. Colonies were screened for biallelic mutation of HCN4, lack of HCN4 protein expression, and propensity to differentiate into cardiomyocytes. We postulate that HCN4-/– cardiomyocytes will display a ventricular gene expression profile and electrophysiological phenotype, resulting in cell populations better suited for use in drug testing and in tissue engineering clinical applications for studying and treating several diverse forms of heart disease.

**Poster Board Number: T-2024**

**ISOLATION AND CHARACTERIZATION OF MOUSE CARDIAC STEM CELLS AS GREEN FLUORESCENT PROTEIN-RETAINING CELLS**

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The heart contains a reservoir of stem and progenitor cells. Cardiac stem/progenitor cells have been characterized by the expression of cell surface markers such as Sca-1 and c-Kit or transcriptional activation of genes such as Isl-1 and Wt1. Alternatively, cardiac stem/progenitor cells can be defined as side population (SP) cells, which are characterized by their unique ability to efflux a DNA-binding dye, e.g. Hoechst 33342. Despite these marker proteins and genes as well as functional definition, the origin and identity of cardiac stem cells remain only poorly understood. In this study, we aimed to identify and characterize cardiac stem/progenitor cells as “label-retaining cells (LRCs).” Tissue stem cells proliferate infrequently and therefore the labels (typically BrdU), once incorporated into their DNA or nucleosomes, are retained for an extended period, while the labels incorporated into transit amplifying cells become quickly diluted as the cells continue to proliferate. Given the difficulty to isolate BrdU-retaining cells without losing their viability, we employed GFP to fluorescently label slow-cycling cells by expressing histone H2B fused-GFP under the control of a tetracycline-responsive regulatory element (TRE). The TRE-H2BGFP transgenic mice (kindly provided by Dr. Elaine Fuchs) were crossed with the mice expressing reverse tetracycline transactivator driven by the ROSA26 promoter, yielding double transgenic mice that express GFP in a wide variety of cell types only when the mice are given doxycycline. The double transgenic mice were given doxycycline through mother’s drinking water for two weeks from E10.5 through P7 and chased for up to 12 weeks. GFP-labeled cells were detected ubiquitously in the heart just after the administration of doxycycline; however, the labeled cells were markedly decreased in number after six weeks of chase and were predominantly localized in the epicardium and near coronary vessels. To characterize the GFP-LRCs, the cells expressing GFP after six weeks of chase were sorted from whole cell suspension of the heart using flow cytometry. The GFP-labeled cells expressed a high level of GFP comprised ~0.5% of whole dissociated cells. Quantitative RT-PCR analyses revealed that the GFP-retaining cells expressed high levels of epicardium-associated markers, i.e., podoplanin, Tbx18 and Wt1, indicating that a majority of GFP-retaining cells are derived from the epicardium. GFP-retaining cells also expressed a panel of cardiac stem/progenitor markers including Sca-1, c-Kit and GATA4, the expression levels of which were equal to or higher than those of SP cells isolated from the adult heart. These results, taken together, indicate that the GFP-retaining cells we isolated represent a major population of cardiac stem/progenitor cells residing in the epicardium and are therefore a promising source for functional characterization of cardiac stem/progenitor cells in myocardial development and regeneration.

**Poster Board Number: T-2025**

**TOWARDS THE LABEL-FREE PURIFICATION OF STEM CELL-DERIVED CARDIOMYOCYTES USING SECOND HARMONIC GENERATION**

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In the translation of regenerative cardiology from bench to bedside, the development of a label-free, sensitive method for analyzing, counting and sorting viable human pluripotent stem cell-derived cardiomyocytes (PSC-CMs) of varied maturity is an important step yet to be taken. In order to use PSC-CMs for transplantation-based regenerative therapies or for engineering cardiac tissue for in vivo use, one must be able to control the phenotype, number and purity of CMs used so that reproducible and clinically acceptable tissue properties are achieved. We have taken the first steps towards realizing a label-free PSC-CM sorting methodology by utilizing second harmonic generation (SHG), a nonlinear optical phenomenon that occurs in the rod domains of sarcomeric myosin. PSC-CMs have a strong capacity to generate the SH signal, while undifferentiated stem cells do not. SHG intensity, when integrated over entire cell volumes, is strongly dependent on the development of sarcomere architecture in PSC-CMs. The effect was observed in PSC-CMs from the same differentiation day that were re-plated for varying lengths of time, as well as in suspension PSC-CMs fixed at different differentiation time points. Additionally, a PSC-CM’s capacity for SHG is retained even after single cells have been retained in suspension for two hours. The potential specificity of using SHG to identify PSC-CMs is demonstrated to be > 97%. Furthermore, it is shown that other cells that arise in a typical cardiac-directed stem cell differentiation protocol, including smooth muscle cells, do not generate SH signals. Based on our observations thus far, we have started to develop a flow cytometer based on SHG by integrating an appropriate optical scheme with microfluidic devices in order to obtain enriched, pure populations of PSC-CMs in a label-free, non-genetic manner.

**Poster Board Number: T-2026**

**A NOVEL CARDIAC EX VIVO CULTURE SYSTEM REVEALED THE ELONGATION OF CELL CYCLE PHASES IN CARDIOMYOCYTES DURING DEVELOPMENT**

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Background: Although cardiomyocyte cell cycle regulatory mechanisms in molecular medicine have been studied, little is known
about the spatial and temporal patterns of cardiomyocyte cell cycle progression in development. One major reason is that prior methods in cell cycle analysis mostly need sample fixation. By interlocking fluorescent probes with antiphase oscillating proteins that mark cell-cycle transitions, novel technique “Fucci” (fluorescent ubiquitination-based cell cycle indicator) effectively labels individual G1 phase nuclei red, S/G2/M phase green and G1/S transition phase yellow in living cells. Therefore Fucci is an useful tool to evaluate native cardiomyocyte cell-cycle, but to our knowledge, there are no standard protocols of live imaging in ex vivo heart model. The purpose of our study was to establish a cardiac ex vivo culture system in order to investigate the native cell-cycle dynamics in cardiomyocytes by applying Fucci. Methods and Results: We first confirmed that Fucci probes effectively highlight the cell cycle in cardiomyocytes. Cardiomyocytes in Fucci-expressing embryos at E18.5 (EO = the day of plug) were immunostained for PCNA and PHH3, which are known as classical markers of S and M phase respectively. Both PCNA and phosphohistoneH3 positive cardiomyocytes were also Fucci green positive. On the other hand, Fucci red positive cardiomyocytes did not show signals of PCNA or phosphohistoneH3. These consistent findings also strongly support Fucci as a novel method to analyze cardiomyocyte cell cycle. For establishing an ex vivo culture system, embryos and neonates were anesthetized by hypothermia, and the heart was transferred to liquefied low melting agarose gel immediately. On an ice bedded condition, myocardial slices (200µm-300µm) were made horizontally, and cell cycle transition was examined by 5-ethynyl-20-deoxyuridine (EdU) staining for 24 hours. The numbers of Edu positive cardiomyocytes showed that our method successfully replicated the in vivo cell cycle transition in our ex vivo culture system (ex vivo 27.4% ± 5.4%, in vivo 29.1% ± 8.9%). Finally we analyzed cardiomyocyte cell cycle dynamics applying the ex vivo culture method to Fucci-expressing transgenic mice. Live imaging visualized the cell cycle progression of cardiomyocytes and we assessed the length of S/G2/M phase in cardiomyocytes. Interestingly, S/G2/M phase length in cardiomyocytes elongated during development (E14.5: 11.4 h ± 1.3 h, post natal day 1: 14.8 h ± 1.5h). Thus, we succeeded in establishing an cardiac ex vivo culture system and by applying new technique Fucci, we elucidated the native cell-cycle dynamics in cardiomyocytes. The elongation of S/G2/M phase in cardiomyocytes could be a sign of maturation during development, and further analysis will lead us to a new approach for investigation of cardiac stem cells.

Poster Board Number: T-2027

NOVEL CHEMICALS POTENTLY INDUCING CARDIOMYOCYTES FROM VARIOUS PROGENITOR POPULATIONS

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Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are promising cell sources for cardiac regenerative medicine. Previously, we established a 2-dimentional culture-based sequential cardiovascular differentiation system from mouse ESC/iPSCs. This method is amenable to assess differentiation efficiencies at each differentiation stage (undifferentiated ESC/iPSCs, mesoderm, cardiac progenitors, cardiomyocytes). Recently, chemical biological approaches are starting to have an increasingly important role in stem cell biology and regenerative medicine. Some small molecules can efficiently regulate cell fates or modulate cell reprogramming. We reported that an immunosuppressant, cyclosporin-A, showed a novel potent cardiogenic effect specifically acting on Flk-1 positive mesoderm cells to increase cardiomyocytes by 10 times. We recently established a high-throughput screening (HTS) based on our ESC cardiovascular differentiation system and chemical approach. Using this HTS of co-culturing mouse ES cells that carry α-myosin heavy chain promoter-driven EGFP gene with OP9 mouse bone marrow-derived stroma cells, we can accurately and efficiently identify chemicals promoting cardiomyocyte differentiation from Flk-1 positive mesoderm cells. Here we report that we successfully discovered several cardiomyocyte differentiation chemicals (CDCs) from chemical libraries. In a natural chemical library derived from marine invertebrates, we identified a couple of natural chemicals (nCDC). Particularly, nCDC1 showed potent cardiomyocyte induction at nanogram/milliliter (ng/ml) level. This active concentration (2 ng/ml) was 1000 times lower than cyclosporin-A. And nCDC1 increased cardiomyocyte percentage and cell number that appeared from Flk-1 positive mesoderm cells approximately 20 times more than control. Next, we examined direct effect of nCDC1 on Flk-1 positive mesodermal cells and ES cell-derived cardiac progenitor cells, Flk1+/CXCR4+/VE cadherin− (FCV) cells. nCDC1 potently induced cardiomyocytes from Flk-1+ cells and FCV cells even in the absence of OP9 stromal cells. We further examined effects of nCDC1 on a somatic cardiac progenitor population, cardiac side population (CSP) cells. nCDC1 induced differentiation of rat neonate CSP cells into cardiomyocytes. These results showed nCDC1 induced cardiomyogenesis from mesodermal cells, cardiac progenitor cells derived from both ES/iPS cells and cardiac tissue. These findings would provide a clue for cardiomyocyte differentiation mechanisms and offer novel cardiac regenerative strategies.

Poster Board Number: T-2028

GENERATION OF CARDIOMYOCYTE-LIKE CELLS FROM NONMYOCYTES BY DEFINED FACTORS IN VITRO AND VIVO

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Cardiomyocytes are terminally differentiated cells and adult hearts have very limited regenerative capacity. Regenerative medicine using iPS cell-derived cardiomyocytes is an attractive therapy but still refinement is needed before clinical application. We recently found that mouse cardiac fibroblasts can be directly converted into cardiomyocyte-like cells by introduction of three cardiac-specific transcription factors, Gata4, Mef2c, and Tbx5 (GTM). We have now investigated whether direct injection of cardiac reprogramming factors into mouse hearts after myocardial infarction can convert endogenous cardiac fibroblasts into cardiomyocytes. Retroviral vectors containing cardiac reprogramming factors were used for gene therapy after coronary artery ligation. We found that GMT treatment induced cardiac gene upregulation and cardiac marker expression in fibroblasts. However, the induced cardiomyocytes by GMT did not have clear sarcomeric structures. Next, we screened additional cardiac reprogramming factors in human fibroblasts.
and found that addition of two factors to GMT enhanced cardiac
generation and maturation. We next used 5 factors (GMT plus two
factors) for in vivo reprogramming and found that the induced car-
diomyocytes got mature and exhibited clear sarcomeric structures.
These results indicate that endogenous cardiac fibroblasts can be
a cell source for new cardiomyocytes by local delivery of defined
factors, but modification of reprogramming factors may be needed
to enhance cardiomyocyte generation.

Poster Board Number: T-2029

A CERTAIN MICRORNA SPECIES INDUCE CELL
CYCLE PROGRESSION AND MITOTIC DIVISION IN
NEONATAL AND ADULT RAT CARDIOMYOCYTES.

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Background: Mammalian cardiomyocytes withdraw from the cell
cycle during postnatal development, which significantly limits the
capacity of the adult mammalian heart to regenerate after injury.
Therefore, proliferation of cardiomyocytes is promising strategy
for treatment of heart disease, such as myocardial infarction.
MicroRNAs are a short, non-coding, single stranded RNA that are
21-25 nucleotides in length and involve in the balance of a variety
of developmental events, such as proliferation and differentia-
tion, in many types of cells. Objective: To identify microRNAs that
can induce proliferation of cardiomyocytes. Results: We screened
a library of human microRNAs in primary culture of neonatal rat
hearts, respectively. Conclusion: miR-148a/152 can induce cell cycle
progression and mitosis in post-mitotic cardiomyocytes. Our results
indicate that miR-148a/152 can induce cell cycle progression
in vivocardiomyocytes. Subsequently, in order to determine whether miR-
148a/152 can induce cell cycle progression in vivo, we injected the
adenoviruses harboring miR148a/152 into the left ventricle of the
adult rat hearts. miR-148a/152 showed the remarkable expression
of Ki-67 positive cardiomyocytes 4 days after infection (0.84±0.36%,
4.15±0.75%, 2.65±0.64% in control, miR-148a and miR-152-treated
hearts, respectively. Conclusion: miR-148a/152 can induce cell cycle
progression and mitosis in post-mitotic cardiomyocytes. Our results
provide new insights into the development of therapeutic strate-
gies for treatment of heart disease by induction of cardiomyocyte
proliferation.

Poster Board Number: T-2030

FETAL EPIGENETIC MODIFIERS STIMULATE
CARDIOMYOCYTE REGENERATION AND PROTECT
FIBROSIS IN MAMMALIAN/AMPHIBIAN MODELS.

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Adult fishes and amphibians have high potential for heart regen-
eration, but major mammalians’ model, mice/rat lose their potential
by 7~10 days after birth. To understand this plasticity, we focused
on several epigenetic factors which have unique expression pat-
terns both in embryogenesis and in cardiac regeneration. SWI/
SNF-cardiac BAF type chromatin remodeling factors and histone
regulators were strongly up-regulated within 12 hours after resec-
tion of ventricle heart in neonatal mice and axolotl, and keep these
expression for one week during regeneration. Whereas in adult
rodents, no-expression of BAFs is disrupted within postnatal 10
days, indicating that they act as early response factors during heart
regeneration and the most important key factors for regeneration
in amphibian as well as in neonatal mice. Stable expression of BAFs
in adult heart (BAF-TG) prevented fibrosis in myocardial infarction
(MI) experiments and led to regenerate their lost parts of heart by
new cardiomyocytes, keeping heart function healthy. Surprisingly, in
vivoChIP and ChIP-seq analyses showed that the major cardiac con-
tracted genes’ promoters of Ctnnt2, Myl7, Nppa, were still opened
in the presence of SWI/SNF-BAFs. In addition, we screened out
repressors for SWI/SNF-BAF, suggesting that these factors controls
cardiomyocyte maturation and reduction of regenerative plastic-
ticy. These data suggest that combination of active/repressive
chromatin modifiers are necessary to stimulate/lose the plasticity
of regenerative response and keep early cardiac gene programs in
mammal/amphibian heart regeneration.

Poster Board Number: T-2031

FUNCTIONAL CHARACTERIZATION OF STEM
CELL-DERIVED CARDIOMYOCYTES USING AN
AUTOMATED PATCH CLAMP SYSTEM

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The development of automated electrophysiology instruments
in the past decade has revolutionized ion channel drug discovery
and safety pharmacology by greatly increasing the throughput
of patch clamp assays. This development enables pharmaceutical
and biotechnological companies to employ direct electrophysi-
ological determination of compound activity on ion channels, with
much larger numbers of compounds, and at earlier stages of the
drug discovery and development process. However, automated
electrophysiology assays require large quantity of highly homoge-
neous cells, which often limits the application of current systems
to recording from recombinant cell lines stably transfected with ion
channel of interest, rather than primary cell preparations that are
more physiologically relevant. As a step forward, recent advances
in stem cell research have shown great promise in providing stem
cell-derived human cells, of all types and in sufficient quantity, for
preclinical studies. In this study we attempt to address the growing
interested in combining stem cell technology and high-throughput automated electrophysiology for ion channel drug development and safety assessment. Specifically, we analyzed the electrophysiological properties of stem cell-derived cardiomyocytes using an automated patch clamp system, in both voltage clamp and current clamp mode. The ability to record action potentials and to also study multiple native ion channel targets from the same cell has significantly increased the sophistication of experimentation. The electrophysiological data is also comparable to that obtained using conventional patch clamp recordings. In conclusion, the combination of stem cell-derived cardiomyocytes with an automated patch clamp system offers a powerful assay platform not only for faster assessment of compound efficacy but also for safety profiling of lead compounds in a more biologically relevant system.

Poster Board Number: T-2032

ES AND IPS CELL DERIVED CARDIOMYOCYTES IN CARDIOVASCULAR DRUG DISCOVERY, SAFETY PHARMACOLOGY AND AS DISEASE MODELS IN CARDIAC DRUG DEVELOPMENT

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Drug induced arrhythmia of the torsade des points type has been the reason for the denial of approval of novel drug candidates. The aim of cardiac safety pharmacology is to detect undesirable pharmacodynamic drug effects within and above the therapeutic range. A special focus is on the identification of potential arrhythmogenic effects within the drug discovery chain. Areas covered: Here, the authors discuss the relevance of induced pluripotent stem (iPS) cell derived cardiomyocytes for safety pharmacology. The technology of obtaining functional cardiomyocytes from somatic cells of healthy donors and patients with inherited diseases is the basis for a variety of disease models in multi-level safety pharmacology screening. Here we compare the properties of iPS cell-derived cardiomyocytes, ES cell derived cardiomyocytes with a non mammalian primary cardiomyocyte based assay and heterologous expression systems. We compare results from cardiac tissue recordings and Langendorff hearts with our datasets obtained from a variety of stem cell derived cardiomyocytes for a panel of reference compounds. The data presented do not just focus on prolongation of the ventricular action potential or the QT interval in the ECG, but also include an analysis of pro-arrhythmic events.

Poster Board Number: T-2033

NOTCH1 REGULATES CARDIOVASCULAR PROGENITOR DIFFERENTIATION

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Cellular decision making of self-renewal or differentiation arises from integration of intrinsic and extrinsic signals. Notch is an evolutionarily conserved transmembrane protein with critical roles in numerous cell fate decisions. The Notch pathway is prominent among those known to regulate stem and progenitor development in multicellular organisms from flies to vertebrates. We recently showed in mice that conditional knockout of Notch1 in cardiovascular progenitor cells (CPCs) leads to a marked expansion of CPCs, but causes early embryonic lethality due to cardiac morphogenetic defects, making it difficult to address the fate and cell-autonomous role of Notch1. To overcome this problem, we have derived embryonic stem (ES) cells from Notch1 conditional mutant embryos with fluorescent markers. We have used these ES cells to study CPC autonomous role of Notch1 in cardiac differentiation and discovered a critical role of Notch signaling for the lineage-specific differentiation of CPCs.

Poster Board Number: T-2034

DE-DIFFERENTIATED FAT AS A CELL SOURCE FOR CARDIOVASCULAR TISSUE ENGINEERING

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The source and availability of the cells for tissue engineering (TE) is critical. Ideally, the cells used for TE should be autologous and have the capacity to proliferate and differentiate in vitro, in a manner that can be reproducibly controlled. Although progress has been made towards differentiating embryonic stem cells as well as iPS cells to specific cell lineages, the efficiency is often poor and the number of cells generated is not efficient for therapeutic application. In the search for a perfect cell source for cardiac repair, we propose to study whether pluripotent adipocyte-derived mesenchymal stem cells, which we refer to as de-differentiated fat (DFAT) cells, can be a potential cell source for cardiovascular TE. Materials and Methods: Mouse DFAT cells were isolated in large numbers from fresh subcutaneous fat and cultured in DMEM supplemented with 20% fetal bovine serum and 0.5% of antibiotic-antimycotic solution as describes before. C57BL6/J mice, 25-30 grams, were subjected to experimental myocardial infarction (MI) and assigned to one of the following groups: 1) DFAT cells in DMEM supplemented with 1% Methylcellulose, 2) DMEM supplemented with 1% Methylcellulose (control), 3) DMEM alone (control), and 4) sham. The left anterior descending artery (LAD) was permanently ligated proximally with a suture. After the LAD ligation, once the heart rate stabilized, a cocktail of 500,000 cells in 10 µl DMEM supplemented with 1% Methylcellulose, or for controls without cells, were injected into the border zone of the infarcted area. Successful ligation was confirmed by decreased myocardial ejection fraction from ~70% before and 40-50% after ligation as measured by echocardiography. MI was further confirmed by the presence of collagen-rich infarcted areas in Masson's trichrome stained heart sections. Preliminary results: It has been shown that DFAT cells develop networks of beating cardiomyocytes (CM)-like cells after 3 weeks in culture. These cells express cardiac-specific markers such as Nkx2.5, troponin C and connexin 43. Intracellular Ca2+ transients, action potentials, and contractions are observed in these networks. In addition, the DFAT cells have the capacity to undergo smooth muscle (expressing SM-actin, caldesmon, calponin and SM-myosin) and endothelial cell differentiation (expressing CD31 and CD144), which contract in response to carbamoylcholine chloride and form tube structures, respectively. Our preliminary results from injection of GFP-DFAT cells into the MI area in mice show the presence of the cells in the vascular structures and the heart muscle in the infarct zone up to 8 weeks post MI. This suggests that DFAT cells may support the formation of new vessels and cardiac cells in vivo. Our preliminary data from left ventricular catheterizations indicate an improved cardiac contractility and relaxation up to 8 weeks post MI, as compared to controls. However, additional studies are required to assess whether the DFAT cells had a beneficial effect on the myocardial function. Our expectation from this study is that the results will contribute to improve cardiac function in mouse models of cardiac disease and ultimately in patients. Our findings, will lead
to new strategies to propagate the desired cell population on a large scale, essential for further development of stem cell based therapies.

**Poster Board Number: T-2035**

**MOUSE MESENCHYMAL STEM CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS DEMONSTRATE MYOCARDIOGENESIS POTENTIAL**

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Introduction: Aging adult donors-derived mesenchymal stem cells (MSCs) are functionally impaired in cardiac regeneration. Although MSCs derived from early embryonic stage such as embryonic stem cells or induced pluripotent stem cells (iPSCs) have great potential of proliferation and differentiation in previous studies, therapeutic capacity of mouse MSCs derived from iPSCs remains elusive. This study is therefore aimed to derive and isolate mouse MSCs from iPSCs for myocardiogenesis potential study in experimental myocardial infarction models. Methods: Mesenchymal-like stem cells were isolated from mouse iPSCs by removing feeder cells and leukemia inhibitory factor (LIF). MSCs were enriched by conditioned medium with basic fibroblast growth factor (FGF2) and epidermal growth factor (EGF) supplements before phenotypical characterization of MSCs by flow cytometry, utilizing phycerythrin (PE)-conjugated antibodies against CD34, CD44, CD73, CD90, CD133, Oct4 and TRA-1-60. Multipotent differentiation studies of MSCs, including adipogenesis, osteogenesis and chondrogenesis have been carried out. Purified MSCs were further engaged to a recombinant cocktail formulated with transforming growth factor-beta 1, bone morphogenic protein-4, activin A, retinoic acid, insulin-like growth factor-1, fibroblast growth factor-2, alpha-thrombin, and interleukin-6. Derived cardiopoietic mouse MSCs were transplanted into myocardial infarcted murine model. Results: The iPSC derived cells were negative for hematopoietic markers CD34 and CD133, pluripotency markers Oct4 and TRA-1-60, while being positive for mesenchymal markers, CD44, CD73 and CD90. These cells were further induced into osteocytes and chondrocytes under differentiation conditioned medium. High expression of homeobox transcription factor Nkx 2.5, ventricular myosin light chain MLC-2V, and sarcomeric alpha-actinin were observed in cardiopoietic mouse MSCs. Conclusion: Mouse iPSCs derived MSCs demonstrated myocardiogenesis potential induced by a recombinant cocktail of growth factors. They hold potential capacity to attenuate ischemic cardiomyopathy.

**Poster Board Number: T-2036**

**COMPREHENSIVE SCREENING OF CELL-SELECTIVE PEPTIDES AND ITS APPLICABILITY TO VASCULAR IMPLANT SURFACE MODIFICATION**

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To reduce risks in long-term implants, especially with vascular implants, tissue engineering technology to design effective scaffold property for managing proper regeneration of surrounding cells is important. The design strategies of the medical implant surface had been mainly focused to provide inhibitory effect on cell adhesion and growths. However, since such inhibitory effect on cells is against the regeneration process, those implants designed with inhibitory function have not yet achieved to eliminate risks of side-effects. To breakthrough such limitations of medical implant surface design, our natural regenerative mechanism has a great potential to be investigated. Extracellular matrix (ECM) is one of the most effective biomaterials to enhance the cellular regeneration. In spite of their promising effect, ECM itself is difficult for industrial production with stable and safe product quality. Animal-driven pathogenic risks are one of the main considerations. To mimic the function of ECM as a regeneration enhancing material, taken together with the feasibility of industrial production, we have been investigating short peptides that provide cell-selectivity as a scaffold modification biomaterial. By combining in silico peptide screening algorithms and high throughput peptide array-based cell assay technique, we have reported some clusters of short peptides that can selectively control the adhesion of different types of cells. Compared to ligand-type peptides, our proposing cell selective peptides modifies the implant material surface by their property. In other words, such peptides are the candidate blocks to interpret the properties of ECMs, and to be replaced by analogue molecules that can be more effective to be manufactured as implant material. We here show the effective screening strategies and results of such cell-selective peptides, and its applicability to medical implants by in vivo study of artificial vessel. We especially focus on controlling the selectivity of cells that critically affect the vascular implant sustainability, such as endothelial cells, smooth muscle cells, and mesenchymal stem cells.

**Poster Board Number: T-2037**

**INDUCTION OF EPICARDIAL PROGENITOR CELL PROLIFERATION AS A REGENERATIVE MEDICINE APPROACH FOR ENDOGENOUS CARDIAC REGENERATION AND REPAIR**

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Post-myocardial infarction cardiac dysfunction and heart failure remain a medical challenge as current therapies do not address the fundamental pathology, i.e. loss of functional cardiomyocytes. Our goal is to identify and develop a phenotypic screening assay for small or large molecules that enhance endogenous regenerative capacity, leading to efficient restoration of cardiac function post-myocardial infarction. Epicardial progenitor cells have been shown to play a major role in both heart development and repair of the myocardium. They are activated, although insufficiently, in adult hearts after injury and undergo an epithelial-to-mesenchymal transition that is regulated by expression of Wilms Tumor protein 1 (WT-1). Taking the population of human epicardial progenitor cells that are WT-1(+), we assessed the basal proliferative capacity of several different donors in a kinetic imaging assay. We then tested a set of compounds from the literature that have been shown to have pro-proliferative effects in stem/progenitor cell populations such as 6-bromoindirubin-3’-oxi. This allowed us to determine the optimal window where a proliferative increase could be seen above basal levels. To enable screening of compounds at high throughput we further developed an endpoint imaging assay, we stained the cells with WT-1, Ki-67, and 5-ethyl-2’-deoxyuridine (EdU) with Hoechst 33342 as a nuclear contrastant. To test the robustness of the assay, we treated the cells with an expanded set of compounds drawn from the literature including neuregulin-1, peristin, lithium chloride, thymosin β4, and VEGF. After assay validation, we moved into screening with a larger number of compounds including sets.
where the mechanism of action is known, which will aid in later deconvolution. Here we apply high-content phenotypic screening to assess the proliferation of epicardial progenitor cells following treatment with small molecules. This assay has the potential to be used as the basis for proliferation screens in other progenitor cell types.

Poster Board Number: T-2038

DYNAMIC MATRICES AND EMBRYONIC STEM CELLS IMPROVE CARDIAC OUTCOMES: A POTENTIAL MYOCARDIAL INFARCTION THERAPY

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Tissue-specific elasticity arises from developmental changes that occur in the environment over time, e.g., ~10-fold myocardial stiffening from E3 to E10 in the chick embryo. Recently, we have shown that pre-cardiac mesodermal cells plated on top of a thiolated hyaluronic acid (HA) hydrogel engineered to mimic this time-dependent stiffening improves cardiomyocyte maturation compared to cells on static compliant matrices. Here we examine in vitro and in vivo cell-matrix interactions, the effect of dynamic mechanical cues on the differentiation and development of mouse embryonic stem cells (mESCs), and in vivo biocompatibility. Improved embryonic cardiomyocyte and ESC distribution and viability were observed when cells were encapsulated and bound to immobilized, thiolated gelatin conjugated to the HA matrix. While mechanical cues alone are insufficient to efficiently induce cardiogenesis, a combination of growth factors (e.g. BMP4, Activin A, DKK-1) and mechanics can enhance maturation of mESC-derived cardiomyocytes. mESC-derived cardiac progenitors most affected by developmentally-relevant mechanical cues are those that naturally observe such variation in vivo. Though not toxic to cells in culture, we assessed HAs local and systemic biocompatibility via subcutaneous and intramyocardial injections. Prior to assembly, HA was injected into Sprague-Dawley rats and samples were removed over a post-injection time course and subject to histological, immunological and mechanical analysis. Histological analysis shows minimal infiltration of host cells and capsule formation for subcutaneously injected rats, indicating a limited local immune response. Hematological analysis shows no significant systemic immune response was elicited in pre- vs. post-injection animals compared to controls. Most importantly, atomic force microscopy (AFM) analysis of samples from subcutaneous injections demonstrates dynamically increasing hydrogel stiffness over time similar to that previously found in vitro. When injected intramyocardially, host cells begin to actively degrade HA within 1 week post-injection and lay down matrix, nearly replacing the gel with host tissue by 1 month. These data indicate that when ESC-derived cardiomyocytes are co-injected with HA, vascularization is likely to occur, supplying nutrients to differentiating ESCs. Altogether, we find that HA is a viable source for therapeutic use in treatment of myocardial infarction, and could enhance cardiomyocyte differentiation in vivo.

Cancer Cells

Poster Board Number: T-2041

FASCIN REGULATES BREAST CANCER STEM CELL DIFFERENTIATION AND FUNCTION.

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Despite great success in treating patients with localized tumor disease, treatment of metastasized tumor remains restricted. This restriction has been attributed to the fact that most chemotherapeutic remedies target bulk of differentiated cancer cells sparing cancer stem cells, which are responsible for tumor relapse and metastasis. The molecular pathway that regulates the maintenance and function of cancer stem cell pool within the tumor remains largely unknown. We have previously demonstrated that the expression of actin-bundling protein (fascin) in breast cancer cells regulates various metastasis-associated genes resulting in increased metastasis and shorter survival. Here, we used gain and loss of function approaches to examine whether fascin play a role in regulating cancer stem cell phenotype and function in breast cancer cells. Fascin was knockdown in MDA-MB-231 cell and expressed in T47-D cells, which are breast cancer cell lines positive and negative for fascin, respectively. Results showed that fascin expression was strongly associated with increased CD44 and reduced CD24 expressions, a phenotype consistent with more stem cell-like breast cancer cells. Fascin regulated breast cancer cell self-renewal capacity as indicated by decrease ALDH and Notch-1 activities when fascin was knockdown. Furthermore, when seeded in low attachment culture dishes, fascin-positive cells formed more mammospheres than the fascin-knockdown cells. Most importantly, fascin-positive cells are more resistance to apoptosis when exposed to chemotherapeutic agents. Altogether, our data support the existence of small subpopulation in breast cancer cells with a stem cell characteristic and fascin play a key role in regulating this cancer stem cell population.

Poster Board Number: T-2042

ERBB/NF-κB SIGNALLING REGULATES BREAST CANCER STEM CELL-LIKE PROPERTIES

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Cancer stem cells, a small proportion of heterogeneous tumor cells, can self-renew and simultaneously produce differentiated tumor cells with strong proliferative activity, and therefore are responsible for tumorigenesis. Although it is important to target cancer stem cells for treatment of cancer patients, it has been difficult to find appropriate target molecules because the mechanisms by which cancer stem cells maintain their ability remain obscure. Here, we identified a molecular mechanism that regulates breast cancer...
stem cell (BCSC)-like properties. We found that heregulin (HRG), a ligand for ErbB3, induced mammosphere formation in breast cancer stem cells (BCSCs) as well as in breast cancer cell lines. HRG-induced mammosphere formation was reduced by treatment with inhibitors for phosphatidyl inositol 3-kinase (PI3K) or NF-κB and by expression of IκBα-Super Repressor (IκBαSR), a dominant-negative inhibitor for NF-κB. Moreover, the overexpression of IκBαSR in breast cancer cells inhibited tumorigenesis in NOD/SCID mice. Furthermore, we found that the expression of IL8, a regulator of BCSC self-renewal, was induced by HRG through the activation of the PI3K/NF-κB pathway. These findings illustrate that HRG/ErbB3 signaling appears to maintain BCSC properties through a PI3K/NF-κB pathway in human breast cancer.

Poster Board Number: T-2045

THE HIPPO TRANSDUCER TAZ CONFERNS CANCER STEM CELL TRAITS ON BREAST CANCER CELLS DOWNSTREAM OF EPITHELIAL-TO-MESENCHYMAL TRANSITION AND THE DEREGULATION OF THE CELL POLARITY DETERMINANT SCRIBBLE

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Cancer Stem Cells (CSCs) are proposed to drive tumor initiation, heterogeneity and progression. Yet, our understanding of the cellular and molecular mechanisms that underlie CSC properties is limited. We present evidences indicating that TAZ, a transducer of the Hippo pathway, is a determinant of key biological traits of breast CSCs. TAZ is required to sustain self-renewal and tumor initiation capacities in cellular models of breast cancer progression. TAZ protein levels are stabilized in prospective CSCs, and gain-of-TAZ in non-CSCs induces them to adopt CSCs-like behaviors. We found that gene-signatures denoting TAZ activity are associated with molecular imprints of “stemness” in breast cancer patients’ datasets. In agreement with the view that an increase in CSCs drives tumor progression in breast cancer (Pece et al., 2010), the proportion of TAZ positive cells is much more abundant in poorly-differentiated tumors than in well differentiated ones. Consistently, raising TAZ levels promotes the transition of experimentally induced tumors toward a less-differentiated status. At the molecular level, TAZ promotes self-renewal of CSCs downstream of Epithelial-to-Mesenchymal Transition (EMT). This is caused by deregulation of the cell polarity determinant Scribble, which forms an endogenous complex with TAZ in nontransformed and tumoral mammary epithelial cells. Remarkably, loss-of-Scribble - or induction of EMT - disrupts the association of TAZ with the core Hippo kinases MST and LATS, allowing TAZ to escape phosphorylation by LATS and association to beta-TrCP ubiquitin ligase complex. This study thus links the
CSCs concept to the Hippo pathway in breast cancer, and reveals a mechanistic basis of the control of Hippo kinases by cell polarity.

Poster Board Number: T-2046

PHENOTYPIC CHARACTERIZATION NORMAL AND CANCER STEM CELLS WITHIN THE DIFFERENT SUBPOPULATIONS OF BREAST CELLS

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Strong evidence supports the existence of stem cells in the normal breast. The phenotype of these cells has been characterized using several unrelated markers. Therefore, the overlap between these different cell populations and their relation to breast cancer cells is not well known. To study the breast cells subpopulations we isolated single cells from breast tissue and analyzed these cells by multi-parametric flow cytometry. Results show that breast mesenchymal cells had the phenotype of CD49fneg/Ep-CAMneg/CD44high/CD24neg. The luminal epithelial cells were Ep-CAMhigh and were distributed between differentiated MUC-1high/CD49fneg luminal cells and a progenitor CD49f+ luminal cells. The basal epithelial cells were Ep-CAMlow/CD44f+ and were distributed between differentiated CD10neg and progenitor CD10+ basal cells. All tested stem cell markers were restricted to CD49f+ cells although some of them did not overlap, for example, the previously described stem cell markers ALDHhigh and CD10 were exclusively expressed by cells in Ep-CAMhigh/CD49f+ luminal progenitor and basal Ep-CAMlow/CD44f+ cell respectively. Other stem cell markers were distributed among the different subpopulations of CD49f+ breast cells as ABCB1 and ABCG2 were mainly expressed by cells in Ep-CAMhigh/CD49f+ luminal progenitor and basal Ep-CAMlow/CD44f+ cell respectively. However, there is now a theory that stem cells which reside in many adult tissues are not only responsible for the normal reparative and regenerative processes, but are also considered to be a prime target for genetic and epigenetic changes, culminating in aberrant differentiation and in vivo, regulator and specific marker of totipotent cells in vivo and in vitro, regulator and specific marker of totipotent cells in vivo, regulator and specific marker of totipotent cells in vivo.

Poster Board Number: T-2048

GENERATION OF A NOVEL CANCER STEM CELL-BASED TRANSGENIC MICE OVEREXPRESSING OCT4B1 VARIANT AS A CANCER MODEL

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Since the first gene transfers into mice were successfully executed in 1980, transgenic technologies offered new approaches for life science researches. Fitting the current biotechnology demands, transgenic methods were dramatically used for the past two decades as outstanding tools for in vivo target validation, drug optimization, and pre-clinical studies. Transgenic animals can provide investigators with valuable in vivo models to study biology and etiology as well as novel approaches in the early detection and targeted therapies of defined cancers without arising strict ethical concerns and restrictions. A growing body of recent studies supports the notion that both tumor initiation and sustaining rely on only a small subset of cells within a tumor, termed tumor-initiating or cancer stem cells (CSCs). Few investigators believe that CSCs reside in different organs and grow upon ideal microenvironment. However, there is now a theory that stem cells which reside in many adult tissues are not only responsible for the normal reparative and regenerative processes, but are also considered to be a prime target for genetic and epigenetic changes, culminating in abnormal conditions leading to generation of CSCs. Whatever the origin of cancer is considered, the importance of CSC research lies on the improved understanding of cancer biology and its potential roles to provide new approaches in cancer early detection and targeted personalized treatment. Recent studies have revealed specific expression of stem cell proteins in cancer cells with it. Oct4, a key regulator and specific marker of totipotent cells in vivo and in vitro, is critically involved in the self-renewal and pluripotency networks of ES and EC cells. Human oct4 gene potentially encode three
different spliced variants; OCT4A, OCT4B and OCT4B1. These have different expression pattern. It was reported that novel alternative variant, OCT4B1, sharply down-regulated during the course of differentiation of human ES/EC cells and suggested correlation between OCT4B1 and pluripotent/ undifferentiated state of human ES/EC cells. Moreover, it is determined that there is a relationship between OCT4B1 expression and apoptosis and carcinogenesis. Thus, generation of the transgenic mice with CSCs in their tissues can provide researches with valuable in vivo models for studying the breast cancer biology, etiology, early diagnosis, and targeted therapies. To this end, we could design and produce transgenic mice for the first time with CSCs under inducible conditions. Our novel CSC-based cancer model may open new insights to improve our understanding of cancer biology and development of more efficient approaches in its early diagnosis and targeted therapies.

Poster Board Number: T-2049

NOTCH SIGNALING IS UPREGULATED IN THE CHRONIC PHASE OF CHRONIC MYELOID LEUKEMIA

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Notch signalling is critical for haematopoietic stem cell self-renewal and survival. Chronic Myeloid Leukaemia (CML) is a stem cell disease characterised by the presence of the Philadelphia (Ph) chromosome, and subsequent expression of the BCR-ABL oncogene. The well established role for Notch signalling in human T-cell acute lymphoblastic leukaemia (T-ALL) and the reported interaction between Notch and ABL in different developmental contexts in Drosophila raise the possibility that Notch signalling may be dysregulated in CML. Therefore, the aim of the study was to investigate whether Notch signalling is altered in CML and to study possible crosstalk between Notch signalling pathway and BCR-ABL in CML. The gene expression patterns of all four human Notch genes and the Notch target gene HES1 were studied in CD34+ stem and progenitor cells isolated from CML patients. Poly-A PCR followed by real time PCR analysis was used to quantitate gene expression levels in comparison with levels in equivalent populations isolated from normal bone marrow (NBM). The expression of Notch1 receptor protein levels expressed on the cell surface was also investigated by flow cytometry. Results showed an up-regulation of Notch1 and Notch2 genes and the target gene Hes1 on the most primitive CD34+ Thy+ subset of CML CD34+ cells as compared with NBM. In addition, Notch1 receptor protein was expressed in distinct lymphoid and myeloid progenitors within the CD34+ population of CML cells. These results suggest that Notch signalling may be highly activated in CML primitive progenitors. To investigate the possible crosstalk between Notch and ABL in vitro human cell line model systems were assessed as possible models to study the interactions between Notch and ABL signalling and the FACS based PCR assay was optimised as a rapid method to assess ABL activity. The data showed that K562 and ALL-SIL cell lines are sufficient model systems to investigate the cross-talk between the Notch and ABL signalling pathways. The imatinib induced inhibition of ABL activity in K562 and ALL-SIL cells resulted in significant up-regulation of Notch activity as assessed by Hes1 expression. Similarly, GSI inhibition of Notch signalling in K562 cells resulted in hyperactivation of ABL kinase activity as assessed by P-crk levels. The antagonistic relationship between Notch and ABL signalling observed in cell lines were further confirmed in CD34+ cells from chronic CML patients. Treatment of CD34+ CML cells with imatinib led to significant up-regulation of Notch activity whereas inhibition of Notch signalling with GSI in CD34+ CML cells resulted in increased ABL activity. It can be concluded therefore, that Notch signalling may be dysregulated in the chronic phase of CML. In addition, the data presented in this project demonstrate for the first time the cross-talk between Notch signalling and ABL signalling in cell line model systems as well as in primary CD34+ CML cells. Future work is required to address the possible mechanisms that underlie the findings observed here and to investigate the biological consequences of the interplay between Notch and ABL signalling in CML.

Poster Board Number: T-2050

ACUTE MYELOID LEUKEMIA STEM CELLS LACKING RAPTOR SELF RENEW BUT HAVE DEFECTIVE LEUKEMIA INITIATING CAPACITY IN MICE

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Although dysregulation of the mammalian target of rapamycin complex 1 (mTORC1) promotes leukemogenesis, it is unclear how mTORC1 affects the behavior of established leukemia. Here we investigated the roles of Raptor, an essential component of mTORC1, in mouse hematopoiesis and leukemia by using an in vivo tamoxifen-inducible deletion system. Raptor deficiency impaired granulocyte and B cell development but did not alter survival or proliferation of hematopoietic progenitor cells. In an MLL-AF9-driven acute myeloid leukemia (AML) model, Raptor deficiency significantly suppressed leukemia progression by causing apoptosis of differentiated, but not undifferentiated, leukemia cells. mTORC1 did not control cell cycle or cell growth in AML cells. Transplantation of Raptor-deficient undifferentiated AML cells in a limiting dilution revealed that mTORC1 is essential for leukemia initiation. Strikingly, however, a subset of AML cells with undifferentiated phenotypes survived long-term in vivo without mTORC1 activity. The reactivation of mTORC1 in those cells restored their leukemia-initiating capacity. Thus, AML cells lacking mTORC1 activity can self-renew as AML stem cells. The findings provide mechanistic insight into how residual tumor cells circumvent anti-cancer therapies and drive tumor recurrence.

Poster Board Number: T-2051

ANDROGEN ABLATION MITIGATES DEFECT OF B CELLS TO A PROSTATE CANCER AND INCREASE CANCER AND INCREASE SURVIVAL RATE OF TRAMP MICE.

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Androgen ablation is the most commonly used therapy for prostate cancer. However, the effects of androgen ablation on immune system, especially B cell distribution are not well understood. We have used transgenic adenocarcinoma mouse prostate (TRAMP) mice to characterize the B cell distribution in periphery blood and spleen upon androgen ablation. We found a decrease of the B cell population in wild type TRAMP mice compared to normal wild type mice. Furthermore, the B cells increased when in castrated TRAMP mice compared to the wild type TRAMP mice. Interestingly, we found an increase in immature B cells in the spleen of castrated TRAMP mice, which might be due to the resistance to apoptosis during B cell
Cancer stem cells (CSCs) are a specific population of therapy-resistant cancer cells that have acquired stem cell properties such as self-renewal, survival, and differentiation, and the ability to home to protective niches. Chronic Myeloid Leukemia (CML) is the first cancer that was proven to originate from a genetic abnormality, the Philadelphia BCR-ABL translocation. Research demonstrated that BCR-ABL tyrosine kinase was necessary and sufficient to initiate chronic phase (CP) CML, while additional mutations in an expanded progenitor population may result in leukemia stem cell (LSC) generation and blast crisis (BC) transformation. The molecular mechanisms driving malignant transformation of progenitors into therapeutically recalcitrant LSC in CML have remained elusive. Generation of self-renewing LSC has been linked to activation of transcriptional programs typical of primitive stem cell populations. We propose that transcriptomic diversity fueled by aberrant RNA editing might promote malignant reprogramming of CML BC progenitors, allowing them to persist and evade therapy. Cumulative whole transcriptome RNA sequencing, hematopoietic progenitor assay, serial transplantation, lentiviral overexpression and shRNA knockdown experiments demonstrate that a key RNA editing enzyme, adenosine deaminase acting on dsRNA (ADAR)-1 regulates LSC cell fate determination and self-renewal capacity of CML BC progenitors. During blastic transformation, BCR-ABL amplification and proliferation markers. Type 1 colonies are large and consist of large loosely packed cells. Type 2 colonies are larger than type 3 and consist of a mixture of small and large cells. Both type 1 and type 2 colonies are able to be serially cloned long term, form spheres and are tumorigenic in mice. Type 3 colonies cannot be cloned or form spheres. Type 1 colonies also have an increased Ki67 positive fraction compared to Type 2 and Type 3 and express the cancer stem cell markers CD44 and qβ1 integrin. Type 1 colonies were previously thought to be derived from stem cells and type 2 from transit amplifying cells. In contrast, this study shows both Type 1 and 2 colonies contain cells with stem cell traits, but they differ in the proportion of these cells.

**Poster Board Number: T-2054**

**INVETIGATION OF OCT4-DERIVED PARACRINE EFFECT IN MODULATING CANCER STEM-LIKE PROPERTY IN COLORECTAL CANCER**

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Cancer stem cells (CSC) or tumor-initiating cells (TICs) are subpopulation of tumor cells to be responsible for resistance to chemotherapy, progression as well as recurrence in a variety of cancers, including colorectal cancers (CRCs). Several evidences have been demonstrated the relationship between inflammation and tumorigenesis in CRCs and proinflammatory cytokines have been suggested to regulate preneoplastic growth during colitis-associated cancer tumorigenesis. Interleukin (IL)-32, a proinflammatory mediator, can induce the expression of various cytokines, such as tumor necrosis factor-alpha (TNF-alpha), IL-1beta, IL-6, and IL-8. Since these cytokines would activate several signaling pathway to promote cell growth or survival like JAK/STAT3 pathway. However, the potential role of cytokines in mediating the CSC-initiated tumor progression or recurrence in CRCs is unclear. In this study, we found Oct4 overexpression enhanced cancer stem-like property in colorectal cancer cell line. Microarray-based bioinformatics showed that higher expression levels of embryonic stem cell (ESC)-specific genes in Oct4-expressing colorectal adenocarcinoma cell line (HT29-Oct4). In addition, the overexpression of Oct4 enhanced stem cell properties, which was contributed by the cytokines like IL-8 and IL-32. Targeting IL-8 and IL-32 in HT29-Oct4 cell with specific antibodies blocked these tumorigenic effects of Oct4 overexpressing CRCs. Furthermore, xenograft tumorigenicity assay revealed that Oct4-overexpressing CRCs also enhanced the metastasis potential in vivo, which could be totally blocked by neutralizing IL-32 and IL-8. These data demonstrate that the cytokines IL-32 and IL-8 play a role in regulating stem-like properties and promoting tumorigenesis in CRCs. This may provide a therapeutic strategy for the treatment of CRCs.
Atoh1 affects the form of MC. So, we aim to elucidate Atoh1 function in colorectal carcinogenesis. Moreover, APC deletion on colorectal carcinogenesis regulates the proliferation and differentiation by the ubiquitin proteasome proteolysis of β-catenin and Atoh1 in sporadic colorectal cancer (CRC). The expression of Oct-4, Nanog, Sox2, Nucleostemin, Bmi, Zfx, Esrrb, Tcl1, Tbx3 and Dppa4 in several tissue samples of colon, prostate and bladder cancer and in cancer cell lines (Lncap, HEPG2, HT-1376) was further determined using real-time polymerase chain reaction (RT-PCR). The expression of Oct-4 and Nucleostemin at the protein level was determined using immunocytochemistry. Results: Oct-4, Nanog, Sox2, Nucleostemin, Bmi, Zfx, Esrrb, Tcl1, Tbx3 and Dppa4 were expressed in bladder, colon and prostate cancer tissues and cell lines. Oct4 and Nucleostemin proteins were expressed in both nuclear and cytoplasmic in cancer cell lines. No immunoreactivity was observed in negative controls, which were incubated in the absence of primary antibody. Conclusion: Collectively, our data confirmed the expression of Oct-4 and Nucleostemin in cancer cell lines and in 20 tumor samples. Total RNA was isolated using the ISOGEN method. RNA integrity was checked with agarose gel electrophoresis and spectrophotometry. The expression of Oct-4 and Nucleostemin at the protein level was further determined using immunocytochemistry. Results: Oct-4, Nanog, Sox2, Nucleostemin, Bmi, Zfx, Esrrb, Tcl1, Tbx3 and Dppa4 were expressed in bladder, colon and prostate cancer tissues and cell lines. Oct4 and Nucleostemin proteins were expressed in both nuclear and cytoplasmic in cancer cell lines. No immunoreactivity was observed in negative controls, which were incubated in the absence of primary antibody. Conclusion: Collectively, our data confirmed the expression of Oct-4 and Nucleostemin in cancer cell lines and in 20 tumor samples. Total RNA was isolated using the ISOGEN method. RNA integrity was checked with agarose gel electrophoresis and spectrophotometry. The expression of Oct-4 and Nucleostemin at the protein level was further determined using immunocytochemistry. Results: Oct-4, Nanog, Sox2, Nucleostemin, Bmi, Zfx, Esrrb, Tcl1, Tbx3 and Dppa4 were expressed in bladder, colon and prostate cancer tissues and cell lines. Oct4 and Nucleostemin proteins were expressed in both nuclear and cytoplasmic in cancer cell lines. No immunoreactivity was observed in negative controls, which were incubated in the absence of primary antibody. Conclusion: Collectively, our data confirmed the expression of Oct-4 and Nucleostemin in cancer cell lines and in 20 tumor samples. Total RNA was isolated using the ISOGEN method. RNA integrity was checked with agarose gel electrophoresis and spectrophotometry. The expression of Oct-4 and Nucleostemin at the protein level was further determined using immunocytochemistry. Results: Oct-4, Nanog, Sox2, Nucleostemin, Bmi, Zfx, Esrrb, Tcl1, Tbx3 and Dppa4 were expressed in cancer cell cell lines and cancer tissues and suggested that their expression can be used as potential tumor markers in diagnosis and/or prognosis of tumors. These results confirm the potential value of the cancer stem-cell theory in cancer therapy.

**Poster Board Number: T-2055**

**EVALUATING THE EXPRESSION OF SEVERAL SELF RENEWAL GENES IN COLON, PROSTATE AND BLADDER CANCER AND IN CANCER CELL LINES (LNCAP, HEPG2, HT-1376)**

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**Background:** Uncontrolled self renewal plays a direct function in different types of carcinoma progression. The same molecular pathway that manages self renewal in normal stem cells also images to be appropriate by cancer stem cells in cancers. Here we examined the expression of self renewal regulatory factors such as Oct4, Nanog, Sox2, Nucleostemin, Zfx, Esrrb, Tcl1, Tbx3 and Dppa4 in several tissue samples of colon, prostate and bladder cancer and in cancer cell lines (Lncap, HEPG2, HT-1376).

**Material & Method:** We used RT-PCR (Reverse Transcriptase Polymerase Chain Reaction) to examine the expression of these genes in cancer cell lines and in 20 tumor samples. Total RNA was isolated using the ISOGEN method. RNA integrity was checked with agarose gel electrophoresis and spectrophotometry. The expression of Oct-4 and Nucleostemin at the protein level was further determined using immunocytochemistry. Results: Oct-4, Nanog, Sox2, Nucleostemin, Bmi, Zfx, Esrrb, Tcl1, Tbx3 and Dppa4 were expressed in bladder, colon and prostate cancer tissues and cell lines. Oct4 and Nucleostemin proteins were expressed in both nuclear and cytoplasmic in cancer cell lines. No immunoreactivity was observed in negative controls, which were incubated in the absence of primary antibody. Conclusion: Collectively, our data confirmed the expression of Oct-4 and Nucleostemin in cancer cell lines and in 20 tumor samples. Total RNA was isolated using the ISOGEN method. RNA integrity was checked with agarose gel electrophoresis and spectrophotometry. The expression of Oct-4 and Nucleostemin at the protein level was further determined using immunocytochemistry. Results: Oct-4, Nanog, Sox2, Nucleostemin, Bmi, Zfx, Esrrb, Tcl1, Tbx3 and Dppa4 were expressed in cancer cell cell lines and cancer tissues and suggested that their expression can be used as potential tumor markers in diagnosis and/or prognosis of tumors. These results confirm the potential value of the cancer stem-cell theory in cancer therapy.

**Poster Board Number: T-2056**

**THE ACQUISITION OF CANCER STEMNESS IN COLON CANCER IS REGULATED BY THE STABILIZATION OF ATONAL HOMOLOG 1 PROTEIN.**

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**Background & Aims:** Atoh1 is one of the essential genes for secretory intestinal epithelial cells (IECs) differentiation. However, Atoh1 protein is not expressed in colon cancer region, despite Atoh1 gene is equally expressed between normal colon and colon cancer region. So, we have reported that GSK3 on Wnt signal reciprocally regulates the proliferation and differentiation by the ubiquitin proteasomal proteolysis of β-catenin and Atoh1 in sporadic colorectal cancer (CRC). Moreover, APC deletion on colorectal carcinogenesis causes Atoh1 protein degradation by GSK3, resulting in maintaining the undifferentiated state of CRC. On the other hand, Atoh1 protein is expressed in the mucinous carcinomas (MC) of colon in which APC is normal. However, it remains unknown whether Atoh1 affects the form of MC. So, we aim to elucidate Atoh1 function in MC. Methods: We constructed mutant Atoh1 fused with mCherry that reconstitutes five Serines of GSK3 target site to Alanin (mCherry-SSA Atoh1). mCherry-SSA Atoh1 induced CRC cells were assessed the differentiation/stem characteristics by RT-PCR and immunofluorescence. Cell growth was evaluated by MTS assay. Cell cycle analysis was performed by Live Cell Imaging and FACS using fluorescent cell-cycle indicator, FuCki (Fluorescent Ubiquitination-based Cell Cycle Indicator) system. In vivo study, nude mice were inoculated with naive CRC cells and mCherry-SSA-Atoh1 cells. Subsequently Oxaliplatin or DMSO was administered biweekly. The characteristics of tumors were assessed by the differentiation/stem markers and chemoresistance. Results: mCherry-SSA-Atoh1 showed the stable expression in CRC cells. Atoh1 protein stabilization up-regulated both E-BOX and TCF4 dependent transcriptional activity, resulting in the expression of Mucin2 (mucinous marker) and Wnt target gene, Lgr5 (cancer stem cell marker). Moreover, mCherry-SSA-Atoh1 inhibited cell growth to suppress the cell cycle by the extension of G0/G1 time. Furthermore, mCherry-SSA-Atoh1 acquired the chemoresistance against Oxaliplatin to avoid the caspase-dependent apoptosis. Interestingly, Wild type Atoh1 (mCherry-WT-Atoh1) inducing CRC cells also acquired the chemoresistance against Oxaliplatin, because we found that Oxaliplatin stabilized Atoh1 protein by the inhibition of GSK3 kinase activity. In vivo study, Tumor constituted by mCherry-WT-Atoh1 inducing CRC cells grew up regardless of the treatment with Oxaliplatin, resulting in the Atoh1 protein stabilization and MC phenotype acquisition. Conclusions: Atoh1 protein stabilization might regulate the form of MC, including cancer stemness and chemoresistance that reflect poor prognosis of MC.

**Poster Board Number: T-2057**

**REAL TIME IMAGING OF THE DYNAMICS OF CELL DEATH INDUCED BY THERAPEUTIC NEURAL STEM CELLS IN MALIGNANT BRAIN TUMORS**

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**Neural stem cells (NSCs) have recently been recognized as efficient delivery vehicles in a number of malignancies including the most aggressive brain tumor type: glioblastoma multiforme (GBM). Therefore, engineering NSCs to express tumor-specific cytotoxic reagents, such as tumor necrosis factor related apoptosis-inducing ligand (TRAIL), offers great potential in the treatment of GBMs. In this study, we engineered TRAIL-sensitive and -resistant GBM cells with fluorescence-based live-cell caspase-reporters and assessed their response to SC-TRAIL in real-time. We show that SC-TRAIL induces caspase-mediated apoptosis in GBM cells, which is correlated with their death-receptor (DR4/5)-induction levels, and the sustained release of TRAIL via NSCs has significant anti-tumor effects in vitro and in vivo. To target TRAIL-resistant GBMs, we have developed DR-reporters that offer an imaging-based screening platform to identify agents that can act in concert with SC-TRAIL. Utilizing both DR4/5 and caspase-reporters, we show that an HDAC inhibitor, MS-275, augments the response of TRAIL-resistant GBM cells to SC-TRAIL in vitro and in vivo. This study demonstrates the efficacy of a combination of real-time reporters of TRAIL-mediated apoptosis pathway in evaluating the fate of tumor-cells in response to SC-TRAIL therapies and may have clinical implications for cancer patients.**
BONE MARROW CELLS TRAFFIC AND FUSE TO DEVELOPING COLORECTAL TUMOR CELLS GENERATING TUMOR INITIATING CELLS

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Background: Bone-marrow-derived-cells (BMDC) can traffic to injured organs, and participate in organ maintenance under certain inflammatory conditions. Subsequently, rare reports suggested that cancers could originate from BMDC. Given the fact that tumors generate an inflammatory environment, we hypothesized that BMDC participate in tumor maintenance. Materials & methods: We obtained BMDC and fresh tumors from patients enrolled in this study after proper consent, approved by the NIH Institutional Review Board. We irradiated severe immunodeficient mice (NSG) and performed bone marrow transplantation (BMT) using human CD34+ cells. After 4 weeks of recovery, we xenotransplanted subcutaneously human colon cancer cells isolated from fresh surgical specimens. In the testing experiment, tumor and BMDC cells carried two different lentivirus reporter genes. In the validating experiment, tumor and BMDC cells were followed using HLA markers, and in a sex-mismatch experiment, we used a Y-chromosome marker. When tumors developed, we extracted the tumors and sorted and tested the cells according to their origins. Results: We show that human BMDC, traffic to sites of developing colorectal cancers, fuse to cancer cells, and generate tumor initiating cancer cells. Fused cells expressed genetic markers both from tumor and BMDC cells, were CD45-negaive and expressed CEA. We confirmed the FACS data using qRT-PCR, DNA sequencing and Karyotyping. Quantitative DNA analysis using SNP specific pyro-sequencing showed cells containing DNA derived from both BMDC and colon cancer cells. Karyotyping demonstrated that cells that expressed both colon cancer-and BM-derived HLA were tetraploid while cells that express colon cancer-derived-HLA alone were aneuploid but not tetraploid. Bone-marrow-tumor-fused-cells expressed the typical colon cancer marker CEA, and maintained the malignant phenotype over several generations. Averaged over multiple experiments fused cells comprised 14%-to-28% of the genetic marker bearing tumor cells. Importantly, testing the tumor initiating capacity of tumor cells versus BM-tumor-fused-cells, the BM-tumor-fused-cells have the exclusive capacity to initiate tumors with only 10 cells generating a more aggressive and less differentiated phenotype with high Ki67 expression tumors. Finally, BM-tumor-fused-cells have unique pluripotency gene signature up-regulating Sox2 (>200 folds), MYC, Notch, Numb, CD44, CDH1, BMP1, CXCL12, FOXA, FGFI-3 and ALDH1-1/2 . Conclusion: Similarly to normal tissue maintenance, this data suggest that tumor maintenance can be supported by BMDC. These findings have potential far-reaching implications to our understanding of tumor progression, maintenance and novel approaches for cancer therapeutics.
ABCG2+ CD24+ CD44+ subpopulation, but it also enhanced invasion and metastatic potential of not only can electively outgrow ABCG2+ CD24+ CD44+ subpopulation, but it also enhanced invasion and metastatic potential of ABCG2+ CD24+ CD44+ subpopulation in vitro and in the orthotopic mouse model. Comparing with the tumor-engraft mice fed with normal diet, fructose-enriched diet (60% fructose) enhanced cancer progression and liver metastasis. Importantly, the enhancement in metastatic potential by fructose replacement was found to associate with upregulation of alpha 2,6-sialylation and evaluated levels of beta-galactoside alpha 2,6-sialyltransferase (ST6Gal-I). The knockdown of ST6Gal-I affected cell motility. RNA-seq and target proteomics further revealed c-Met is the substrate of ST6Gal-I. The loss of alpha 2,6-sialic acid on c-Met in ST6Gal-I knockdown abolish cell motility of ABCG2+ CD24+ CD44+ subpopulation. In conclusion, c-Met is required for self-renewal for pancreatic stem cells and CSCs. And our current work identified metabolic reprogramming can enhance metastatic potentials of pancreatic cancer stem cells by increasing functionality of c-Met via alpha 2,6-sialylation indicating sialylated c-Met is a potential target for metastatic cancer stem cells of pancreatic ductal adenocarcinoma.

Poster Board Number: T-2061
TARGETING MOUSE GLIOMA CANCER STEM CELLS USING CPG-SiSTAT3
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Glioblastoma multiforme (GBM), an aggressive grade IV glioma, is known to have high probability of recurrence after therapeutic challenge. Several lines of evidence suggest that cancer-stem cells (CSCs; also called “tumor-initiating cells”) underlie GBM recurrence and resistance to current therapies. Furthermore, glioma cancer stem cells suppress innate and adaptive immune responses while creating an immunologically environment that limits the therapeutic benefit of immunotherapy. It has been demonstrated that the Signal Transducer and Activator of Transcription-3 (Stat3) is critical for glioma tumor-initiating cells’ stem-like phenotype and creating an immunosuppressive microenvironment. We recently developed a platform to deliver siRNA synthetically linked to CpG in vivo; so-called CpG-siRNA. It has been shown that CpG-Stat3siRNA may be used to initiate robust anti-tumor immune responses in multiple cancer types; including melanoma, colon carcinoma, and lymphoma. Here, we demonstrate a powerful approach to curb CSCs in glioma by using CpG-Stat3siRNA, shifting the phenotype of the glioma cancer stem cells in vivo and in vitro. This induced shift would allow for the glioma cancer stem cells to become sensitive to conventional treatment protocols. Additionally, we demonstrate how the CpG-Stat3siRNA might guard against glioma recurrence in vivo. Finally, we show how silencing Stat3 may shift the glioma’s immune microenvironment towards an anti-tumor response. Thus, we consider CpG-Stat3siRNA as a multi-pronged therapeutic against glioma by selectively targeting the resistant phenotype of the glioma tumor-initiating cells, as well as causing an anti-tumor immune response against glioma tumor cells. These findings may lead to a more effective therapy against glioblastoma multiforme. (Supported by the California Institute of Regenerative Medicine and the National Institutes of Health)

Poster Board Number: T-2062
EVALUATION OF CD44 VARIANT EXPRESSION AS A RECURRANCE MARKER OF GASTRIC CANCER AFTER ENDOSCOPIC SUBMUCOSAL DISSECTION
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Background: As for the treatment of early gastric cancer (EGC), endoscopic therapy is widely used and have shown a good prognosis, although some of EGCs often show metachronous recurrence after initial endoscopic treatment. Recently, cancer stem cells having a stem cell-like ability such as multilineage potential and the self-renewal potential in a cancer tissue has been identified, and it is suggested that they are linked to tumor recurrence and metastasis. CD44 has recently been identified as one of the cell surface marker associated with cancer stem cells in various solid tumors. In addition, it was reported that human gastrointestinal cancers with a high level of CD44 variant (CD44v9) showed an enhanced capacity of glutathione synthesis, defence against reactive oxygen species (ROS) and reduced activation of p38MAPK, a downstream target of ROS (Cancer Cell 19, 387-400, 2011). In the present study, we investigated whether the expression of CD44v9 could become the marker of the recurrence of the gastric cancer. Method: A case-control study was conducted to identify CD44v9 as a recurrence marker of gastric cancer after initial endoscopic submucosal dissection (ESD) at the Keio University Hospital (Tokyo, Japan) from February 2008 to February 2009 within a follow-up period (33.6±7.9 months). The study compared 12 cases who had undergone curative resections for EGC and recurrence was identified after initial ESD with 13 age- and sex-matched controls. The expression of CD44v9 and activated p38MAPK (phospho-p38) were evaluated in immunohistochemistry. Quantification of the proportion of CD44v9-positive area (CD44v9 IHC score) was calculated by ImageJ (US National Institutes of Health, Bethesda, MD). The clinical features of the patients, such as age, sex, body mass index (BMI), H. pylori infection, smoking history, and location and differentiation of the tumors were retrospectively obtained from medical records. Result: The average of CD44v9 IHC score were 1.47±0.58% (median 0.63%). We divided patients into two groups of CD44v9 higher group (n=13) and lower group (n=12) based on a cut-off level which was decided with 0.61% by ROC analysis (sensitivity 75.0%, specificity 69.2%). In the analysis of recurrence rate using the Cox proportional hazard model, recurrence rate was significantly higher in CD44v9 higher group in comparison with CD44v9 lower group (p=0.015). Furthermore, a reduced expression of phospho-p38 was identified in CD44v9 positive cells. Conclusion: In the CD44v9 higher group, recurrence rate was significantly high as compared with the CD44vp lower group. Our result suggest that the expression of CD44v9, which up-regulates intracellular ROS resistance, is one of major predictive factors for the recurrence in the EGCs.
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Poster Board Number: T-2063

SUPPRESSION OF CANCER STEM-LIKE PROPERTIES IN Glioblastoma by DELIVERY OF MicroRNA145 SHORT BRANCH PEI
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Glioblastoma (GBM) the most common primary brain tumor bearing dismal prognosis. CD133 has been considered a putative marker of cancer stem cells (CSCs) in malignant cancers, including GBM. Micro RNAs (miRNAs), highly conserved small RNA molecules, may target oncogenes and become a therapeutic strategy against cancer. However, the involvement of miRNAs in GBM-associated CSCs remained mostly unclear. Using miRNA/micro RNA microarray analysis, we identified that miR145 (a tumor-suppressive miRNA) expression is inversely correlated with the levels of Oct4 and Sox2 in GBM-CD133+ cells and specimens of malignant glioma. Consistently, we demonstrated that miR145 negatively regulates tumor growth of GBM by directly targeting Oct4 and Sox2 in GBM-CD133+ cells. We therefore evaluated the treatment effect of polyurethane-short branch polyethylenimine-mediated miR145 (PU-PEI-miR145) on GBM-CSCs. PU-PEI-miR145 delivery in GBM-CD133+ significantly inhibited their tumorigenic and CSC-like abilities, and facilitated their differentiation into CD133− non-CSCs. Furthermore, PU-PEI-miR145 delivery in GBM-CD133+ effectively suppressed their expressions of drug-resistance and anti-apoptotic genes, and dramatically increased the sensitivity to radiation and chemotherapeutic drugs, including temozolomide. Finally, in vivo alone delivery of PU-PEI-miR145 significantly suppressed tumorigenesis, and synergistically improved survival in orthotopic GBM-CD133+--transplanted immunocompromised mice, combined with radiotherapy and temozolomide. Therefore, PU-PEI-miR145 is potential therapeutic approach for malignant brain tumors.

Poster Board Number: T-2064

ROLE OF A7 Nicotinic Acetylcholine Receptor in Cancer Stem Cells
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Epidemiological studies have suggested that cigarette smoking is related to increased cancer risk. Nicotine, a major component of cigarette smoking, is believed to be responsible for the pathogenesis of smoking-related malignancies. However, the mechanisms by which nicotine promotes cancer development are not fully understood. Growing evidence suggests that cancer cells are heterogeneous and are originated from cancer stem cells (CSCs), which are a minor population of cancer cells. In the present study, we investigated the effects of nicotine on the size of CSC population in human cancer cell lines. CSC population was analyzed by flow cytometry with a CSC functional marker aldehyde dehydrogenase. We examined whether nicotine increased a CSC population in human cancer cell lines such as A549 (lung), MCF-7 (breast), PC-3 (prostate) and U-251 (glioma). We found that nicotine increase CSC population in MCF-7 cells, not in A549, PC3 and U251 cells. The nicotine-treated MCF-7 cells formed more mammospheres than nicotine-treated normal cells, confirming that nicotine increases the CSC population. These data suggest that the effect of nicotine is selective in breast cancer. We next investigated the subtype of nicotinic acetylcholine receptor (nAChR). The effect of nicotine was blocked by the α7 subunit-selective antagonist of nAChR α-Bungarotoxin. In addition, the α7-selective nAChR agonist PHA543613 increased the CSC population in a dose-dependent manner. These data suggest that nicotine increases the CSC population via α7-nAChR. To investigate whether the effect of nicotine is mediated through a stem cell-dependent pathway, we examined the Notch pathway, which is a feature of CSC. We found that nicotine induced an expression of Notch target gene Hes1. DAPT, which inhibits cleavage of activated Notch receptors by γ-secretase and thereby prevents Notch signaling, reduced the nicotine-induced Hes1 expression and the nicotine-induced CSC population. Taken together, these data suggest that nicotine increases the CSC population via the α7-nAChR- and Notch-dependent pathway in MCF-7 cells. These findings reveal a relationship between nicotine and the CSCs in human breast cancer. This might explain the development of breast cancer in cigarette smokers.

Poster Board Number: T-2065

NOVEL GRP78 - TARGETING PEPTIDES FOR THERAPY AND IMAGING OF CANCER
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We had developed a strategy of structure-based optimization of cancer-targeting peptides to target human GRP78. Based on computer modeling, we designed peptide GRP78 composed of two major structural domains, a peptide-binding and an ATPase domain. GRP78 was reported to reside on the outer surface of cancer cells, but only in the cytoplasm of normal cells. Using our newly developed software, HotLig, for predicting protein-peptide interactions, a series of novel cancer-targeting peptides against GRP78 were designed in silico with molecular docking. The abilities of these peptides to bind to peptide-binding domain of GRP78 were further confirmed via Biacore analysis. We also demonstrated their capacities to target cancer cells through in vitro binding with a variety of cancer cells including clinical cancer specimens, and in vivo tumor imaging and targeted chemotherapeutic studies using peptide-linked liposome. Based on these analysis, we delineated a cancer-targeting motif Pro-X1-Leu-X2, where X1 could be amino acids with a hydrophobic side chain including Leu, His, Phe, and Tyr, and X2 could be Pro and aromatic amino acids Phe and Trp. Peptides possessing this motif, such as P-6, P-12 and P-13, target the peptide-binding domain of human GRP78, and could bind to a broad spectrum of cancer cells but not to normal cells. In addition, the novel peptide-linked Lipo-Dox displayed significant increases in therapeutic efficacy for human breast and lung cancer compared with the Lipo-Dox without targeting peptides. Besides, the microSPECT/CT imaging showed significantly greater uptake of radioactivity with 188Re-peptide-linked liposome compared with 188Re-liposomes alone, opening up a valuable application of these novel peptides for cancer diagnosis and targeted radiotherapy. Notably, these new cancer-targeting peptides possess the potential to target drug delivery to cancer initiating/stem cells, which might also provide a new paradigm for cancer stem cell-targeted therapy. In our recently established inducible KrasG12D-driven lung adenocarcinoma model in CCSIP-Tet-O-Cre/LSL-KrasG12D mice, we have identified bronchiolar Clara cells as the origin of cells which initiated Kras-induced tumor formation in the lung (Cancer Res. 71 7250 2011). Now we also found that the expression of GRP78 in the tumorigenic EpCAM+ MHCII+ subpopulation of cells in this in vivo lung cancer model was increased at least six folds, especially on the outer surface of cancer initiating cells. Similarly, the expression of GRP78 in the breast-cancer stem cell subpopulation in xenografts of human primary breast cancer was also significantly increased. Of interest, these peptides could bind to breast-cancer stem cell subpopulation...
in xenografts of primary breast cancer as well as CD44+CD24- cells from clinical specimens of breast cancer. Therefore, these newly found and optimized cancer-targeting peptides were expected to enhance “tumor selectivity” for imaging and targeted therapy of cancers, including cancer initiating/stem cells, thereby increasing the therapeutic index of the anticancer drugs.

Poster Board Number: T-2066

ROLE OF RAT MESENCHYMAL STEM/STROMAL CELLS AS A CO-CONSPIRATOR FOR TUMOR PROGRESSION IN A RAT OSTEOSARCOMA MODEL: A COMPARATIVE ANALYSIS OF GENE EXPRESSION PROFILES IN RELATION TO TUMOR PROGRESSION

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INTRODUCTION: Mesenchymal stem/stromal cells (MSCs) are proposed as a source of tumor stromal cells during the progression of various cancers such as breast cancers. However, the role of MSCs still remains uncovered in most sarcomas, although the MSCs are considered to be the cell of origin for most of sarcomas. We will propose the role of MSCs in tumor growth and metastatic ability with comparative analysis of gene expression profiles based upon the results of preliminary experiments in rat osteosarcoma model. MATERIALS and METHODS: Two components of experimental design have been performed using the cell lines of rat osteosarcoma COS1NR established from the tumor chemically induced by 4-hydroxy(amino)quinoline 1-oxide in F344 rats and rat MSCs isolated from syngeneic rat femur bone marrow. In vivo animal experiments were performed as the simultaneous co-implantation of both cells into subcutaneous tissues and the subcutaneous inoculation of osteosarcoma cells followed by subsequent intravenous injection of MSCs intermittently. Upon those experiments, we have analyzed the gene expression profiles using Agilent gene expression array comparing MSCs and osteosarcoma cells to identify the factors possibly involved in the process of tumor progression. RESULTS: The data of animal experiments indicated that MSCs could promote tumor engraftment and metastatic colonization in a rat osteosarcoma model. Briefly, simultaneous co-implantation of MSCs and osteosarcoma cells showed higher incidence of tumor formation and tumor growth rate in early phase compared to osteosarcoma cell inoculation alone. Intravenous MSCs injections after subcutaneous inoculation of osteosarcoma cells enhanced the formation of lung metastatic nodules in the group with MSCs injection compared to the group without MSCs, while no difference was observed in subcutaneous tumor growth between those groups. The pathway analysis from comparative gene expression profiles between syngeneic MSCs and osteosarcoma cells identified that pathways involved in cell cycle acceleration including CDKs-cyclins and DNA repairs were up-regulated in osteosarcoma cells, while genes involved in focal adhesion, cytokines and chemokines, and MMPs in MSCs may be involved in cell-cell and cell-matrix interaction to promote tumor progression, potentially compensating the tumor cell ability and cultivating the microenvironment for tumor cells.

Poster Board Number: T-2068

CD138-NEGATIVE CLONOCGENIC CELLS ARE PLASMA CELLS BUT NOT B CELLS IN SOME MULTIPLE MYELOMA PATIENTS

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Clonogenic multiple myeloma (MM) cells reportedly lacked expression of plasma cell marker CD138. It was also shown that CD19+ clonotypic B cells can serve as MM progenitor cells in some patients. However, it is unclear whether CD138-negative clonogenic MM plasma cells are identical to clonotypic CD19+ B cells. We found that in vitro MM colony-forming cells were enriched in CD138- CD19- CD38++, plasma cells, while CD19+ B cells never formed MM colonies in 16 samples examined in this study. Next we used the SCID-rab model, which enables engraftment of human MM in vivo. CD138-CD19-CD38++ plasma cells engrafted in this model rapidly propagated MM in 3 out of 9 cases, while no engraftment of CD19+ B cells was detected. In 4 out of 9 cases, CD138+ plasma cells propagated MM, although more slowly than CD138- cells. Finally, we transplanted CD19+ B cells from 13 MM patients into NOD/SCID IL2rg-/- mice, but MM did not develop. These results suggest that in at least some MM patients CD138-negative clonogenic cells are plasma cells rather than B cells, and that MM plasma cells including CD138- and CD138+ cells have the potential to propagate MM clones in vivo in the absence of CD19+ B cells.

Poster Board Number: T-2069

A PUTATIVE CD34+ CANCER STEM CELL LINE WITH KUPFFER CELL CHARACTERISTICS PRODUCES A COMBINED HEPATOCELLULAR CHOLANGIOCARCINOMA

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CD34+ stem cells play an important role during liver development and regeneration. We hypothesized that human liver carcinomas (HLC) might be derived from oncogenically mutated or epigenetically aberrant CD34+ cells. We determined that a population of CD34+ stem cells isolated from PLC/PRF/5 hepatoma cells (PLC) and clonogenically expanded on mouse feeder layers appears to function as liver cancer stem cells (LCSC) by forming HLC xenografts in NOD/SCID/IL2rg mice after injecting only 500 CD34+ stem cells; whereas unsorted parental PLC required at least 100,000 cells to produce HLC. Thus, a small number of CD34+ stem cells had tumor initiation capacity. We then attempted to characterize the origin of these LCSC. 12 subpopulation of CD34+ stem cells (CD34+CD44+, CD34+CD133+, CD34+CD31+, CD34+CD90+, CD34+EpCAM+, and CD34+OV6+) were sorted and injected into NOD/SCID/IL2rg mice. HLC were formed in mice from all 12 subpopulations. By
using flow cytometry, we found that the phenotype of the tumor cells produced by injecting CD34+ stem cells and its subpopulations were similar to tumours produced by injecting parental PLC. Hep Par 1 was expressed in a high percentage of parental PLC cells; however, only a few cells were positive for CK19 and CD68. The tumour cells produced from the injection of parental PLC or from the CD34+ stem cells both co-expressed Hep Par 1 and CK19 in a high percentage of the populations, showing the phenotype of combined hepatocellular cholangiocarcinoma (CHC). Interestingly, CD34+ double positive cells (except CD34+OV6+, and CD34+CD133+) expressed CK19 in a higher percentage of the populations when compared to those from the corresponding group for CD34+ negative for CD31, CD90, CD44, and EpCAM. Moreover, the tumour cells produced after the injection of either parental PLC or CD34+ stem cells expressed Kupffer cell (KC) markers: CD68, lysozyme, and CD14 in a very high percentage of the population, and did not express liver sinusoidal endothelial cell (LSEC) markers except CD31. Oval cells, LSEC and KC are all bone marrow-derived liver cells and express CD34; however, LSEC and KC express CD31, but oval cells do not. Moreover, oval cells are positive for OV6, whereas LSEC and KC are not. Thus, we were surprised by the finding that our putative LCSC were positive for OV6 and KC markers. In the adult liver, oval cells are thought to be an unhomogenous population of primary liver stem/progenitor cells, which are activated during progenitor-dependent regeneration. The isolated CD34+OV6+ population from parental PLC or cultured CD34+ stem cells also expressed CD68 as well as CD133, which is not expressed in normal liver cells, and did not express CD31, EpCAM, CD90, or CD44. Therefore, we conclude that the CD34+ stem cells that originated from CD34+OV6+ oval cells have KC characteristics, and function as LCSC with CD34+OV6+CD133+CD68+ stemness. The CD34+ double positive cells derived from these CD34+OV6+ cells show tumor initiation capacity and produce well-differentiated CHC. The tumor cells produced from injecting these CD34+ derived cells by serial transplantation co-expressed hepatocyte, cholangiocyte, and Kupffer cell markers as a homogenous population, and the xenografts yielded a well-differentiated CHC tumor with KC markers. This is the first report that a human CHC appears to be initiated and developed from CD34+ LCSC which have characteristics common to both oval cells and KC.

Poster Board Number: T-2070

PURIFICATION AND DEPLETION OF CANCER INITIATING CELLS CULTURED ON BIOMATERIALS HAVING NANOSEGMENTS

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Tumors contain a small subpopulation of cells, i.e., cancer stem cells (CSCs, cancer-initiating cells), which exhibit a self renewing capacity and are responsible for tumor generation. The cancer stem cells and not normal cancer cells persist in tumors as a distinct population, and cause relapse and metastasis by giving rise to new tumors. It is necessary to eliminate only a tiny subset of cells (0.0001-0.1%, cancer stem cells) that have the ability to generate a new tumour in cancer patients. If we succeed to develop biomaterials where CSCs are depleted or purified from tumor cells, it should be useful in clinical application. The purified CSCs can be used for the development of the specific anti-cancer drugs targeting only the cancer stem cells. We can save tumor patients with low side effects of medicine and avoid relapse and metastasis. On the other hand, the depletion of CSCs from tissue should be useful for the isolation of mesenchymal stem cells or bone marrow cells (hematopoietic stem cells) from patient tissue or blood. The mesenchymal stem cells or bone marrow depleting CSCs can be used for the stem cell therapy for the patients. Currently, surface markers and/ or gene expression of CSCs are unknown, although CD34+, CD44+, CD133+, CD166+, Sca-1, Lgr5, and Muc2, etc are suggested. There are several contrary data suggesting those surface markers and/ or genes are not specific to CSCs. The most promising method to quantify and identify CSCs is in vivo experiments where the sample cells are injected into mice subcutaneously, and to evaluate the tumour generation speed by the injection of the sample cells. In this study, several colon cancer cell lines (LoVo, Colo205, etc) and primary colon cancer cells from patients are cultured on tissue culture dishes (TCPs), extracellular matrix (ECM, collagen type I, fibronectin, vitronectin, or laminin) coated dishes, and pluronic-grafted dishes. The pluronic is polyethylene oxide (PEO)-polypropylene oxide (PPO)-PEO triblock copolymers where it is reported that hematopoietic stem cells efficiently preserved on pluronic-grafted dishes. It is found that tumour generation of colon cancer cells was enhanced after the colon cancer cells were cultured on ECM-coated dishes, which indicates CSCs are enriched. On the other hand, tumour generation of colon cancer cells decreased after culture of colon cancer cells on pluronic-grafted dishes. Remarkably, tumor generation did not observed when primary colon cancer cells were cultured on pluronic-grafted dishes, which indicates CSCs in primary colon cancer cells are depleted after culture on pluronic-grafted dishes. The cells from fat cancer tissues are cultured on pluronic-grafted dishes, and adipose-derived stem cells (ADSCs) were isolated from the fat cancer tissues. These cells did not generate tumor on mice, while the cells can differentiate into osteoblasts, which indicates ADSCs are safely isolated without contamination of CSCs after culture on the pluronic-grafted dishes. On the other hand, ADSCs isolated from fat cancer tissues, which cultured on conventional TCPs or ECM-coated dishes generated tumor on mice, which indicates ADSCs have contamination of CSCs when the fat cancer cells are cultured on TCPs or ECM-grafted dishes. It is concluded that the pluronic-grafted surface deplete cancer-initiating cells (CSCs) from colon cancer cell lines and primary cancer cells, while CSCs in colon cancer cells are enhanced by culture on conventional TCPs and ECM-grafted dishes promote CSCs.

Poster Board Number: T-2071

PROLIFERATION OF HUMAN PLURIPOTENT EMBRYONAL CARCINOMA STEM CELLS IS INHIBITED BY CO-CULTURE WITH THEIR DIFFERENTIATED PROGENY

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The 'cancer stem cell theory' proposes that undifferentiated tumour-initiating cells behave akin to stem cells to both self-renew and produce the differentiated cells that form the bulk of the tumour. A high proportion of undifferentiated cells is the main determinant in defining a high grade tumour with poor prognosis. In vivo, undifferentiated and differentiated tumour cell types reside in the same environment. Undifferentiated and differentiated cancer cells have been isolated from several tumour types and comparatively analysed in isolation. However, our understanding of how these different populations may interact in vivo is poor. This was addressed through PASCA (www.pasca.eu), a European Union 7th Framework funded project aiming to characterise the heterogeneous nature of cancers. Ntera2 human pluripotent embryonal carcinoma stem cells were retinoic acid-differentiated for one week. These cells were then co-cultured with undifferentiated Ntera2 cells at ratios of 1:1 or 9:1 to broadly model the in vivo tumour.
After co-incubation, undifferentiated and differentiated cells were separated via flow cytometry based on the expression of Stage Specific Embryonic Antigen 4 (SSEA4). Proliferating undifferentiated (SSEA4+) cells were expected to outgrow terminally differentiated (SSEA4-) cells unless inhibited by the co-culture. Differentiated and undifferentiated cells mixed at 1:1 and 9:1 ratios could be immediately correctly discriminated by flow cytometry to approximately 1:1 or 9:1 proportions based on SSEA4 expression. In 1:1 co-cultures, SSEA4+ cells outgrew SSEA4- cells over time as expected. However, two weeks of 9:1 co-culture was sufficient to substantially slow this proliferation of SSEA4+ cells. Co-culture with two-week differentiated cells amplified this inhibition, which now took place earlier. Next, the requirement for cell-cell contact in this inhibition was assessed. SSEA4+ cells were incubated with conditioned media from either SSEA4+ or SSEA4- cells. SSEA4+ conditioned media had little effect on the cells or their SSEA4 flow cytometry profile. However, after one week, cells treated with SSEA4- conditioned media had reduced proliferation and increased size compared to cells treated with SSEA4+ conditioned media. Additionally, incubation with SSEA4+ cell conditioned media shifted the SSEA4 profile between that of undifferentiated and differentiated cells. Collectively, this data indicates that differentiated cells communicate with and regulate the growth of their parental undifferentiated cell in a cancer stem cell model of tumour biology. Regulation requires an excess of differentiated cells and is amplified as differentiated cells mature. This effect is partially due to products secreted by differentiated cells but requires the presence of differentiated cells for maximum effect. Mechanistic analysis continues and will be reported upon. The intriguing question of whether embryonic stem cells are regulated by the differentiated cells in their in vivo environment remains.

Poster Board Number: T-2072

PS3 DEFICIENCY IN MESENCHYAL STEM CELLS CONTRIBUTES TO TUMOR DEVELOPMENT BY SHIFTING IMMUNE MICROMENVIRONMENT

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Tumor development is often associated with mutations in tumor suppressor genes. Most studies focus on the alterations by these mutations in tumor cells, while the changes in stromal cells are rarely investigated. Mesenchymal stem cells (MSCs) are continuously attracted to tumors and become a key component of the tumor microenvironment. Recent studies have demonstrated that MSCs play a critical role in regulating immune responses. However, whether mutations in tumor suppressor genes in MSCs could affect tumor progression is not known. We isolated MSCs from wild type and p53 deficient mice and investigated their effect on tumor growth. We found that wild type MSCs had no effect on tumor growth. MSCs with the deficiency in p53 significantly enhanced tumor growth in mice with normal immune system, but not in NOD/SCID mice. Moreover, neither p53 deficient MSCs nor wild type MSCs affected the proliferation of tumor cells, indicating that the tumor promoting effect on p53-/- MSCs is exerted through immune reactions. Furthermore, p53 deficiency in MSCs leads to elevated iNOS expression and nitric oxide production when cells were stimulated with inflammatory cytokines interferon-γ and tumor necrosis factor-α. This increase in NO production leads to more prominent immunosuppression, which could be abolished by iNOS inhibitor L-NMMA. Taken together, p53 deficiency in MSCs promotes tumor growth through inhibiting immune response. Modulation of p53 expression in stromal cells is a novel target for cancer therapy.
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Poster Board Number: T-2074
REGULATION OF CANCER STEM CELL ACTIVITIES BY IMMUNOLOGICAL NICHE

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Research background: Tumor microenvironments play a critical role in regulation of activities and survival of cancer stem cells (CSC) that are responsible for rendering tumors to resist various anticancer modalities. Recent studies have been unveiled that indicate tumor-associated macrophages (TAM) regulate tumor progression and metastasis, but it remains unclear whether interplay between cancer stem cells and immunological niche regulate CSC activities. Therefore, we evaluated the contribution of TAM in the regulation of tumorigenic activities and anticancer drug sensitivities of CSC.

Experimental results: We used several soluble/cell surface proteins, such as MFG-E8, IL-6, arginase-I, CCL-2, IDO, TIM-3, TIM-4 as functional markers produced from TAM because they are highly produced from TAM and play a critical role in triggering anticancer drug resistance and tumorigenesis. We verified in this study that CSC, but not other tumor subsets, could upregulate TAM-derived tumorigenic factors (referred as TAM-TF) from normal macrophages. Furthermore, in vitro adaptive cell transfer model revealed that TAM-TF are responsible for maintaining tumorigenic activities of CSC, such as self-renewal capacities and anticancer drug resistance. The pharmacological inhibition of several TAM-TF with neutralizing antibodies significantly reduced tumor burden and increased anticancer drug sensitivities of primary human and murine CSC in preclinical models. Through screening oncogenic signals responsible for TAM-mediated activation of cancer stem cells, we found that Stat3 and Hedgehog pathways are responsible for enriching and activating cancer stem cells in bulk tumor populations. Significance: We identified positive feedback mechanisms whereby CSC is responsible for converting normal macrophages into those with tumor-promoting activities. In turn, CSC-modified macrophages produced several tumorigenic factors that are critical to activate self-renewal and tumorigenic activities of CSC. The molecular targeting of these positive-feedback pathways provides the new therapeutic strategy to eradicate treatment-resistant tumors across the broad spectrums of different genetic and epigenetic alterations.

Germline Cells

Poster Board Number: T-2075
MIR-372/LET-7 AXIS IN HUMAN PRIMORDIAL GERM CELL SPECIFICATION

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The embryonic stem cell-specific cell cycle-regulating (ESC) microRNA (miRNA) family including miR-372 promotes while the let-7 family inhibits pluripotency. Here we show that during differentiation of human embryonic stem cells (hESC), somatic lineages activate let-7 and silence the ESC miRNA family. In contrast, induced primordial germ cells (iPGC) keep let-7 silenced while retaining expression of miR-372. Introduction of let-7 into differentiating hESC block iPGC specification, in part due to its ability of to suppress Pردm1. In contrast, miR-372 promotes differentiation of hESC into iPGCs by suppressing targets in multiple cellular processes including cell cycle, epithelial-mesenchymal transition (EMT), and epigenetic regulation. These same targets were shown previously to promote reprogramming of human fibroblasts to induced pluripotent stem cells (iPS) uncovering common pathways of miRNA regulation in these two transitions. These findings identify a novel role for the ESCC and let-7 miRNAs in regulating cell fate choice between somatic and germline lineages.

Poster Board Number: T-2076
DERIVATION OF POST MEIOTIC GERM CELLS FROM HUMAN EMBRYONIC STEM CELLS

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Investigating the mechanisms of human primordial germ cell (PGC) and gamete development is important for understanding the causes of infertility, effects of chemicals on reproductive development and cancer; and may eventually lead to new clinical applications. The aim of this study was to investigate a monolayer method to isolate PGCs and later germ cells. Shef hESC lines (Shef 2 and 4) were cultured on mouse embryonic feeders (MEFs) and allowed to undergo spontaneous differentiation in medium supplemented with retinoic acid. Differentiation to germ cells was monitored using cell surface markers and mRNA expression by Q-PCR. There are six novel gene meiosis marker being introduced to determine the differentiated primordial germ cells using Q-PCR. Fluorescent in situ hybridization (FISH) has been done to detect the cell undergoing meiosis with random probe. After 4-7 days in culture with retinoic acid, up to 20% of hESCs displayed a PGC phenotype as determined by antibody markers (ctk, SSEA-1, VASA, DAZL) although there was batch to batch variation. After 7 days of culture, there showed an increase of genes meiosis marker expression in culture supplemented with retinoic acid. The expression of germ cell-specific mRNA correlated with proportion of cells exhibiting appropriate surface marker. We manage to get a small population of cells undergoing meiosis using FISH. The cells were then re-cultured using neonatal mouse testis conditioned media (NMTCM) for 45 days. After Q-PCR analyses and staining with specific post-meiotic markers, we managed to identify the positive population of post-meiotic cells. The culture have been continued using NMTCM added with spermagenesis hormones: Follicle Stimulating Hormone, Luteinizing Hormone, and Testosterone for 15 days. The development of post meiotic cells were increased significantly using this approach.

Poster Board Number: T-2077
EVIDENCE OF PRIMORDIAL GERM CELL IN HUMAN OVARIAN ENDOMETRIOTIC LESIONS

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Endometriosis is a common gynecological disorder affecting 10% of all women. This pathology is characterized by dysmenorrhea, dyspareunia, pelvic pain, and infertility. In some cases, malignant transformation occurs in the lesion. Although this disorder was described in 1860, it still remains an enigmatic disease, mainly in its etiology. Sampson’s implantation theory is the most accepted explanation on the origin of endometriosis proposing that endometrial cells exfoliated during menstruation reflux through the uterine tubes, adhere to and proliferate at ectopic sites. Recently, the presence of stem cells in ovarian endometriosis was demonstrated; these cells could be involved in the progression of the disease and its malignant transformation. We propose that ovarian stem cells,
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lying in the gonad to renew the germinal mass, initially recruited to develop into primordial germ cells (PGC), act afterwards in the disease progression as a consequence of the hormonal environment of the endometriotic lesion. The main objective of this work was to search for the existence of PGC at ovarian endometrioma. Five samples were collected from patients, selected from those informed consent, according to the following criteria histopathological diagnosis of ovarian endometriosis, women aged 18 to 35 years old, normal menstrual cycle and no history of any hormonal therapy. As control samples we used endometrial tissue both in proliferative and secretory phase (n=2 for each). Samples were processed by immunohistochemistry and immunofluorescence for: DDX4, IFITM1, IFITM3, OCT4, SSEA4, Progesterone receptor (PGR), Estrogen receptor alpha (ESR1), PCNA and CD45. Germ cell markers IFITM3 and DDX4 were strongly immunostained in clock face-arranged cells with central nucleolus. These cells also displayed nuclear signal for ESR1, PCNA and SSEA4 but were negative for PGR, OCT4 and CD45. Positive cells for PGR, ESR1, OCT4 and PCNA were found in the stroma of all the endometriotic lesions and endometriotic control samples. The expression of PGR, DDX4, IFITM1 and IFITM3 was analyzed by RT-PCR from formalin-fixed paraffin embedded tissue. DDX4, IFITM1, and IFITM3 mRNAs were expressed in all endometriotic samples but not in endometrial tissues. The presence of DDX4, IFITM1 and IFITM3 indicates the existence of PGC in ovarian endometriotic lesions. Additionally, the expression of ESR1 suggests that these cells were probably recruited due to estrogenic stimulation from ovarian stem cells, with loss of OCT4 activity while maintaining the expression of SSEA4. These results outline ovarian endometriosis as a model for studying the renewal of the germlinal mass in the female gonad.

Poster Board Number: T-2078

FUNCTIONAL ANALYSIS OF PIWIL1 IN COMMON
MARMOSET

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Piwi-interacting RNAs (piRNAs) are a distinct class of germel-specific small non-coding RNAs, piRNAs associate with germ line-specific Argonaute proteins, or PIWI proteins, to form piRNA-induced silencing complexes (piRISCs) and function in transposon silencing. In mice, MIWI (PIWIL1), MILI (PIWIL2) and MIWI2 (PIWIL4) are classified as PIWI proteins. Mutations in the Piwi genes cause de-repression of transposons in testes and male sterility. Interestingly, however, each PIWI protein shows distinct expression pattern through development. MILI expression can be observed starting from primordial germ cells to round spermatids. MIWI expression is found in pathytheine spermatocytes to round spermatids. MIWI are only found from E12.5 to 3 days after birth in G1-phase arrested gonocytes. In embryonic testis, MILI and MIWI2 are localized in the pi-bodies and piP-bodies, respectively, both of which are known to be nuage, germ line-specific, high electron dense granules appearing around the nucleus. On the other hand, in adult testis, MIWI and MILI co-localize in pi-bodies. In addition, each PIWI associates with different piRNA populations. Evidence has shown that the piRNA pathway associates with DNA remethylation in the transposable elements and also in the paternal imprint loci in the gonocytes, like Dnmt3l mutants. Thus, the PIWI-piRNA pathway in mice is essential for spermatogenesis. Unlike rodents, primates including humans encode four members of the PIWI genes (through PIWIL1 to PIWIL4). This may suggest that the PIWI-piRNA-mediated silencing mechanism in primates differs from that in rodents. However, the PIWI-piRNA pathway remains to be elucidated. Here, we study the primate PIWI-piRNA pathway in common marmoset, Callithrix jacchus. We produced a monoclonal antibody against marmoset PIWIL1 (an ortholog of MIWII), which we named as MARWI. Immunofluorescence analysis showed that MARWI is expressed in spermatocytes to round spermatids in the juvenile and adult testis, but not in the neonatal testis. Furthermore, immunoelectron microscopic (iEM) analysis revealed that MARWI is localized nuage-like, high electron dense granules in the cytoplasm. Immunopurified MARWI associates with small RNAs of ~30 nucleotides. Currently, we are engaged in determining sequences of MARWI-associated piRNAs. We will report characterization of MARWI-associated piRNAs.

Poster Board Number: T-2079

DERIVATION OF PRIMORDIAL GERM CELLS FROM
MOUSE EMBRYONIC STEM CELLS IN A
GROUND STATE PLURIPOTENCY.

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Recently, successful derivation of germ cells which showing capacity of fertilization has been reported. However the successful rates of germ cells are not uniform between each study. In order to apply the technology of in vitro germ cell production for research of infertility or clinical treatments, development of the method with high reproducibility is essential. Now, it has been demonstrated that the mouse embryonic stem cells (mESCs) show heterogeneous populations, in which the gene expressions could be varied in each individual cell. This characteristic of the mESCs could lead to susceptibility by culture conditions in undifferentiated status, and it also could result in low reproducibility when the cells are induced differentiation into germ cell lineage. In this study, we first produced a mES cell line containing Oct4deltaPE-GFP construct, which enables monitoring primordial germ cell derivation by GFP expression, cultured it in the condition administered with a GSK3b specific inhibitor CHIR99021 and a MEK specific inhibitor PD0325910 (referred as 2i), which forcibly stabilized the mESC pluripotency in a ground state (referred as 2i-ESCs), and then efficiency of the germ cell differentiation was evaluated following spontaneous differentiation. When observed at day-9 from differentiation induction, increasing GFP-positive fractions that expressed Mvh and Stella were observed in the derivative cells from the 2i-ESCs. However, in the derivatives from control ESCs without 2i treatment at this time-point, rates of the GFP-positive fraction were significantly varied, and marker gene expressions in it were significantly lower than that of the derivatives of the 2i-ESCs. When transplanted the day-9-derivative cells from the 2i-ESCs into seminiferous tube of B6WB-W/Wv mice, successful engraftment and contribution into the region of a spermatogonia-stage cells could be observed. These results suggested that the preliminary treatment of the ESCs in the 2i-added condition to induce ground state pluripotency could improve the efficiency of the method for germ cell differentiation induction.
In early germ cell development, primordial germ cells (PGCs) are should be regulated by transcriptional and post-transcriptional level with tight spatial and temporal control for the unique characteristics distinguishing themselves from other somatic cells. MicroRNAs (miRNAs) are small non-coding RNAs, being between 18~25-nucleotides length, that post-transcriptionally regulate transcripts and function by binding with their target mRNA through base-pairing with the 3'untranslated region (3'UTR). Moreover, in mouse, it has been known that miRNA biogenesis is critical for the PGCs development. However, the complex networks of developmental processes especially in miRNA are less understood in chicken PGCs. Thus, we identified specifically expressed miRNAs at the post-transcriptional levels in chicken PGCs and investigated a PGC-specific miRNA and its function. Here, we generated microarray analysis for miRNA expression by 6-day embryonic primordial germ cells and gonadal somatic cells (GSCs) sorted by FACS. Based on microarray, we identified ten miRNAs that are highly expressed in PGCs. Among them, miR-181a* showed the most significant expression in PGCs development. Interestingly, by repression of miR-181a*, we observed a decrease in Erk and Akt phosphorylation pathway as indicated by increase of Erk and Akt phosphorylation. Spermatogonia differentiation does not proceed beyond the prophase of the first meiotic division due to massive apoptosis of these germ cells. In our study, we have demonstrated that miRNA contribute to modulating differentiation and maintaining of undifferentiated characteristics in PGCs.

**Poster Board Number: T-2080**

**MIRNA REGULATION ON THE UNDIFFERENTIATED STATE OF CHICKEN PRIMORDIAL GERM CELLS**

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The successful cultivation of germline stem cells (GSCs) from testis has opened up the possibility of the molecular analyses of mammalian spermatogonial stem cells. In contrast, primordial germ cells (PGCs) transform to the pluripotent state in culture, which has prevented a detailed investigation of their properties. This study demonstrates the in vitro expansion of presumptive PGCs derived from mouse induced pluripotent stem cells (iPSCs), without the re-acquisition of pluripotency and tumorigenicity. The cells expressed both migratory and postmigratory PGC marker genes, and retained the parental genomic imprint. Interestingly, the imprint was erased in the neonatal testicular environment, suggesting that the developmental potential of the iPSC-derivatives reflected that of PGCs which had colonized the genital ridge. The induction and propagation of cells with PGC characteristics should serve as a conventional strategy for understanding mammalian germ cell development in the fetal gonad.

**Poster Board Number: T-2082**

**IN VITRO PROPAGATION OF PRESUMPTIVE GERM CELLS WITH PRIMORDIAL GERM CELL CHARACTERISTICS DERIVED FROM MOUSE INDUCED PLURIPOTENT STEM CELLS**

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Dazl is a key regulator of pluripotency in the germline

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Embryonic germ cell lines (EGCs) derived from primordial germ cells (PGCs) are known to exhibit hallmarks of pluripotency, including teratoma and chimera formation. This potential is maintained from E8.5 up to E12.5 in mouse development, at which the PGCs begin to undergo sexual differentiation. Dazl orthologues are essential for oogenesis and spermatogenesis across vertebrate species, and in mice, Dazl was found to be important in multiple steps of germ cell development, although the underlying molecular mechanisms are still unclear. Dazl belongs to the DAZ family, which is comprised of three mammalian members: DAZ (Deleted in Azosperma), Dazl and Boll (Drosophila boule-like). Each of these members contains an N-terminal domain with RRM motifs, which presumably interact with RNAs, and a C-terminal domain containing one or multiple DAZ motifs with unknown molecular function. Previous biochemical studies of Dazl were restricted to...
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adult testicular lysates, as the inaccessibility and paucity of the primordial germ cells (PGCs) and gonocytes present a major hurdle to our understanding of early germ cell development. We have developed employed an in vitro germ cell differentiation system to study the protein-RNA and protein-protein interactions of Dazl in early PGC development. We have identified mRNA transcripts that interact directly with Dazl and have found that Dazl specifically regulates the expression of core pluripotency factors, during in vitro as well as in vivo PGC development. We hypothesise that Dazl functions as a molecular switch, limiting germline pluripotency and thus allowing the initiation of meiosis.

Poster Board Number: T-2084
ELUCIDATION OF THE PRIMARY PIRNA PATHWAY IN DROSOPHILA OVARIAN SOMATIC CELLS
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PIWI proteins, germline-specific members of Argonaute family, function in RNA silencing in the gonad by associating specifically with PIWI-interacting RNAs (piRNAs). piRNAs arise mainly from intergenic repetitive elements including transposable elements (TEs) and their remnants. The PIWI-piRNA complex therefore is able to silence parental TEs, and also other genes when they show complementarities to the piRNA sequences. Without the functions of PIWI proteins and piRNAs, TEs become active, which has great potential to injure the genome; thus, both PIWI proteins and piRNAs are essential for maintenance of the genomic integrity and germ cell development. piRNAs in Drosophila ovarian somatic cells (OSCs) are generated only through the primary processing pathway and are loaded onto Piwi, a member of PIWI proteins. We have previously shown that primary piRNA biogenesis occurs through cytoplasmic granules called Yb bodies in OSCs. Both Armitage (Armi) and fs(1)Yb (Yb) proteins, core components of Yb bodies, are required for piRNA biogenesis. Armi guides nascent Piwi to Yb bodies by bridging the two proteins. This association leads the Armi-Piwi complex to be localized to Yb bodies. Recently, we found that Armi and Yb individually associate with piRNA intermediates and that Yb is required for assembly of the Armi-piRNA intermediate complex, further indicating the functional relevance of Armi/Yb in the piRNA biogenesis. We also found that Zucchini (Zuc), a member of the phospholipase D family, is required for primary piRNA biogenesis. Zuc localizes to mitochondria and has a non-redundant role in Yb body formation. To further understand the molecular functions of Yb bodies, we performed mutation analysis of Yb, a putative RNA helicase. A point mutation introduced in the Yb helicase ATP-binding site affected Yb body formation and primary piRNA production, suggesting that the interaction between Yb and putative piRNA precursors is a crucial step in the primary piRNA pathway. We also performed RNA fluorescence in situ hybridization (RNA FISH) to determine the intracellular localization of piRNA precursors. A piRNA cluster, flamenco (flam), gives rise to abundant piRNAs in OSCs. An RNA FISH probe recognizing the flam transcript detected dot-like flam signals in the cytoplasm. Double staining with anti-Yb antibodies showed that the flam signals were often localized adjacent to Yb bodies. These results suggest that primary piRNA precursors (or intermediates) are stored near Yb bodies until piRNA maturation finally occurs in Yb bodies. We are currently investigating how the flam signals in OSCs are affected by depletion of Armi, Piwi, Zuc and Yb. Pluripotent stem cells can differentiate early germ cells in vitro, providing a model to study germ lineage differentiation and a potential avenue to generate gametes from infertility donors. However, differentiation beyond early germ cell stages is challenging and requires the three-dimensional testicular environment. Isolated testicular somatic and germ cells are able to recapitulate testicular morphogenesis and form a functional germ line stem cell niche upon transplantation into an immunodeficient mouse; however, currently the efficiency of de novo formed tissue supporting spermatogenesis is low and needs to be improved to allow study of the stem cell niche, testis development and to support spermatogenesis. This study tested the hypotheses that enrichment of spermatogonia prior to grafting or the presence of a scaffold would improve the formation of seminiferous tubules supporting spermatogenesis. Methods: Cells were harvested from the testes of 1 week old piglets by two step enzymatic digestion followed by a 3-step differential adhesion culture over 72 hours to attain germ cell enrichment. Cell recovery and viability were recorded and immunocytochemistry for UCH-L1 and vimentin was used to identify germ cells (UCH-L1 +, vimentin -) and somatic cells (UCH-L1-, vimentin +). The enriched germ cell population (48.7±4.2%) was combined with the initial cell suspension in six different groups and grafted under the dorsal skin of castrated SCID mice (6 mice with 1 pellet per treatment group). Group 1: 5x106 cells containing 5% spermatogonia; Group 2: 50x106 cells containing 25% spermatogonia; Group 3: 50x106 cells containing 5% spermatogonia in Matrigel with reduced growth factor; Group 4: 10x106 cells containing 5% spermatogonia; Group 5: 10x106 cells containing 25% spermatogonia in Matrigel; Group 6: 10x106 cells containing 25% spermatogonia. The degree of reconstitution of spermatogenic tissue was assessed by immunohistochemical localization of UCH-L1 and vimentin in all cross sections of tubular structures formed 24 weeks after transplantation. Results: OSC enrichment and the presence of the scaffold providing anchorage to the cells contributed to increased tubule formation and tubules supporting germ cell development when compared to Group 1 (Control group). Total number of de novo formed tubules and percentage of tubules supporting spermatogenesis per group was Group 1: 53.6±63.0, 20.8±9.9%; Group 2: 193.5±245.8, 35.1±24.3%; Group 3: 370.0±468.8, 36.4±13.8; Group 4: 122.6±23.8, 36.6±8.6%; Group 5: 89.3±92.2, 18.3±21.1%; Group 6: 61.3±40.6, 33.5±20.3, respectively. Despite the large variability encountered in the recovered newly formed tissue from all groups, the overall results supported the hypotheses. Therefore, enrichment of germ cells and presence of an extracellular matrix scaffold improve the efficiency of this bioassay. Given the high number of cells required to attain enrichment of the initial cell population the use of treatment 2 may be impractical, but our results indicate that the presence of MRGF can overcome germ cell loss and promote sufficient support to improve tubule formation. Consequently, this approach will provide an efficient bioassay system to investigate germ cell differentiation from germ line stem cells as well as from pluripotent cells.
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**Poster Board Number: T-2086**

**HISTONE H3K27 DEMETHYLASE JMJD3 REGULATES THE TURNOVER OF SPERMATOGONIAL STEM CELLS.**

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During spermatogenesis, epigenetic modifications of the genome, such as covalent modifications of histone residues, regulate chromatin structure and transcription and ensure appropriate gene activation and repression. Recent studies have provided evidence that modifiers of histone methylation are important for self-renewal and/or differentiation of stem cells. The renewable population of cells for spermatogenesis are the spermatogonial stem cells (SSCs). However, the roles of histone demethylases in spermatogenesis as well as spermatogonial stem cell renewal and differentiation remain elusive. We have discovered that histone H3 lysine 27 (H3K27) specific histone demethylase, JMJD3 (also known as KDM6B), is expressed higher in spermatogonial stem cells in culture than in neonate and adult testis. Expression of JMJD3 in spermatogonial stem cells is decreased by removal of glial cell line derived neurotropic factor (GDNF), an essential growth factor for both self-renewal of spermatogonial stem cells. Immunofluorescent staining showed that JMJD3 localizes to PLZF-positive spermatogonia. Tri-methylated and di-methylated histone H3K27 that are methylation targets of JMJD3 formed punctate foci in PLZF-positive spermatogonia. In addition, H3K27me3 increased in differentiating spermatogonial stem cells in culture. When JMJD3 levels were knocked-down in spermatogonial stem cells, spermatogonial stem cells, surprisingly, formed more colonies than control. Most colonies formed single, 2- and 4-aligned colonies when JMJD3 was knocked-down, whereas there are many 8-16 aligned colonies in the control culture. We also found some cells were not connected with neighboring cells, even if they were in same colony. To address the in vivo role of JMJD3 in spermatogonial stem cells, JMJD3 conditional knockout mice were generated. Unexpectedly, germ cell specific JMJD3 knockout mice were fertile and produced pups even at one year after mating. When spermatogonia in seminiferous tubule were visualized by PLZF staining, there were more single spermatogonia in JMJD3 deficient tubules than in control tubules. Cell surface marker analyses revealed that undifferentiated spermatogonia were increased in JMJD3 null testes. Molecular analyses showed that some genes expressed in undifferentiated spermatogonia as well as some genes expressed in differentiating spermatogonia were increased in the spermatogonia-enriched fraction from JMJD3-deficient testes. These results suggest that JMJD3 could regulate turnover of spermatogonial stem cells by repression of dedifferentiation of spermatogonial stem cells. We believe that our results provide further information on epigenetic regulation of stem cells as well as unique regulation of spermatogonial stem cells.

**Poster Board Number: T-2087**

**NICHE-DEPENDENT REGULATION OF GERM CELL DEVELOPMENT**

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The transmission of genetic information from one generation to the next depends on the production of functional male and female gametes, the sperm and egg, respectively. Primordial germ cells (PGCs) are the embryonic precursors of gametes and during development, interact with a continuously changing microenvironment or “niche”. The ability to generate PGCs and subsequently differentiate them to functional gametes in vitro has been challenging, and identification of niche signals that regulate key maturation steps in PGC development may facilitate germ differentiation in vitro. Previous attempts to differentiate embryonic stem cells (ESCs) into germ cells suggest that development in embryoid body culture reaches the equivalent of a migratory e9.5 PGC by gene expression and epigenetic profiling. Using ESCs carrying the Oct4(APE)-GFP transgene, we similarly obtained a PGC-like (esPGC) population that was highly enriched for transcripts of PGC-specific genes Stella, Blimp1, and Nanos3, but late PGC genes Dazl, and Mvh were low at the transcript level and absent by immunostaining in this population. Interestingly, Dazl and Mvh upregulation in esPGCs did not result even after prolonged embryoid body culture, suggesting that this microenvironment cannot support the completion of PGC differentiation. In an attempt to drive further PGC differentiation, we co-cultured esPGCs with somatic gonad & mesenchephos cells (SGM) from embryos. After 3 days in co-culture, esPGCs up-regulated both DAZL and Mvh, indicating that differentiation of esPGCs to mature PGCs is possible and dependent on the presence of SGM cells. In vivo, migratory PGCs arrive at the developing SGM beginning ~e10.5, which is followed by the upregulation of DAZL and Mvh. To determine whether expression of these PGC maturity markers requires interaction with developing gonads, we cultured PGCs isolated from e9.75 and 10.5 embryos in the absence of somatic cells. After 12 or 24 h of culture, DAZL immunostaining could be detected in 54% of PGCs isolated at e10.5, but only 21% of e9.75 PGCs, suggesting that the SGM environment confers PGC competence or directly induces expression of mature markers in vivo. Through analysis of gene expression in PGCs and SGM, we have identified candidates for these required maturation cues from the SGM microenvironment, and are screening these candidates in embryo-derived as well as esPGCs. Together, the results indicate an important role for supporting niche cells in regulating PGC development both in vivo and vitro.

**Hematopoietic Cells**

**Poster Board Number: T-2088**

**ERYTHROPOIETIC POTENTIAL IN HUMAN CORD BLOOD AND ADULT PERIPHERAL BLOOD FOR TRANSFUSION**

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Blood transfusion is the process of receiving blood products into one’s circulation intravenously. There are many patients around the world whose survival depends on safe blood transfusion. Nevertheless blood transfusion saves lives, but the transfusion of donated unsafe blood puts lives at risk because of HIV, hepatitis or other infections. As a countermeasure, hematopoietic stem cells from several human organs can be used for certain therapeutic uses as alternative safe blood source. There are many productive manuals to obtain mature RBCs from CD34+ hematopoietic stem cells via in-vitro culture. Among them human cord blood (CB) and adult peripheral blood (PB) are common adult stem cells for allogeneic transplantation. Owing of their largest, easily availability, addition of which contains large amounts of stem cells can be used to induce RBC maturation and transplantation. Our present study focused on comparison of CB and PB derived stem cell characterization and function test on in-vitro culture system. For experiment

**Poster Board Number: T-2087**

**NICHE-DEPENDENT REGULATION OF GERM CELL DEVELOPMENT**

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The transmission of genetic information from one generation to the next depends on the production of functional male and female gametes, the sperm and egg, respectively. Primordial germ cells (PGCs) are the embryonic precursors of gametes and during development, interact with a continuously changing microenvironment or “niche”. The ability to generate PGCs and subsequently differentiate them to functional gametes in vitro has been challenging, and identification of niche signals that regulate key maturation steps in PGC development may facilitate germ differentiation in vitro. Previous attempts to differentiate embryonic stem cells (ESCs) into germ cells suggest that development in embryoid body culture reaches the equivalent of a migratory e9.5 PGC by gene expression and epigenetic profiling. Using ESCs carrying the Oct4(APE)-GFP transgene, we similarly obtained a PGC-like (esPGC) population that was highly enriched for transcripts of PGC-specific genes Stella, Blimp1, and Nanos3, but late PGC genes Dazl, and Mvh were low at the transcript level and absent by immunostaining in this population. Interestingly, Dazl and Mvh upregulation in esPGCs did not result even after prolonged embryoid body culture, suggesting that this microenvironment cannot support the completion of PGC differentiation. In an attempt to drive further PGC differentiation, we co-cultured esPGCs with somatic gonad & mesenchephos cells (SGM) from embryos. After 3 days in co-culture, esPGCs up-regulated both DAZL and Mvh, indicating that differentiation of esPGCs to mature PGCs is possible and dependent on the presence of SGM cells. In vivo, migratory PGCs arrive at the developing SGM beginning ~e10.5, which is followed by the upregulation of DAZL and Mvh. To determine whether expression of these PGC maturity markers requires interaction with developing gonads, we cultured PGCs isolated from e9.75 and 10.5 embryos in the absence of somatic cells. After 12 or 24 h of culture, DAZL immunostaining could be detected in 54% of PGCs isolated at e10.5, but only 21% of e9.75 PGCs, suggesting that the SGM environment confers PGC competence or directly induces expression of mature markers in vivo. Through analysis of gene expression in PGCs and SGM, we have identified candidates for these required maturation cues from the SGM microenvironment, and are screening these candidates in embryo-derived as well as esPGCs. Together, the results indicate an important role for supporting niche cells in regulating PGC development both in vivo and vitro.
we obtained five cases of each CB and PB from normal human with informed consent. Selected CD34+ cells were cultured and charac-
terized by common Giemsa staining, flow cytometry assay, quan-
titative PCR and hemoglobin electrophoresis; we also confirmed their functions using Hemox Analyzer. Our results from the study clearly reports that, CB and PB derived CD34+ hematopoietic stem
cells have similar character in same culture conditions, however
they show definitely differences in various gene expression level
and final hemoglobin development. The results support the idea
that CB and G-CSF mobilized PB possess different erythropoietic
potential in-vitro culture system williess they express nearly same
characters in some test events.

Poster Board Number: T-2089

CLINICAL OBSERVATION OF 24 CASES OF PATIENTS UNDERGOING AUTOLOGOUS PURIFIED BLOOD STEM CELL CD34+ FOR TREATMENT OF ANKYLOSING SPONDYLITIS

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Background: Ankylosing spondylitis (AS) is one kind of chronic and
progressing disease which can relate to the central axis joints and
cause arthropathy and mainly affect pelvic sacroiliac joint, vertebral
progressing disease which can relate to the central axis joints and
vertebral column joint and latero-vertebral tissues and is very difficult to
be restrained. Objectives: In order to find a new way to treat AS, we
use purified autologous peripheral blood stem cell CD34+ as treating
method and evaluate its effect. Methods: From November 2008
To October 2011, we collected 24 patients with AS, whose condi-
tions were all consistent with the New York AS diagnostic criteria
in 1984. We got the agreement with patients and his or her family.
There were 19 male and 5 female patients. The patients' ages were 20-40,
median 31.5. The courses of disease had been 3 months
to 25 years. In the 24 patients, 20 cases were HLA-B27 positive.
4 cases were HLA-B27 negative, and 15 cases had been treated with
methotrexate, sulfasalazine, diclofebac sodium without good effect
before our treatment. (1) The preparation of purified autologous
peripheral blood stem cell CD34+: We added recombinant human
granulocyte stimulating factor(G-CSF, made in Northern China)
150μg-300μg/d for 3 days, hypodermic. After G-CSF mobilizing, using
COBE machine, we collected peripheral blood 100ml, then from
the blood purified CD34+ stem cells by CliniMACS machine, made
the CD34+to (1.0-10.6) ×107, and the positive rate 85 per cent
tested by flow cytometry.(2)Treatment method: Took the purified
CD34+ stem cells about (1-3)×107, volume 40ml, infused separately
into the patients' double sides sacroiliac joint cavities (each for
5ml) and along the patients’ two sides of erector muscle of spine
in punctiform injected about 30ml . After treatment we instructed them
to enhance article functional training. Except 5 patients
were still orally administrated small amount of diclofenac sodin,
sulfasalazine,all the other patients did not use any medicine. Re-
sults: Following up to visit the patients for 3-26 months, we found
the patients that mainly in the cervical part, the waist, the sacroiliac
joints appeared easement of pain, in vertebral column the active
limits were expanded and the consciousness turned much better
than before. Before treatment, 24 cases of patients BASDAI:1.8-13.8,
mean value 4.46±2.66, BASFI:8-78 points,mean value 28.67±18.25.
After treatment , following up for 3 months, we found that
the 24 cases of patients BASDAI:0.4-4.5, mean value 2.47±1.30;
BASF1:2-66 points,mean value 14.29±13.59. We applied EXCELT-TEST
software procedure to do matched-pairs analysis, all the P values
were less than 0.01, post-treatment was obviously better than
prior-treatment.Imaging test of the vertebral column, sacroiliac
joints did not show clearly change between post- treatment and
prior-treatment. Following up for more than half a year, 14 cases of
patients, we found the above index was still stable. Following up
for more than a year, we found, 4 cases patients that the disease
states were still in stable phase, 20 cases HLA-B27 positive without
turning to negative. After treating all the 24 patients for 3 months,
we tested their blood routine, liver function and kidney function all
appearing normal and no other bad reaction. Conclusion: That us-
ing purified autologous peripheral blood stem cell CD34+ to treat
the patients with AS could relieve the patients’clinical symptoms,
improve their life quality, and would be a safe and effective method
for treating AS.

Poster Board Number: T-2090

DETERMINING THE EFFICACY OF HUMAN-TRIM5-
CYP IN INHIBITING A BROAD SPECTRUM OF HIV
VIRUSES WHEN STABLY EXPRESSED IN CD34-
DERIVED MACROPHAGES

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Hematopoietic stem cells genetically modified to express anti-HIV
genes can repopulate the immune system, replacing HIV target
cells such as CD4+ T lymphocytes and macrophages. If such cells
express therapeutic genes that halt early steps in the viral life cycle,
HIV replication can be suppressed prior to reverse-transcription
and integration. The HIV host restriction factor, huTrimCyp, was
recently developed and does not occur naturally in humans [1]. It
is believed that huTrimCyp inhibits HIV shortly after viral entry by
binding to capsid proteins and sequestering them for degradation
[2]. My preliminary data indicates that T-cell lines transduced with
a lentivirus vector expressing huTrimCyp showed no short term or
long term cytotoxicity, as well as exhibited a stable gene expression
for a period greater than six weeks in vitro. Furthermore transduced
T-cell lines showed an inhibition of R5- tropic HIV strain greater
than 5 fold when compared to cells expressing 2sGreen alone as
determined by flow analysis of HIV-reporter infected MOLT4 T-cell
line. To that end, I have initiated experiments in CD34+ hematopo-
ietic stem cells in order to determine the feasibility of pursuing
huTrimCyp as an anti-HIV gene therapy candidate in vivo. I will also
investigate the ability of vector transduced HIV target cells (e.g. CD4
t lymphocytes, macrophages) to resist infection and inhibit viral
replication in vitro, using X4- and R5-tropic strains of HIV. 1.Neagu,
M.R. et al. Potent inhibition of HIV-1 by TRIM5-cyclophilin fusion
proteins engineered from human components. J. Clin. Invest. 119,

Poster Board Number: T-2091

CHARACTERIZATION OF DENDRITIC CELLS
INDUCED FROM HUMAN CORD BLOOD
HEMATOPOIETIC STEM CELLS IN THE SERUM-FREE
CONDITION

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Dendritic cells (DCs) are the most powerful antigen presenting cells
(APCs) and play a pivotal role in initiating the immune response,
which differentiated from CD133+ hematopoietic stem cells (HSCs).
Hence, we used the ex vivo expanded of hematopoietic stem cells
as a source of DCs, and developed the optimal DCs induction
medium. In the previous study, we had developed a serum-free he-
Liver transplantation is the only effective treatment for decompensated liver cirrhosis. Several factors, such as non-availability of donors, operative-risks, complications associated with rejection, usage of immunosuppressive agents, and high cost of treatment, make this strategy available to only a few people. Hepatic progenitor stem cell transplantation (HSCT) using human fetal liver-derived stem cells have been shown to be a good alternative to manage end-stage liver diseases. In this retrospective study, we investigated safety and efficacy of HSCT by monitoring the T-cell, NK-cell and cellular immune response following transplantation in case of decompensated liver cirrhosis.

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Lever transplantation is the only effective treatment for decompensated liver cirrhosis. Several factors, such as non-availability of donors, operative-risks, complications associated with rejection, usage of immunosuppressive agents, and high cost of treatment, make this strategy available to only a few people. Hepatic progenitor stem cell transplantation (HSCT) using human fetal liver-derived stem cells have been shown to be a good alternative to manage end-stage liver diseases. In this retrospective study, we investigated safety and efficacy of HSCT by monitoring the T-cell, NK-cell and cytokines which play major role in cellular immune response and rejection of chronic decompensated liver cirrhosis patients. A total of 5 patients with decompensated liver cirrhosis were enrolled in the study. After giving human fetal liver-derived EPCAM positive cell transplantation, T-cell (CD3, CD4 and CD8), NK-cells (CD16) by flowcytometry and cytokine levels (IL-2, TNFα, IFNα, IFNβ and IFNγ) by ELISA were monitored four times within a month. Present study demonstrated that after HSCT patient showed marked clinical recovery and decline in the MELD score and there was no significant variation found in cell mediated response and cytokine levels between pre and post transplantation. Hence this preliminary study demonstrated human fetal liver-derived EPCAM positive stem cell transplantation is safety for end stage liver cirrhosis.

Poster Board Number: T-2093

INTEGRATION-FREE HUMAN INDUCED PLURIPOTENT STEM CELLS FROM PERIPHERAL BLOOD MONONUCLEAR CELLS

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Human induced pluripotent stem cells (iPSCs) can be derived from somatic cells by ectopic expression of key factors and are highly similar to human embryonic stem cells (ESCs). This discovery led to the generation of patient-specific iPSCs which have unprecedented value for regenerative medicine, disease modeling and drug screening. However, iPSC derivation commonly utilizes skin fibroblasts, which involves donor skin biopsy and is time-consuming. In addition, human skin fibroblasts may have been exposed to UV irradiation and have increased risk of harboring genetic abnormalities. Sampling of blood is one of the least invasive but most routinely performed clinical procedures, making it an ideal cell source for reprogramming. Additionally, blood cells that are derived from hematopoietic stem cells (HSCs) in marrow before being released into circulation may be less exposed to environmental mutagens. Of the types of blood cells used for reprogramming, CD34+ blood cells come to mind first because they are enriched for HSCs and have high proliferation potential. However, CD34+ blood cells are very rare and the purification is cumbersome. Another concern is that they may not be feasible for certain diseases like aplastic anemia in which patients have a shortage of CD34+ blood cells. Thus the generation of iPSCs from a small amount of peripheral blood mononuclear cells (PB MNCS), without need of fractionation, would be a big leap. iPSCs can be established from terminally differentiated circulating T cells expanded from one milliliter of whole blood. However, the pre-existing T cell receptor rearrangement may limit iPSC differentiation ability to the full spectrum of cell linages. Other researchers also found mice generated from T cell reprogrammed by human bone marrow cells for generating GMP-compliant iPSC lines for clinical applications. The data showed reprogramming efficiencies were in similar level although they were ~50x less than CB cell types. iPSCs derived from two starting colonies may not be feasible for certain diseases like aplastic anemia in which patients have a shortage of CD34+ blood cells. Thus the generation of iPSCs from a small amount of peripheral blood mononuclear cells (PB MNCS), with no need of fractionation, would be a big leap. iPSCs can be established from terminally differentiated circulating T cells expanded from one milliliter of whole blood. However, the pre-existing T cell receptor rearrangement may limit iPSC differentiation ability to the full spectrum of cell linages. Other researchers also found mice generated from T cell somatic nuclear transfer are prone to lymphomagenesis, which may complicate the usage of T cells as a reprogramming source. To find an alternative, we chose a condition to stimulate myeloid-erythroid growth from un-fractionated MNCS. After 8-9 days of culture, cells were under proliferative status, showed erythroblast antigen expression and were enriched for colony-forming erythroid progenitor cells (erythroblasts). To assess the reprogramming potential of expanded erythroblasts, we first compared them with cord blood (CB) CD34+ cells using both retroviruses or episomal vectors. Similar high efficiencies of iPSC derivation were achieved from either cell type. Hundreds of TRA-1-60+ colonies can be identified from one million starting cells in just two weeks by either type of vectors. To be more definitive, we reprogrammed CD34+ cells and expanded erythroblasts from the same adult donor by episomal vectors. The data showed reprogramming efficiencies were in similar level although they were ~50x less than CB cell types. iPSCs derived from two starting blood cell sources are both indistinguishable from ESCs with respect to cell morphology, expression of surface markers, and the ability to differentiate in vitro and in vivo. Detailed analysis also indicated they are integration-free. Characterization of gene expression profile, epigenetic signature and re-differentiation capability to blood cells is currently underway and will be discussed during the meeting. Taken together, our data suggest that erythroblasts from a small volume of PB MNCS are suitable for reprogramming by integration-free episomal vectors, and may provide an advantage for generating GMP-compliant iPSC lines for clinical applications.
we show that Fev is an important regulator of HSC development in vitro either from embryonic or induced pluripotent stem cells has not yet been successful. Therefore, it is critical to fully understand molecular mechanisms of HSC development in vivo. Recent evidence suggests that ETS transcription factors play important roles in HSC development and functions. Fev (Fifth Ewing Variant) is a member of the ETS family and required for the differentiation and maintenance of serotonergic neurons in zebrafish and mammals. However, its role in HSC development remains to be explored. Here we show that Fev is an important regulator of HSC development in vertebrates. In fev-deficient zebrafish embryos, HSCs were compromised and fewer T cells were found in the thymus. Genetic analysis and chemical treatment experiments support a mechanism whereby Fev regulates HSC development through a novel Foxc1-ERK pathway. Moreover, experiments with human cord blood show that Fev is also required for the maintenance and expansion of primitive HSCs/progenitor cells in human, indicating its conserved role in higher vertebrates. Finally, we demonstrated that Fev function in HSCs is distinct from its role in serotonergic neurons in both zebrafish and mouse systems. Our data indicate that Fev-Foxc1-ERK signaling is essential for haemogenic endothelium-based HSC development.

Poster Board Number: T-2096

DEPLETION OF HUMAN HEMATOPOIETIC STEM CELLS WITH A HUMANIZED ANTI-HUMAN CD117 ANTIBODY: A NON-TOXIC CONDITIONING REGIMEN FOR ALLOTRANSPLANTATION

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Allogeneic hematopoietic cell transplantation (AHCT) is an important treatment modality for many malignant and non-malignant hematologic disorders. Most patients who undergo AHCT are prepared with cytotoxic chemotherapy and/or radiation to eliminate barriers to donor engraftment. For patients with malignancies, toxic conditioning agents also play a role in eradicating residual cancer cells. For patients with non-malignant disorders, conditioning regimens with no or little toxicity would be preferable. Our group previously demonstrated that an anti-CD117 (c-kit) monoclonal antibody (mAb) eliminates endogenous hematopoietic stem cells (HSC) and facilitates engraftment of purified donor HSC in a mouse model of severe combined immunodeficiency (SCID) (Czecowicz et al., Science, 2007). Patients with SCID are highly susceptible to life-limiting infections, and while AHCT is the only effective therapy for most forms of SCID, treatment-related sequelae provide an urgent need to improve methods for achieving donor HSC engraftment. Based on our pre-clinical model, we believe anti-CD117 mAb therapy would be ideal, minimally-toxic conditioning regimen for AHCT in SCID children. To that end, we identified a humanized anti-human CD117 mAb (anti-hCD117) as a potential candidate for clinical application in AHCT preparation. When human cord blood or bone marrow derived HSC (Lin-CD34+CD38-CD90+CD45RA-) were cultured in serum-free media with Flt3 ligand, stem cell factor, thrombopoietin, IL-3, and IL-6, profound inhibition of cell division was observed in the presence of 0.1 - 100 µg/mL anti-hCD117. Anti-hCD117 also inhibited HSC growth and differentiation in a methylcellulose colony assay. To assess in vivo activity of the anti-hCD117 mAb, we determined mAb pharmacokinetics in NOD/scid/IL2Rγ-/- (NSG) mice. We determined that doses between 0.5mg/kg and 3mg/kg provided appropriate maximal concentrations (5.8 - 38.3 µg/mL) and half-lives (10.4 - 12.4 days) for pre-clinical modeling. NSG mice were hematopoietically humanized on day 2 of life via facial vein injection of 2000-4000 human HSC following conditioning with 100cGy. After permitting hematopoietic stabilization for 6 months, we evaluated xenochimerism in the peripheral blood (PB) and bone marrow (BM; via femoral aspirate in anesthetized mice). Overall human leukocyte chimerism in the BM of 32 mice was a median 4% (range 0.1 - 51%) with median differential composition: 13% (0-96%) B lymphocytes, 16.4% (0-95%) T lymphocytes, 0.02% (0-1%) NK cells, 2.5% (0-36%) monocytes, and 3.7% (0-49%) granulocytes, indicating engraftment of pluripotent HSC. After treatment with 3mg/kg or 0.5mg/kg anti-hCD117 IV, mice were depleted of total human chimerism a median 60% (35-100%) in the PB and 100% (84-100%) in the BM at 6 weeks after treatment, with >99% depletion of myeloid chimerism in both compartments. Human HSC and progenitor cells (Lin-CD34+CD117+) in the bone was significantly increased at day 7 and remained high at day 10. Conclusion: Non-mesenchymal mononuclear cells from the bone marrow have a potential in induction of functional differentiation in SH-SYSY cells, but the effect is time-dependent.
DENDRITIC CELLS ARE POTENT SOURCES FOR THE GENERATION OF DENDRITIC CELLS

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Background: In utero transplantation (IUT) of hematopoietic stem cells (HSCs) has been pursued as a treatment for congenital hematologic and genetic disorders. Although the engraftment of HSCs following IUT has been achieved, the levels of donor-cell engraftment have been too low for the treatment of most target diseases. In order to achieve clinically relevant levels of HSC engraftment, we have examined two methods in the setting of sheep IUT. One is the transduction of HSCs with the HoxB4 gene before transplantation, ie, the expansion of HSCs. The other is the myeloablation of recipients with busulfan (BU), ie, the expansion of niche. Here, we report long-term follow-up data regarding the engraftment of human HSCs in the two (HoxB4 and BU) groups. Methods: We performed IUT of human cord blood (CB) CD34+ cells (0.32 to 2.4 million) into the liver of fetal sheep at 45-49 gestational days (full term, 147 days) at which the immune system is premature. In the HoxB4 group (n = 4), human CB CD34+ cells were transduced with HoxB4 by the P gene-defective Sendai virus vector for 4 days, and the cells were transplanted into the fetuses. In the BU group (n = 4), BU at 3 mg/kg (calculated by maternal body weight) had been intravenously administered to pregnant ewes at 6 days before performing IUT. Results: The two methods (the HoxB4-transduction and BU-conditioning) similarly enhanced short-term engraftments of human HSCs in sheep (up to 3% in terms of the bone marrow CFUs for 5 months post-IUT). At further time points, human CFUs were not detectable either in the BU or control group. However, in the HoxB4 group, they were still detected in 2 out of the 4 lambs at 15 months post-IUT (1-2%), and in 1 out of the 4 lambs at 40 months post-IUT (2%). No leukemia developed in any of the sheep in the HoxB4 group at present (at 40 months post-IUT) in our study, unlike high incidence of leukemia in other large animal studies using a HoxB4-expressing retroviral vector (Zang et al, JCI 2008;118:1502-1510). Conclusion: In the context of IUT, although the two methods enhance the short-term engraftment of human HSCs in sheep (for 5 months), the transduction of HoxB4 results in longer-term engraftment of those (up to 40 months) compared to the conditioning with BU. These results imply that a cell-autonomous action would be more effective than a non–cell-autonomous action for long-term engraftment of HSCs at least in sheep.
Detailed Program and Abstracts — Thursday, June 14

Poster Board Number: T-2099

HUMAN LEUKEMIC STEM CELLS ESCAPE CHEMOTHERAPY BY FORMING SUBCLONES IN DISTINCT NICHEs
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Failure to eliminate leukemic stem cell (LSC) is presumed to be the reason why leukemia relapses after chemotherapy. The mechanism in which LSC escapes chemotherapy remains much unknown. It has been demonstrated in the previous studies that LSC develops from pre-leukemic stem cell (pre-LSC) and continually evolve into multiple sub-clones in concert with disease progress (Hong D, et al. Science 2008; Ford A, et al. JCI 2009; Anderson K, et al. Nature 2011). To reveal in the current study how does LSC form sub-clone in the bone marrow niches and escape chemotherapy, the xenograft leukemic model was established by transplanting acute lymphoblastic leukemic (ALL) cells into sub-lethally irradiated NOD-SCID IL2Rγnull mice or anti-CD122 antibody-treated NOD-SCID mice. By applying dynamic technologies of ex-vivo imaging and immunoassaying, GFP-labeled leukemic cells were observed to home into the endosteal niche in the recipient bone marrow in 24h after intravenous injection and clonally grow. When the recipients presented clinically manifest disease, these mice were treated with chemotherapy medicines. Ara-C or/and Daunorubicin, for two to seven days. Majority of leukemic cells in the bone marrow were killed and consistently minimal cells resided in the BM niches. Interestingly, we observed that LSC exploited or created distinct niches for refuge in response to chemotherapy. Leukemic cells preferentially usurped the normal HSC niche given it was still intact, whereas leukemic cells created an abnormal stromal niche when the normal HSC niche was destroyed by leukemic cell infiltration. And the process was associated with the interaction between leukemic cells and chemotherapeutic drugs. The residual leukemic cells in the stromal niches isolated from the recipient BM were capable to reconstitute leukemia in the recipients of serial transplantation, thus were proved to be LSCs. By analyzing the biopsy samples of ALL patients who only achieved partial remission after induction chemotherapy, the abnormal stromal niche was observed surrounding leukemic cells and associated with poor prognosis. Therefore these studies have implication for therapeutics of leukemia depending on LSC eradication.

Poster Board Number: T-2100

STIMULATION OF POOLS OF MURINE HEMATOPOIETIC AND INTESTINAL STEM CELLS BY PERORAL INTAKE OF NANJDIAMONDS COMPLEXES WITH THE CONDITIONED MEDIUM FROM ALLOGENIC AND XENOGENIC MESENCHYMAL STEM CELLS CULTURE
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Nanodiamonds with the sizes of 5-10 nm have been intensively studied lately as prospective agents for the anticancer drugs delivery into tumors. A possibility of nanodiamonds application for the delivery of other biologically active substances to normal tissues is not excluded, either. We have found that peroral intake of a suspension containing complexes of nanodiamonds with the conditioned medium (CM) from the cultures of mesenchymal stem cells (MSC) by F1(CBAxC57Bl/6) mice activates the pool of hematopoietic stem cells (HSC). This can be observed by the method of spleen endocolonies after the total irradiation (γ-rays of Co⁶⁰) of animals in sublethal doses. We assume that the complexes of nanodiamonds with CM taken by mice may arrive into the HSC “niches” in the hematopoietic tissues and activate (by yet unknown way) HSC being mainly in the state of proliferative rest. This may result in the temporary increase of the HSC pool, as it is observed for a large group of radioprotective preparations with the “biological mechanism of action”. We also studied the radioprotective effect of the complexes of nanodiamonds with CM from allogenic and xenogenic MSC cultures by the 30 days post-irradiation survival test of mice undergone the lethal dose of γ-irradiation. The same stimulation effect of the perorally-taken complexes of nanodiamonds with CM was also revealed by the test of “microcolonies” production by the intestinal stem cells of lethally irradiated mice and by the survival test of animals during development of the “intestinal form” of radiation death. Since the experimentally observed radioprotection effects are undoubtedly accounted for by the stimulation of regenerative activity of adult stem cells, then the task of primary importance is to study possible effects of such complexes using other nonradiation tests, in particular, using their influence on the stem cell-dependent efficiency of reparative processes accompanying various types of damages of different organs and tissues.

Poster Board Number: T-2101

CLONAL EXPANSION AND LINEAGE BIAS OF MOUSE HEMATOPOIETIC STEM CELLS ARE INDUCED BY PRE-TRANSPLANTATION CONDITIONING
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Hematopoietic stem cells (HSCs) are identified as cells capable of reconstituting the entire hematopoietic system upon transplantation into lethally irradiated recipients. Irradiation conditioning is used in the vast majority of HSC studies to facilitate HSC engraftment by depleting the recipient’s hematopoietic system. While the massive loss of hematopoietic cells can be reconstituted by donor HSCs, it is unclear whether HSC regulation after this severe damage still resembles that under steady state conditions. Here, we compare HSC lineage commitment at the clonal level after lethal irradiation mediated transplantation, after unconditioned transplantation, and after c-kit antibody clearance based transplantation, which eradicates hematopoietic progenitor cells while leaving other hematopoietic cells intact. Clonal tracking of HSCs was performed using our recently developed single cell tracking system based on genetic barcoding and high-throughput sequencing. This experimental system offers high throughput and high sensitivity that allows for the direct examination of HSC clonality and for tracking the low numbers of HSCs that engraft after unconditioned transplantation. Our data suggests that HSCs are regulated differently after lethal damage of different organs and tissues.
B cells and T cells. This clonal expansion occurs at every step of the HSC lineage commitment process at week 22 post transplantation. Moreover, this expansion is unbalanced between myeloid and lymphoid lineages. In irradiated mice and ckit antibody treated mice, HSC clones exhibit differential contributions to granulocytes and B cells, a phenomenon previously identified as “lineage bias”. However, this myeloid versus lymphoid lineage bias is not present after unconditioned transplantation. In addition, it is not exhibited at the progenitor level in irradiated mice. In these mice, HSC clones that are balanced at the progenitor level exhibit lineage bias at the mature blood cell stage. This indicates that lineage bias is induced by clonal expansion downstream of the oligopotent progenitors. In order to determine whether clonal expansion and lineage bias involve HSC clones that expand immediately after transplantation, we examined granulocytes in the peripheral blood at week 4 and week 8 after irradiation mediated transplantation. These early-expanded HSC clones are under represented by HSC clones that undergo expansion at week 22 and are over represented by HSC clones with lymphoid bias. This suggests that lineage bias is induced by pre-transplantation conditioning may be related to time dependent clonal expansion post transplantation. In summary, HSCs exhibit clonal expansion and lineage bias after conditioned transplantation but not after unconditioned transplantation. These two induced features are associated with each other throughout the HSC lineage commitment process and are intertwined over time following conditioned transplantation.

**Poster Board Number: T-2102**

**IGF2 ACCELERATE ERYTHROID DIFFERENTIATION IN THE MOUSE YOLK SAC**

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In vertebrates, both primitive and definitive hematopoeisis first occurs in yolk sac (YS), which consists of hematopoietic cells, capillary blood vessels, and endodermal cells. Although hematopoietic cell fate is extrinsically determined by niche cells, little is known about its regulation at YS. There was no specific marker to recognize YS capillary blood vessel. We successfully found that lymphatic endothelium hyaluronan receptor (Lyve-1) is expressed on YS capillary blood vessels (micro-vessels), but not on arteries and veins (non-micro-vessels). Single YS cells at E10.5 were prepared by collagenase treatment and analyzed by flow cytometry. Ter119+ and/or CD45+ cells as “hematopoietic cells”, Ter119+/CD45-/CD31+/Lyve-1+ cells as “micro-vessels”, Ter119-/CD45-/CD31-/Lyve-1+ cells as “non-micro-vessels” and Ter119-/CD45-/CD31-/Lyve-1- cells as “unclassified cells”, which are likely endodermal cells we sorted out, following gene expression of hematopoietic cytokines was examined by real-time PCR. Among several cytokine genes, Insulin-like growth factor 2 (lgf2) was predominantly expressed at E10.5 YS. In addition, expression level of lgf2 in unclassified cells was 7.0, 9.6 and 15.9 times higher than micro-vessels, non-micro-vessels and hematopoietic cells, respectively. To further investigate the function of lgf2 in YS hematopoiesis, neutralizing antibody against lgf2 was added in vitro culture of YS cells for 6 days. The production of erythroid progenitor cells (c-Kit+/CD71+), erythroblasts (CD71+ /Ter119+) and mature erythrocytes (CD71+/Ter119+) were decreased after inhibition of lgf2. Taken together, endodermal cells likely secrete lgf2, which functions in erythroid differentiation in YS.

**Poster Board Number: T-2104**

**ALCAM MEDIATED CELLULAR INTERACTION REGULATES MOUSE HEMATOPOIETIC STEM CELL REPOPULATION AND SELF-RENEWAL**

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Alcam, which encodes the activated leukocyte cell adhesion molecule (CD166), is a cell surface immunoglobulin superfamily member mediating homophilic adhesion as well as heterotypic interactions with CD6. It has recently been shown that Alcam+ endothelial cell subset in the bone marrow contain hematopoietic niche cells able to support hematopoietic stem cell (HSC) activity. We use an Alcam null mouse model to assess the function of Alcam in HSC differentiation and self-renewal. We found that Alcam is highly expressed on the cell surface of long-term repopulating HSC (LT-HSC), multipotent progenitors (MPP), and is expressed on a subset of granulocyte/macrophage progenitors (GMP). The frequency and cellularity of bone marrow LT-HSCs are not altered in Alcam-deficient mice. We show that the serial-replating potential of Alcam-deficient LT-HSCs is reduced using a clonogenic colony-forming progenitor (CFU) assay. We examined the differentiation potential of individual phenotypic LT-HSC using an in vitro single-cell differentiation assay. Our results show that Alcam-deficiency leads to a decreased granulocytic differentiation. We performed competitive repopulation assays in vivo followed by secondary transplantation. We show that Alcam-deficient cells show a transient engraftment enhancement, however, the engraftment is significantly lower upon secondary transplantation. These results suggest that the self-renewal capacity of Alcam-deficient HSC is compromised. We performed a limiting-dilution transplantation assay and determined that the frequency of long-term repopulating cells in Alcam-deficient bone marrow is significantly reduced. We further assessed the engraftment efficiency of limiting numbers of phenotypically purified LT-HSCs and show that the engraftment efficiency of Alcam-deleted LT-HSCs is significantly reduced compared to wild type LT-HSCs. We observed that Alcam-deficient progenitors are significantly more responsive to SDF-1 chemo-attractant. Collectively, these studies implicate Alcam mediated cell-cell interaction in the regulation of HSC self-renewal and migration.

**Poster Board Number: T-2105**

**ANALYSIS OF HEMATOPOIETIC AND MESENCHYMAL STEM CELL MARKERS FROM MOUSE YOLK SAC IN DIFFERENT GESTATIONAL STAGES**

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Yolk Sac (YS) is an embryonic attachment present during vertebrate development, responsible for embryonic feed during development, protein synthesis, phagocytic activity, material transfer and hematopoiesis. YS derived cells from dogs fetuses were cultured for a long-term without plasticity lost in our lab. SV cells retain the ability to rapid proliferate and can be differentiated in blood cells, including lymphocytes, granulocytes, monocytes, erythrocytes and
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megakaryocytes. Thus, they can have a wide spectrum of applications, including rescuing or recovering of hematopoietic system, transgenic animal for blood and antibody production, vaccine and immunotherapy production and other applications. Therefore, in this project we aim to establish and characterize YS stem cells from mice in different gestational stages in order to analyze particularly the pluripotency, mesenchymal and hematopoietic stem cell markers in these cells, morphology and behavior in cell culture. Swiss mice with 10, 15 and 19 days of gestation were euthanized and SV were collected in a tube containing PBS plus 5% of antibiotics. After washing, tissues were minced into very small pieces and submitted to four different culture conditions: 1) DMEM supplemented with 20% of FBS, 200 U/ml penicillin, 200 μg/ml streptomycin, 200 μg/ml L-glutamine, 1% MEM NEAA, 1% L-glutamine, 1% NEAA, 10-4M of β-mercaptoethanol; 2) DMEM/F12 supplemented with 15% of FBS, 200 U/ml penicillin, 200 μg/ml streptomycin, 1% L-glutamine, 1% MEM NEAA, 1% L-glutamine, 1% NEAA, 10-4M of β-mercaptoethanol and 4) α-MEM supplemented with 15% of FBS and endothelial progenitor cells, suggesting that this cell population is positive for CD45 and CD90 using flow cytometry assay.

We analyzed a variety of genomic features to find that the most significantly associated CpGs were associated with hypermethylation in aging HSCs. Overall, out of 1,777 differentially methylated CpGs, 92.8% showed age-related hypermethylation and 7.2% showed age-related hypomethylation. Gene ontology analyses have revealed that differentially methylated CpGs were significantly enriched near genes associated with alternative splicing, DNA binding, RNA-binding, transcription regulation, Wnt signaling and pathways in cancer. Most interestingly, over 579 splice variants were detected as candidates for age-related hypermethylation (86%) and hypomethylation (14%) including Dnmt3a, Runx1, Pbx1 and Cdkn2a. To quantify differentially expressed RNA-transcripts across the entire transcriptome, we performed RNA-seq and analyzed exon arrays. The Spearman’s correlation between two different methods was good (r=0.80). From exon arrays, we identified 586 genes that were down regulated and 363 gene were up regulated with aging (p<0.001). For the RNA-seq analysis, we focused first on annotated transcripts derived from cloned mRNAs and we found 307 genes were down regulated and 1015 gene were up regulated with aging (p<0.05). Secondly, we sought to identify differentially expressed isoforms and also novel transcribed regions (antisense and novel genes). To characterize the genes showing differential regulation, we analyzed their functional associations and observed that the highest scoring annotation cluster was enriched in genes associated with translation, the immune network and hematopoietic cell lineage. We expect that the results of these experiments will reveal the global effect of DNA methylation on transcript stability and the translational state of target genes. Our findings will lend insight into the molecular mechanisms responsible for the pathologic changes associated with aging in HSCs.

Poster Board Number: T-2107

PRE HSCS ARE LOCATED IN INTRA-AORTIC HEMATOPOIETIC CLUSTERS BEFORE HSC PRODUCTION IN THE MOUSE EMBRYO

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Hematopoietic Stem Cells (HSCs) are at the foundation of all blood cells. They are first detected in the dorsal aorta at day (E)10.5 of the mouse embryonic development. HSCs derive from specialized endothelial cells capable of hematopoietic cell production and therefore named hemogenic. HSCs most likely reside in Intra-Aortic Hematopoietic Clusters (IAHCs) that are attached to the endothelial layer of the aorta. The very low number of HSCs compared to the very high number of IAHCs, and the earlier appearance of the IAHCs during development raises several questions. Here, we performed an in-depth analysis of the IAHCs to determine their exact cell composition and function. We tested the hematopoietic potential of the IAHC cells isolated from the aorta of E10.0 embryos in vitro. We specifically isolated the IAHC cells after intra-aortic injection of antibodies against c-kit and flow cytometry sorting. c-kit+IAHC cells were further sub-fractionated based on the differential expression of CD45 and Ly6A(Sca1)+GFP. The four populations (c-kit+CD45+Ly6A−, c-kit+CD45−Ly6A−, c-kit−CD45+Ly6A+, c-kit−CD45−Ly6A+) were tested for B lymphoid, erythroid and myeloid potentialities. We found that IAHCs contain very few erythroid-myeloid progenitors. Most progenitors are in the circulating blood and in the c-kit+CD45+Ly6A+ population (similarly to the progenitors found in the yolk sac, fetal liver and placenta). Moreover, no B progenitors were present in the IAHCs. We then tested in vivo the pre-HSC potential of the IAHC cells. Pre-HSCs, capable of long-term and multilineage reconstitution after injection into the liver of newborn recipients, are present in E9 embryos. Using this assay, we found that IAHC cells isolated from E10.0 embryos contain...
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Poster Board Number: T-2108

JAK2 V617F SUBVERTS MOUSE HEMATOPOIETIC STEM CELLS BY INCREASING PROLIFERATION AND DIFFERENTIATION WHILE REDUCING LONG-TERM SELF RENEWAL CAPACITY

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Hematopoietic stem cells (HSCs) are a tightly regulated cell population ultimately responsible for providing the correct numbers and types of blood cells throughout life. Imbalances in this regulation can perturb normal blood cell production and result in malignancy. Clonal myeloproliferative neoplasms represent an early stage of such imbalances and identification of the highly prevalent JAK2 V617F mutation provides a powerful tool for observing the process of stem cell subversion. Recently, our group has generated a conditional knock-in mouse in which a single copy of the human JAK2 V617F is expressed under the control of the mouse Jak2 locus. These mice show modest increases in hemoglobin and platelet levels, reminiscent of a mild human JAK2 V617F–positive essential thrombocythemia, together with a 10% transformation rate to more severe disease (polythemia vera or myelofibrosis). Surprisingly, both competitive and non-competitive whole bone marrow transplantation experiments revealed a significant decrease in JAK2 mutant stem cell activity, evidenced most strikingly in secondary transplantation studies. Here, we undertook experiments to characterize the HSC compartment and to understand the stem cell defect evidenced by whole bone marrow transplantations. The frequency of HSCs (EPCR+/CD150+/CD48-/CD45+) in JAK2 V617F mutant animals is reduced and individual HSCs have qualitative defects when studied in vitro and in transplantation experiments. Specifically, JAK2 V617F alters the balance of HSC fate choices in vitro and in vivo, skewing progeny toward differentiation and proliferation resulting in a loss of HSCs. In aged cohorts, HSCs from JAK2 V617F mice are not expanded and the relative proliferative advantage of their progeny is restrained, however the tendency to differentiate is maintained. Approximately 10% of mice progress to more severe disease, and together these data suggest that the JAK2 V617F mutation leads to HSC exhaustion, but exposes cells to the accrual of additional genetic lesions and eventual transformation.

Poster Board Number: T-2110

ROLE OF THE CLASP2 MICROTUBULE PLUS-END TRACKING PROTEIN DURING MOUSE ADULT HEMATOPOIESIS

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Hematopoietic Stem Cells (HSCs) are at the foundation of the hematopoietic system in vertebrates. They are multipotent and capable of self-renewal, which allow the constant production of all blood cell types throughout life without exhaustion of the HSC pool. HSCs reside in the bone marrow in specific areas or niches. HSC fate is controlled by the surrounding microenvironment via cell-cell or cell-extracellular matrix (ECM) contacts and by the production of growth factors. Therefore, attachment, migration and (a)symmetric division are very important for a proper HSC homeostasis. It is known that the microtubule (MT) cytoskeleton plays an important role in these processes. However, little is known about the organization and function of the MT network in HSCs. Mammalian CLASPs are MT plus-end tracking proteins that play essential roles in the local regulation of MT dynamics. To determine the function of CLASP2 in hematopoiesis, we have generated Clasp2 knockout mice. Adult knockout mice are viable but develop a severe pancytopenia. We observed, after performing long-term transplantations, a strong HSC defect in the bone marrow of the knockout mice, which is partially due to defective homing of hematopoietic cells to the bone marrow. We also found that the MT network is impaired in the Clasp2 knockout hematopoietic stem and progenitor (HSPCs), which results in a cell attachment defect in vitro, and possibly also in vivo in the bone marrow niche. Finally, we found that HSC-enriched populations sorted from the bone marrow of Clasp2 knockout mice (Lin-Sca-1-c-kit+) contain reduced mRNA levels of c-Mpl (which encodes the thrombopoietin receptor) and Meis1 (transcription factor regulated by c-Mpl). Both factors are known to be essential for HSC maintenance. Altogether, we show that CLASP2-mediated stabilization of MTs is required at multiple levels for HSC activity, including homing, attachment, and signaling (via the transcription of essential HSC genes).

Poster Board Number: T-2110

POLYCOMB REPRESSIVE COMPLEX-1 COMPOSITION DETERMINES MOUSE HEMATOPOIETIC STEM CELL FATE

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Adult stem cells have the unique capacity to undergo both symmetric and asymmetric divisions, leading to their self-renewal or differentiation. A precise coordination of these cell fate decisions is essential to sustain tissue homeostasis. Here we show that different CBX-associated Polycomb Repressive Complex-1 (PRC1) complexes regulate the switch between self-renewal and differentiation divi-
sions in hematopoietic stem cells (HSCs). Among all Cbx proteins, Cbx7 showed to be highest expressed in long-term HSCs, and its expression decreased upon differentiation. Overexpression of Cbx7 specifically induced self-renewal divisions of HSCs, and resulted in development of multiple leukemia-subtypes upon transplantation in mice. Abrogating its binding to methylated histon 3 or Ring1b, completely impaired the effect of Cbx7 on HSC self-renewal. Modulation of the PRC1 composition in HSCs via overexpression of Cbx4 or Cbx8, resulted in enhanced differentiation and subsequent HSC exhaustion.

Using global chromatin occupancy analyses (ChIP-seq) we found that two alternatives might account for the functional differences between Cbx7 and Cbx8. First, Cbx8 unique targets showed to be significantly enriched in genes involved in transcription, signal transduction, and apoptosis. Second, we did not find unique Cbx7 targets and Cbx7 showed to have lower chromatin binding affinity than Cbx8 in general. The binding of Cbx7 showed to be significantly higher than Cbx8 at only a few genomic positions. These correspond to transcription start sites of genes involved in transcription, cell cycle, and development. Together, our results show that the Cbx-subunit determines PRC1 target selectivity which provides a key molecular switch between HSC self-renewal and differentiation.

Posters Board Number: T-2111

HOMING OF BONE MARROW AND PURIFIED TRANSPLANTED STEM CELLS IN MARROW ABLATED MICE.

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Stem cell-based therapy has been one of the best documented approaches in regenerative medicine, promising cures for treatment of a multitude of diseases and disorders. The whole body irradiation of mice by lethal or sublethal doses represents a model for elimination of endogenous hematopoiesis and demonstration of efficacy of transplantation with hematopoietic cells. Homing is a simple process and it is the first and essential step in clinical cell transplantation. A special feature of the intravenously transplanted HSCs and HPCs is their migration from peripheral blood (PB) to bone marrow (BM), a process referred to as homing. In our experiments we have used positively selected bone marrow cells Lin−/CD117+ cells that are presented less heterogeneous than the whole bone marrow. We have performed a histochemical analysis of GFP+ cell engraftment in the recipient’s tissues. Tissue analyses were performed on day 30 and day 70 after exposure to 9 Gy irradiation and transplantation of bone marrow cells or Lin−/CD117+ (GFP+) cells. After 30 days in the bone marrow, the transplanted cells were frequently observed in the vicinity to the endostem of bone trabeculi. Large numbers of grafted cells entered the thymic parenchyma. Lot of GFP+ cells engrafted the lamina propria of small intestines and splenic parenchyma. On the other hand, GFP+ hematopoietic cells were rarely observed in the liver. After 70 days, numerous transplanted cells occupied the core of intestinal villi and splenic nodules in the splenic white pulp. In the liver, most GFP+ cells differentiated into hepatic Kupffer cells. Transplanted cells differentiated into hematopoietic cells, lymphocytes and Kupffer cells. We detected GFP+ gene expression in the recipient’s tissues using qRT-PCR. Data confirm colonisation of recipient tissues with transplanted cells on day 30 and day 70 post-transplantation. After bone marrow transplantation, we detected increased levels of GFP+ in the spleen on day 30, and also in the thymus. On the other hand, transplantation of Lin−/CD117+ cells resulted in the largest accumulation of engrafted cells in the spleen on day 30 followed by a transient decrease; next peaks were detectable on day 70 in the spleen and thymus. The bone marrow, small intestine and liver showed engraftment with GFP+ cells although their levels did not reach those observed in the spleen and thymus. Transplantation of lethally irradiated mice with bone marrow-derived cells supports hematopoiesis and leads to colonization of diverse organs with grafted cells. Engraftment efficiency depends on variety factors including number and type of transplanted cells.

Poster Board Number: T-2112

ROLE OF BMI-1 IN NORMAL MOUSE HEMATOPOIETIC STEM CELLS AND LEUKEMIC CELLS

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Hematopoietic stem cells (HSCs) are well-characterized stem cells, which are able to replenish cells of all lineages in the hematopoietic system. It is known that Bmi-1, as a core component in polycomb repressor complex 1, helps to sustain self-renewal and proliferation of HSCs. Bmi-1 was previously reported as negative regulator of Ink4a-Arf tumor suppressor gene locus, which is involved in the regulation of cell cycle in hematopoietic cells. Apart from the Ink4a-Arf locus, little is known about the cellular targets of Bmi-1, which presumably play crucial role in self-renewal and differentiation of HSCs. Moreover, aberrant expression of BMI-1 was observed in human leukemias, suggesting the deregulation of BMI-1 is highly associated with leukemogenesis, yet its function remains unclear. In this study, hematopoietic stem cells with LinSca-1−c-Kit− (LSK) phenotype were isolated from mouse bone marrow and were subjected to Bmi-1 gene knockdown. Q-RT-PCR analysis showed that knockdown of Bmi-1 gene leads to de-repression of p16Ink4a and p19Arf genes, without alteration of other cell cycle regulators and hematopoietic lineage genes, in LSK cells, suggesting that Bmi-1 has limited function on stem cell differentiation. Interestingly, cell cycle analysis showed that depletion of Bmi-1 drives cells from G0/G1 phase to S phase, which overturns the effect of elevated p16Ink4a and p19Arf expression in blocking the entry to S phase, suggesting an alternative mechanism of cell cycle regulation is involved. Colony forming assay further demonstrated reduced colony forming ability of Bmi-1 knockdown LSK cells, which indicates defects in their self-renewal ability. On the other hand, Bmi-1 was over-expressed in HL-60 promyelocytic leukemia cells. Cell proliferation study demonstrated an increased in total cell number with a higher proportion of cells in S and G2/M phases. We also found that overexpression of Bmi-1 increased p14Arf and p19Arf gene expression, which explains the observed S/G2/M phase progression. Ongoing investigation is to find out the epigenetic regulation mediated by Bmi-1 on its target genes for the self-renewal pathway in both normal HSCs and leukemic cells. By revealing the functional role of BMI-1 in hematopoietic system, it provides insight into the development of novel therapeutic strategies which could potentially eradicate leukemic stem cells.
MOUSE EMBRYONIC ERYTHROPOIESIS

DOK2 REGULATES GLOBIN GENE EXPRESSION IN MOUSE EMBRYONIC ERYTHROPOIESIS

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Downstream of tyrosine kinase 2 (Dok-2) reportedly plays an important role in myeloid cell homeostasis particularly leukemia suppression and is also one of the novel signaling cascade proteins stimulated in the activated platelets. The Dok-2 gene has been obtained from our database in micro-array analysis of c-myc deficient embryos which impaired fetal liver erythropoiesis. As Hematopoietic Stem Cells (HSCs) differentiate into erythrocytes, Dok-2 gene expression was highest in the HSC (CD45+, Sca1+ and c-kit+) fraction, but declined in erythroid lineage, such as BFU-E, CFU-E, erythroblasts and mature erythrocytes, suggesting that Dok-2 gene is involved in erythroid differentiation. We also found that Dok-2 gene was down-regulated in both HSC and BFU-E fractions in heterozygous c-myc mutant mice as compare to wild type. To investigate the role of Dok-2 in erythroid differentiation, HSC and BFU-E fraction (CD45-, Sca1- and c-kit-+) from fetal liver cells at 14.5 dpc were transfected with Dok-2 siRNA. After 24 hours of siRNA transfection, gene expressions of beta-major-, beta-minor- and epsilon-globins were up-regulated in BFU-E fraction, but slightly down-regulated in HSC fraction. Taken together, Dok-2 regulates globin gene expression during erythroid differentiation in the mouse fetal liver. Key words: Dok-2, Hematopoietic Stem Cells, Erythropoiesis

SIRT1 IS DISPENSABLE FOR FUNCTION OF HEMATOPoietIC STEM CELLS IN Adult MICE

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SIRT1 is an NAD+-dependent histone deacetylase implicated in the establishment of the primitive hematopoietic system during mouse embryonic development. However, investigation of the role of SIRT1 in adult hematopoiesis has been complicated by high perinatal mortality of SIRT1−/− mice. Herein we perform a comprehensive in vivo study of the hematopoietic stem cell (HSC) compartment in adult SIRT1−/− animals and show that, apart from anemia and leukocytosis in older mice, production of mature blood cells, lineage distribution within hematopoietic organs and frequencies of the most primitive HSC populations are comparable to those of wild type littermate controls. Furthermore, we show that SIRT1−/− deficient bone marrow cells confer stable long-term reconstitution in competitive repopulation and serial transplantation experiments. Taken together, our results rule out an essential physiological role for cell-autonomous SIRT1 signaling in maintenance of adult HSC compartment in the mouse.

ACTIVATED GS SIGNALING IN IMMATURE OSTEOBLASTS ALTERS THE HEMATopoietIC STEM CELL NICHE IN MICE

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Adult hematopoiesis occurs primarily in the bone marrow space where blood cells closely interact with stromal niche cells, including osteoblastic lineage cells. Despite this close association, little is known about the specific roles of osteoblasts in supporting hematopoietic stem cell (HSC) function, and how conditions affecting bone formation influence hematopoiesis. Here, we use a constitutively-active engineered Gs-coupled G-protein coupled receptor (GPCR) to activate Gs-GPCR signaling in osteoblasts and assess how the concomitant increase in bone formation impacts HSC function and blood homeostasis. The ColII(2.3)+/Rs1+ transgenic mouse model of fibrous dysplasia of the bone showed a 5-15 fold increase in trabecular bone mass with near complete loss of the normal bone marrow cavity. The mice showed BM aplasia with progressive loss of up to 85% of HSC numbers and impaired megakaryocyte and erythrocyte development with defective recovery after myeloablation with 5FU. These blood phenotypes developed without compensatory extramedullary hematopoiesis. Surprisingly, the loss of HSCs occurs despite a paradoxical expansion of HSC-supportive niche cells, including osteoblasts, mesenchymal stem cells, and endothelial cells. Expression analysis showed that the ColII(2.3)+/Rs1+ osteoblasts had decreased expression of key HSC-maintenance genes, including Sdf1, Vcam1, and Angpt1, accounting for the impaired ability to support HSC function. These findings indicate that long-term activation of Gs-GPCR signaling in osteoblasts of the HSC niche decreases HSC-supportive activity and leads to lineage-specific hematopoietic defects. Our results also suggest that long-term activation of Gs-GPCR signaling, such as in medical conditions of excess parathyroid hormone, may lead to
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adverse effects on endogenous HSCs and contribute to hematopoietic disorders such as anemia.

Poster Board Number: T-2118

AGE-SPECIFIC ALTERATIONS IN A NUMBER AND EFFICIENCY OF CLONING THE STROMAL CELLS-PRECURSORS OF HEMATOPOIETIC AND LYMPHOID ORGANS FROM EXPERIMENTAL ANIMALS

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The analysis of specific differences in age-related alterations in the number of colony-forming cells-precursors of fibroblasts (CFU-F) in bone marrow, thymus, and spleen of CBA mice, guinea pigs, and Wistar rats, as well as the efficiency of their cloning (CE-F) was carried out with the use of mathematical method of gradient decline. In addition, those parameters were examined in SAMP (rapidly aging) animals and SAMR (normal rate of aging) murine strains and in tests with the use of cross heterotopic transplants of bone marrow and spleen between the differently aged animals. The study revealed that reliable decrease in CFU-F number, the morphological change in their colonies being formed and the efficiency of stromal cells-precursors' cloning in all the animals tested were observed in the course of bone marrow, thymus and spleen aging. Most significant and rapid decrease in CFU-F amount was observed in guinea pig and mouse thymus namely by 75% and 12-fold, respectively. The age-specific alteration in CFU-F quantity and their CE-F vary in the extent of expressiveness, age of the different strains of experimental animals and in animals of one strain in different organs that seems to depend on the physiological characteristics, peculiarities of body aging, life time of animals and functional role of the organs. Data obtained on the earlier age-specific decrease in the number of CFU-F in SAMR murine strain (16-19 mo.) and experiments with cross heterotopic transplants of bone marrow and spleen between the differently aged animals. The study revealed that reliable decrease in CFU-F number, the morphological change in their colonies being formed and the efficiency of stromal cells-precursors' cloning in all the animals tested were observed in the course of bone marrow, thymus and spleen aging. Most significant and rapid decrease in CFU-F amount was observed in guinea pig and mouse thymus namely by 75%, and 12-fold, respectively. The age-specific alteration in CFU-F quantity and their CE-F vary in the extent of expressiveness, age of the different strains of experimental animals and in animals of one strain in different organs that seems to depend on the physiological characteristics, peculiarities of body aging, life time of animals and functional role of the organs. Data obtained on the earlier age-specific decrease in the number of CFU-F in SAMP murine strain (9-12 mo.) as compared with the SAMR murine strain (16-19 mo.) and experiments with cross heterotransplants demonstrate that alongside with self age-related alterations in stromal tissue, the body as a whole influences it while aging. As far as it is well-known that CFU-F population in spleen and thymus involves the inducible osteogenic cells and bone marrow comprises determinate CFU-F; therefore the results obtained indicate the possibility of reducing the number of both categories of stromal precursors due to aging that could be considered as one of the reasons for age-related osteoporosis. This work was supported by RFBR grant 11-04-96037r_ural_a and administrative body of Perm Region.

Poster Board Number: T-2119

HOXB4 OVEREXPRESSION INCREASES PRIMITIVE CORD BLOOD CD34 POSITIVE HEMATOPOIETIC PROGENITORS CELLS

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Background: homeobox B4 (HOXB4) overexpression induces enhanced self-renewal of murine hematopoietic stem cells (HSCs) without compromising differentiation or homeostatic regulation of the HSC pool size. However, the effects of HOXB4 overexpres-
high concentration of CS1 showed much higher fold expansion of HSCs and colony-forming units (CFU-GEMM) than those on other dishes, such as fibronectin-grafted and polyamine-grafted dishes. These data suggest that the specific interaction between HSCs and CS1 helps to maintain the pluripotency of HSCs during the ex vivo expansion of HSCs. CS1 is one of the cell-binding domains of fibronectin. However, the ex vivo expansion and CFU expansion of HSCs cultured on fibronectin-grafted dishes were less than those on PS-CS1-H dishes, although the ex vivo expansion and CFU expansion of HSCs on PS-FN dishes were higher than those on PS or TCP5. The presentation of specific binding site number of CS1 is 500 times (440,000/880) higher than that of fibronectin at the same grafting weight density of CS1 and fibronectin on dishes, because of low molecular weight of CS1 (approximately 880 dalton from 110 [average molecular weight of amino acids] x 8 [No. of amino acids in CS1]) than fibronectin (440,000 dalton). The presentation of specific cell-binding domains of CS1 at high concentrations seems to be important for the signal transduction that promotes the ex vivo expansion of HSCs.

Poster Board Number: T-2121
FROM HSC-CD34+ CELLS TO OSTEOCLAST IN A MODIFIED LTC ASSAY
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Osteoclasts (OCs) are multinucleated giant cells responsible for bone resorption and play an important role in normal skeletal remodeling. Recent studies suggest that osteoclasts are important component of stem cells niche and are involved in the regulation of haematopoiesis. Since several lines of evidence demonstrate that osteoclasts derive from haematopoietic CD34 cells, there is not clear information about the real osteoclast progenitor and its differentiation. In this study, to investigate osteoclastogenesis, we developed a 5 week 2-phase culture of human CD34 cells to induce mature osteoclastogenesis. At the same time we assessed the effect of a co-culture system of mouse stromal cell line MS-5 and CD34+ cells on osteoclastogenesis. In the first 2 week, CD34 cells from PB donors were isolated by flow cytometry. CD34+ cells were placed in either hematopoietic supporting media (Stem cell factor, IL-3, IL-6, Flt3L) or osteoinductive media (Bone morphogenetic protein 2). Differentiation of OCs was detected using TRAP staining, and RT-PCR which assessed the expression of f-rrns, RANK, MMP-9. Resorption pit formation was also evaluated. After 5 weeks the output of cell population displayed the full range of OC differentiation markers. Stimulated CD34 generated functional OCs that formed extensive resorption lacunae on mineralized surface. To investigate if the OCs are derived from the monocyte-macrophage fusion, we analyzed the kinetics of OCs generation during culture (the CD34 expression and the acquisition of CD14, M-CSF and RANK-L) by flow cytometry and in-vitro imaging was performed during the 4-5 weeks of the modified LTC assay. In this study, we show that MS-5 is able to induce osteoclastogenesis. Our system provides a new, robust, and simple methods for investigating the mechanism of osteoclast development (commitment and differentiation) from CD34 hematopoietic cells.

Poster Board Number: T-2122
MAINTENANCE AND EXPANSION OF HEMATOPOIETIC STEM CELLS THROUGH REGULATION OF WNT AND MTOR PATHWAYS
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Hematopoietic stem cells (HSCs) are able to self-renew and differentiate into all blood cell lineages. The signaling pathways that regulate HSC self-renewal and differentiation are not well understood. In the bone marrow, HSC self-renewal and lineage commitment also depend on complex interactions with the microenvironment. Recent evidence suggests that HSCs reside in a low perfusion, reduced nutrient niche and that nutrient sensing pathways contribute to HSC homeostasis. Our published work demonstrated that GSK3 plays critical roles in HSC homeostasis and is involved in the Wnt/b-catenin and mTOR pathways. Here we report that suppression of the mammalian target of rapamycin (mTOR) pathway, an established nutrient sensor, combined with activation of canonical Wnt/b-catenin signaling, allows the ex vivo maintenance of human and mouse long-term HSCs under cytokine-free conditions. We also show that combining two clinically approved medications that activate Wnt/b-catenin signaling and inhibit mTOR lead to expansion of long-term HSCs in vivo.

Poster Board Number: T-2123
MIR 92A REGULATES THE DIFFERENTIATION OF CD34+ CELLS VIA CONTROLLING CIRCADIAN TARGET GENES
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The endogenous circadian system via a set of autoregulatory clock genes drives the rhythm of many physiological and behavioral processes. Abnormal expression of clock genes is implicated in many pathologic disorders like metabolic syndrome, obesity, premature aging and abnormal sleep cycle. Previously, we reported that the defects in clock gene expression of endothelial progenitor cells (EPCs) from Type II diabetic rats are involved in development of diabetic retinopathy. In the present study, we hypothesized that the differentiation of EPCs towards endothelial cells is under the control of clock genes and that in diabetes this pattern is altered. CD34+ cells (~95% starting purity) obtained from healthy or diabetic donors were isolated by flow cytometry. CD34+ cells were placed in either hematopoietic supporting media (Stem cell factor, IL-3, IL-6, Flt1) or endothelial differentiation media (VEGF, Serum and other growth factors) and 1) evaluated at 0 hr and 96 hr using a combination of CD133 and CD144 markers by flow cytometry; 2) sampled every 4 hr for 4 days to evaluate the expression pattern of the clock genes (Clock, Bmal1, Per1, Per2, Cry1 and Cry2) using qRT-PCR. Using microarrays, the miRNA signature of differentiating CD34+ cells was compared to early EPCs and outgrowth endothelial cells (OECS). Data was analyzed using Ingenuity pathway analysis software. CD34+ cells under hematopoietic supporting media rapidly began expressing the stem cell marker, CD133 (p<0.05) with a significant decrease in expression of the endothelial marker CD144 (p<0.05) while exposure to the endothelial supporting media showed a reverse trend with a dramatic increase (p<0.05) in CD144 expression. The mRNA pattern of clock genes, in CD34+ cells sampled every 4
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hr showed an increase in amplitude of Bmal1, Per1, Per2, Cry1 and Cry2 of oscillations upon differentiation while expression of Clock remained suppressed. Using specific siRNA to Per2 (2 fold; p<0.05) and Cry2 (1.5 fold; p<0.05) we observed a reduction in CD133 expression under hematopoietic supporting conditions. Per2 and Cry2 mRNA showed a complete suppression in OECs, the most differentiated population of the endothelial progenitor cells examined. Microarray analysis mapped 59 miRNAs in non-diabetic and 56 miRNAs in diabetic ECs regulating 1372 miRNAs among the three different populations of ECs. Further analysis of miRNA target filter revealed unique 25 miRNAs targeting clock genes. qRT-PCR to confirm miRNA targets identified in microarray analysis showed a 50% reduction (p<0.05) in the expression of miR92a in diabetic CD34+ cells. In conclusion, clock genes play a pivotal role in differentiation of CD34+ cells and correcting the period and amplitude of clock gene oscillations in dysfunctional CD34+ cells may impact their reparative function in disease states such as type 2 diabetes.

Interventional approach restoring proper miRNA regulation paves a new therapeutic option for optimizing progenitor cell function prior to their use in autologous cell therapy.

Poster Board Number: T-2124

TISSUE CHIMAERISM AFTER BONE MARROW CELL TRANSPLANTATION.

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Bone marrow (BM) cell transplantation represents a life-saving treatment for syndromes involving BM failure and thalassemia. To trace distribution of transplanted cells in the recipient organism, we utilized transgenic eGFP mice as donors whose cells were tagged to endogenous vectors. BM ablation in recipient C57Bl6/J mice was induced by whole body lethal irradiation 9.5 Gy that permitted to endogenous vectors. BM ablation in recipient C57Bl6/J mice was induced by whole body lethal irradiation 9.5 Gy that severely damages haemopoiesis. Irradiated splenectomized or non-splenectomized mice were injected i.v. with cell suspension of donor BM. Our data demonstrate BM transplantation induces a stable post-transplantation chimaerism in several recipient tissues.

Poster Board Number: T-2125

ZINC-FINGER NUCLEASE MEDIATED CORRECTION OF ALPHA-THALASSEMIA IN IPS

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IPS technology has the potential to provide cells for treatment of alpha-thalassemia and beta-thalassemia, and correction of the mutation causing sickle cell anemia using homologous recombination. The gene corrections were demonstrated at the genetic level and evidence was provided that the correction was functional at the protein or mRNA levels by differentiating the IPS into erythroid cells. However, levels of expression were very low because current IPS differentiation protocols yield erythroid cells that express mostly embryonic and fetal hemoglobin and only trace amounts of beta-globin. We report here correction of alpha-thalassemia major hydrops fetalis using zinc finger nuclease mediated insertion of therapeutic globin transgenes in the AAVS1 site within the PPP1R12C gene on human chromosome 19. Four types of constructs were tested. With the best one, which contains the beta-globin miniLCR driving the alpha-globin promoter and the alpha-globin gene with its 2 introns, homozygous insertion in the two copies of the PPP1R12C genes led to complete correction of chain imbalance in erythroid cells differentiated from the corrected IPS (alpha-like/beta-like ratio = 1 and 0.9 respectively in basophilic and orthochromatic erythroblasts). Because the alpha-globin genes are expressed at very high level in cells derived from IPS, globin chain measurement could be made precisely by HPLC in cells where the globin gene is expressed at their maximum level. ZFN-mediated site specific insertion has very attractive characteristics for gene therapy in IPS. Firstly, the risk of insertional mutagenesis is eliminated provided that a “safe harbor” is identified. Secondly, and equally importantly, the use of this technology should eventually simplify clinical implementation because a single set of validated constructs and reagents could be used to correct the large variety of mutations that cause the hemoglobinopathies. This presents considerable practical and economic advantages over having to design and validate custom constructs for each particular mutation. To determine if AAVS1 is a safe harbor, we analyzed expression of the 10 closest genes to the site of integration. We found that transgene insertion at AAVS1 had no effects on neighboring genes in undifferentiated cells but that it activated four genes in erythroid cells derived from IPS. Because the level of activation were relatively small, it is unclear if that disqualifies the site as a safe harbor. Nevertheless, these results suggest that additional landing pad should be developed and tested. The major conclusion of the study is that ZFN-mediated gene insertion is a very effective method to obtain high-level, quasi normal expression of globin therapeutic cassettes.
Myst-type histone acetyltransferases, MOZ (MONocytic leukemia Zinc finger; Myst3, KAT6A) and its related MORF (MOz Related Factor; Myst4, KAT6B), are involved in chromosome translocations associated with FAB-M4/5 subtypes of acute myeloid leukemia. We have reported that MOZ is essential for hematopoietic cell development and self-renewal of hematopoietic stem cells. To explore the possibility MORF also plays important roles in hematopoiesis, we generated Morf-deficient mice with homologous recombination methods. Morf-/- mice were smaller than their wild-type littermates and died within 4 weeks after birth on C57BL/6 background. In Morf-/- fetal liver, Flt3-negative KSL (c-Kit+, Sca-1+, Lineage-) cells containing hematopoietic stem cells were slightly decreased. Defects in megakaryopoiesis were also observed. Transplantation analysis showed that Morf-/- fetal liver cells less efficiently reconstituted hematopoiesis when compared to wild-type cells. Especially, contribution of Morf-/- cells to KSLs and myeloid compartments were decreased in recipient mice. To reveal relationship between MORF and MOZ in hematopoiesis, we generated double heterozygous (Moz+/-, Morf+/-) mouse. Double heterozygous mice were smaller than wild-type littermates and died at least 4 weeks after birth. Number of KSL cells, especially Flt3- KSL cells and common myeloid progenitors were decreased in the double heterozygous embryos. The double heterozygous fetal liver cells also displayed less activity to reconstitute hematopoiesis than Moz+/+ or Morf+/+ cells. Since Morf-/- mice were alive at a mixed C57BL/6; DBA2 genetic background, we investigated adult hematopoiesis in these mice. Morf-/- mice were smaller than their wild-type littermates and had small numbers of thymocytes and splenocytes. In Morf-/- bone marrow, CD34-negative KSLs including hematopoietic stem cells were decreased. Aged or 5-FU treated Morf-/- mice had reduced number of hematopoietic stem cells. MORF as well as MOZ interacts with AML1 and PU.1 that were important for hematopoiesis. The mechanisms regulating stem cell aging hypothesis postulates that aging is caused, in part, by the loss of functional adult stem cells required for tissue regeneration and repair. The mechanisms regulating stem cell stress resistance and its contribution to age-related degenerative changes, however, remain unclear. Understanding cellular signaling that can protect the stem cells from chronic toxicity and from being depleted or exhausted after constantly replenishing damaged tissues may shed a light on stem-cell aging hypothesis and augment existing chemotherapy and stem-cell transplant therapies. Damping of IGF-I signaling, via either dietary intervention or genetic modification, is known to promote longevity in organisms ranging from yeast to mammals. In this study, we reported the potential anti-aging and/or rejuvenation effect of low circulating IGF-1 in the murine hematopoietic system. As in aging, multiple-cycles of chemotherapy-induced chronic oxidative stress caused long-term stem cells/progenitors impairment, in addition to the immediate tissue damages. We hypothesize that GH/IGF-I deficiency can also benefit the hematopoietic system in overcoming the cumulative long-term toxicity associated with chemotherapy. Mice with either regular or manipulated low IGF-I levels were challenged with multiple-cycle cyclophosphamide (CP) treatments for 4-6 months and their hematopoietic system was examined. Preliminary results suggest improved hematopoietic protection or recovery possibly supported by the enhanced long-term repopulation capacity of the protected hematopoietic stem cell/progenitors in mice with reduced circulating IGF-I. Interestingly, a newly discovered type of pluripotent stem cells, Very-small-embryonic-like (VSEL)(Oct+4+ SSEA-1+Sca-1+Lin-CD45-) stem cells, was also found to respond to the changes of IGF-I level. VSEL stem cells are known play an important role in repair of various tissues. Whether the low-IGF-I recruited VSEL directly supply the HSCs pool or indirectly repair the stem cell niche and eventually enhance hematopoietic regeneration, however needs to be further investigated. The protective effect of low IGF-I was then confirmed with human amniotic fluid stem cells (hAFSC), as an in vitro model, to investigate the therapeutic potential of manipulating IGF-I signaling in human stem cells. Here we present a study linking stem cell aging with stem cell exhaustion under chronic toxicity of chemotherapy and demonstrate that reducing IGF-I signaling may augment cellular stress response and therefore improved hematopoietic recovery from long-term chemotoxicity.
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nuclear cells by magnetic activated cell sorting with the use of erythocyte lineage markers (anti-CD71 and anti-glycoporphin A antibodies). Phenotypic characteristics of the transformed erythroid cells were subsequently determined by cell morphology and the expression profiles of several erythroid and megakaryocyte lineage markers as well as their ability to form megakaryocyte colonies under appropriate culture condition. Our results showed that the percentages of Erg1- and FLI1-overexpressed erythroid cells which express megakaryocyte-lineage marker CD41 was 27-folds higher than those of non-transformed erythroid cells (35.7 ± 2.8 % vs. 1.3 ± 0.6 %, P<0.05) after 5 days of culture in megakaryocyte inducing medium. In addition, the morphology of the overexpressed FLI1 and Erg1 erythroid cells was change from small cells with high nuclear/cytoplasmic ratio (which are typical for erythroid) to large multi-nucleated cells that expressed megakaryocyte specific marker, glycoprotein IIb/IIIa while the morphology of non-transformed erythroid cells were remain the same. Moreover, the transformed erythroid cells could also generate several megakaryocyte colony-forming units under collagen-based megakaryocyte culture condition while non-transformed erythroid could not generate any megakaryocyte colonies under the same condition. In conclusion, our study indicates that both FLI1 and Erg1 genes are sufficient to reprogrammed committed erythroid cells to megakaryocytes as determined by morphology, the expression profile of megakaryocyte specific marker and their ability to form megakaryocyte colonies under appropriate culture condition. The function of these reprogrammed erythroid cells is currently investigated.

**Poster Board Number: T-2129**

SAFETY AND SEIZURE CONTROL IN PATIENTS WITH MEDICALLY REFRACTORY MESIAL TEMPORAL LOBE EPILEPSY TRANSPLANTED WITH AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS

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Temporal lobe epilepsy (TLE) is a highly prevalent syndrome in patients with seizures and it is usually refractory to drug treatment. Anatomical and physiological changes are often present in patients with TLE such as mesial temporal sclerosis (MTS) characterized by extensive cell loss, reactive gliosis, mossy fiber sprouting and neurogenesis in the hippocampus and para-hippocampal structures. Hippocampal sclerosis can be verified by magnetic resonance imaging (MRI). Stem cells are proven to have the ability to reduce seizure frequency and to preserve the hippocampal neuronal networks in experimental model of chronic epilepsy. The aim of this study is to verify the safety and the therapeutic potential of bone marrow mononuclear cells (BMMC) transplantation on seizure control in patients with medically refractory mesial temporal lobe epilepsy (MTLE). To be eligible, patients had to have at least 18 years old and have to be diagnosed as MTLE according the Commission report of the International League Against Epilepsy. They were submitted to neurological evaluation, MRI study with hippocampal volumetry, 72 hour VideoEEG for epileptic seizure recording and laboratory tests. Also, patients were evaluated with a standardized neuropsychological test battery, focusing on verbal and nonverbal memory domains, consisting of the Wechsler Memory Scale-Revised, the Rey Auditory Verbal Learning Test, and the Rey Visual Design Learning. Also, patients were evaluated with a standardized neuropsychological test battery, focusing on verbal and nonverbal memory domains, consisting of the Wechsler Memory Scale—Revised, the Rey Auditory Verbal Learning Test, and the Rey Visual Design Learning. All patients who fulfilled selection criteria were invited to take part. After informed consent those with seizures originating in one temporal lobe that had consistent data from MRI and neuropsychological tests showing convergence for the same side, underwent BMMC transplant by selective posterior cerebral artery catheterism. This study was approved by Ethics Committee from our institution and National Committee on Ethics in Research and it is registered on www.clinicaltrials.gov (NCT00919266). All patients who fulfilled selection criteria were invited to take part. After informed consent those with seizures originating in one temporal lobe that had consistent data from MRI and neuropsychological tests showing convergence for the same side, underwent BMMC transplant by selective posterior cerebral artery catheterism. This study was approved by Ethics Committee from our institution and National Committee on Ethics in Research and it is registered on www.clinicaltrials.gov (NCT00919266). Twenty patients have already undergone this procedure. Fourteen patients were submitted to 3 and 6 months follow-up. After a follow-up of 6 months, nine patients (64.3%) were free of disabling seizures (Engel’s Class I). There were no complications related to the procedure. No additional lesions were detected on MRI. Also, no significant hippocampal volumetric change was observed. The EEG analysis showed a decrease of theta activity and spike density. Given these data we believe that BMMC autologous transplant for the treatment of MTLE is indeed feasible and seems to be safe so far. Seizure control achieved in this first experience gives us a very promising path in therapeutic potential of stem cells transplant in this population.

**Poster Board Number: T-2130**

NERVE GROWTH FACTOR IN COMBINATION WITH COLLAGEN HAS NOVEL ACTIVITIES IN PRESERVING GROWTH FACTOR-ACTIVATED HEMATOPOIETIC STEM CELL SURVIVAL AND EXPANSION IN VITRO

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Background: Many conditions have been found to stimulate the expansion of cells with long term hematopoietic repopulating ability, but conditions that preserve durable self-renewal remain elusive. We have previously shown that hematopoietic stem cells (HSC) from adult mouse bone marrow stimulated to divide in single-cell serum-free cultures execute a limited number of self-renewal divisions in the presence of 300 ng/mL Steel Factor + 20 ng/mL IL-11. However, only 40% of cells in highly purified HSC suspensions survive and form clones, and the yield of HSCs with durable self-renewal activity after one week is <10%. Medium conditioned by murine UG-26 fibroblasts (CM) improves both of these endpoints. It was therefore of interest to identify the responsible factors. Methods: Affymetrix array analysis was performed on RNA obtained from highly purified HSCs (CD45+PECR+CD150+CD48, “E-SLAM” cells, ~ 50% HSCs) before and after their incubation for 6 hours in CM only, in CM+100 ng/mL SF+20 ng/mL IL-11 (+GFs), or +GFs only. We then used the GO classifier to identify “receptor activity” transcripts in fresh ESLAM cells and “extracellular region” transcripts in UG26 cells (data online at GSE11589) to identify factors secreted
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by UG26 cells that had a putative receptor on ESLAM cells. Evidence of differential pathway activation in the presence of factors in CM was obtained by comparing ESLAM cells stimulated ±CM (±GFs). For clonogenic assays, single ESLAM cells were sorted into each well of a 96-well plate ±GFs and one or more candidate CM factors. Input cells were then scored for their ability to generate >=10 colonies after >=1 week. HSCs in 7-day cultures initiated with 30 ESLAM cells were quantified in limiting dilution transplant assays using congeneric, sublethally irradiated W41/W41 recipients (3 dilutions/ experiment) and detection of positive mice 16 weeks later (mice with >1% donor-derived circulating WBCs and a >1% donor contribution to the myeloid WBCs). Results: We identified transcripts for 172 "extracellular region" proteins in the UG26 cells for which there were also matching transcripts in the fresh ESLAM cells for one or more interacting proteins. Of the 430 tested Reactome pathways surveyed, we found 250 for which some members showed significantly altered transcript expression (p<0.05) between the tested conditions. Within this subset were a number of pathways for which a cognate cytokine gene was expressed in the UG26 cells. These included "signalling by NGF" and a "collagen-mediated activation cascade". From these data, a number of factors were selected and clonogenic assays used to identify those that promoted ESLAM cell survival and clone formation in cultures ±GFs. Of the factors tested, we found collagen, NGF (nerve growth factor), and Matrigel increased ESLAM cell cloning efficiency to 72%, 86%, and 100%, respectively (holm-adjusted p values <=0.01). Of the defined factors, NGF + collagen gave the highest clonogenicity (97%). Transplants with >1% donor-derived circulating WBCs and a >=1% donor contribution to the hematopoietic lineage showed a number of factors were selected and included HSC, predominantly express PKM2. In order to understand the role of PKM2 and glycolysis in normal hematopoiesis as well as in hematological malignancy, we have generated a genetically modified mouse strain that allows conditional deletion of the PKM2 specific exon. Our data show that PKM2 deletion does not compromise normal HSC function under homeostatic conditions. Rather, loss of PKM2 results in a competitive advantage over wild-type HSC, as evidenced by enhanced bone marrow reconstitution in a competitive transplantation assay. The altered cellular function by PKM2 deletion is accompanied with decreased glycolysis and increased oxidative phosphorylation specifically in the primitive stem/progenitor cell population. Notably, however, the absence of PKM2 appears to markedly disadvantage the establishment of leukemia in mice when hematopoietic cells express human leukemogenic alleles associated with either acute or chronic myeloid leukemia. Such a distinction in response between normal and malignant cells is unprecedented to our knowledge and may offer a highly unique opportunity to advantage normal while disadvantaging malignant cells of the hematopoietic lineage by targeting a single metabolic enzyme.

Poster Board Number: T-2132

GNAT-LIKE PROTEIN DOWN-REGULATES GLOBIN SYNTHESIS BY BINDING ACETYL COENZYME A

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Globin synthesis is crucial event in erythroid differentiation and regulated by histone acetylation. We identified novel molecule that regulates histone acetylation through our database of anemic zebrafish induced by cold-exposure. This molecule belongs to the family of GCNs-related N-acyetyltransferase (GNAT), which functions in histone acetylation. Mouse GNAT-like gene was up-regulated as hematopoietic stem cells differentiate into mature erythrocytes synchronizing with increase of globin gene expression. Although this GNAT-like protein could bind to acetyl coenzyme A (acetyl CoA), it lacked histone acetylation activity. Ectopic expression of mouse GNAT-like gene in erythroleukemia cells decreased histone acetylation and globin gene expression, whereas knock down of GNAT-like gene in zebrafish embryos increased gene and protein expressions of globin. Taken together, this GNAT-like down-regulates globin gene synthesis likely through competing with other GNATs for acetyl CoA binding.

Poster Board Number: T-2133

TRANSCRIPTOME ANALYSIS WITHIN THE IMMGEN DATABASE IDENTIFIES REGULATORS OF PROGENITORS AND STEM CELLS.

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Although hematopoietic stem cells are the best characterized of adult tissue-specific stem cells, surprisingly little is known about the mechanisms that regulate their central properties of self-renewal and multi-lineage potential. Towards improving the molecular understanding of HSCs and their proximal multi-potent and oligo-potent progenitor subsets, we performed expression profiling of these primitive progenitors and analyzed the data within the context of the ImmGen microarray database comprised of over 700
Disruption of TET2 leads to enhanced self-renewal and competitive repopulating capacity of fetal liver hematopoietic stem cells

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TET2 (ten-eleven-translocation 2) gene has been reported to be frequently mutated in various human myeloid and lymphoid malignancies. These observations suggest critical roles of TET2 dysfunction in molecular pathogenesis of hematologic malignancies. Recent studies using conditional knockout mouse model indicated that mouse TET2 loss leads to clonal dominance of adult hematopoietic stem cells (HSCs) in competitive repopulation assay. However, self-renewal capacity of adult HSCs has never been addressed precisely by serial transplantation assay. In addition, the effect of TET2 loss on HSCs was never examined in the fetal livers (FLs). Since FL HSCs and adult HSCs differ in several aspects of their phenotypes and functions, we speculated that TET2 might be involved differentially in the regulation of FL and adult hematopoiesis. To address this issue, we analyzed E14.5 FL cells from TET2−/− mice. RT-PCR analysis showed that over 99% of TET2 mRNAs from endogenous promoter were trapped by the gene-trap cassette in Tet2−/− mice, showing that Tet2−/− allele can be considered as a null allele. Initial analysis showed that TET2−/− embryos developed normally, but most TET2−/− mice were perinatally lethal. Interestingly, TET2−/− embryos displayed significant increase in lineage (Lin) Sca-1+ c-Kit+ (LSK) fraction compared to wild type (WT) (TET2+/−) littermate (1.45±0.62% vs. 0.85±0.34%, p=0.0027).

DISRUPTION OF TET2 LEADS TO ENHANCED SELF-RENEWAL AND COMPETITIVE REPOPULATING CAPACITY OF FETAL LIVER HEMATOPOIETIC STEM CELLS

Poster Board Number: T-2134

Increasing evidence suggests tumors are maintained by cancer stem cells; however, their nature remains controversial. In a HoxA9-Meis1 (H9M) model of acute myeloid leukemia (AML), we found that tumor-initiating activity existed in three, immunophenotypically distinct compartments, corresponding to disparate lineages on the normal hematopoietic hierarchy, stem/progenitor cells (Lin−), and committed progenitors of the myeloid (Gr1−kit+) and lymphoid lineages (Lym−). These distinct tumor-initiating cells (TIC) clonally recapitulated the immunophenotypic spectrum of the original tumor in vivo (including cells with a less-differentiated immunophenotype) and shared signaling networks, such that in vivo the original tumor suppressor signaling networks were retained. Collectively, H9M AML is organized as an atypical hierarchy that defies the strict lineage marker boundaries and unidirectional differentiation of normal hematopoiesis. Moreover, this suggests that in certain malignancies tumor-initiation activity (or “cancer-stemness”) can represent a cellular state that can exist independently of distinct immunophenotypic definition.

DECOUPLING OF TUMOR-INITIATING ACTIVITY FROM STABLE IMMUNOPHENOTYPE IN HOXA9-MEIS1 DRIVEN AML

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TIM-3 expression in the various types of hematological malignant leukemic stem cells

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Acute myeloid leukemia (AML) originates from self-renewing leukemic stem cells (LSCs), an ultimate therapeutic target for AML. We identified T-cell immunoglobulin mucin-3 (TIM-3), as a surface marker of FL HSCs. Moreover, donor-derived HSC fraction (CD34+ LSK cells) was significantly expanded in the recipients of Tet2−/− FL cells, suggesting that increased self-renewal capacity is cell intrinsic to Tet2−/− HSCs. We have also examined differentiation of Tet2-mutant FL cells in the recipients’ peripheral blood, and found that Tet2−/− cells displayed impaired differentiation to Gr1+CD11b+ mature granulocytes. Liquid culture of FL cells with cocktails of cytokines in vitro demonstrated that Tet2−/− FL cells retained higher percentage and number of LSK, Lin− and c-Kit− cells compared to WT cells, showing enhanced resistance of Tet2−/− cells to differentiation stimuli in vitro culture. It is of note that Tet2−/− mice showed a significant increase in LSK compartment in the BM compared to wild type littermate (0.48±0.11% vs. 0.32±0.04%, p=0.04). However, they presented no signs of extramedullary hematopoiesis such as splenomegaly and expansion of LSK cells in spleens during an observation up to 35-weeks. Taken together, we demonstrate that Tet2 critically regulates self-renewal and long-term repopulating capacity of FL HSCs and has pleiotropic functions in hematopoietic differentiation. In addition, enhanced HSC self-renewal, expansion of HPC and myeloid progenitors and perturbed differentiation induced by TET2 ablation likely to set molecular basis for malignant transformation, which explains high incidence of loss-of-function mutations of TET2 in hematologic malignancies.

TIM-3 EXPRESSION IN THE VARIOUS TYPES OF HEMATOLOGICAL MALIGNANT LEUKEMIC STEM CELLS

Poster Board Number: T-2136

Microarrays that encompass the vast majority of immune cell types. Principle component analysis positioned all hematopoietic stem and progenitor subsets (HSPCs) in a cluster at a central position in relation to downstream effector cells showing the relatedness of HSPC subsets and consistent with their role as undifferentiated precursors of all blood lineages. Gene set enrichment analysis revealed that HSPCs were most enriched for cell growth and cell cycle pathways in line with their role as transit amplifying cells.

Analysis of the multipotent subsets (MP) identified a novel group of genes encoding KRAK-domain containing proteins, predicted to be transcriptional repressors- suggestive of a role in suppressing lineage commitment in these otherwise uncommitted cells. Genes involved in transcriptional regulation were identified in HSCs and co-expression analysis of these factors showed that those with established roles in HSC quiescence and self-renewal clustered separately from a set of factors of unknown function in HSCs.

Finally, enforced expression of one transcription factor, Hlf was able to imbue stem and progenitors with a sustained primitive immune-phenotype, and extensive self-renewal and multi-myeloid lineage potential. This work, within the ImmGen project, presents a reliable reference of whole-transcriptome data across the immune system, finding novel genes as candidate regulators of adult HSCs.
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molecule expressed on LSCs in most types of AML except for acute promyelocytic leukemia, but not on normal hematopoietic stem cells (HSCs). TIM-3+ but not TIM-3- AML cells reconstituted human AML in immunodeficient mice, suggesting that the TIM-3+ population contains most, if not all, of functional LSCs. We established an anti-human TIM-3 mouse IgG2a antibody having complement-dependent and antibody-dependent cellular cytotoxic activities. This antibody did not harm reconstitution of normal human HSCs, but blocked engraftment of AML after xenotransplantation. Furthermore, when it is administered into mice grafted with human AML, this treatment dramatically diminished their leukemic burden, and eliminated LSCs capable of reconstituting human AML in secondary recipients (Kikushige et al, Cell Stem Cell, 2010). We extended the analysis of TIM-3 expression in the various types of human hematological malignancies, and found that human TIM-3 was expressed in the vast majority of CD34+CD38- leukemic stem cell fractions of human myeloid malignancies including chronic myeloid leukemia, chronic myelomonocytic leukemia and myelodysplastic syndrome (MDS). Especially in MDS, the expression level of TIM-3 on CD34+CD38- leukemic stem cells is dramatically up-regulated along with disease progression to AML, suggesting the important role of TIM-3 during leukemogenesis. We also intensively investigated the function of TIM-3 signaling in human hematological malignancies. In this presentation, we would like to discuss the further details of human TIM-3 function in myeloid leukemic stem cells.

Poster Board Number: T-2137

EFFECT OF NANOSEGMENTS ON EX VIVO EXPANSION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS CULTURED ON SURFACE HAVING SEVERAL NANOSEGMENTS

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Umbilical cord blood (UCB) is an attractive source of hematopoietic stem and progenitor cells (HSPCs) for HSPC transplantation. However, the low number of HSPCs obtainable from a single donor of UCB limits direct transplantation of UCB to the treatment of pediatric patients. In this study, we investigated the ex vivo expansion of HSPCs cultured on biomaterials grafted with several nanosegments, i.e., polyamine, fibronectin, RGDS, and CS1 (EILD-VPST), at several surface densities. No direct correlation was found between fold expansion of HSPCs and physical parameters of the culture dishes, i.e., surface roughness and water contact angle of the culture dishes. However, a small amount of grafted amino groups, less than 0.8 residual mol/cm², on the dishes was effective for the ex vivo expansion of HSPCs. A high amount of grafted amino groups hindered the ex vivo expansion of HSPCs on the dishes. HSPCs cultured on dishes with a high concentration of CS1 (2.40 residual mol/cm²) showed greater expansion of HSPCs and more pluripotent colony-forming units (i.e., colony-forming unit-granulocyte, erythroid, macrophage, and megakaryocyte (CFU-GEMM)) than those on fibronectin-grafted and polyamine-grafted dishes. These data suggest that the specific interaction between HSPCs and CS1 helps to maintain the pluripotency of HSPCs during the ex vivo expansion of HSPCs. This is the first study on the effect of the surface density of nanosegments immobilized on culture dishes on the ex vivo expansion of HSPCs. The amount of grafted amino groups less than 0.80 residual mol/cm² on the dishes was effective for the ex vivo expansion of HSPCs. An amount of grafted amino groups on the dishes hindered the ex vivo expansion of HSPCs. There was no direct correlation between fold expansion of HSPCs and physical parameters of the culture dishes, i.e., RMS surface roughness and water contact angle, while HSPCs cultured on dishes containing a high concentration of CS1 showed much higher fold expansion of HSPCs and colony-forming units (CFU-GEMM) than those on other dishes, such as fibronectin-grafted and polyamine-grafted dishes. These data suggest that the specific interaction between HSPCs and CS1 helps to maintain the pluripotency of HSPCs during the ex vivo expansion of HSPCs. CS1 is one of the cell-binding domains of fibronectin. However, the ex vivo expansion and CFU expansion of HSPCs cultured on fibronectin (FN)-grafted dishes were less than those on PS-CS1-H (CS1-grafted polystyrene (PS) with high concentration of CS1) dishes, although the ex vivo expansion and CFU expansion of HSPCs on PS-FN (fibronectin (FN)-grafted polystyrene (PS)) dishes were higher than those on PS or TCPS (tissue culture polystyrene) dishes. The presentation of specific binding site number of CS1 is 500 times (440,000/880) higher than that of fibronectin at the same grafting weight density of CS1 and fibronectin on dishes, because of low molecular weight of CS1 (approximately 880 dalton from 110 [average molecular weight of amino acids] x 8 [No. of amino acids in CS1]) than fibronectin (440,000 dalton). The presentation of specific cell-binding domains of CS1 at high concentrations seems to be important for the signal transduction that promotes the ex vivo expansion of HSPCs.

Poster Board Number: T-2138

IN VIVO IMAGING OF DYNAMIC INTERACTIONS WITHIN THE HAEMATOPOIETIC STEM CELL NICHE

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The stem cell microenvironment (or niche) is a critical regulator of stem cell function. Haematopoietic stem cells (HSC) reside within the bone marrow and therefore have been traditionally inaccessible to direct observation. For this reason, despite numerous functional studies demonstrating a role of several bone marrow stroma cells in regulating HSC function and number, still very little is known about the anatomical location of HSC during steady state and in response to several types of stimuli. Many functional studies over the years have pointed to osteoblasts, bone-lining cells responsible for matrix deposition during bone growth and remodelling, as regulators of the HSC pool. Moreover, several studies have indicated that multiple perivascular stroma cells actively influence HSC fate. It is an open question whether HSC firmly localise within a specific niche or whether they migrate between different niches, defined by distinct bone marrow microenvironments, where they receive different stimuli leading to their quiescence vs. proliferation, self-renewal vs. differentiation. Another open question is whether leukaemia development affects HSC-niche interactions and whether leukaemia stem cells (LSC) and HSC compete for niche space. Confocal and two-photon hybrid microscopy allows visualisation of single transplanted HSC in the calvarium bone marrow of live mice. FACs-purified, ex-vivo labelled haematopoietic stem and progenitor cell (HSPC) populations can be visualised upon their initial arrival in the niche (homing) and following the first divisions (early engraftment). We showed that long-term repopulating (LT) HSPC selectively localize proximal to osteoblasts within a few hours from transplantation, whereas their progeny are more distal. By analysing homing and early engraftment patterns of mutant HSPC known to dominate or fail when in competition with normal HSC for bone marrow engraftment, and of wild type HSC transplanted...
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into environments known to improve or hinder their function, we monitored dynamic interactions between HSC and the bone marrow microenvironment and collected further evidence that HSC and HSC progeny localization near osteoblasts correlates with positive transcriptional outcome. Finally, we used a mouse model of acute myeloid leukemia based on retroviral transduction of the MLL-AF9 oncogene and observed that leukemic granulocyte-monocyte precursors (L-GMP), an established LSC population, share the same homing pattern of normal GMP rather than that of LT-HSC. This indicated why MLL-AF9 LSC are insensitive to niche-derived signals that otherwise affect normal HSC function.

Poster Board Number: T-2139

DUAL FUNCTION OF CX32 IN HEMATOPOIESIS: MAINTENANCE OF CELL QUIESENCE AND SUPPORT OF PROLIFERATION OF HEMATOPOIETIC STEM/PROGENITOR CELLS

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Membrane channel connexin (Cx) forms gap junctions that are implicated in the homeostatic regulation of various cellular functions, including growth control and differentiation, apoptosis, and the synchronization of electrotonic and metabolic functions. Our studies of the role of Cx32, a member of the Cx family, in steady-state hematopoiesis and its potential protective role against leukemogenesis were reported previously (Exp Biol Med 232:700-12, 2007; J Membr Biol 217:105-13, 2007). Namely, in wild-type mice, Cx32 expression was detected in primitive hematopoietic stem/progenitor cells by immunocytochemistry and reverse transcriptase-polymerase chain reaction analysis, although it was not detected in unFractionated bone marrow cells. Cx32-knockout (KO) mice showed decreased numbers of peripheral mononuclear cells and various progenitor cell compartments, an increased number of cells in primitive stem cell fractions, such as the lineage marker (-)/c-kit (+)/stem cell antigen-1 (+) (LKS) fraction, and a clearly delayed regeneration of progenitor cells after chemical abrasion. Furthermore, the incidence of methyl nitro-urea (MNU)-induced leukemia was markedly high in the Cx32-KO mice after a single administration of MNU. Subsequent cell-cycle analysis of the LKS fraction using a cell sorter with Hoechst 33342, a conditional Ebf2 knockout mouse line. At this point, we were successful in generating such a line, as ubiquitous deletion of the Ebf2flox allele fully recapitulates the phenotype of Ebf2-mutant animals. Surprisingly, deletion of Ebf2 in adipocytes (Ap2-Cre) does not influence HSC frequency to a detectable level, whereas deletion in immature osteoblastic cells (Osterix-Cre) fully recapitulates the phenotype of the conventional deletion of Ebf2. Furthermore, deletion of Ebf2 using the Prrx1-Cre line, which supposedly is active in mesenchymal stem cells, does not lead to any phenotypic alterations. Taken together, the data show that Ebf2 is required for HSC maintenance specifically via its expression in immature osteoblastic cells, thereby defining these cells as components of a niche for HSC. As Ebf2 is a transcription factor, this finding also opens up the possibility of systematically analysing the transcriptional profile and target genes of Ebf2 in primary niche cells.

Poster Board Number: T-2140

EXPRESSION OF EBF2 IN IMMATURE OSTEOSTEAL CELLS DEFINES A NICHE FOR HEMATOPOIETIC STEM CELLS

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Hematopoietic stem cells (HSC) need a specialised microenvironment to maintain hematopoiesis life-long. Several cell types like osteoblasts, osteomacs, adipocytes, CXCL 12 abundant reticular (CAR) cells and nestin-positive mesenchymal stem cells have been implicated in the support of HSC. Among those, the strongest evidence for a functional involvement exists for osteoblastic cells, but the exact nature of HSC supporting osteoblastic cells is not clear. Early B cell factor 2 (Ebf2) is a transcription factor expressed specifically by mesenchymal stem cells, immature osteoblastic cells (IEO) and adipocytes of the bone marrow. Deficiency for Ebf2 does not alter the number of these cells, but their ability to support HSC and immature hematopoietic progenitors. In vitro studies suggest that the HSC supporting function is mediated via its expression in osteoblastic cells, but the differentiation stage as well as the contribution of other cell lineages like MSC and adipocytes is unclear. To analyse the cellular contribution to HSC support exactly, we generated a conditional Ebf2 knockout mouse line. At this point, we were successful in generating such a line, as ubiquitous deletion of the Ebf2flox allele fully recapitulates the phenotype of Ebf2-mutant animals. Surprisingly, deletion of Ebf2 in adipocytes (Ap2-Cre) does not influence HSC frequency to a detectable level, whereas deletion in immature osteoblastic cells (Osterix-Cre) fully recapitulates the phenotype of the conventional deletion of Ebf2. Furthermore, deletion of Ebf2 using the Prrx1-Cre line, which supposedly is active in mesenchymal stem cells, does not lead to any phenotypic alterations. Taken together, the data show that Ebf2 is required for HSC maintenance specifically via its expression in immature osteoblastic cells, thereby defining these cells as components of a niche for HSC. As Ebf2 is a transcription factor, this finding also opens up the possibility of systematically analysing the transcriptional profile and target genes of Ebf2 in primary niche cells.

Mesenchymal Stem Cell Differentiation

Poster Board Number: T-2141

MECHANICAL DERIVATION OF FUNCTIONAL MYOTUBE FROM ADIPOSE DERIVED STEM CELLS

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In recent years, ECM stiffness and resulting cell contractility have been identified as potent stem cell differentiation regulators. Suc-
successful stem cell-based therapies will require acclimating cells to
the abnormally stiff ECM of muscular dystrophy while inducing
and/or maintaining myogenesis, fusion, and dystrophin delivery.
Here we directly compare ASC to BMSC stiffness responsiveness
and show myotube formation derived from ASCs on matrices that
mimic skeletal muscle. ASCs are shown here to not simply reflect
the qualitative stiffness sensitivity of bone-marrow-derived
stem cells (BMSCs) but to exceed BMSC myogenic capacity (40-
fold higher myogenic marker expression on myogenic stiffness),
expressing the appropriate temporal sequence of muscle trans-
criptional regulators on muscle-mimicking extracellular matrix in
a focal adhesion- and contractility-dependent manner. 2% of ASCs
formed multi-nucleated myotubes with a continuous cytoskel-
eton (10-fold higher than chemical induction) that was not due to
misdirected cell division; microtubule depolymerization severed
myotubes, but after washout, ASCs re-fused at a rate similar to pre-
treated values. BMSCs never underwent stiffness-mediated fusion.
ASC-derived myotubes, when replated onto non-permissive stiff
matrix, maintain their fused state. Fusion frequency was increased
by a contractile agonist, lysophosphatidic acid and decreased by
a myosin inhibitor, blebbistatin. ASCs generated higher tangen-
tial force than BMSCs and showed more non-muscle myosin IIb.
Mechanical induction was mediated via focal adhesions; vinculin
assembled earlier in ASCs. Inhibiting fibronectin-integrin binding
using alpha 5 or V integrin siRNA blocked mechanosensing process
as ASCs fail to ‘feel’ myogenic 10kPa gel and to show myogenic
mRNA expression. Together these data imply enhanced mechano-
sensitivity for ASCs, making them a better therapeutic cell source
for fibrotic muscle.

Poster Board Number: T-2142

CONTROLLING STEM CELL FATE WITH SPATIALLY
VARYING EXTRACELLULAR MATRIX STIFFNESS

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Regenerative musculoskeletal applications have been plagued
with setbacks owing in part to the fibrosis present in degenerative
muscle disorders, which increases collagen density and transgluta-
minease activity that stiffens the extracellular matrix (ECM). Injection
of undifferentiated stem cells into diseased muscle, i.e. cellular
myoplasty, was thought to ameliorate the disease, but instead
causes cell calcification due at least in part to the abnormally stiff
environment. Here, we have developed a patterned hydrogel that
can assess stem cell phenotype maintenance and engrafment potential
in a fibrotic-like environment in vitro. This “Zebraxis”
bio-reactor contains alternating regions of firm (10 kPa; myogenic)
and stiff (34 kPa; fibrotic or osteogenic) or soft (1 kPa; neurogenic)
and firm that mimic fibrotic muscle or innervated, healthy skeletal
muscle, respectively. In the non-fibrotic model (1 vs. 10 kPa), C2C12
myoblasts migrate onto 10kPa stripes after day 1 and formed myo-
tube by day 7 in lower serum conditions. Adipose-derived stem
cells (ASCs), which we have previously shown are myogenic on firm
ECM, also migrated onto firm stripes and fused on the non-fibrotic
model. Supporting cells types on soft regions, e.g. PC12 neurons,
should help this process. With the fibrotic model (10 vs. 34 kPa),
ASCs and C2C12 myoblasts segregated on 34 and 10 kPa make
direct contact and will mimic cellular myoplasty in vitro. Using
ASC-derived myotubes-rather than undifferentiated stem cells-this
in vitro system highlights our ability to monitor engrafment poten-
tial improvements in vitro using novel fibrosis-mimicking tissue
equivalents. Our efforts to develop tissue engineered musculoskel-
etal systems hinge on the understanding gained here about the
stem cell-microenvironment interaction so that we may overcome
the fibrosis problem.

Poster Board Number: T-2143

RAPMYCIN ATTENUATES OSTEOGENIC
PROPERTIES OF DAXAMETHASONE ON HUMAN
BONE MARROW DERIVED MESENCHYAL STEM

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Introduction: Application of allograft Mesenchymal Stem Cells in
combination with immunosuppressive drugs which have osteo-
egenic properties, would be a very good option to regenerate bone
defects in clinics. Rapamycin, and Dexamethasone in particular, are
two commonly used immunosuppressive drugs in clinics which
have been shown to possess osteogenic properties. The aim of
this study was to assess osteogenic properties of Rapamycin in
combination with dexamethasone on promotion of osteogen-
esis in human bone marrow-derived Mesenchymal Stem Cells.

Methods: To do that, Bone marrow-derived mesenchymal stem
cells were cultured in osteogenic medium supplemented with
10nM Dexamethasone without rapamycin(Control) and with 1, 10,
and 100nM rapamycin, then evaluated at 7, 14 and 21 days post
induction. Osteoblastic differentiation was characterized quanti-
tatively by Alkaline Phosphatase Activity and Alizarin Red Staining
Assay. Results: Our pilot study results showed that rapamycin alone
showed no remarkable osteogenic differentiation effect on human
mesenchymal stem cells, therefore we evaluated the synergistic
effect of rapamycin and dexamethasone in promotion of osteo-
egenesis. Our results showed that at day 7, Alkaline Phosphatase
enzyme activity significantly increased in groups treated with Dexam-
ethasone and 1nM rapamycin rather than dexamethasone alone (Control). Two weeks post induction, the enzyme activity increased
significantly in all groups in comparison with the same groups at
day 7; however, the enzyme activity was remarkably decreased in
groups treated with Rapamycin in combination with Dexametha-
sone alone, and there was not a remarkable difference in enzyme
activity in groups treated with 1, and 10 nM Rapamycin. At day
21, no significant difference was observed between groups which
received both rapamycin (1 & 10nM) and Dexamethasone in one
hand, and the control group which received dexamethasone alone
on the other hand; however in presence of 100nM of rapamycin
the enzyme activity was the least among he others. Evaluation of
mineralization by alizarin red-based osteogenesis assay showed no
difference between either control group(Dexamethasone alone)
or other treated groups (Rapamycin & Dexamethasone) at day 7.
However, one week later, matrix mineralization increased signifi-
cantly in control group(Dexamethasone alone) in comparison with
groups which received 10 & 100 nM of Rapamycin. At third week of
induction, control group(Dexamethasone alone) showed a remark-
able increase in matrix mineralization in comparison with other
groups. Conclusion:Our results showed that Rapamycin would not
be a good stimulator for promotion of osteoblastic differentiation
of human bone-marrow-derived mesenchymal stem cells in com-
bination with Dexamethasone. Not only have any synergistic effect
in induction of osteogenesis with Dexamethasone, rapamycin
attenuates the inductive effect of Dexamethasone in human bone
marrow-derived mesenchymal stem cells.
Background: The osteogenic differentiation capacity of human mesenchymal stem cells for bone formation and regeneration, leads to establish different strategies in order to provide reproducible systems for induction and enhancement of osteogenesis in these cells. Besides growth factors, some small molecules, such as purmorphamine serve as chemical tools in promotion of osteogenesis; however, there has been no exact evaluation on the appropriate dose and the response time of this molecule for promotion of osteogenesis. The aim of this ongoing study is to define the most appropriate dose and the response time of Purmorphamine, to evaluate its efficacy in the promotion of osteogenesis in human Bone Marrow Mesenchymal Stem Cells in vitro and in vivo.

Methods: To do that, Bone marrow derived mesenchymal stem cells were cultured in high glucose Dulbecco’s modified eagle medium supplemented with 10 nM Dexamethasone without purmorphamine (control) and with 1, 3, 5, and 10 µM of this small molecule and evaluated at 7, 14 and 21 days post induction. Results: Although ALP activity was low 7 days post induction, it increased significantly (p< 0.05) in cells treated with 3 µM of purmorphamine in comparison with control group. Interestingly, one week later, this enzyme activity remarkably increased in all treated groups, especially in groups which received 3 and 5 µM doses of this small molecule. The enzyme activity decreased again one week later at day 21, and no significant difference was observed between purmorphamine- treated groups and the control one. Interestingly, our Real-time PCR results showed that 5µM of purmorphamine significantly(p< 0.05) increased the expression level of Runx-2, an early transcription factor of osteogenesis, Osteocalcin and Osteopontin, two important matrix protein transcripts at day 14 post induction; however, the groups which received 10 µM of purmorphamine showed significant over expression of osteocalcin and osteopontin, not earlier than the end of the third week. We also analyzed mineralization capacity of the cells with and without (control) purmorphamine. Our results showed that although a significant increase in matrix mineralization was observed in all treated and non- treated groups from day 7 up to 21 days post induction; this augmentation was not significant in comparison with control group at each day. Conclusion: As whole, this part of our study indicates that (1) events related to osteoblast differentiation, including increased ALP activity and bone specific gene expression are enhanced in hBM-MSCs at 2nd week post induction by 5 µM purmorphamine. (2) Due to high activity of ALP and over expression of bone specific genes such as RUNX-2 in the presence of 5µM of purmorphamine at day 14 and subsequent decrease in ALP activity and RUNX-2 expression at day 21; in order to in vivo study, we recommend to treat the cells with 5 µM purmorphamine at least for 14 days in transplantation.
EVALUATION OF DIFFERENT CONCENTRATIONS OF RETINOIC ACID IMPACTION ON GERM CELL SPECIFIC GENES EXPRESSION IN RAM MARROW DERIVED MESENCHYMAL STEM CELLS

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Introduction: Mesenchymal stem cells (MSCs) can be isolated from several sources and have high capacity for differentiation to different cell types of all three germ layers. In recent years some studies have discussed about the application of MSCs for germ cells (GCs) production in vitro and infertility treatment especially in males. Some researchers have suggested that some materials like some hormones, growth factors, retinoic acid and ([Unsupported Character - &lt;#8943;)]) can induce GCs differentiation from MSCs in vitro. In this study we evaluated and compared effects of three different concentrations of retinoic acid (RA) treatment on male GC-specific genes expression in bone marrow-derived MSCs in sheep. Our main goal was to obtain germ cells from MSCs by RA treatment. Material and methods: MS like cells was isolated from an aspiration of bone marrow from tibia of a newly mature male sheep under deep sedation condition that was induced by Ketamine and Xylazin injection. The cells mesenchymal stemness characteristics confirmed by test of their osteogenic, adipogenic and chondrogenic capacity of differentiation. Passage 3 MSCs were cultured in complete media culture containing three different concentrations of RA for 21 days. RA concentrations were 1 µM (RA1), 5 µM (RA2) and 10 µM (RA3). At the end of the experiment time the cells were analyzed with RT-PCR and real-time RT-PCR for expression of male GS-specific genes and were immunostained for PGP9.5 - spermatogonia specific gene - expression. The genes examined for RT-PCR and real-time RT-PCR were OCT4, Vasa, β-1integrin, PGP9.5, maybe it could be concluded that RA can induces germ cell differentiation in sheep marrow-derived MSCs and the maximum effect was seen in 10 µM RA concentration.

THE EFFECT OF THREE-DIMENSIONAL PILLAR SURFACES ON CELL ADHESION AND OSTEOGENIC DIFFERENTIATION

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Introduction The hypothesis was that compared to conventional two-dimensional (2D) cultures, substrates containing 3D nano- and micron-size pillars would allow cultured cells to grow at two or more different levels and help them attach to and extend e.g. from top-to-top or top-to-bottom, activating their cytoskeleton to promote osteogenesis. Materials and methods Fibroblasts, osteoblast-like SaOS-2 cells and human mesenchymal stem cells (MSC) were studied. Plain substrates were compared to 200 nm, 5 µm and 20 µm high pillars fabricated from Si or Ormocomp®. Some of the Si pillars were coated with diamond-like carbon (DLC) or TiO2. Adhesion of cells was evaluated with scanning electron microscopy (SEM) and staining of actin cytoskeleton. Activation of cytoskeletal tension was studied by staining extracellular signal-regulated kinases (ERK) and Rho-associated protein kinase (ROCK). Osteogenic markers, alkaline phosphatase (ALP), osteopontin (OP) and mineralization (Alizarin Red, Osteoimage™) were stained to visualize the effect of pillars on osteogenesis. Results and discussion SEM and immunofluorescence staining of actin cytoskeleton of hMSCs and fibroblasts showed adhesion to high pillar edges at 7.5 hours and stretching between adhesion contacts at different levels well over 100 µm distances at 5 days. SaOS-2 cells, which are about half the size of hMSCs or fibroblasts, adhered flatly and individually on horizontal and vertical substrate surfaces without showing respect for contact inhibition. ERK and ROCK immunostaining verified the activation of the cytoskeleton in the stretched cells at 14 and 21 days. In contrast to expectations, the success of induced osteogenesis was thoroughly dominated by the cytocompatibility of the substrate, with early ALP intermediate OP and late mineralisation markers, together with bone nodule formation, being seen in plain and low profile TiO2 pillars, but being poor in the 20 µm landscape. Only cell-high 5 µm pillars slightly enhanced induced osteogenesis. In 2D cultures, MSCs grew to confluence, with extensive cell-to-cell contact on their periphery allowing intercellular communication, whereas in 3D pillar surroundings cells were stretched between their two or more substrate contact points, with most of their cellular surface bathing in cell culture fluid. This lack of intercellular cell-cell contacts seems to suppress the osteogenesis promoting effects of cytoskeletal organization and tension, which osteogenesis promoting effects have otherwise been shown in many other experimental settings.
**PULSED ELECTROMAGNETIC FIELD PROMOTES PROLIFERATION OF HUMAN MESENCHYMAL STEM CELLS AND DEHYDROEPIANDOSTERONE ENHANCES OSTEOGENESIS**

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Introduction: The stem cell fate is determined by soluble factors, physical forces, cell-cell and cell-extracellular matrix contacts. We studied the effect of pulsed electromagnetic field (PEMF) and dehydroepiandrosterone (DHEA) on the proliferation of human mesenchymal stem cells (hMSC) and osteoblastic SaOS-2 cells and on the osteogenic differentiation of hMSCs. The ability of stem cells to differentiate into bone cells is critical for fracture healing and integration of implants into bone. PEMF has been clinically used since food and drug administration approval in 1979 to promote bone fracture healing and others bone disorders for at least three decades its applications include osteoporosis, bone fractures, pseudarthroses and osteotomies. The cellular mechanism by which the PEMF exerts its effects is still mainly unknown. DHEA is a pro-hormone produced in the reticular zone of the adrenal cortex, but only in man and other primates. It is in peripheral cells and tissues metabolized by steroidogenic, intracrine enzymes to various active sex steroids, e.g. 17β-estradiol (E2) or dihydrotestosterone (DHT). Such functionally active sex steroids play key roles in bone metabolism and their failure is a well-recognized cause of post-menopausal osteoporosis in women and osteoporosis in hypogonadism in men.

Materials and methods Human osteogenic sarcoma SaOS-2 (ECACC 890500205) cells and bone marrow derived hMSCs (PoieticsTM, Lonza, Basel, Switzerland) were cultured. PEMF were produced by OSSATEC® Bone growth stimulation device (Uden, The Netherlands) using continuous stimulation. To test the effect of DHEA, the cells were cultured in 1, 10 μM or 100 μM DHEA (Sigma Aldrich, St. Louis, MO, USA), with and without 100 nM dutasteride (Avodart®, GlaxoSmithKline), which inhibits type I and II 5α-reductase. Proliferation was studied using the MTT-method at days 4-21 and differentiation at days 1-28 with quantitative real time polymerase chain reaction and staining of osteogenic markers. Results and discussion PEMF promoted the proliferation rate of both cell types and DHEA decreased it in a concentration dependent manner. PEMF stimulation increased hMSC proliferation by 16% at day 14 and SaOS-2 proliferation by 30% at day 11 compared to non-stimulated cells. DHEA treatment decreased the proliferation rate of hMSCs by 58% at day 21 and proliferation of SaOS-2 cells by 35% at day 14 compared to non-stimulated cells (p < 0.001 for all comparisons). DHEA clearly promoted the osteogenesis of hMSCs, which were induced to osteogenesis with beta-glycerophosphate, ascorbate and dexamethazone. Alkaline phosphatase, SMAD1, RUNX2, osteocalcin (OC) and osteopontin (OP) RNA levels were increased and Alizarin red S and hydroxyapatite specific OsteoImageTM stainings showed mineralization. In addition, DHEA increased OC and OP mRNA levels of non-induced hMSCs. At this low seeding density, 3000 cells/cm², PEMF did not have major effects on osteogenesis, which is logical as proliferation and differentiation are generally considered opposite and mutually exclusive states in cell biology, with proliferation being followed by differentiation. Conclusions PEMF stimulated the proliferation of hMSCs and SaOS-2 cells and DHEA stimulated the osteogenic differentiation of hMSCs.

**EXPANSION AND DIFFERENTIATION OF HUMAN MESENCHYMAL STROMAL CELLS**

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Human mesenchymal stromal cells (hMSC) are candidates for clinical use because they are readily expanded in culture, have immuno-modulatory potential and can differentiate into the osteogenic, chondrogenic and adipogenic lineages. Their therapeutic potential is currently studied as part of clinical trials to treat diseases such as graft-versus-host disease and osteoarthritis, as well as in the regeneration of cardiac muscle following myocardial infarcts. Whether the requirements are for clinical or research use, obtaining a substantial number of cells can constitute a bottleneck for the investigator. hMSC display some plasticity in their culture conditions, but several investigators report a higher growth index and increased differentiation potential at lower seeding densities. We present here a protocol enabling the clinician or researcher to rapidly expand a population of hMSCs on Thermo Scientific Nunclon Delta cell culture treated surface utilizing the potential of Thermo Scientific HyClone AdvanceSTEM Mesenchymal Stem Cell Basal Medium, developed specifically for the optimal expansion and maintenance of undifferentiated hMSCs. A definite test of multi-potency is a functional test. In consequence, we subjected the expanded hMSC to differentiation. The cells were differentiated into osteoblasts or adipocytes in Nunc 48 well multidishes.
EFFECT OF T3 ON CHONDROGENESIS IN VITRO OF HUMAN MESENCHYMAL STEM CELLS FROM UMBILICAL CORD STROMAL SOURCE

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There are evidences about the thyroid hormone role into chondrogenesis but its molecular implication is not clear. The current study treats to answer this question working about a new in vitro model of chondrogenesis using mesenchymal stem cells (MSCs) from human umbilical cord stroma tissues. The umbilical cord tissues were obtained from caesareans from healthy women in the Maternity Faculty at Complejo Hospitalario Universitario de A Coruña under the supervision of the hospital ethical committee. MSCs were isolated from umbilical cord stroma tissue using an enzymatic digestion and cultured the cells adhered to the plastic plate. Chondrogenic process was performed using our model which consisted in growing the cells during two days in medium with FCS 10% in DMEM. After 2 days the medium was replaced by a medium with 10% fetal bovine serum (FBS) and TGF-β3 which induces the chondrogenesis through spheroid formation. Different amounts of human T3 (1, 10 or 100 ng/mL) were adding to the medium to test their role into chondrogenesis process, also different experiments with T3 alone or together with M151, a specific steroid covalent receptor type 2 (SCR2) inhibitor, in the chondrogenic medium, were performance to check which was the T3 receptor which had an active role in the chondrogenesis. Spheroids made by this method were collected after 7, 14 and 28 days in culture and storage at -20°C for their posterior analysis. Immunohistochemistry analysis of spheroids against COL2, COL1 and aggrecane stain was performed to check chondrogenic differentiation stage and quantitative RT-PCR to check expression of COL2, COL1 and Sox9 genes also was made. Furthermore, proteins involved into Wnt (β-catenin and GSK3-β) and Notch (Notch2 and Jagged) pathways were analyzed by western. Immunohistochemistry analysis of the tissues from the spheroids demonstrated that 100 ng/mL of T3 improved significantly (P<0.05) the COL2 and aggrecane staining after 14 days into chondrogenic medium versus chondrogenic medium without T3. This improving was totally withdrawn when the SCR2 inhibitor, M151, was added to the medium at 2.5 ng/mL dose. Quantitative RT-PCR analysis confirm the immunohistochemistry results, improving significantly (P>0.05) the COL2 and aggrecane gene expression after 14 days into chondrogenic medium when 100 ng/mL of T3 was present and revealed that expression of COL1 was significantly increased (P<0.05) when M151 (2.5 ng/mL) was added to this medium. Western analysis indicated that β-catenin was increased in the spheroids when T3 (100 ng/mL) was in the medium after 14 days (2 fold) with respect to medium alone and was decreased until basal levels found in spheroids growth into medium without T3, when M151 (2.5 ng/mL) was present in the medium. Notch2 was increased (2 fold) during the chondrogenesis when T3 was present in the medium with respect to medium alone and the opposite effect was found when M151 was present in the medium. T3 is improving the chondrogenic differentiation of mesenchymal stem cells from umbilical cord tissue in our in vitro model. That effect is mediated through SCR2 as well as T3 is associated the expression of β-catenin and Notch2 in our chondrogenic model.

MACROMOLECULAR CROWDING AND HUMAN MESENCHYMAL STEM CELL DIFFERENTIATION

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The ex vivo expansion of stem cells is an important step towards realizing the great potential of stem cell-based therapy in modern medicine. Stem cells are promising therapeutic agents because they could serve as a source of replacement tissues and organs. Cell-based therapies involve removing cells from a physiological environment and propagating them in an artificial cell culture system. A challenge in stem cell research today is ensuring that therapeutically adequate numbers of cells are produced for subsequent transfer to a patient. The non-physiological conditions to which cells are exposed during their lengthy ex vivo cultivation compromise their phenotype. Cells function less efficiently, resulting in sub-optimal proliferation and differentiation. The ex vivo differentiation of hMSCs into the adipogenic lineage is enhanced dramatically when a Ficol mixture is used as a macromolecular crowder in the differentiation protocol. Macromolecular crowding is known to affect molecular diffusion and biochemical reaction rates. Fluorescence correlation spectroscopy (FCS) is a powerful single-molecule-sensitive technique that has been used extensively to study dynamic molecular processes in living cells and organisms. We have used FCS to probe diffusive events in different experimental systems. Our goal is to understand how Ficol interacts with hMSCs and improves their proliferation and differentiation. We have found that molecular diffusion in the cell surface membrane and in supported lipid bilayers (SLBs) is not changed significantly by the crowder, suggesting that Ficol does not directly alter membrane properties and cell surface signalling. We have measured the diffusion of fluorophore-labelled Ficol 70 and Ficol 400 in the cytoplasm of hMSCs as well as in solutions of physiologically relevant concentrations of unlabelled Ficol 70 and Ficol 400. By using an appropriate data fitting model, we are able to describe the mode of diffusion occurring in the cytoplasm and solutions. Ficol 70 and Ficol 400 undergo anomalous subdiffusion (ASD) in the cytoplasm of hMSCs. In the solutions, however, the degree of anomalous subdiffusion is lower than it is in cytoplasm. However, we have data that conclusively show that the same concentrations of Ficol 70 and Ficol 400 increase actin polymerization reaction rates in vitro. We have also determined that Ficol increases the rate of in vitro collagen aggregation. As collagen is a principal component of the extracellular matrix (ECM), we hypothesize that the excluded volume effect caused by Ficol in the stem cell culture media directly affects the rate of deposition of ECM. Based upon our data, we conclude that Ficol crowders have a greater effect on biochemical reaction kinetics than on molecular diffusion. Ficol is thus an effective modulator of the ex vivo behaviour of hMSCs.

ISOLATION AND CHARACTERIZATION OF MESENCHYMAL-LIKE STEM CELLS FROM HUMAN SALIVARY GLAND

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Salivary glands produce and secrete saliva. Stem cells of salivary gland are known to regenerate and ameliorate on their function after damage. Here we report that salivary gland stem cells were isolated from human salivary glands. In order to effectively isolate and...
amplify stem cells in large amounts from submandibular glands, we developed a culture system (lasting four to five weeks) without any selection. After five passages, we found adherent cells which have expressed MSC surface antigen markers such as CD44, CD49f, CD90 and CD105, not including CD34 and CD45 hematopoietic stem cell marker. As we expected, these cells were differentiated into adipogenic, osteogenic, and chondrogenic cells with tissue-specific induction medium. Finally, the mesenchymal-like stem cells were isolated from submandibular gland and have great potential to be used to regeneration of other organ.

Poster Board Number: T-2154

ANALYSIS OF THE PROSPECTIVE CELL SURFACE MARKERS FOR HUMAN MESENCHYMAL STEM CELLS THAT READILY DIFFERENTIATE INTO CARDIOMYOCYTES.

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Myocardial infarction caused by chronic atherosclerosis of the coronary artery could induce heart failure with high mortality. Because myocardium has very limited ability of regeneration, heart transplantation is currently considered to be the most effective treatment for severe heart failure. In the case of patients with some remaining heart functions, ventricular assist devices can be used to complement their lost functions. For severe patients who need to replace their heart, artificial hearts are used in order to bridge the time to heart transplantation for a limited period of time. However, at present these devices cannot be used forever, and long-time antithrombogenicity, biocompatibility, reliability and durability are needed to be improved. On top of that, limited number of donor for heart transplantation causes serious issues of this methodology. Recently, stem cell therapy is expected to be the alternative regenerative medicine for heart failure. In addition to pluripotent embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, Mesenchymal stem cells (MSCs) are one of the most promising stem cell sources for regenerative medicine. MSCs have been studied extensively and shown to differentiate into various cells including bone, cartilage, fat, neuron, skeletal muscle, and cardiac muscle lineages in vitro. MSCs can readily differentiate into osteoblasts, chondrocytes and adipocytes, but they do not differentiate into the other type of tissue cells, such as cardiomyocytes, at high efficiency. Recent reports suggested that delivery of adult MSCs into ischemic tissue augments the recovery of cardiac function after ischemia in vivo. The effects induced by MSCs are generally temporary and limited.

In this study, we investigated prospective cell surface markers that enable to enrich cardiomyogenic progenitors from MSCs derived from human bone marrow or adipose tissues. We analyzed the cell-surface expressions of trans-membrane protein markers that are essential during heart development using various human MSCs and various human MSC cell lines were co-cultured on mouse cardiomyocytes. These differentiated cells expressed terminally differentiated cardiomyocyte-specific markers, such as α-actinin, cardiac troponin T, and connexin-43. We found that cell-surface expression of N-cadherin shows the best correlation to the transdifferentiation potential into cardiomyocytes. In contrast, MSC cell lines with low expression of N-cadherin showed apparently less differentiation abilities toward cardiomyocytes. Similar correlation was also observed with primary human MSCs derived from bone marrow and adipose tissue. We further demonstrated that the cell population with higher cardiomyogenic potential can be isolated from heterogeneous primary MSCs using N-cadherin antibody immunoaffinity beads. N-cadherin-high MSC population separated by anti-N-cadherin antibody beads revealed significantly higher expression of Nkx2.5 and GATA4 mRNAs and higher differentiation abilities into cardiomyocytes than those of N-cadherin-low expression. Our results suggested that N-cadherin could be a prospective cell surface marker for MSCs that have higher cardiomyogenic potential.

Poster Board Number: T-2155

CHARACTERIZATION OF OSTEOPROGENITORS FUNCTIONALLY ISOLATED FROM HUMAN MESENCHYMAL STEM CELLS BY A RUNX2-RESPONSIVE REPORTER ADENOVIRUS

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Objective: Mesenchymal stem cells (MSC) are a heterogeneous cell population characterized by their self-renewal capability and their multidifferentiation potential. Current isolation methods of MSCs are still rudimentary due to the lack of a unique marker. Here, we report a novel method for the isolation of osteoprogenitors from human MSCs, along with the characterization of the resulting cell populations. A subpopulation of MSCs was functionally identified and isolated by coupling the expression of the key osteogenic transcription factor Runx2 to the expression of enhanced green fluorescent protein (EGFP) via a Runx2 reporter adenovirus. On that basis, fluorescing cells can be selected by means of fluorescence activated cell sorting (FACS). Material and methods: MSCs were obtained from bone marrow aspirates by Ficoll separation and cell attachment to plastic. MSCs were expanded in the presence of bFGF. Cells were infected with the Runx2 reporter adenovirus using lant hodofection at 100 MOI. Cells were then subjected to osteogenic induction for 3 days and sorted by means of FACS. The resulting cell populations, namely Runx2 GFP+, Runx2 GFP-, and the unsorted cells, were separately expanded in the presence of bFGF, and thereafter subjected to comparative investigation of their in vitro osteogenic differentiation potential. Characterization of the cell populations was substantiated with the assessment of their proliferative capacity. Osteogenic differentiation was evaluated by alkaline phosphatase (ALP) activity at d7, 14, and 21, as well as 45Ca incorporation at d21. To perform the functional analysis of the Runx2 binding sites, a Runx2-driven luciferase reporter plasmid as well as Runx2 expressing and non-expressing effector plasmids were prepared. Results: Colony forming unit (CFU) analysis at d14 revealed that Runx2 GFP+ cells proliferate at a slower rate than the other two cell populations. This suggests that Runx2 GFP+ cells show a more committed/differentiated phenotype than the other two cell populations. ALP activity of Runx2 GFP+ cells was shifted towards earlier timepoints of osteogenic differentiation, showing highest ALP activity at d7 as opposed to d14 for the other two cell populations. 45Ca incorporation to assess matrix mineralization was massively higher for osteogenically differentiated Runx2 GFP+ cells than for the other cell populations treated with the same medium. Results of both assays are in accordance with each other, indicating that Runx2 GFP+ cells are more osteogenic than Runx2 GFP- and unsorted cells. Further, we investigated the functionality and specificity of the reporter by means of luciferase assay at 48 hours post-transfection. Upon cotransfecting HeLa cells (which do
not express Runx2) with a Runx2 expressing effector plasmid and a luciferase reporter plasmid driven by the same Runx2 binding sites used in the Runx2 reporter adenovirus, a clear transactivation can be seen compared to the Runx2 non-expressing cotransfection control. These findings indicate that the Runx2 binding sites are responsive to Runx2 protein. Conclusion: We have utilized a Runx2-responsive reporter adenovirus to sub-divide osteogenically induced human MSCs. Reporter-positive subpopulation displays characteristics appropriate for osteoprogenitors: (1) a slower proliferation rate, and (2) a more osteoblast-like phenotype upon in vitro osteogenic differentiation, as compared with reporter-negative as well as original cell population.

Poster Board Number: T-2156

MODULATION OF HUMAN MESENCHYMAL STEM CELL BEHAVIORS BY NANOSCALE GROOVE PATTERN TOPOGRAPHY

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Cell-substrate interactions are crucial for controlling diverse cellular behaviors. In particular, surface topographical effects of culture substrate are known to modulate adhesion, alignment, proliferation, and differentiation of stem cells. In this study, we investigated the effects of nanoscale topographies on the behaviors of human mesenchymal stem cells (hMSCs). Human adipose tissue-derived MSCs were cultured on polyurethane acrylate (PUA) nanopatterned surfaces (150-300 nm in width, 300-600 nm in pitch, and 200 nm in depth) grafted with glycidyl methacrylate (GMA) by initiated chemical vapor deposition (iCVD) technique. The surfaces of PUA substrates were modified with GMA group for potential grafting of bioactive molecules with functional groups that have the binding affinity to GMA. Live/Dead staining assay revealed that there was no significant difference in HMSC viability and proliferation between GMA-coated and uncoated PUA nanopatterned groups, indicating that iCVD-mediated GMA coating did not cause significant cytotoxicity. The morphological changes of hMSCs cultured on the GMA-coated PUA groove nanopatterned surfaces were examined by F-actin cytoskeleton (phalloidin) staining and compared with those of hMSCs on the GMA-coated PUA flat surfaces. Interestingly, the culture on the groove nanopatterned surfaces significantly promoted elongation and alignment of hMSCs along with the axis, whereas hMSCs cultured on the flat surfaces did not exhibit such elongated and oriented morphology. Given that GMA contains epoxy group that can readily bind to amine functional group, diverse bioactive molecules including growth factors and adhesion peptides can be easily immobilized onto the GMA-PUA nanopatterned surfaces. Therefore, combinatorial effects of surface topography and bioactive molecules will be examined for enhancing proliferation and lineage-specific differentiation of hMSCs in future study. Acknowledgment: This study was supported by grants (2010-0025982, 2010-0022037, and 2011-0027538) funded by the National Research Foundation of Korea.
Detailed Program and Abstracts — Thursday, June 14

Poster Board Number: T-2158

MITOCHONDRIAL ALTERATIONS DURING ADIPOGENIC DIFFERENTIATION OF HUMAN ADULT MESENCHYMAL STEM CELL
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Mesenchymal stem cells (MSCs) received increased attention due to their invaluable potential to differentiate into all kind of cell lineages that can replenish the cell pool. However little effort has been made in studying the metabolic aspects of stem cells. This importantly involves mitochondrial alteration i.e. mitochondrial number, membrane potential, ROS production and cellular redox status.

We studied the mitochondrial alteration including their membrane potential (MMP), cellular load, production of superoxides and peroxides during adipogenic induction of adult human mesenchymal stem cells. Human adult mesenchymal stem cells were isolated from adult human bone marrow after consent. These MSCs were characterized for the presence of stem cell surface marker i.e. CD90, CD105 and CD 73 along with the absence of hematopoietic lineage makers CD34 and CD45. They were successfully differentiated them into hepatocytes, oseocytes and adipocyte and characterized using lineage specific stains (glycogen storage using PAS stain and ICG uptake, alizarin-red, Oil Red-O stain respectively). For targeted study, MSCs were induced with adipogenic medium (dexamethasone, insulin, IBMX, indomethacin) and the mitochondrial alterations (peroxide and superoxide levels, mitochondrial load and mitochondrial membrane potential) was analysed from day of induction to 21 days. All the mitochondrial alterations were analyzed by flowcytometry. Cells were incubated with DCFH-DA for peroxide and DHE for superoxide for 45mints at 37°C. For mitochondrial load and MMP cells were incubated with NAO for 30 mints and JC-1 for 10 mints at 37°C respectively within 24 hrs of induction. Peroxide level decreased by 12% and mitochondrial load by 17%. Peroxide production gradually decreased after 2nd day of differentiation whereas mitochondrial load remained unchanged throughout the differentiation period of 21 days. The superoxide level and mitochondrial membrane potential increased by 7% and 9% respectively within 24hrs of induction, after that there was continuous decrease in the superoxide level with the progression of differentiation while the mitochondrial membrane potential decreased up to day 4 and after it become stable. Conclusion: Adipogenic induction decreased the peroxide levels, which are complemented by decreased mitochondrial load, and increased superoxide levels. The significance of these changes needs further studies.

Poster Board Number: T-2159

ENHANCEMENT DIFFERENTIATION OF HUMAN INFRAPATELLAR FAT PAD STEM CELLS IN A HYALURONAN-ENRICHED MICROENVIRONMENT
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Microenvironment plays a critical role in guiding stem cell differentiation. We investigated the effect of a hyaluronal (HA)-enriched microenvironment on chondrogenesis of human infrapatellar fat pad stem cells (IFPSC). The IFPSC were obtained from patients undergoing arthroscopy. HA-coated culture wells were used as HA-enriched microenvironment. The mRNA expression of chondrogenic, adipogenic and osteogenic marker genes were quantified by real-time polymerase chain reaction. Sulfated glycosaminoglycan (sGAG) deposition was detected by Alcian blue assay. The IFPSC cultured in HA-coated wells (HA concentration from 25 to 100%) showed enhanced aggregation and increased sGAG deposition. The migration of IFPSC was promoted under chondrocyte condition medium. Our results suggest that HA-enriched microenvironment induces chondrogenesis in IFPSC, which may be beneficial in articular cartilage tissue engineering.

Poster Board Number: T-2160

UTILIZING COMPARATIVE GENOME-WIDE EPGENETIC AND TRANSCRIPTONAL ANALYSIS TO IDENTIFY AND CHARACTERIZE NOVEL REGULATORS OF INFLAMMATION IN HUMAN PRIMARY ADIPOSE TISSUE.
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Over the past several years it has been increasingly recognized that obesity is a strong contributing factor to a number of human diseases including cardiovascular disease, cancer, and most notably, type II diabetes (T2D). One of the underlying features of the obesity-related insulin resistance observed with T2D is chronic low-grade inflammation of adipose tissue. This inflammation is characterized by a marked increase in macrophage infiltration into adipose tissue with concomitant pro-inflammatory cytokine secretion. While several recent studies have highlighted the link between obesity and inflammation, the signals and mechanisms that trigger and maintain the inflammatory response are not well understood. In order to better understand the role obesity is playing in inflammation-mediated T2D, it is imperative to better understand the regulation and function of immune modulating factors in adipocytes. Unfortunately there are currently no robust in vitro model systems to study adipose inflammation. A well-established model of adipocyte function is based on the use of cultured adipose derived mesenchymal stem cells (ADMSCs). ADMSCs can be physically transitioned from a fibroblast-like state to an adipocyte-like state using hormones and mitogens. Although this tool has been very helpful for studying the development and function of fat cells in vitro, ADSMC-derived adipocytes are not exposed to the immune factors found in vivo, therefore studying ADMSC-derived adipocytes alone is not an ideal model for understanding mechanisms of adipose tissue inflammation. To bridge this gap between in vitro and in vivo adipocytes we have devised a genome-wide epigenetic and transcriptional screen to identify novel regulators of adipose inflammation. By comparing sets of historic H3 lysine 4 methylation (H3K4me1, active promotor/enhancer mark) and whole genome transcriptional profiles between primary human adipocytes from several obese donors to donor-matched ADMSC-derived adipocytes we have identified a novel transcription factor which may play a role in regulation of inflammation-mediated insulin resistance. Ectopic expression of this single transcription factor in ADSMC-derived adipocytes leads to upregulation of several transcriptional pathways characteristic of an immune response. In addition, these adipocytes exhibit several robust phenotypes characteristic of inflamed adipose tissue in vivo, including lipid droplet hypertrophy, pro-inflammatory cytokine release, down-regulation of lipolysis, and insulin resistance. In conclusion, by integrating H3K4me1 Chip-Seq data with microarray
Introduction: Dental pulp stem cells (DPSCs) have received much attention as a promising population of stem cells in regenerative endodontics. Securing a good blood supply during regeneration is a challenging task due to the constricted apical canal opening is a formidable task. We aimed to investigate the synergistic effects of dental pulp stem cells (DPSCs) and endothelial cells (ECs) on osteo/odontogenic differentiation and vasculogenesis; and to fabricate prevascularised 3-dimensional pulp constructs in vitro. Methods: Different ratios of DPSCs and ECs were cultured in direct-contact in optimized medium and were induced for osteo/odontogenic differentiation up to 3-weeks. Alkaline phosphatase (ALP) activity, expression levels of ALP, bone sialoprotein (BSP), dentin sialophosphoprotein (DSP), genes and Alizarin Red staining for mineralization at different time points were analyzed. Tubular network formation on Matrigel and gene expression levels of CD117, VEGF, CD34, Flk-1 were used to analyse vasculogenesis. Three-dimensional microtissue-spheroids of DPSCs and ECs were fabricated in agarose 3D petri dishes made using 12-series micro-molds (MicroTissues Inc.). Briefly, the cell suspensions were added to the each 3D petri dish and allowed 10 minutes for cells to settle into the features. After incubating at 37°C in 5% CO2 atmosphere for 3 days, the petri dishes were inverted and centrifuged at 500 rpm for 5 minutes to harvest microtissues under immunohistochemical analysis for the EC-specific marker CD31. All experiments were conducted in triplicate using DPSCs from three different donors and statistically analysed using one-way ANOVA. Results: The quantification of ALP revealed significantly greater activity in DPSC:EC co-cultures compared to DPSC-alone cultures (p<0.05). DPSCEC, 1:1 and 1:5 co-cultures had a greater amount of calcification under Alizarin Red staining compared to other cultures (p<0.01). The higher expression levels of ALP, BSP and DSPP genes further confirmed the greater osteo/odontogenic differentiation in co-cultures compared to those of DPSC-alone cultures. Matrigel assay showed that the addition of DPSCs stabilized pre-existing vessel-like structures formed by ECs and increased their longevity. Compared to DPSC-alone cultures, the co-cultures expressed higher levels of vasculogenic markers. DPSC-EC macrotissues showed a significantly higher amount of extracellular matrix and mineralization compared to DPSC-alone macrotissues in 3-D. In contrast to DPSC-alone macrotissues, a dense-network of ECs was found throughout the DPSCEC macrotissues under immunohistochemical analysis for the EC-specific marker CD31. Conclusion: DPSCs and ECs synergistically enhance vasculogenesis and osteo/odontogenic differentiation; and prevascularize pulp-like macrotissues in vitro.

**Hepatic Differentiation of Human Dental Pulp Stem Cells: Transplantation into Rats with Liver Injury and Cirrhosis**

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Objectives: The stromal mesenchymal tissues offer a large therapeutic potential in the regenerative medicine. In this study our goal is to differentiate stem cells cultures from human tooth pulp to hepatic-like cells with serum-free medium and to prove that after transplantation into F344 nude rats' spleens, human hepatic tissues, including blood vessels and bile ducts, were reproduced. Methods: CD117+ cells were isolated from deciduous tooth pulp stem cells (SHED) cultures by magnetic separation. The CD117+ cells were purified at every 4 passages to keep the undifferentiated stem cells phenotype. Cultures were characterized for expressing a panel of stem cell markers by immunofluorescence. Cells were grown in DMEM supplemented with 50ng/ml insulin-transferrin-selenium-x (ITS-x), 100μg/ml embryo-trophic factors (ETF) and 20ng/ml hepatocyte growth factor (HGF) for hepatic commitment (5 days). For hepatic differentiation the medium was changed to IMDM supplemented with 50ng/ml ITS-x, 100μg/ml ETF, 10ng/ml oncostatin, 20ng/ml HGF and 10nM dexamethasone (15 days). Human specific hepatic markers albumin, α-feto-protein, insulin-like growth factor-1 (IGF-1), hepatic nuclear factor 4-α (HNF4- α), carboxamyl phosphate synthase 1 (CP1), c-Met and prothrombin expression was tested after differentiation by immunofluorescence. Urea concentrations were measured calorimetrically in the culture media by ELISA. Glycogen storage was visualized by PAS reaction. Immediately after twelve F344-Nude rats were subjected to 90% liver resection, hepatic cells from SHED were transplanted in the spleens (approx. 107 cells /animal) of six of the animals. Twelve F344-Nude rats were subjected to bile duct ligation in order to induce cirrhosis. Sixty days later hepatic cells from SHED were transplanted in the spleens of six animals. Non-transplanted rats from both groups were used as negative control. Animals were sacrificed 40 days after transplantation. Previous human specific hepatic markers were tested by immunohistochemistry. Expression of human-specific albumin in rats' livers was proven by in situ hybridization. Concentrations of human-specific albumin, α-feto-protein and IGF-1 in rats' serum were analyzed respectively by Bromcresol Green, chemiluminescence immunoassay and radioimmunoassay. Results: After in vitro hepatic differentiation α-feto-protein, albumin, IGF-1 and HNF-4α were all shown to be positive by immunofluorescence. Around 90% of cells are deemed as hepatic-like. The concentration of urea in the media increased (p<0.05). Glycogen accumulations in the cells' cytoplasm were observed, whereas no accumulation was found in the control. In transplantation study clusters of human-specific hepatic markers expressing tissues, including blood vessels and bile ducts were found in the rats' livers and spleens by immunofluorescence. Human specific albumin, α-feto-protein and IGF-1 were found in rats' serum. Conclusions: Hepatocyte-like cells differentiated in SFM were transplanted into the rats and functioned as human hepatocytes. SHED may therefore be ideal source for cell-therapy of patients requiring liver transplantation.
DENTAL PULP REGENERATION: THE ROLE OF PREAMELOBLAST-CONDITIONED MEDIUM ON HUMAN DENTAL PULP STEM CELLS

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Objectives: Regeneration therapy of pulp using human dental pulp stem cells (hDPSCs) has been emerged to maintain the tooth vitality against the conventional endodontic therapy. Preameloblast-conditioned medium (PA-CM) from murine apical bud cells induces the odontogenic differentiation of hDPSCs and promotes dentin formation. The present study aims to evaluate the effect of PA-CM with hDPSCs on pulp regeneration in vivo. Methods: hDPSCs were seeded in the pulp cavity of 5mm-thick human tooth slice with or without PA-CM treatment, and then they were transplanted into immunocompromised mice subcutaneously. Results: In the control group, skeletal muscle with pulp-like tissue was regenerated in the pulp cavity. Reparative dentin-like structure with the entrapped cells was lined on the existing dentin wall. However, in the PA-CM treated group, only pulp-like tissue was generated without muscle and reparative dentin-like structure. Moreover, human odontoblast-like cells exhibited palisade arrangement around the pulp and typical odontoblast processes elongated into the dentinal tubule. Conclusion: These findings suggested that PA-CM induced vascularized pulp-like tissue regeneration of hDPSCs.

IMMUNOLOGICAL EVALUATION OF HUMAN DENTAL PULP STEM CELLS DIFFERENTIATION WITH EMPHASIZE ON TOLL-LIKE RECEPTOR 4

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Background: Maintaining homeostasis is highly regulated at cellular and molecular level in all tissues including dental pulp. One of the important key regulators of the pulp tissue is human Dental Pulp Stem Cells (hDPSCs), which supports pulp repair / regeneration by their proliferation & differentiation capacity. On the other hand, inflammatory mediators such as cytokines and/or chemokines are pulp tissue molecular regulators. It has been suggested that the inflammatory mediator gradient is controlled by kind of innate immune receptor called Toll-Like Receptor (TLR). TLRs are expressed on various immune cells and even non-immune cells. Interestingly, there are some evidences indicated that TLRs are also expressed in Adult Stem Cells. Among ten functional members of the TLR family in human, TLR4 recognizes very divergent collection of exogenous and endogenous ligands involving in the sterile & pathogen-associated inflammations. It has been recently suggested that TLR4 has multi-functional activities in human mesenchymal stem cell (hMSC) including stem cell proliferation, migration & also differentiation. Here we investigate the comparative expression of TLR4 in hDPSCs and differentiated odontoblasts during the differentiation process. Methods: hDPSCs were enzymatically isolated from wisdom teeth (n=5) and characterized in terms of surface epitopes (hMSC markers CD90/CD73/CD44/CD105 & hematopoietic/endothelial markers CD34/CD45/CD11b/CD31) as well as differentiation capacity into bone, cartilage & adipose cells. Then, hDPSCs were differentiated into odontoblast for 21 days in differentiation medium. Expression of TLR4 gene was analyzed on day 0, 7th, 14th & 21st of odontoblast’s differentiation by RT-PCR & Q-PCR. Results: hDPSCs showed MSC phenotype & differentiation capacity into three mesenchyme lineages. Immuno-phenotyping results confirmed the existence of MSC markers and the lack of hematopoietic/endothelial markers. RT-PCR results show the expression of odontoblast differentiation markers dentin matrix acidic phosphoprotein 1 (DMP1) & dentin sialophosphoprotein (DSP) in differentiated cells. The results of RT-PCR indicated the expression of TLR4 gene in hDPSCs on day 0, 7th, 14th & 21st of differentiation. Interestingly, Q-PCR results showed significant increasing expression of TLR4 gene during the differentiation. Conclusion: Immunological evaluation of human adult stem cells has been always considered in stem cell therapy & regenerative medicine. Different expression of TLR4 in differentiated & non-differentiated hDPSCs may shed light on the correlation of innate immunity & differentiation process. Up-regulation of TLR4 may prompt DPSCs to undergo differentiation. Since this difference was significant, less expression of TLR4 may block differentiation process.

STROMAL PHENOTYPE OF DENTAL FOLLICLE STEM CELLS.

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It has been suggested that stem/progenitor cells exist in dental tissue. This study identified adult mesenchymal stem/stromal cell-like populations in the dental follicle of human impacted third molars. The immunohistochemical analysis, of dental follicle using known stem-cell markers: Cytokeratins (AE1-AE3), Smooth Muscle Actin, Ki-67, CD34, CD44, CD45, CD56, and CD113. A positive reaction for at least one of the markers typical of stromal phenotype (CD56, CD44 and CD271) was observed in seven cases. Interestingly, all positive cases showed coexpression of CD44 and CD56, except for one case which was CD56 positive and CD44 negative. Immunohistochemical reaction was negative in all 27 cases for Ki-67, Cytokeratins, Smooth Muscle Actin, CD34, CD113 and CD45. The association: negative for CD34, CD45, CD113, and positive for CD44, CD56 (markers of a subpopulation of stem cells from bone marrow) suggests these may be quiescent mesenchymal stem cells, a hypothesis supported by the negativity of Ki-67 (proliferative index). Our results are compatible with the identification of immature fibroblast cells with phenotypic features of stromal stem cells in the dental follicle. Acknowledgments: Funding for Stem Cell work was awarded from Ministero dell’Istruzione, dell’Università e della Ricerca -PRIN 2009 (Progetto di Ricerca d’Interesse Nazionale-Grant 2009)
EFFECT OF TRANSIENT HYPOXIC STIMULATION ON CULTURED HUMAN PERIODONTAL LIGAMENT DERIVED-CELLS

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Background: Several studies have reported effective cell culture conditions for the isolation and expansion of stem cells that reside in dental pulp and periodontal ligament tissues. A proportion of these stem cells are known to be derived from the cranial neural crest. There are also reports that hypoxic stimulation during culture influences the stem cell function and differentiation. Specifically, hypoxic stimulation was reported to promote the survival rate of neural crest cells, and induced pluripotent stem cell factors in cultured dental pulp-derived cells. Therefore, this "hypoxic treatment" can contribute to isolating more stem cells or to induce the reprogramming of cells with a higher potential for growth and plasticity. However, little is known about the effect of hypoxic treatment on human periodontal ligament-derived cells (PDLCs). Objective: In this study, we investigated whether the surviving human PDLCs cultured under two hypoxic conditions acquired the high growth and plasticity potential. Methods: Human PDLCs were obtained from extracted teeth, digested with collagenase, and the isolated cells sub-cultured until passage 3 for experiments herein. PDLCs were then exposed to either hypoxic (O2<5%) or severe hypoxic/anoxic (O2<0.1%) conditions in low glucose/serum-free media for 6 or 24 hours. Surviving PDLCs were evaluated for the cell viability, alkaline phosphatase (ALP) activity, and gene/protein expressions to determine their stem cell characteristics. Furthermore, multi-lineage differentiation assay was performed to assess their stemness. Results: The severe hypoxic/anoxic treatment (O2<0.1%) led to significantly more cell deaths and reduced ALP levels, when compared with the hypoxic treatment (O2<5%). Expression of Stro-1, CD105 and CD166 was increased in surviving PDLCs of the severe hypoxic treatment group. In particular, stem cell related-genes, such as nanog, oct4, p75 and bmi1, were up-regulated markedly in these surviving cells after 24 hours of severe hypoxic treatment. These data suggest that the transient hypoxic stimulation may influence stemness properties of cultured PDLCs. We are currently investigating the plasticity of these surviving cells in more details, and the usefulness of transient hypoxic stimulation for the expansion of stem cells in PDLCs culture.

DIFFERENTIAL IMMUNOSUPPRESSIVE PROPERTIES OF MOUSE MSCS ON TH1 CELLS

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In recent years, many authors have described that Mesenchymal Stem Cells (MSCs) are able to suppress the proliferation of T-lymphocytes although the mechanism by which MSCs exert this effect is still unclear. The aim of our study was to determine if MSCs were able to suppress the proliferation and the differentiation of TH1 cells in vitro and to evaluate dependency on T-cell activation state, cell-to-cell contact and T-cell/MSCs ratios. Mesenchymal stem cells were obtained from mice bone marrow and characterized by their surface antigen expression pattern and their capability of multilineage differentiation. T-CD4+ cells were obtained from mice splenocytes and differentiated into Th1 cells. MSCs were added to Th1 cultures either at early (day 0) or late (day 2 and 4) time points in the presence or absence of a transwell system. After 6 days of co-culture, Th1 populations were measured by flow cytometry using intracellular IFN-gamma. We demonstrate that MSCs effectively suppress Th1 cells in spite of their activation state and T-cell/MSC ratios. MSCs are able to diminish IFN-gamma secretion up to an 85% (p<0.5) when they are added early on cultures and 26% (p<0.05) when added at later time points. Previous reports have shown that MSCs IL6-/- are unable to suppress T cell activation and proliferation, therefore we investigated if this factor was responsible for Th1 suppression. Surprisingly, and in contrast to what was observed on non-differentiated T-cell, MSCs IL6-/- were also able to suppress Th1 cells. Importantly, the mechanisms used by MSCs to suppress different subtypes of T-cells are different since differentiated Th1 cells are suppressed in the absence of IL-6 while non-differentiated T cells are not. Our findings suggest that the mechanisms of MSC-mediated immune suppression are more complex and dynamic.
Toward Understanding the Balance Between Osteoblastogenesis and Adipogenesis in Bone Marrow Microenvironment.

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The majority of conditions associated with bone loss, including osteoporosis, were shown to be accompanied by increased marrow adiposity due to shifting in the balance between osteoblast and adipocyte differentiation of bone marrow skeletal stem cells (MSC). However, little is known about how the balance between osteogenesis and adipogenesis is regulated in the bone marrow microenvironment. Recently, we proposed a novel molecular model for lineage fate determination of MSC that is regulated through the cross-talk between the two unique populations of pre-committed osteoblasts (MSC-OB) and adipocytes (MSC-Adipo) rather than through changes in the differentiation pathway of multipotent MSC (Taiapalennäki H, Abdallah BM. Exp Cell Res. 2011; S. Post, B.M. Abdallah. Bone. 2008). Thus, we aimed in this study to identify novel secreted factors that mediating the cross-talk between osteoblasts and adipocytes in bone marrow. For that purpose, we used a combination of microarray (GeneChip® MG430A 2.0 Array) and mass spectrometry-based proteomics (secretome) to identify secreted proteins by MSC-OB (only can differentiate into osteoblast) versus MSC-Adipo (only can differentiate into adipocyte), Transcriptome and secretome profiles of these two cell lines were compared using different criteria including bioinformatics, function annotation analysis, gene expression analysis and extensive literature survey. After excluding all previously studied factors in MSC differentiation, we identified four novel secreted proteins by MSC-Adipo (three proteins by MSC-Adipo and other 4 by MSC-Adipo). Studying the molecular function of these proteins in MSC cells revealed a selection of 3 secreted factors with the potential to control the commitment of MSC into either osteoblast or adipocyte lineage. In conclusion, cross-talk exists between different populations of pre-committed osteoblast and adipocyte cells in bone marrow to regulate differentiation of multipotent MSC through novel secreted factors.

Poster Board Number: T-2170

Differentiation of Mouse Umbilical Cord Derived Mesenchymal Stem Cells In Vitro Into Hepatocyte Like Cells

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Objective: The purpose of this study is to differentiate mesenchymal stem cells into hepatocyte-like cells by adding liver extract on mesenchymal stem cells (MSCs), which manifest the morphology of hepatocyte cells. To prove this similarity, we use Periodic Acid-Schiff test. Methods: MSCs were isolated from NMRI mice on days 14-17 and cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% FBS (Fetal Bovine Serum). We investigated the differentiation potential of mouse mesenchymal stem cells into hepatocytes in vitro by using liver extract every 4 days. We cultured mesenchymal stem cells without additional factors or late differential markers of hepatic development. Periodic Acid Schiff test was used to indicate this differentiation. Results: Our results have shown that mesenchymal stem cells were changed to hepatocyte-like cells after 30-35 days. These cells have polygonal morphology of hepatocyte-like cells with 1 or 2 obvious nuclei. Periodic Acid-Schiff (PAS) test was used for detecting the amount of glycogen synthesis in differentiated hepatocyte-like cells. The red colour confirmed this differentiation. Conclusion: The proposed procedure for hepatocyte cells differentiation by using mesenchymal stem cells from mouse umbilical cord and diluted liver extract demonstrates that mouse mesenchymal stem cells can change to hepatocyte-like cells with liver extract treatment. Keywords: Mesenchymal Stem Cells (MSCs), in vitro, Differentiation, liver extract, Periodic Acid-Schiff, hepatocyte-like cells

Poster Board Number: T-2171

Proteomic Analysis of Integrin-Associated Complexes from Multipotent and Early Differentiated Mesenchymal Stem Cells

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Stem cell differentiation is an important process in maintaining bone homeostasis. Bone marrow mesenchymal stem cells (MSCs) are multipotent cells with the potential to differentiate into adipocytes and osteocytes and are responsible for maintaining bone homeostasis. It has been shown that a shift of MSC differentiation toward adipogenesis leads to reduced osteogenesis. The process that drives MSCs into an adipogenic versus an osteogenic lineage in the bone marrow is poorly understood. Numerous factors in the MSC microenvironment have been identified to be involved in MSC function, such as cell-extracellular matrix (ECM) adhesion, cell-cell interactions and soluble factors. Adhesion of cells to the ECM is mediated by integrin adhesion receptors. Following adhesion, integrins cluster at the cell membrane, and protein complexes called focal adhesions (FAs) build up inside the cells. FA assembly is likely to induce signalling events in cells and affects cell fate. In this study, a proteomics-based system has been developed to isolate, identify and quantify integrin-associated complexes in undifferentiated MSCs and MSCs induced to an adipocyte lineage (short-term induction, 3 hours). The aim of this study was to compare the composition of FAs and the abundance of their components in these two cell lines to identify proteins that may be involved in MSC differentiation to an adipocyte lineage. MSCs were allowed to adhere to surfaces coated with defined ECM proteins. Following incubation, equal populations of MSCs were subjected to two different treatments for 3 hours: 1) the adipogenic induction medium; or 2) adipogenic maintenance medium (control). Ligand-induced integrin-associated complexes were stabilized using crosslinker. Cells were lysed, and isolated complexes were subjected to downstream proteomic analysis. Mass spectrometry (MS) analysis showed the enrichment of key proteins, such as integrin β1, integrin α5, vinculin, talin, α-actinin-4, zyxin, tensin, filamins, kindlin-2, integrin-linked kinase (ILK) and the actin-binding proteins PDZ and LIM domain 1 and 7 (Pdlim1 and Pdlim7) in ECM-bound complexes from non-induced and induced MSCs compared to complexes isolated from a non-ECM ligand (control). These data were validated by immunoblotting. Furthermore, the comparison of spectral counts (used as a measure of protein abundance) between the integrin-associated complexes in non-induced and induced MSCs showed a remarkable difference in protein abundance in these two cell lines. These data demonstrate that integrin adhesion complexes can be isolated in a reproducible manner from non-
induced and induced MSCs in a way suitable for MS analysis. They also indicate that the early induction of an undifferentiated cell line (MSCs) into a certain lineage (adipogenesis) increased the abundance of key FA proteins, specifically in integrin-associated complexes induced by ECM ligands. Future work will be directed towards obtaining further in-depth MS analyses, which, followed by bioinformatics evaluation, will implicate molecules involved in the integrin-mediated regulation of stem cell maintenance and differentiation in non-induced and induced MSCs.

**Poster Board Number: T-2172**

**EVALUATING THE EFFICACY OF ENDOTHELIAL CELLS DIFFERENTIATED FROM ADIPOSE DERIVED MESENCHYMAL STEM CELLS FOR IN VITRO INTIMA CONSTRUCTION**

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Adipose tissue is an attractive candidate to become a major stem cell source for regenerative medicine and tissue engineering applications. Adipose-derived mesenchymal stem cells (AdMSCs) possess multilineage differentiation potential. Differentiation into mesodermal cell types, i.e. into adipocytes, chondrocytes, osteoblasts, and myocytes has been shown in several studies. They also can be induced to differentiate into ecto and endodermal origin, i.e. hepatocytes, pancreatic islet cells, neural cells, epithelial cells, and endothelial cells (ECs). The objective of this study was to investigate the differentiation capacity of human (h) AdMSCs into vascular endothelial-like cells, and to evaluate the efficiency of their use for in vitro construction of a functional tunica intima layer into an engineered-vascular scaffold. Methods. hAdMSCs were isolated from human lipoaspirates, cultured, then immunophenotypically characterized by FACS and by their tri-lineage differentiation potential. Passages 4-6 were used in all experiments. AdMSCs were seeded on collagen type IV-coated surfaces and cultured for 7 and 14 days in the EC-GM containing FGF2, VEGF, heparin, IGF-I, EGF, hydrocortisone, and ascorbic acid. hAdMSCs were also seeded inside a nanofibrous tubular polymer scaffold and differentiated into ECs either in static or flow conditions. As positive control, human internal mammary artery endothelial cells (hIMAECs) were isolated and expanded on bovine skin gelatin-coated tissue culture plates in EC proliferative medium (EC-PM); hAdMSCs cultured in standard medium without differentiation inducers served as the negative control in all experiments. To evaluate EC characteristics, immunohistochemistry studies were performed using antibodies against six EC-specific proteins. Additionally, acetylated-low density lipoprotein (ac-LDL) uptake and expression of endothelial cell nitric oxide synthase (eNOS) were determined for endothelial cell function analysis. Results: Human AdMSCs had CD 90+, CD105+, CD73+, CD29+, CD44+, CD34-, CD45-, and CD133- immunophenotype and demonstrated tri-lineage differentiation potential. Immunohistochemistry demonstrated that hAdMSCs on nanofibrous tubular construct differentiated into endothelial-like cells, and expressed CD31 (PECAM-1), CD34, von Willebrand factor (vWF), VE-cadherin (CD144), tie-2 and flk-1 (VEGFR2) at day 7. Overall, the expression of these endothelial specific proteins significantly increased after 14 days which were comparable with that of the hIMAECs. While the expressions of eNOS and ac-LDL uptake were found to be quite low in static culture, a positive influence was observed under in vitro biomimetic shear conditions. Conclusion. As the conclusion, human adipose mesenchymal stem cells can be differentiated into functional vascular endothelial-like cells inside a nanofibrous tubular scaffold under flow conditions that express EC-specific proteins. Findings support the notion that the tunica intima could be constructed in vitro using ECs derived from hAdMSCs. Currently, we are testing the possibility of increasing expression levels of eNOS and ac-LDL uptake by constructing a media layer containing smooth muscle cells in close contact with the neointima layer inside a real time computer-controlled bioreactor system.

**Poster Board Number: T-2174**

**GLUTATHIONE DEPENDENT OSTEOSTEROGENIC DIFFERENTIATION OF SKIN MESENCHYMAL PROGENITORS OCCURS THROUGH MAPK SIGNALING**

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Mesenchymal stem cells (MSCs) are mesodermal precursors located at the dermis, capable of osteogenesis. Increased reactive oxygen species (ROS) levels have been implicated in delayed bone development in mouse embryo and hFOB pre-osteoblasts, but little is known about the impact of ROS in stem cell osteogenic differentiation. We induced murine skin MSCs to osteocyte differentiation for 7, 14 and 21 days (terminal differentiation) and in short periods...
IL1BETA IS A POTENTIAL STROMAL GROWTH FACTOR

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Here we demonstrate, for the first time, the ability of IL1-beta - a well known proinflammatory cytokine, - to stimulate the development of hematopoietic microenvironment in vivo. Mesenchymal stem cells (MSCs) are capable to transfer hematopoietic microenvironment. After implantation of femur bone marrow plug under the renal capsule of syngeneic recipients the ectopic hematopoietic foci are formed. Stromal cells in such foci derived from donor MSCs while hematopoietic cells have recipient’s origin. The size of the foci formed (estimated by nucleated cell number) is proportional to the femur equivalent transplanted. This can be used for semi-quantitative determination of MSCs number. In this work we show that IL1-beta injections during ectopic foci formation lead to the enlargement of hematopoietic territory within such foci. Methods: The bone marrow plug was implanted under the renal capsule of syngeneic (C57Bl x CBA) F1 recipients. Six weeks later the ectopic foci of hematopoiesis were analyzed. IL1-beta have been injected in a dose of 0.1, 0.5, 1, 2.6 and 24h (commitment). These cells were successfully differentiated, producing calcium/phosphate matrix (Alizarin-Red, Von-Kossa stain) and increased mRNA levels of alkaline-phosphatase, osteopontin and osterix. We then analyzed the contribution of the canonical and glutathione related antioxidant system along the differentiation through qRT-PCR, WB and enzymatic activity. The canonical antioxidant pathway apparently does not play an important role in this model, since no significant differences in the levels of catalase and Cu/ZnSOD were found, except for MnSOD (n=3, p≥0.05). For the glutathione-dependent system, we found increased levels of glutathione peroxidase isofoms and glutathione reductase. In agreement with these data, the thioredoxin/peroxiredoxin system is also upregulated (n=3, p<0.001), and the balance between GSH/GSSG altered (HPLC), suggesting a role for the glutathione-dependent system in the differentiation process. We then set out to evaluate which pathways could perform this redox-regulated signaling. The kinases p38, ERK1/2 and JNK were differentially phosphorylated during commitment of differentiation and displayed differential patterns when the differentiation was performed in the absence of GSH (depletion through BSO) or in a more reduced-versus-oxidized intracellular environment (DTT/H2O2). Also, the expression of some of the glutathione-related enzymes was abrogated when these MAPKs were pharmacologically inhibited. In brief, the relative levels of GSH/GSSG and related antioxidant system are differentially regulated through a MAPK axis during skin MSC differentiation leading us to propose a central role for this thiol in the maintenance/commitment of stem cells during osteogenesis.

Poster Board Number: T-2175

Detailed Program and Abstracts — Thursday, June 14

DIABETES CAUSES ABNORMALITIES IN THE BONE MARROW NICHE BETWEEN OSTEOSTATIC NICHE CELLS AND LONG-TERM RECONSTITUTING HEMATOPOIETIC STEM CELLS

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Interaction between hematopoietic stem cells and bone marrow niche cells through chemokines and their receptors as well as cell adhesion molecules maintains the balance in hematopoietic stem cells (HSCs) between cell division/proliferation and quiescence. Although it is well accepted that diabetes induces functional abnormalities in hematopoietic stem/progenitor cell mobilization and proliferation, the interaction between bone marrow niche cells and their partner long-term reconstituting HSCs (LT-HSCs) has not been extensively investigated. Here we isolated osteoblastic niche cells (Lin− OPN+) and LT-HSCs (Lin− Sca-1+ c-Kit+ CD135− CD34−) from STZ-diabetic mice and examined the expression of molecules essential to maintain bone marrow niche functions. To mimic the microenvironment in the bone marrow niche, we established in vitro co-culture between LT-HSCs and osteoblastic niche cells at normoglycemic or high glucose conditions. In diabetic mice osteoblastic niche cells and LT-HSCs were decreased in number, and expressions of N-cadherin and β-catenin on LT-HSCs and osteoblastic niche cells at normoglycemic or high glucose conditions. In diabetic mice osteoblastic niche cells and LT-HSCs were decreased in number, and expressions of N-cadherin and β-catenin on LT-HSCs as well as β1-integrin on osteoblastic and vascular niches in the bone marrow. The results obtained indicate that IL1-beta possesses new unexpected properties - to stimulate the formation of hematopoietic microenvironment and bone tissue in vivo by presumably acting on mesenchymal progenitor cells while one can not exclude its effect on MSCs. Such novel stromal growth factor may be useful for treatment of several diseases affecting either hematopoietic microenvironment or bone defects. Properties of IL-1beta as stromal growth factor require further detailed investigations. One of the most interesting questions is to determine target cells in the hierarchy of mesenchimal stem cells for this cytokine which is still obscure.

Poster Board Number: T-2176

In the group which was injected with 100 pg of IL1-beta the size of hematopoietic foci was 1.7 times larger than in control group and that was statistically significant (P = 0.001). Thus the effect was dose-dependent with the optimal dose of IL1-beta for stimulating hematopoietic environment being 100 pg. The weight of bone shells was 2.8 ± 0.1 mg for control group, 3.7 ± 0.1, 4.8 ± 0.1, 3.5 and 2.3 ± 0.3 mg for each experimental group, respectively. These results demonstrate that injecting 100 pg of IL1-beta lead to the formation of larger foci of hematopoiesis with both higher amount of nucleated cells and larger bone shells. The actual size of hematopoietic territory may depend not only on the number of MSCs but also on more mature mesenchymal progenitor cells. These cells, as they differentiate, create new bone and stroma thus providing new niches for hematopoietic cells which seem to be analogous to the osteoblastic and vascular niches in the bone marrow. The results obtained indicate that IL1-beta possesses new unexpected properties - to stimulate the formation of hematopoietic microenvironment and bone tissue in vivo by presumably acting on mesenchymal progenitor cells while one can not exclude its effect on MSCs. Such novel stromal growth factor may be useful for treatment of several diseases affecting either hematopoietic microenvironment or bone defects. Properties of IL-1beta as stromal growth factor require further detailed investigations. One of the most interesting questions is to determine target cells in the hierarchy of mesenchimal stem cells for this cytokine which is still obscure.
exposed to diabetic osteoblastic niche cells. Conversely, reduced expression in these molecules on diabetic LT-HSCs was reversed into normal levels when exposed to nondiabetic osteoblastic niche cells in co-culture system. By these experiments we succeeded to reverse diabetes-induced abnormality in LT-HSCs by replacement of osteoblastic niche cells and this may provide the future perspectives for treatment of diabetes-induced complications in hematopoietic cells.

Poster Board Number: T-2177

MIR-124 PROMOTES NEURONAL DIFFERENTIATION OF ADULT MESENCHYMAL STEM CELLS BY TARGETING THE ANTI-NEURAL REST/SCP1 PATHWAY AND SOX9

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Adult mesenchymal stem cells (MSCs) isolated from the bone-marrow and adipose tissue exhibit self-renewal and differentiation into multiple lineages including bone, cartilage and adipose cells. Recent studies suggest that these cells can also acquire neuronal phenotypes under certain growth conditions. MicroRNAs (miRs) are small non-coding RNAs that act as important post transcriptional regulators by either inducing their target mRNA cleavage or by translational repression. Specific miRs have been implicated in various differentiation processes and in cell transformation and their ability to regulate large numbers of target genes implicate them as important regulators of stem cell differentiation. In this study we examined the role of the neuronal-related miR-124 in the neuronal differentiation of adult bone-marrow and adipose-derived MSCs. miR-124 significantly decreased cell proliferation, induced a neuronal-like morphology and increased the expression of voltage-dependent sodium channels and the neuronal markers TuJ1 and Neun. On further characterization, we found that the MSCs preferentially expressed tyrosine hydroxylase and additional dopaminergic markers. miR-124 decreased the expression of its potential targets, STAT3, CDK6, ROCK, Sox9, Jagged 1, PTBP1 and the phosphatase SCP1 which exhibits anti-neural function. miR-124 directly targeted the Sox9 and SCP1 -3’ UTRs and overexpression of SCP1 and Sox9 partially abolished the proneural effect of miR-124 in the MSCs, suggesting that additional pathways may be involved in this effect. We conclude that miR124 promotes the neuronal differentiation of MSCs by targeting multiple signaling pathways and could be employed to generate a potential cellular source for cell replacement therapy in various neurodegenerative disorders.

Poster Board Number: T-2178

MICRORNA-145 REGULATES OSTEogenic LINEAGE DIFFERENTIATION BY TARGETING TRANSCRIPTION FACTOR CBFB

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Bone marrow derived mesenchymal stem cells are self-renewing multipotent cells that can differentiate along several lineages, including the osteoblast lineage, in response to diverse factors. Understanding the regulatory mechanism of osteoblast differentiation is a prerequisite for treatment of bone loss diseases such as osteoporosis. MicroRNAs (miRNAs) are small non-coding RNAs that significantly inhibit the translation of target genes. miRNAs have emerged as important regulators in various developmental, physiological and pathological conditions. Previous reports implicated some miRNAs in the differentiation of osteoblasts and osteoclasts. However, their importance in the regulation of osteogenic differentiations remains to be elusive. In the present study, we screened for differentially expressed miRNAs during osteoblast differentiation of MC3T3-E1 cells and identified miR-145 as a potential regulator of osteogenesis. Overexpression of miR-145 in MC3T3-E1 cells inhibits ALP activity and osteoblast specific gene expression. To understand the molecular mechanisms that underlie the miR-145 mediated regulation, we searched for potential targets of miR-145 implicated in osteoblast differentiation using in silico analysis. Among the predicted targets, we identified core binding factor beta (Cbfb), a heterodimeric partner for Runt-related transcription factor 2 (Runx2) in which the essential transcription factor in skeletal development by regulating osteoblast differentiation. To determine whether miR-145 inhibits Cbfb gene expression by binding to the predicted target site in the 3’-UTR, we used a luciferase reporter analysis. Co-transfection of Cbfb 3’-UTR containing reporter with miR-145 resulted in significantly decreased luciferase activity compared with control. Furthermore, ectopic expression of miR-145 down-regulated endogenous Cbfb mRNA and protein expression. Taken together, these results suggested that miR-145 regulates osteogenic differentiation at least in part by inhibiting the Cbfb expression. Our finding suggested that pharmacological inhibition of miR-145 may lead to the therapeutic strategy for bone degenerative diseases such as osteoporosis.

Poster Board Number: T-2179

TAUROSODEOXYCHOLIC ACID ATTENUATES ADIPOGENESIS OF ADIPOSE-DERIVED STROMAL CELLS BY DECREASING ENDOPLASMIC RETICULUM STRESS

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Human adipose-derived stromal cells (ASCs) are a heterogeneous group of multipotent progenitor cells that are able to differentiate into various types of cells such as adipocyte, chondrocyte, osteoblast etc. Therefore, it is critical to appropriately induce ASC differentiation with high efficiency toward target cells. Tauroursodeoxycholic acid (TUDCA) is a commercially available bile acid derivative to treat cholestatic liver disease and cholelithiasis. Recently, it has been reported that TUDCA not only has the ability to decrease endoplasmic reticulum (ER) stress, also plays a role as leptin-sensi-
Detailed Program and Abstracts — Thursday, June 14

Progenitors by RhoA-Kinase Inhibition

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Current advances in stem cell biology have brought much hope for therapy of neuro-degenerative diseases. However, neural stem cells (NSCs) are rare adult stem cells, and the use of non-NSCs requires efficient and high-yielding lineage-specific differentiation prior to transplantation for efficacy. We report on the efficient differentiation of embryonic stem cell-derived mesenchymal progenitors (EMPs) into a neural phenotype with use of Y-27632, a clinically compliant small molecular inhibitor of Rho kinase (ROCK). EMPs are a population of mesenchymal progenitors derived from human embryonic stem cells (hESC) which are capable of multilineage differentiation but do not form teratomas. These early mesenchymal progenitors are an ideal stem cell source since they are non-tumorigenic and immunomodulatory. Similar to adult bone marrow mesenchymal stem cells, EMPs do not easily senesce after *in vitro* culture, and are able to be regenerated indefinitely along from ESCS and other pluripotent stem cells including induced pluripotent stem cells. We treated EMPs with Y-27632 and investigated for differentiation capacity and neural lineages by gene expression, and protein expression. Y-27632 induced EMPs into a neural-like morphology, with rapid development of cell extensions and processes within 24 hours. Y-27632-treated EMPs express several neural lineage genes at the RNA and protein level, such as Nestin, MAP2, TuJ1 and GFAP. EMPs can differentiate into a neural phenotype via inhibition of RhoA/ROCK pathway by Y-27632. Further investigation of the detail mechanisms of neural differentiation is needed to improve the efficiency of differentiation for clinical therapeutic use.

Poster Board Number: T-2181

Expression of HOX Genes in the Bone Marrow Derived Mesenchymal Stromal/ Stem Cells from Fanconi Anemia Patients Changes Following Bone Marrow Transplantation

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HOX transcription factors are encoded by thirty-nine genes clustered in four different chromosomes in mammalian and designated as HoxA, HoxB, HoxC, HoxD. HOX code provides important intrinsic regulatory program for cell fate. Whether HOX code has a role as an extrinsic factor and how HOX code changes in hematopoietic niche in disease states are interests of our research group. In this study, we investigated HOXOME profile of bone marrow derived mesenchymal stromal cells (MSC) from Fanconi anemia patients before and after bone marrow transplantation. Fanconi anemia (FA) is a cancer predisposition disorder characterized by progressive bone marrow failure. Bone marrow derived mesenchymal stromal cells (MSC) from donors and FA patients (before and after transplantation) were expanded and characterized for their stromal cell surface markers and differentiation capacity to adipocytes and osteoblasts. HOXOME profile was determined by real-time RT-PCR using Taqman probes. Expression of Hox genes in the third passage of MSCs mostly showed decrease in FA patients comparing to donors. Among down-regulated genes, HoxA5, HoxA6 and HoxA10 were obtained to be rescued after the bone marrow transplantation.

Poster Board Number: T-2180

Induction of Neural Phenotype in Embryonic Stem Cell-Derived Mesenchymal Progenitors by RhoA-Kinase Inhibition

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Human mesenchymal stem cell (hMSC) differentiation has been examined extensively in two dimensional environments, but most materials examined to date do not mimic the adhesive heterogeneity of native extracellular matrix; as such, it is unclear if the spatial arrangement and size of these adhesives regions play any role in guiding stem cell fate. Here, we report on a process where porous foams are fabricated by high internal phase emulsion (HIPE) templating using amphiphilic copolymers. The different copolymers assemble at the oil-water interface and undergo confined phase separation. This creates foams’ surface topology with nanoscopic domains of cell inert and active chemistries detected by the chemical force spectroscopy based on the atomic force microscopy. These results qualitatively resemble native matrix, and results show localized protein deposition in domains of active chemistry. hMSC adhesion then occurs through specific copolymer domains. Interestingly, without the induction media, hMSCs are likely to express lineage specification corresponding to these ‘patchy’ matrices, and their expression depends on stem cell origin: marrow-derived and mesenchymal progenitor cells exhibit fundamentally different differentiation patterns, adipogenic and osteo-genic, respectively. Together these data implicate adhesion as a complex regulator of cell fate.

Poster Board Number: T-2183

Stem Cell Differentiation Can be Directed by Scaffolds With Adhesive Domains

Tudca Inhibits Adipogenic Differentiation. In this study, we examined extensively in two dimensional environments, but most materials examined to date do not mimic the adhesive heterogeneity of native extracellular matrix; as such, it is unclear if the spatial arrangement and size of these adhesives regions play any role in guiding stem cell fate. Here, we report on a process where porous foams are fabricated by high internal phase emulsion (HIPE) templating using amphiphilic copolymers. The different copolymers assemble at the oil-water interface and undergo confined phase separation. This creates foams’ surface topology with nanoscopic domains of cell inert and active chemistries detected by the chemical force spectroscopy based on the atomic force microscopy. These results qualitatively resemble native matrix, and results show localized protein deposition in domains of active chemistry. hMSC adhesion then occurs through specific copolymer domains. Interestingly, without the induction media, hMSCs are likely to express lineage specification corresponding to these ‘patchy’ matrices, and their expression depends on stem cell origin: marrow-derived and mesenchymal progenitor cells exhibit fundamentally different differentiation patterns, adipogenic and osteo-genic, respectively. Together these data implicate adhesion as a complex regulator of cell fate.
EVALUATION OF HEPATOCENIC DIFFERENTIATION POTENTIAL OF MENSTRUAL BLOOD DERIVED STEM CELLS

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Introduction: Recently, menstrual blood has been identified as an easily accessible and renewable stem cell source. However, the challenge remains to develop robust protocols to generate different lineages such as hepatocyte from MenSCs. In this study, hepatocenic differentiation capacity of menstrual blood derived stem cells (MenSCs) was investigated in presence of different combination of growth factors and cytokines. Methods: MenSCs were isolated of menstrual blood samples by discontinuous density gradient centrifugation and plastic adherence. After karyotypic and immunophenotypic analysis, differentiation ability of cultured cells into hepatocyte using three-stage methods developed by different combination of hepatocyte growth factor (HGF), oncostatin M (OSM), epidermal growth factor, basic fibroblast growth factor, insulin-like growth factor, dexamethasone and nicotinamide was evaluated. Results: Flow cytometric analysis illustrated that MenSCs can typically express CD29, CD44, CD73, CD105, OCT-4 and CD10, while lacking CD34, CD38, CD113 and CD45. Immunofluorescence staining and enzyme-linked immunosorbent assay showed that unlike undifferentiated MenSCs, albumin accumulation and secretion was significantly detectable in differentiated cells in different combination of growth factors and cytokines. However, positivity rate of albumin expression was dependent to concentration of HGF and OSM. The cytochemical observations were corroborated with the extent of mRNA expression of albumin and another hepatic specific markers such as cytokeratin-18, cytokeratin-19 and alphafetoprotein evaluated by real-time PCR analysis. Conclusion: The evidence presented here introduces MenSCs as unique population of cells possess bi-directional differentiation potentials towards both osteoblastic and osteoclastic lineages, whereas Sca-1+/PDGFRa+ progenitor cells differentiate into the osteoblastic lineage unidirectionally. When treated with PPARγ agonist, Sca-1+/PDGFRa+cells preferentially differentiated to osteoclasts. Sca-1+ calcifying progenitor cells in the artery originated from bone marrow (BM) and can be clonally expanded. Vessel-resident BM-derived Sca-1+ calcifying progenitor cells showed non-hematopoietic, mesenchymal characteristics. In order to test their capability to modulate in vivo calcification, we next preformed two kinds of experiments, ectopic and atherosclerotic calcification, CT scoring revealed that Sca-1+ progenitor cells increased the volume and calcium score of ectopic calcification. But, Sca-1+/PDGFRa+ cells when treated with PPARγ agonist decreased the bone formation. Systemic infusion of Sca-1+/PDGFRa+ cells into Apoe−/− mice increased calcified atherosclerotic plaques. However, Sca-1+/PDGFRa− cells with PPARγ activation markedly deceased plaques, indicating bi-directional fate in vivo. Conclusions: These findings suggest that a subtype of BM-derived and vessel-resident progenitor cells offer a therapeutic target for the treatment of vascular calcification, and the PPARγ activation may be an option to reverse calcification.

A NOVEL BONE MARROW-DERIVED, VESSEL-RESIDENT CALCIFYING PROGENITOR CELL POSSESSES BI-DIRECTIONAL (OSTEOBLASTIC/OSTEOCLASTIC) DIFFERENTIATION POTENTIALS.

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Dept of Internal Medicine, Seoul Nat’1 Univ Hospital, Seoul, Korea, Republic of Background: Vascular calcification is an advanced feature of atherosclerosis. But, currently no effective therapy is available. To modulate or even reverse the process of calcification, we aimed to identify calcifying progenitor cells and investigated calcifying/decalcifying potentials of these cells. Methods and Results: Cells from aortas of mice were sorted into 4 groups using Sca-1 and PDGFγα markers. Sca-1+ (Sca-1+/PDGFγα+ and Sca-1+/PDGFγα−) progenitor cells showed greater osteoblastic differentiation potentials than Sca-1− (Sca-1−/PDGFγα+ and Sca-1−/PDGFγα−) cells. Interestingly, among Sca-1+ progenitor populations, Sca-1+/PDGFγα− progenitor cells possess bi-directional differentiation potentials towards both osteoblastic and osteoclastic lineages, whereas Sca-1+/PDGFγα+ progenitor cells differentiate into the osteoblastic lineage unidirectionally. When treated with PPARγ agonist, Sca-1+/PDGFγα+ cells preferentially differentiated to osteoclasts. Sca-1+ calcifying progenitor cells in the artery originated from bone marrow (BM) and can be clonally expanded. Vessel-resident BM-derived Sca-1+ calcifying progenitor cells showed non-hematopoietic, mesenchymal characteristics. In order to test their capability to modulate in vivo calcification, we next preformed two kinds of experiments, ectopic and atherosclerotic calcification, CT scoring revealed that Sca-1+ progenitor cells increased the volume and calcium score of ectopic calcification. But, Sca-1+/PDGFγα+ cells when treated with PPARγ agonist decreased the bone formation. Systemic infusion of Sca-1+/PDGFγα− cells into Apoe−/− mice increased calcified atherosclerotic plaques. However, Sca-1+/PDGFγα− cells with PPARγ activation markedly deceased plaques, indicating bi-directional fate in vivo. Conclusions: These findings suggest that a subtype of BM-derived and vessel-resident progenitor cells offer a therapeutic target for the treatment of vascular calcification, and the PPARγ activation may be an option to reverse calcification.
The freshwater planarian Dugesia japonica has high regenerative ability, by which it can regenerate complete individuals from even tiny fragments from almost all parts of its body. This ability depends on adult somatic pluripotent stem cells called “neoblasts.” Interestingly, these stem cells have some morphological and molecular features similar to those of germ-line cells in other animals. For instance, neoblasts have cytoplasmic chromatoid bodies, which are electron-dense structures morphologically similar to germ-line granules in other animals. Neoblasts express many genes homologous to genes encoding germ-line granule components in other organisms, especially RNA-binding proteins, such as cbc-1 (a Me31B homolog), vasa and tudor. One of those genes, piwi, is predominantly expressed in the neoblasts in planarians. piwi was originally identified as a gene which was required for the maintenance of germ-line stem cells in the ovary and testis in Drosophila. Generally, it is thought that Piwi proteins suppress transposable elements to protect the integrity of the genome by acting at the transcriptional level in the nucleus and/or the translational level in the cytoplasm by interacting with short non-coding RNA (piRNA) in sequence-specific manners. Therefore, determination of the sequences of piRNAs would help to identify candidate target genes of Piwi protein. In planarians, piwi genes have important roles in the maintenance and differentiation of the neoblasts. Especially, DjpiwiB(RNAi) animals show serious defects in their regeneration. However, the target genes of DjpiwiB and the molecular mechanism of its function in pluripotency of the neoblasts remain unclear. In this research, we aimed to identify DjpiwiB targets to clarify the molecular function of DjpiwiB in the pluripotency of the neoblasts. Among the four DjPiwi proteins expressed in the neoblasts, only DjPiwiB protein was a nuclear-localized protein, which was observed in almost all cells. In DjpiwiB (RNAi) animals the DjPiwiB protein specifically disappeared from the nuclei of the neoblasts. Although the neoblasts in DjpiwiB (RNAi) animals could proliferate, they lost differentiative ability. These facts indicate that DjPiwiB protein has essential roles in the maintenance and/or exertion of the pluripotency of the neoblasts. To identify the targets of DjPiwiB, we performed immunoprecipitation using anti-DjPiwiB antibody and thereby obtained small (around 32 nt) DjPiwiB-interacting RNAs. Then we sequenced them using an Illumina sequencer and obtained 4,177,817 reads, which showed some typical features of piRNA. We focused on the piRNAs corresponding to our EST database, which were 5% of all piRNAs reads. About 34% of these piRNAs corresponded to transposable elements, for example, Polintont, Gypsy and Penelope, whose expression levels tended to be up-regulated in DjpiwiB (RNAi) animals, suggesting that the main targets of DjPiwiB might be transposable elements, and that DjPiwiB as well as nucleus-localized Piwi proteins in germ-line cells of other animals might suppress transposable elements at the transcriptional level in the nucleus to protect the integrity of the genome. Furthermore, we are now performing transcriptome analysis of DjPiwiB (RNAi) animals to identify additional targets of DjPiwiB which should be strongly suppressed in intact animals.

Here, we will mainly discuss on target genes of PiwiB protein, which are predicted to influence the pluripotency of the neoblasts.

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**Chromatin in Stem Cells**

**Poster Board Number: T-2188**

**TRANSCRIPTIONAL CONTROL OF ADULT NEURAL STEM CELL QUIESCENCE**

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The capacity to produce neurons throughout life with the demand of stem-cell maintenance is central to normal development and aging. How neural stem cell homeostatic processes in the adult mammalian brain are coordinated is not well understood. Errors in this process can disrupt mature neural circuits and cause defects that affect learning, olfaction and behavior. In our studies aimed at identifying novel regulators of neural stem cell self-renewal and differentiation, we focused on the transcription factor REST (Restrictive Element 1 Silencing Transcription factor), which mediates gene repression by recruiting histone deacetylases, demethylases and methyltransferases to cause epigenetic remodeling of chromatin architecture around the REST target genes. More than 1300 genes are confirmed REST targets; moreover, REST targets are highly enriched in neurons, consistent with the wide scope of REST’s influence. In recent work, we demonstrated that: 1) REST is expressed in adult neural stem/progenitors and downregulated in committed neuroblasts, 2) it is required cell-autonomously to prevent precocious neuronal differentiation and maintain the adult neural stem cell pool, and 3) co-binding of REST and its effector proteins control the epigenetic chromatin landscape in adult neural stem cells. To gain mechanistic insight into how REST maintains the neural stem cell pool, we have developed an *in vitro* model of neural stem cell quiescence and found that REST is required to prevent proliferation and transition to the neuronal phenotype. These results suggest that studies of REST in our *in vitro* quiescence model could potential reveal the mechanism controlling adult neural stem cell quiescence and what triggers their transition to proliferative stages.

Ongoing studies are aimed at identifying the downstream targets of REST that mediate quiescence and co-regulators of REST and elucidating their roles in quiescence.

**Poster Board Number: T-2189**

**IDENTIFICATION OF USP16, A CHROMATIN REGULATOR ABLE TO ANTAGONIZE BMI-1 AND LIMIT MOUSE AND POSSIBLY HUMAN STEM CELL EXPANSION IN DOWN SYNDROME**

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Bmi-1 is one of the most important players in the maintenance of self-renewal of stem cells in many adult tissues, including brain, breast and bone marrow. Bmi1 has also shown to be important for the maintenance of cancer and cancer stem cells in many tumors. We identified a gene, USP16, expressed at low levels in normal hematopoietic stem cells, but highly expressed in non self-renewing progenitor cells. USP16 is a chromatin regulator, able to remove ubiquitin moieties from histone H2A, antagonizing the effect of the Polycomb complex (that includes Bmi-1). We found that USP16 can antagonize the function of Bmi-1 in the bone marrow...
and in mouse embryonic fibroblasts, by reactivating the expression of downstream genes with tumor-suppressor functions, such as p16INK or p19Arf. Expression of USP16 in mouse hematopoietic stem cells reduces the self-renewal ability of these cells. Interestingly, the human USP16 gene is located on chromosome 21, and it is expressed at abnormal high levels in cells from Down Syndrome (DS) patients, presenting three copies (trisomy) of chromosome 21. In line with our expectations, bone marrow samples from human DS patients show a reduced number of hematopoietic stem cells. Moreover, DS mice models trisomic for USP16 (Ts65Dn) have stem cell defects in both the bone marrow and the breast. Conversely, another DS mouse model disomic for USP16 (Ts1cje) show a normal development of both stem cell compartments. Ts65Dn DS mice have reduced numbers of stem cells with reduced ability to proliferate in vitro and to engraft in vivo. The overexpression of USP16 in normal mice tissues mimic this effect. Since DS patients develop considerably less solid tumors than expected, we also hypothesize that USP16 is reducing the self-renewal potential not only of stem cells, but also of cancer stem cells, providing a protection from tumor development. Indeed, preliminary data show that USP16 overexpression in human and mouse breast cancer models reduces tumor cell growth. Future studies are directed towards understanding the precise role of USP16 in cancer stem cells and its use as a possible therapeutic strategy.

Poster Board Number: T-2190
DISTINCT ROLES OF TET1 AND TET2 IN MOUSE EMBRYONIC STEM CELLS
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The TET proteins (TET1, TET2 and TET3) constitute a new family of dioxygenases that utilize molecular oxygen and the cofactors Fe(II) and 2-oxoglutarate to oxidise 5mC to 5-hydroxymethylcytosine (5hmC) in DNA. The functions of these proteins have been intensively investigated in mouse embryonic stem cells (mESC), which express Tet1 and Tet2 and contain high levels of 5hmC. The 5hmC and mRNA levels of Tet proteins are tightly controlled during mESC differentiation, with decreasing of 5hmC, downregulation of Tet1 and Tet2 and upregulation of Tet3 mRNA respectively. Depletion of Tet1 in mESC skewed differentiation towards the trophectoderm lineage displayed by mESC; moreover Tet1 depletion resulted in decreased expression of the Nodal antagonist Lefty, thereby skewing mESC differentiation toward mesendoderm both in culture and in teratoma assays. However, Tet1 depletion in mES cells resulted in only a partial loss of 5hmC. Tet2 mRNA is expressed at lower levels than Tet1 mRNA in mESC, and Tet2-depleted mESC show a different behavior in teratoma assays compared to Tet1-depleted mESC. We have examined gene expression and 5hmC distribution regulated by Tet1 and Tet2 in mESC, and find that these proteins have distinct roles. Tet2 regulates 5hmC at gene bodies of highly expressed genes, whereas Tet1 regulates 5hmC at both gene bodies and TSS. Genes with Tet1-regulated 5hmC at TSS tend to be expressed at lower levels and to be associated with bivalent marks and polycomb repression complex (PRC); whereas genes with Tet2-regulated 5hmC at TSS are expressed at higher levels and show less overlap with bivalent marks and PRC. Moreover, Tet1 and Tet2 play distinct roles in regulating the splicing pathway. Together, these data suggest that Tet1 and Tet2 play different roles in mESC.

Poster Board Number: T-2191
PONTIN IS ESSENTIAL FOR MURINE HEMATOPOIETIC STEM CELL SURVIVAL
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Pontin is a highly conserved DNA helicase/ATPase, which is a component of several macromolecular complexes with functions that include DNA repair, telomere maintenance and tumor suppression. While Pontin is known to be essential in yeast, fruit flies and frogs, its physiological role in mammalian organisms remains to be determined. We here find that Pontin is highly expressed in embryonic stem cells and hematopoietic tissues. Through germline inactivation of Ruvbl1, the gene encoding Pontin, we found it to be essential for early embryogenesis, as Ruvbl1 null embryos could not be recovered beyond the blastocyst stage, where proliferation of the pluripotent inner cell mass was impaired. Conditional ablation of Ruvbl1 in hematopoietic tissues led to bone marrow failure. Competitive repopulation experiments showed that this included the loss of hematopoietic stem cells through apoptosis. Pontin is therefore essential for the function of both embryonic pluripotent cells and adult hematopoietic stem cells.

Poster Board Number: T-2192
EPIGENETIC SWITCH ON THE DIFFERENTIATION OF MOUSE VASCULAR ENDOTHELIAL CELLS
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During the last decades, most studies of vascular development have consisted of gene knockout and knockdown studies using mice and zebrafish. Although these works led to the discoveries of some important factors in vascular development, they could not sufficiently identify the conditions required for vascular formation. To clarify the molecular and epigenetic mechanisms underlying vascular development, we have developed a novel embryonic stem (ES) cell differentiation system. Using this system, we can systematically induce vascular cells in vitro and dissect their differentiating processes in detail. 96 hours after induction of differentiation from mouse ES cells, Flk (VEGF, vascular endothelial cell growth factors, receptor 2)-positive mesoderm cells are sorted by MACS using anti-Flk antibody. If these cells are stimulated by VEGF (50ng/ml), cells have commitment to endothelial cells (EC). On the other hand, cells differentiate to smooth muscle cells (SMC) without VEGF stimuli. To elucidate comprehensive gene expression profiles during EC or SMC differentiation, we performed sequential DNA microarray experiments 6, 12, 24, 48 hours after with or without VEGF stimulation. Then we statistically calculated each probe signal value about VEGF (+) / VEGF (-), then selected significantly expression genes at each time point. At a result, transcription factors (Gata2, Etv2, Sox18) were induced at early time points, cell-specific markers (Icam2, Ve-Cadherin, Endomucin) were induced at late time points. For example, Etv2 was early induced at 6h VEGF (+), then downregulated, which is consistent of previous reports. Other ets family protein, FlI1 was induced at 12h VEGF (+), Erg was induced at 48h VEGF (+). Our data have suggested that precise mechanisms of temporal and special different ets protein expressions might be necessary for EC differentiation. Next, in order to depict epigenetic landscape
about vascular development, we conducted ChiP-seq (Chromatin immunoprecipitation with next generation sequencing) using H3K4me3 and H3K27me3 specific antibodies. In general, H3K4me3 marks represent active promoter regions or H3K27me3 marks represent repressive regions and bivalent (both positive H3K4me3 and H3K27me3 marks) mean master transcription factors for differentiation. From ChiP-seq, we found out H3K4me3 have strongly correlation with gene expression profiles such as endothe-

**Poster Board Number: T-2193**

**SET NUCLEAR ONCOGENE IS ESSENTIAL FOR MOUSE EMBRYONIC STEM CELL DIFFERENTIATION**

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Embryonic stem cells (ESCs) are unique in their ability to self-renew and differentiate into all cell types of the organism. A core set of transcription factors in concert with chromatin regulators maintains the ‘stem cell state’. However, this list is not exhaustive and our knowledge about proteins that play important roles in ESCs biology is still limited. To identify novel regulators involved in stem cell maintenance and differentiation, we performed a screen for proteins that are specifically down-regulated during early stages of ES cell differentiation. To this end, we created a library of ESC clones using a gene-tagging approach, with each clone expressing a YFP tagged protein under the control of its own endogenous pro-
motor. Retinoic acid (RA) induced differentiation of gene-tagged clones was used as a model system in combination with time-lapse imaging to measure changes in protein levels that are significantly decreased during differentiation. Using this approach, we identi-
cified candidate genes and systematically investigated their role in ESC function. One of the candidates we identified is SET nuclear oncogene (SET), a multifunctional linker histone chaperone. SET has been implicated in a wide array of cellular processes including histone acetylation, DNA replication and cell cycle regulation. Here we show a novel role for SET as a repressor of developmental genes in ESCs. By using various biochemical and genetic methods, we show that SET is essential for active proliferation and differentia-
tion of ESCs. Interestingly, SET function is isoform-specific. During differentiation, SET isoform-α levels decline rapidly with concomi-
tant increase in the levels of isoform-β. Hence, SET joins the league of recently identified proteins with alternative functional isoforms in ESCs and differentiated cells. Microarray analysis revealed that expression of important pluripotency markers was not efficiently silenced in SET knockdown cells upon differentiation. We further show that SET is required for proper differentiation of ESCs. Knock-
down of SET in ESCs results in upregulation of mesodermal lineage genes, indicating that SET acts as a mesodermal repressor in ESCs. Furthermore, SET depleted ESCs fail to differentiate into neuronal lineage upon induction with RA, suggesting a role in facilitating ectodermal differentiation. Taken together, our data identify a novel chromatin regulator of differentiation in ESCs.

**Poster Board Number: T-2194**

**GLOBAL ANALYSIS OF HIGHER-ORDER CHROMATIN STRUCTURE IN PLURIPOTENT STEM CELLS**

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Global analysis of histone modifications and electron micro-

scopic observations have shown that induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) have a more open ( euchromatic ) chromatin structure than somatic cells. This fact indicates that the reprogramming process involves dynamic changes of chromatin structures. However, it remains unclear how chromatin conformation affects the pluripotency. Here, we report the characterization of chromatin interactions in pluripotent stem cells. First, we investigated the differences in chromatin interactions among mouse embryonic fibroblasts (MEFs), mouse iPSCs and mouse embryonic stem cells (ESCs) by the chromosome conformation capture (3C), which can detect chromatin loci in close physical proximity. As a result, the interactions between Nanog promoter region and its upstream regions occurred at higher frequencies in mouse ESCs and iPSCs than in MEFs. The different interactions were also observed between human dermal fibroblasts (HDFs) and human iPSCs. These results were consistent with a previous report showing that the chromatin interaction at the Nanog locus in ESCs is dependent on Oct4. Next, we performed the Hi-C, which can determine the genome-wide interactions of chromatins by combining the 3C method with massively parallel sequencing. We identified several different intrachromosomal interactions between MEFs and mouse pluripotent stem cells. In both mouse pluripo-
tent stem cells and MEFs, the probability of the intrachromosomal interaction was higher than that of interchromosomal interactions. In addition, we found that the mouse ESCs and iPSCs had more diverse intrachromosomal interactions than MEFs. These findings suggested that the chromatin interactions in somatic cells are dynamically changed during reprogramming process. This study will provide new insights into the molecular basis underlying the iPSCs generation.

**Poster Board Number: T-2195**

**INTEGRATED ANALYSIS OF LNCRNA-CHROMATIN INTERACTION**

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Long noncoding RNAs (IncRNAs) are key regulators of chromatin state, yet the nature and sites of RNA-chromatin interaction are mostly unknown. Furthermore, the full cast of IncRNA chromatin regulators remains to be defined. Here we introduce an integrated analysis pipeline that consists of two techniques: Chromatin RNA-IP (ChRIP) and Chromatin Isolation by RNA Purification (ChIRP). While the former technique allows us to systematically discover all IncRNAs stably associated with the chromatin and likely serving regul-
atory roles, the latter identifies their binding sites on the genome. Using this pipeline, we have found more than 20 stably expressed chromatin-associated IncRNAs in primary human fibroblast, including well-documented examples such as XIST and KCNQ1OT1. The chromatin content is significantly enriched in IncRNAs. In addition, the genomic binding sites of three well-characterized IncRNAs, roX2, TERC and HOTAIR have also been enumerated, which provided critical insights into their mechanisms of action. The ChRIP-
Two distinct Polycomb complexes, PRC1 and PRC2, collaborate to maintain epigenetic repression of key developmental loci in embryonic stem cells (ESCs). PRC1 and PRC2 have histone modifying activities, catalyzing mono-ubiquitination of histone H2A (H2AK119u1) and trimethylation of H3 lysine 27 (H3K27me3) respectively. Compared to H3K27me3, localization and role of H2AK119ub1 is not fully understood. Here we present genome-wide H2AK119u1 maps in ESCs and identify a group of genes at which H2AK119u1 is deposited in a Ring1A/B, core PRC1 components, dependent manner. These genes are a distinctive subset of genes with H3K27me3 enrichment and are the central targets of Polycomb silencing that are required to maintain ESC identity. We further show that the H2A ubiquitination activity of Ring1 is dispensable for its target binding and activity to compact chromatin at Hox loci, but is indispensable for efficient repression of target genes and thereby ESC maintenance. The Ring1 ubiquitination activity is also indispensable for the maintenance of epiblast stem cells (EpiSCs). These data demonstrate that H2A ubiquitination is an essential step for PRC1-dependent repression of genes that are crucial for the maintenance of ESC and EpiSC identities.
**GENE REGULATION BY POLYCOMB GROUP PROTEINS IN THE NEOCORTICAL NEURAL PRECURSOR CELLS**

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During neocortical development, neural precursor cells (NPCs, or neural stem cells) produce various types of neurons and glial cells in a sequential manner. Although the timing of the fate switch during this sequential process is critical for determining the number of each cell type, the mechanisms are not fully understood. We have previously shown that the polycomb group complex (PcG) restricts neurogenic competence of NPCs and promotes the transition of NPC fate from neurogenic to astrogligenic. We also found that the level of histone H3K27 trimethylation (H3K27me3) increases at the promoter of the proneural gene neuromedin 1 over time during development and that inactivation of PcG by knockout of the Ring18 or Ezh2 gene in the late stage of neocortical development resulted in the increase of ngn1 expression. In the present study, we carried out a genome-wide analysis of H3K27me3 and gene expression in different stages of NPCs during the course of neocortical development. Comparison of H3K27me3 levels and gene expression levels among these different stages of NPCs revealed that PcG regulates a specific subset of neuronal differentiation-related genes. Based on these studies, we will discuss PcG mediated gene regulation during development, in relation to the regulation of RNA polymerase II.

**IDENTIFICATION OF A PREDICTIVE MOLECULAR SIGNATURE FOR EX VIVO MIGRATION OF HUMAN MESENCHYMAL STROMAL CELLS**

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Bone marrow-derived mesenchymal stromal cells (MSC) are promising candidate cells in regenerative therapies as they are easily obtained, have the potential to differentiate into several cell types, and show immunomodulatory properties. However, previous studies have demonstrated that human MSC exhibit poor homing capacity to bone following i.v. infusion, possibly due to the heterogeneity within a MSC population, constituting cells with different ability for homing. We have recently studied the cellular heterogeneity of MSC in relation to bone forming ability by isolating several single MSC cell clones based on their ectopic in vivo high- (HBF) and low bone-forming (LBF) capacity. To investigate whether MSC clones would also maintain a predictive molecular signature for better migration and engraftment in vivo, we performed Illumina®-based microarray analysis comparing three HBF versus three LBF clones. Among 746 differentially up-regulated genes (p value < 0.01, 1.5-fold cut off), around 14 percent of genes were annotated as potentially involved in stem cell homing (chemoattraction, adhesion, migration), including chemokine (C-C motif) ligand 8 (CCL8), CCL13, chemokine (C-X-C motif) ligand 16 (CXCL16), CXCR receptor 7, insulin-like growth factor receptor (IGFR), alpha 1 integrin, interleukin 8, vascular cell adhesion molecule 1, stromal cell-derived factor 1 (SDF1), platelet-derived growth factor receptor (PDGFR) A and B, and several members of the tumor necrosis factor super family. In consistence with this molecular signature, HBF-clones showed significant in vitro transwell migration toward SDF1, IGF1, and PDGFb as compared to LBF-clones (116.2±12.1% vs 101.2±1.9%; 124.9±8.6% vs 106.4±3.5%; 160.8±8.1 vs 139.0±5.2%, respectively). In conclusion, our data demonstrated the existence of cellular heterogeneity in relation to the migration ability of human bone marrow derived MSC and a positive correlation between responsiveness towards chemoattractants and bone forming capacity. Furthermore, we identified a predictive molecular phenotype that may enable isolation of MSC sub-populations with high bone homing capacity suitable for bone regeneration.

**THE HUMAN MESENCHYMAL STEM CELLS DERIVED INDUCED PLURIPOTENT STEM CELLS MAY BE A PROMISING CELL SOURCE FOR ALLOGENEIC iPSCS TRANSPANTATION THERAPY**

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Background: Placental tissue is readily available, easily procured without invasive procedures, and does not elicit ethical debate.
Human amniotic mesenchymal stem cells (hAMSCs) are isolated from amniotic membrane underlying the chorion of placental. The hAMSCs demonstrated immunomodulatory properties known to suppress host immune responses. As reported in several studies, hAMSCs’ immunosuppressive effects were confirmed with positive findings for CD59 and HLA-G. Soluble HLA-G molecule, produced by the placenta induced apoptosis of activated CD8+ T-cells and inhibited CD4+ T-cell proliferation. CD59, a complement regulatory protein, prevented complement-mediated cell damage through inhibition of the complement membrane attack complex. Immunologic reaction is a critical issue in stem cell-based therapy when using non-matched stem cell therapy. To address this issue, the immune characteristics of hAMSCs and iPSCs derived from hAMSCs (MiPSCs) were investigated in vivo and in vitro in this study. Method and Results: hAMSCs were isolated from human placentas obtained from healthy subjects at the Stanford University Medical Center, Stanford, CA. This population of hAMSCs demonstrated high expression of HLA-G and CD59 by immunohistology and RT-PCR assays. A single polyclonal lentivirus was introduced into hAMSCs to generate MiPSCs. The MiPSCs also expressed high level of HLA-G and CD59 by immunohistology and RT-PCR assays. The immunological property characterized by CD59 +, HLA-G + may suggest that the MiPSCs retain the immunosuppressive properties of the hAMSCs. In in vitro study, the leukocyte-mediated cytoxicity experiments revealed that MiPSCs and hAMSCs both inhibited the immune rejection of leukocytes in vitro. To investigate the post-transplantation survival in vivo, the mESCs, hAMSCs and MiPSCs with luciferase reporter gene were injected into hind limbs of immunocompetent SVJ mouse. The in vivo study demonstrated robust BLI survival signal by the luciferase-transduced mESCs and hAMSCs at week 1, whereas MiPSCs didn’t survive in the SVJ mouse. Conclusions: The generation of induced pluripotent cells (iPSCs) from differentiated adult cells has vast therapeutic implications in regenerative medicine, then which is hampered by immune rejection of post-transplantation. Our data revealed that hAMSCs were able to be survival in immunocompetent SVJ mice, which suggested HLA-G and CD59 might play an important role in immunosuppressive effects. Although the hAMSCs derived MiPSCs didn’t show the survival signals in vivo as well as hAMSCs, MiPSCs retained the unique immune properties of hAMSCs and inhibited immune rejection in vitro that both have been verified in our study. The reasons of poor survival of MiPSCs are not clear, but one of which may be due to changes of immune profiles of MiPSCs in differentiation process. This represents a major challenge for iPSCs transplantation therapy, which may be addressed by the immunosuppressive effects of HLA-G and CD59. In our future works, the MiPSCs’ immune properties will be modified by enhancing expression of HLA-G and CD59 to improve survival of MiPSCs in immunocompetent SVJ mice. The study could make allogeneic iPSCs transplantation therapy possible.

EVALUATING THE DIFFERENTIATION POTENTIAL OF HUMAN SOMATIC CELLS IN MOUSE FOETUSES.

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We employed a two-phased ex vivo culture protocol in which cells from adult human olfactory mucosal biopsies were enriched to apparent homogeneity. The resulting cells exhibited a bipolar, mesenchymal morphology and were amenable to stable genetic modification by electroporation with plasmid DNA. Transcriptome analyses of Phase 1- and Phase 2-cultured cells revealed that signaling through the interferon pathway was active in Phase 1 cells, but was silenced in Phase 2 cells, and the expression of chromatin-modifying genes was induced concomitantly with repressors of transcription in Phase 2 cells, whereas the opposite was true in Phase 1 cells. 19 of 175 pluri potency network gene transcripts were significantly more abundant in Phase 2 cells, including CD44, a marker of mesenchymal stem cells. In contrast, CDK7 and SNRPN were preferentially expressed in Phase 1 cells. The karyotypes of Phase 2 cells of both genetic sexes were normal, suggesting that the two-stage culture conditions did not cause detectable chromosomal abnormalities. The plasticity of cultured human olfactory cells in vivo, was tested by blastocyst complementation. C57BL/6J host foetuses were recovered at 16.5 days post coitus (dpc), the designated experimental endpoint. At this stage, the tissues were functionally immature; however body morphogenesis and tissue architecture were well established. Immunohistochemical analysis confirmed that cultured adult human olfactory cells contributed to the developing midbrain and structures elsewhere in the CNS, myocardin, skin, eye primordium, skeletal muscle, connective tissue, adipose tissue, chondrocytes, lung, chorionic villi, placenta and the developing testis. Adult human olfactory cells were not detected in the liver of foetuses that were examined. Thus, the foetal mouse would appear to be a useful model in which to evaluate human somatic cell lineage commitment and early differentiation in vivo.
decreased, while senescence-associated β-galactosidase (SA-β-gal) activity of the cells was increased, indicating that exposure of MSCs to H2O2 induced premature senescence of the cells. Premature senescence of MSCs in response to H2O2 induced the decrease of SIRT1 activity (indirectly identified by measuring acetylated Lys-9 of histone H3) as well as the decrease of SIRT1 expression. p21 and pERK1/2 in the cells in response to H2O2 were increased, while ppRb was decreased in a dose-dependent manner. On the other hand, RSV pretreatment decreased premature senescence of MSCs. In addition, RSV pretreatment before exposing the cells to H2O2 alleviated the alteration of the proteins (SIRT1, p21 and pERK1/2) sensitive to H2O2 treatment. Conclusion: Our results suggest that oxidative stress including H2O2 exposure induces not only premature senescence of MSCs in vitro but also alteration of senescence-associated proteins. Further, MSCs may exhibit an increased tolerance against oxidative stress via senescence-associated proteins regulated by RSV.

**Poster Board Number: T-2205**

**MICRONRNA PROFILE ANALYSIS OF HUMAN MESENCHYMAL STEM CELLS FROM DIVERSE ORIGINS**

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Most miRNAs are evolutionarily conserved in related species and many miRNAs have well-defined developmental and cell-type-specific expression patterns. Mesenchymal stem cells (MSCs) have been isolated from various human tissues and been used as powerful resources for further clinical therapies. However, the differences between MSCs-derived from different sources are still understudied. Here, we show the miRNA expression profiles and analyze the diverse pattern of MSCs derived from bone marrow (BM), umbilical cord blood (UCB), adipose tissue (AT), amniotic membrane (AM) and amniotic fluid (AF). Our results indicate that within each group of MSCs from the same origin, the variability of the miRNA expression levels is smaller than that between groups of different origins.

**Poster Board Number: T-2206**

**GENE EXPRESSION ANALYSIS OF HUMAN MULTIPOTENT MESENCHYMAL STROMAL CELLS DERIVED FROM BONE MARROW OF OSTEOGENESIS IMPERFECTA PATIENTS DURING OSTEOBLAST DIFFERENTIATION.**

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Osteogenesis imperfecta (OI) is characterized as a genetic disorder in which a generalized osteopenia leads to short stature, bone fragility and serious skeletal deformities. MSCs are precursors present in adult bone marrow that can differentiate into osteoblasts, adipocytes and myoblasts that have been given great importance as a source cell therapy. The aim of this study was to analyze the gene expression profile during osteogenic differentiation from mesenchymal stem cells from bone marrow taken from patients diagnosed with Osteogenesis Imperfecta and control subjects. Samples were collected from three normal individuals and five samples from patients with Osteogenesis Imperfecta. Mononuclear cells (MON) were isolated to obtain mesenchymal cells that were expanded until third passage when the stimulus for osteogenic differentiation was induced. Analyses were also conducted to count the CFU-F and for four of the five samples from patients with OI, the number of CFU-F observed was lower than generally found for normal samples. Cells were collected for analysis of cell immunophenotyping by flow cytometry and RNA was extracted from the resulting sample called T0. Remaining cells were stimulated for osteogenic differentiation. After a day in culture with stimulation, cells from another bottle had their RNA extracted (T1), and the same procedure was performed on days 2 (T2), 7 (T7), 12 (T12), 17 (T17) and 21 (T21). All samples have shown potential of in vitro differentiation into osteoblasts and adipocytes. Immunophenotyping of mesenchymal cells was performed and samples of all patients had immunophenotypic profile consistent with previous works. We identified mutations in COL1A1 and / or COL1A2 responsible for developing the disease for four of five patients. For the patient with Osteogenesis Imperfecta and Bruck Syndrome, coding region of the gene PLOD2 was also sequenced, but no mutations were found. The gene expression analysis was performed by microarray and identified several genes with differential expression. Some genes of fundamental importance in osteoblast differentiation showed lower expression in samples from patients with OI, suggesting a minor involvement of MSCs of patients with osteogenic lineage. Other genes also confirmed their differential expression by Real Time PCR. We observed an increased expression of genes related to adipocytes, suggesting an increased adipogenic differentiation at the expense of osteogenic differentiation. The expression of PLOD2 gene variants proved to be different between normal samples, OI and the patient with Bruck Syndrome. There was also evidence of differential expression of 29b microRNA, with established role during osteogenic differentiation, suggesting a mechanism dependent regulation of miRNA abundance of its gene target, COL1A1.
DNA METHYLATION PROFILING OF HUMAN BM-MSCS IN LONG-TERM CULTURE

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Human bone marrow mesenchymal stem cells (MSCs) expanded in vitro exhibit not only a tendency to lose their proliferative potential, homing ability and telomere length but also genetic or epigenetic modifications, resulting in senescence. We compared differential methylation patterns of genes and miRNAs between early-passage (passage 5 (P5)) and late-passage (passage 15 (P15)) cells and estimated the relationship between senescence and DNA methylation patterns. When we examined hypermethylated genes (methylation peak ≥ 2) at P5 or P15, 2,739 genes, including those related to fructose and mannose metabolism and calcium signaling pathways, and 2,587 genes, including those related to DNA replication, cell cycle and the PPAR signaling pathway, were hypermethylated at P5 and P15, respectively. There was common hypermethylation of 1,205 genes at both P5 and P15. In addition, genes that were hypermethylated at P5 (CEP81, GMPPA, CDKN1A, TBX2, SMAD9 and MCM2) showed lower mRNA expression than did those hypermethylated at P15, whereas genes that were hypermethylated at P15 (MAML2, FEN1 and CD4) showed lower mRNA expression than did those that were hypermethylated at P5, demonstrating that hypermethylation at DNA promoter regions inhibited gene expression and that hypomethylation increased gene expression. In the case of hypermethylation on miRNA, 27 miRNAs were hypermethylated at P5, whereas 44 miRNAs were hypermethylated at P15. These results show that hypermethylation increases at genes related to DNA replication, cell cycle and adipogenic differentiation due to long-term culture, which may in part affect MSC senescence.

HUMAN BLOOD-DERIVED MESENCHYMAL STEM CELLS ARE ASSEMBLED

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We have found that a group of RNA-containing particles in human umbilical cord blood and mouse blood. These particles contain RNA, but not DNA, fragmented less than 200 nt and; more than 30% of these RNAs are micro-RNA. After relocate into lipid membrane vesicles, these particles grow from less than 2 μm up to 10 μm and become living organisms, which we termed Aidars. Video records show that mature Aidars have a flexible membrane and amoebic-like movement. They increase their size by taking materials through a process similar to endocytosis and phagocytosis. Mature Aidars also take materials from the nuclear areas of the adjacent eukaryotic cells, however, without harming these cells. Further, mature Aidars fuse together to increase their body sizes and become eukaryotic cells. FACS analysis and immunofluorescent studies demonstrate that most Aidars express integrin β1, Sox-2 and VASA. Electron microscopy and stains also confirm that Aidars do not have nucleus. Our data provide the evidence that circulation RNA particles together with cellular membrane vesicles and proteins can self-assemble into a group of living organisms. These organisms fuse and, after taken the genetic materials of the eukaryotic cell, transform into blood-derived mesenchymal stem cells.

A MODIFIED METHOD FOR ISOLATION AND PROPAGATION OF MOUSE BONE MARROW DERIVED-MESENCHYMAL STEM CELLS

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Bone marrow derived-mesenchymal stem cells (MSCs) also, called mesenchymal stromal cells have captured substantial scientific and public interest because of their suitability in the context of physical and immune injuries, to regenerate tissues either by producing bioactive molecules such as growth- and differentiation factors or by differentiating themselves into damage-specific cells/tissues. MSCs have been cultured in different media supplemented with various cytokines and growth factors as well as on various surfaces modified with extracellular components like collagen, fibronectin, poly-L- l-lysine etc., aiming to improve and understand their biology of self-renewal, proliferation and differentiation. In similar direction, we present a novel method for culture of mesenchymal stem cells derived from the mouse bone marrow on an extracellular matrix-independent, scratch-based modified surface in Dulbecco’s modified Eagles medium with high glucose (DMEM-HG) and 15 % fetal bovine serum (FBS) in a 37°C, 5% CO₂ incubator. On comparison with the cells grown on the unmodified surface, we observed three to four fold increase in the cell yield. Apart from the surface modification, we also observed trypsinization and cell harvesting to be very easy by using a pre-warm trypsin and phosphate buffered saline (PBS) solution. A higher number of the pure population of spindle-shaped cells expressed the MSC-specific markers like CD29, CD44, Sca-1 and were devoid of hematopoietic cells as they were negative for the markers, CD34, CD45 and CD11b. The MSCs also demonstrated full potential for differentiation into mesodermal cells like adipocytes, osteocytes and chondrocytes in culture. This simple method can be used for isolation, culture and propagation of MSCs from the bone marrow.

GROWTH AND PHENOTYPIC CHARACTERISTIC OF MURINE NAIL MATRIX CELL CULTURE

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Objective. The search for new sources of stem cells that can be used in regenerative medicine is the topic issue for today. Nail matrix probably contains cells with high regenerative potential, because growth and regeneration of nails occurs throughout life. It is known, that the nail matrix can stimulate regeneration of the phalanges of mammals. Moreover, allogenic cells from blood or bone marrow transplants can contribute to formation of humans’ nails. But stem cell cultures and localization of these cells in nails are still not enough characterized. Material and methods. Nail matrixes of mice were isolated by microdissection technique under aseptic conditions. Cells were obtained using 0,1% collagenase and plated into dish coated with BD Matrigel. Cells were cultured in DMEM:F12 supplemented with 15% fetal calf serum (FCS), refreshing the medium every 4th day. Subcultivation was performed when monolayer reached approximately 80% confluence. After passage 3 the culture medium was changed to neurobasal medium with 10% FCS and 0,3 ng/ml β-FGF. Phenotyping of the cells was performed using BD FACS Aria cell sorter. Results. Cells underwent 6 passages
and survived in vitro for up to 12 weeks. First adhered cells were observed on the day 12 after plating. Primary cell culture consisted of different cell types. There were fibroblast-like cells which did not form dense monolayer and were oriented along longer axes. Polygonal cells formed on the surface many cobblestone-like plaques with typical epithelial morphology. Spindle shaped cells formed net like structures contacting with each other with their long sprouts. Number of these cells increased after passing. After passage 3 in some cells lipid granules (Oil Red staining) were formed which indicates their spontaneous adipogenic differentiation. Phenotypic of these cells at the 2nd passage showed high level expression of CD44 and CD73 (about 90% both), whereas expression of CD90 was about 45%, and expression of haematopoietic markers was performed at low levels: CD34 - less than 25%, CD 117 - about 13% and CD45 - only 6%. On passage 3 expression of CD44 (100%), CD73 (98%) and CD90 (nearly 90%) increased while expression of CD34, CD 117 and CD45 was less than 2%. Conclusion. We showed that nail matrix cell culture is rather heterogenous and consists of cells with different morphology. Phenotypic characteristic of this culture represented high expression of stromal stem cell markers and low level of haematopoietic markers. We suggest that the nail matrix can be a transient niche for different types of stem cells and can be considered as an alternative repository of adult cells with regenerative potential.

Poster Board Number: T-2211

EFFECTS OF LONG-TERM SEQUENTIAL PASSAGE ON CHARACTERISTICS OF CANINE ADIPOSE DERIVED MESENCHYMAL STEM CELLS

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Adipose-derived mesenchymal stem cells (AD-MSCs) have demonstrated the feasibility in therapeutic application, due to ease of isolation and abundance in the tissue without age barrier with the great potential of differentiation into multi-lineage cells. However, the promising potential is not predictable and may be lost during proliferation of the cells in a large number and sequential passage in a long-term. Therefore, this study was conducted to investigate effect of sequential passage on characteristics of canine AD MSCs. In this study, canine mesenchymal stem cells (cAD-MSCs) were isolated from adipose tissues (n=5) and subjected to 9 sequential passages. The cells at each passage were characterized for properties associated with multipotent MSCs such as proliferation kinetics, expression of MSCs-specific surface markers, expression of molecules associated with self-renewal and differentiation capabilities into mesodermal lineage cells. In results, the proliferation of the cells were peaked at passage 1 and stopped after passage 7, which was evaluated by CPDL, while doubling time was increased significantly after passage 6. Expression of MSCs surface markers (CD44, CD90, and CD105) and molecule (Oct-4, Sox-2, Nanog and HMGa2) associated with self-renewal was decreased significantly after P6. Regarding differentiation capabilities, cells at passage 1 to 9 could be stimulated to undergo adipogenic and chondrogenic differentiation under specific culture conditions. However, the level of adipogenic and chondrogenic differentiation was negatively correlated with the number of subpassage. Conclusively, the present study suggested that sequential passages affect multipotent properties of cAD-MSCs, which should be considered in therapeutic trial of cAD-MSCs.

Poster Board Number: T-2212

CHARACTERIZATION OF COMMON MARMOSET BONE MARROW DERIVED STEM CELLS

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Mesenchymal stem cells and hematopoietic cells, which are present in bone marrow, are expected to be applied to regeneration medicine. In this study, we harvested bone marrow-derived cells from the femur of newborn common marmoset (Callithrix jacchus) males and investigated the characterization of the cells with an aim to develop the research field of regenerative medicine. The cells were cultured in DMEM containing 10% FBS, and cell morphology was observed to resemble that of fibroblasts. Immunofluorescence and western blotting revealed that the cells expressed the mesenchymal markers CD73 and CD90. In addition, in vitro differentiation analysis showed that the cells had the potency to differentiate into adipocytes and osteocytes. These results indicate that the cells had characteristics of mesenchymal stem cells. In the future, we will investigate whether the cells retain the characteristics of mesenchymal stem cells with increasing passage number.

Poster Board Number: T-2213

MSCS-INDUCED IMMUNOSUPPRESSION OF TH17 CELLS IS CELL-TO-CELL CONTACT MEDIATED AND DOSE DEPENDENT

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Mesenchymal stem cells (MSCs) are multipotent stem cells with immune-modulatory properties. MSCs produce soluble factors such as IL-6, IL10, TGF-β1 and PGE2 that are involved in their suppressive abilities. In addition, IL-6 and TGF-β1 are also involved in the development of the proinflammatory T helper 17 (Th17) cells. The aim of this study is to determine the impact of MSCs on the proliferation and differentiation of Th17 cells in a co-culture system, with particular interest on unraveling the mechanism behind it. MSCs isolated from mice bone marrow were characterized according to their surface antigens expression and by their multilineage differentiation potential. Mouse CD4+T cells were differentiated into Th17 cells and co-cultured with MSCs at different ratios and in the presence or absence of a transwell culture system. At 6 day post-coculture, the expression level of IL-17 by Th17 cells was measured by flow cytometry. We first show that MSCs exert a dose-dependent suppressive and proinflammatory effect on Th17 cells depending on MSCs/CD4+T ratio, where a significant inhibition was only observed at the ratio of 1:10 and not in the case of 1:100. The suppression of the differentiation of Th17 cells was cell-to-cell contact mediated as in the presence of a transwell culture system prevented the observed inhibition effect on Th17. All this data demonstrate, that MSCs exert an immunosuppressive effect on Th17 differentiation, however, the molecules involved in this mechanism are still under investigation and needs to be elucidated to be able to use their properties for the treatment of autoimmune diseases.

Poster Board Number: T-2214

DOSE DEPENDENT CELL-TO-CELL CONTACT MEDIATED IMMUNOSUPPRESSION OF TH17 CELLS IS CELL-TO-CELL CONTACT MEDIATED AND DOSE DEPENDENT

Alcayaga-Miranda, Francisca A.1, Luz-Crawford, Patricia1, Fernandez, Ximena1, Djouad, Farida2, Kurne, Monica2, Contreras, Rafael1, Angualuz, Maria Fernanda1, Jorgensen, Christian1, Khoury, Maroun1, Figueroa, Fernando1, Carrion, Flavia1

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Mesenchymal stem cells (MSCs) are multipotent stem cells with immune-modulatory properties. MSCs produce soluble factors such as IL-6, IL10, TGF-β1 and PGE2 that are involved in their suppressive abilities. In addition, IL-6 and TGF-β1 are also involved in the development of the proinflammatory T helper 17 (Th17) cells. The aim of this study is to determine the impact of MSCs on the proliferation and differentiation of Th17 cells in a co-culture system, with particular interest on unraveling the mechanism behind it. MSCs isolated from mice bone marrow were characterized according to their surface antigens expression and by their multilineage differentiation potential. Mouse CD4+T cells were differentiated into Th17 cells and co-cultured with MSCs at different ratios and in the presence or absence of a transwell culture system. At 6 day post-coculture, the expression level of IL-17 by Th17 cells was measured by flow cytometry. We first show that MSCs exert a dose-dependent suppressive and proinflammatory effect on Th17 cells depending on MSCs/CD4+T ratio, where a significant inhibition was only observed at the ratio of 1:10 and not in the case of 1:100. The suppression of the differentiation of Th17 cells was cell-to-cell contact mediated as in the presence of a transwell culture system prevented the observed inhibition effect on Th17. All this data demonstrate, that MSCs exert an immunosuppressive effect on Th17 differentiation, however, the molecules involved in this mechanism are still under investigation and needs to be elucidated to be able to use their properties for the treatment of autoimmune diseases.
Detailed Program and Abstracts — Thursday, June 14

Poster Board Number: T-2214

THE ANCESTRAL ORIGIN OF MESENCHYMAL STEM CELLS IS REVEALED BY PERIVASCULAR SDF1 EXPRESSION

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Mesenchymal stem cells (MSC) are being used in clinical trials to provide immune suppression or assist in tissue repair. Although MSC are mesodermal in origin and function as a "niche cell" in the bone marrow, their definitive origin remains elusive. Stromal derived factor-1 (SDF1), a chemokine critical for hematopoietic stem cell homing, was first discovered in cultured bone marrow stromal cells, presumably MSC. To identify an in vivo ancestor to MSC, we utilized the zebrafish model to create an SDF-1 transgenic animal. A 4 kb promoter region of sdf1a upstream of DsRed was cloned to create transgenic zebrafish harboring sdf1a expressing cells identified by DsRed fluorescence. Not unexpectedly, a wide range of tissues expressed SDF1. Prominent perivascular cells expressed DsRed. DsRed positive cells were sorted, positively selected cells were flow sorted and a culture system was established for in vitro propagation. DsRed positive cells expressed elevated levels of transgelin, smooth muscle actin and cadherin 5 compared to flow sorted endothelial cells by qRT-PCR; results consistent with a perivascular cell origin. Cultured perivascular cells showed a fibroblastoid morphology and a doubling time of ~48 hours. Cultured cells were positive for CD90, CD73, CD105 and negative for CD45 by RT-PCR, characteristic for MSC. Cultured perivascular cells were differentiated into the osteoblastic, chondrocytic and adipocytic lineages over 4 weeks. Cell histochemical staining patterns and multi-gene expression appropriate for each lineage by RT-PCR was observed (exampled by osteocalcin, leptina, and collagen10a1 respectively). Cultured perivascular cells expressed fgfr and responded to bFGF in a dose dependent manner. Genes important hematopoietic cell maintenance including Notch-Delta family members, angiopoietin, and kit-ligand also were expressed. Cultured cells supported zebrafish hematopoietic cells in co-culture conditions and in vitro co-cultured cells engrafted into myeloid differentiated recipient fish. Microarray expression analysis as was performed since such data are not available for perivascular cells of any type. Genes associated with vascular differentiation and vascular repair were found to be expressed. Tandem mass-spectrometry-based proteomic studies on the cultured perivascular cells revealed proteins also found to be expressed in human MSC. In conclusion, we have isolated cells with many of the characteristics of MSC whose origin was perivascular in the correct anatomical location in zebrafish. Such bona fide perivascular MSC will be useful in high throughput experiments. Thus, the sdf1:DsRed zebrafish will be useful tool to study both the role of perivascular cells in vascular function as well as their interaction with hematopoietic cells in future work taking advantage of the power of the zebrafish especially, that of in vivo microscopic visualization of cell trafficking.

Poster Board Number: T-2215

BETA-CATENIN IS ESSENTIAL FOR REGENERATIVE ROLE OF MYELOID LINEAGE CELLS DURING WOUND REPAIR.

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Three percent of the population suffers from disordered cutaneous wound repair. This causes morbidity from loss of function, negative psychosocial effects from disfigurement, or even mortality from the loss of the skin’s barrier function. During the proliferative phase of wound repair, β-catenin mediated Tcf-dependent activation is active in a subset of dermal cells. β-catenin mediates the effects of growth factors and regulates the number of fibroblast like cells that accumulate during healing, controlling scar size. Cells in which β-catenin is activated potentially derive from several sources, but the source of these cells and their functional contribution is unknown. Here we showed that Tcf transcriptionally active cells express genes characteristic of myeloid lineage cells during skin healing. Lineage tracing studies showed that myeloid lineage cells contribute 18% of dermal cells during the proliferative phase of murine wound repair. A subpopulation of these cells showed characteristics of mesenchymal cells only in the presence of β-Catenin. Thus, β-Catenin is necessary for myeloid lineage cells to attain characteristics of mesenchymal cells. Mice whose macrophages lack β-Catenin show a significant impairment in wound healing. Injection of wild-type macrophages, but not macrophages lacking β-Catenin, improves deficient wound repair in irradiated skin. Taken together, our results show β-Catenin plays an essential role in myeloid lineage cells during wound repair, suggest a novel cell source and targetable mechanism which can be utilized to improve disordered wound healing.

Poster Board Number: T-2216

REPROGRAMMING TO A PLURIPOTENT STATE MODIFIES MENSTRUAL BLOOD-DERIVED MESENCHYMAL STEM CELL RESISTANCE TO OXIDATIVE STRESS

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Aim: Recently, mesenchymal stem cells (MSCs) were obtained from menstrual blood and proved to be useful to treat diseases in which tissue damage is linked to oxidative stress (OS). Reactive oxygen species (ROS) play a key role in the regulation of cell adhesion, migration, proliferation and it has been demonstrated that ROS inhibit cellular adhesion of transplanted stem cells. Thus, the aim of this work was to evaluate whether human menstrual blood-derived mesenchymal cells (MBMC) are resistant to OS and compare to human embryonic stem cells (hESC) and induced pluripotent stem cells derived from MBMC (iPS-MBMC). Methods and Results: Human menstrual blood was collected from twelve healthy female subjects when menstrual flow initiated. All experiments below were approved by our local institutional review board (HUCFF, UFRJ, RJ, Brazil) protocol no: 056/09. MBMC described in this work...
were obtained based on the criteria defined by The International Society for Cellular Therapy position statement. Besides this, MBMC expressed the core embryonic stem (ES) cell regulators Oct4, Sox2, nanog and Klf4. MBMC and human dermal fibroblasts were cultured with increasing H2O2 concentrations ranging from 0 to 10000 µM and cell viability was evaluated by MTT assay 24 hours later. The dose (IC50) which kills 50% of the cells was 1812 µM for MBMC and 816 µM for fibroblasts, showing that MBMC are extremely resistant to OS-induced death. Moreover, MBMC produce three times more extracellular H2O2 than iPS-MBMC and h9 (5.76; 2.35; 2.09 nmol H2O2/h/10^6 cells, respectively), which were detected by Amplex red/HRP assay. This extracellular production of H2O2 by MBMC was inhibited by diphenylidionium (DPI), in a non-dose-dependent way, indicating that NADPH oxidases are responsible for this production. To measure intracellular ROS production, cells were incubated with 10 µM of CM-H2DCFDA and fluorescence was measured by flow cytometry. No differences were found in the amount of ROS produced in the cytoplasm of MBMC, iPS-MBMC and h9 in baseline conditions. However, when exogenous H2O2 was added, iPS-MBMC and h9 had a significant increase in the cytoplasmic amount of ROS, which did not occur in MBMC. In addition, expression of genes coding for antioxidant enzymes was assessed by real-time RT-PCR. Catalase, SOD2, SOD3 expression was five fold higher in MBMC than in iPS-MBMC and h9, and SOD1 was two fold higher in MBMC than in the other cells. However, GPx1 was only 50% lower in iPS-MBMC when compared to MBMC. Curiously, GPx 3 was approximately 25% higher in h9 cells. In contrast, GPx activity was similar in MBMC, iPS-MBMC and ES cells. Conclusion: MBMC are mesenchymal stem cells and express pluripotency markers, suggesting that they are a powerful source of progenitors and could potentially be differentiated in different mesodermal tissue types. Moreover, MBMC presented high resistance to OS, probably because they are capable of producing ROS, leading to a greater expression of antioxidant enzymes. In contrast, after reprogramming to pluripotent stage, iPS generated from MBMC were not OS resistant and were similar to embryonic stem cells. Therefore, MBMC might be considered a source for cell therapy strategy aimed to treat patients with diseases in which onset and progression is associated with OS.

**Poster Board Number:** T-2218

**GENE TRANSFECTION TO MSCS BY CHITOSAN NANOPARTICLES**

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1Medical biotechnology, Qazvin Uni. of medical sci., Tehran, Iran, Islamic Republic of, 2Stem Cells, royan, Tehran, Iran, Islamic Republic of, 3stem and a second population entitled stromal stem cell. Stromal stem cells also known as mesenchymal stem cells (MSC), were considered a source for cell therapy strategy aimed to treat patients with diseases, not only by their capacity of differentiation, but also by their immunomodulatory properties.

**Detailed Program and Abstracts — Thursday, June 14**

**SOX2-POSITIVE AND -NEGATIVE DERMAL PAPILLA CELLS MAINTAIN THEIR INTRINSIC DIFFERENCES, INDEPENDENT OF THE MICROENVIRONMENT**

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In neonatal mouse skin two types of dermal papilla (DP) can be distinguished on the basis of Sox2 expression. We describe a three-dimensional hydrogel culture system that supports clonal growth of neonatal dermal cells. Disaggregated cell populations formed spheres that expressed DP markers alkaline phosphatase, alpha-8-integrin and CD133. Nevertheless, spheres formed by CD133-cells could not support hair follicle formation in skin reconstitution assays. Sox2+CD133+ and Sox2-CD133+ spheres contributed to the DP of both GAA and ZZ hairs. There was no correlation between sphere size and hair type. Sox2 expression was maintained in culture, but was not induced in Sox2- cells in vitro or in vivo, suggesting that Sox2+ cells are a distinct cellular lineage. Although Sox2+ cells were least efficient at forming spheres they had the greatest ability to contribute to DP and non-DP dermis in reconstituted skin. Since the culture system supports clonal growth of DP cells and maintenance of distinct DP cell types, it will be useful for further analysis of the intrinsic and extrinsic signals that control DP function.

**Poster Board Number:** T-2220

**ISOLATION AND CHARACTERIZATION OF MESENCHYMAL STEM CELL FROM REUSABLE AND DISPOSABLE BONE MARROW COLLECTION FILTER AN ETHICAL SOURCE OF CELLS.**

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Have been described the presence of at least 2 types of stem cells deriving from bone marrow; a population termed hematopoietic stem and a second population entitled stromal stem cell. Stromal stem cells also known as mesenchymal stem cells (MSC), were firstly described more than 30 years ago, and there has been an increasing interest on studying this cells, not only by their capacity of differentiation, but also by their immunomodulatory properties.
Isolation of MSC has been reported from several tissues, but since bone marrow derived MSC are the most commonly used cells in clinical trials, these cells probably will be the main target of further studies, in addition an important control for other MSC sources. Bone Marrow mesenchymal stem cells obtainment for research is complex, due to the harvest procedure morbidity. In this study, we investigated the possibility of MSC isolation from washing the bone marrow disposable and reusable collection sets. The isolated cells were characterized according to ISCT criteria. There was obtained 3 samples from disposable and 3 samples from reusable collection set. All the samples obtained from bone marrow disposable set successfully derived MSC, but only 2 obtained from the reusable set effectively derived MSC. Despite the fact that reusable filter sets presented smaller pore size which should retain a higher number of cells, we were able to acquire more cells from disposable filter sets. Possible, due to the fact that MSC are plastic adherent and disposable filter sets are enclosed in plastic, in addition to larger areas to be washed. Therefore, this study presented evidences that MSC can be derived from reusable collection kits, which are usable for research as a very ethical source.

**Poster Board Number: T-2221**

**JAGGED-1 INTRACELLULAR DOMAIN EXPRESSED IN MESENCHYMAL STROMAL CELLS SUPPRESSES THE SUPPORT OF HEMATOPOIETIC STEM/PROGENITOR CELLS IN-VITRO**

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1, Thieme, Sebastian2, Wobus, Manja1, Brenner, Sebastian1, Bornhäuser, Martin1

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In-vitro expansion of human hematopoietic stem and progenitor cells (HSC) is an active area of research. Promising candidates for in-vitro expansion in co-culture systems are mesenchymal stromal cells (MSC). Among a plethora of signalling and structural molecules provided by MSC are ligands of Notch receptors. Immobilized on the surface they were shown to enhance HSC self-renewal in-vitro. In our study we examined if human bone marrow-derived MSC over expressing Jagged-1 could be more effective in the in-vitro expansion of HSC providing Notch ligands simultaneously with molecules physiologically expressed by MSC. Therefore, human bone marrow-derived MSC were transduced with a lentiviral construct containing the open reading frame of Jag-1/dnMAML1. Over production of Jagged-1 protein was confirmed with immunostaining and Western blot analysis of total cell lysate from JICD MSC revealed the protein being recovered at the same level as the supposed JICD fragment in Jag-1 MSC. Immunostaining of JICD MSC showed a predominant intranuclear localization. Additionally, some HSC supportive genes, Angiopoietin-1 and SDF-1, were down-regulated in Jag-1, Jag-1/dnMAML and JICD MSC in comparison with control-transduced MSC. This could explain, at least partially, the impaired support of HSC by Jag-1 MSC. In summary, MSC over expressing Jagged-1 protein do not support in-vitro expansion of HSC and this is probably due to formation of JICD and its influence on regulation of gene expression, including HSC supportive genes. These findings require further investigations to understand which role the Jagged-1/JICD pathway may play in human MSC/osteoblasts and which effect it has on the regulation of HSC self-renewal and differentiation in-vivo.

**Technologies for Stem Cell Research**

**Poster Board Number: T-2222**

**AUTOMATED ANALYSIS AND SORTING OF HUMAN INDUCED PLURIPOTENT STEM CELL (HIPS) CLUSTERS USING LARGE PARTICLE FLOW CYTOMETRY.**

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Biomedical methods based on stem cells are currently one of the most dynamic areas in life science and biomedicine. Of particular importance is the development of cellular test systems for disease modeling and pharmacological and toxicological screenings. The reprogramming of somatic cells into human induced pluripotent stem cells (iPS cells) has opened unique perspectives for producing disease and patient specific human cell products. To take full advantage of this technology there is a need (i) to produce a high number of iPS cell lines using high throughput techniques, (ii) to standardize the respective protocols and (iii) to deliver fully characterized cells. Accordingly, the StemCellFactory project aims to fully automate by robotics (i) the generation of human iPS cell lines and (ii) the differentiation of iPS cells into cardiomyocytes and neuronal cells. Currently, standard procedures for iPS cell production are mainly based on manual processing, but automatic procedures are now being developed. This includes, for example, cell picking devices that recognize and transfer iPS cells and/or clusters from a culture plate to another based on morphology. So far one limitation of this approach is that selection of iPS cells and/or cell clusters requires operator intervention and does not occur automatically. Alternative approaches use immunomagnetic beads and column-based selection of single cells, which however results in loss of iPS cell clonality. We have explored a third approach, one using Union Biometrica’s large particle flow cytometry technology (COPAS and BioSorter). This technology offers a high throughput technique, which is fully automatable and capable of selecting and sorting cell clusters. The results of our analysis are provided. Human iPS cell colonies were stained with the FITC-labeled pluripotency marker TRA-1-60, collagenase treated and cell clusters were subjected to sorting by flow cytometry with the COPAS device. Cell clusters were sorted according to size and TRA-1-60 expression and distribution in clusters, and single cell clusters were deposited in 96 well format. The analysis was fast and dispensing to wells of multiwell plates was automated. Sorted cells were efficiently expanded as clonal iPS cell lines and used in further studies. This establishes Biometria’s
large particle flow cytometry technology as a versatile device for analysis and sorting of iPS cell colonies.

**Poster Board Number: T-2223**

**ENCAPSULATION IN MONODISPERSE HYDROGEL MICROSHERES ENABLES FAST AND SENSITIVE PHENOTYPIC ANALYSIS USING LARGE PARTICLE FLOW CYTOMETRY**

Bongaarts, Rico
Union Biometrica, Geel, Belgium

Detection and characterization of microorganisms usually involves culture during more than 20 generations in order to achieve the formation of macro colonies on solid media. Alternatively, microencapsulation allows the detection of microbial growth by monitoring the development of micro colonies from encapsulated individual cells. Microbial proliferation inside the microcapsules can be detected using COPAS™ large particle flow cytometry. Here we show the successful application of the Flow Focusing® technology to the microencapsulation of different types of cells in monodisperse hydrogel microspheres. Using a Cellena® Flow Focusing microencapsulator, we managed to produce monodisperse alginate microparticles containing individual bacteria, yeast and human stem cells. Alginate particle sizes were reproducibly selected from less than 100 µm to over 600 µm, by just replacing the disposable nozzle. Sterility was preserved during the microencapsulation procedure, preventing undesired contaminations. Microencapsulated microorganisms were utilized for a variety of applications: from characterizing secreted enzymes to detection of thermosensitive mutants. Proliferation inside the particles was monitored by COPAS large particle flow cytometry without requiring fluorescent labeling.

**Poster Board Number: T-2224**

**SIMULATED MICROGRAVITY INITIATES A CHANGE IN CYTOSKELETON TO PROLIFERATE HUMAN MESENCHYMAL STEM CELLS.**

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Physical stimuli such as gravity, electrical field, and magnetic field etc., are common environments during human evolution, which has gone through about 3.5 billion years. The 3D-clinostat, simulated microgravity machine, produced by Mitsubishi Heavy Industries, Ltd., is a multi-directional gravity device, by controlled rotation of two axes, and makes $10^{-9}$ G average over time (patented: undifferentiated pluripotent stem cell proliferation/differentiation regulation method and system, Japanese patent, publication number P2001-197182A, date of filing June 28, 2001, P2003-9852A, date of filing January, 14, 2003, and overseas patents, WO2004/061092 A1 PCT [U.S.A., Canada, China, and Korea], P/E [Italy, U.K., Sweden, Germany, and France], 2004). We reported simulated microgravity, one of physical stimuli, inhibited cell differentiation such as myoblasts, osteoblasts, bone marrow stromal cells, and stem cells. Therefore, simulated microgravity is considered as safety physical stimulation for stem cell proliferation. However, it is unclear why stem cells can proliferate or maintain stemness in microgravity environment than in normal 1G environment. We examined human mesenchymal stem cells (hMSCs) cultured in 1G environment and simulated microgravity environment. The number of hMSCs expressed surface markers increased in microgravity. DNA microarray showed the hMSCs in microgravity showed different gene expression, such as cytoskeleton and metabolism, compared to the hMSCs in 1G. The lower expressing genes in microgravity were mostly related to cytoskeleton component. Moreover, hMSCs in microgravity stained with vinculin and F-actin stress fiber showed decreasing number of focal contact and stress fiber network. Focal contact is possibly considered as gravity sensor in space biology. These results suggested the cytoskeleton changing in hMSCs is associated with stem cell proliferation and differentiation in simulated microgravity. Microgravity can provide us novel technology into stem cell biology.

**Poster Board Number: T-2225**

**SYNTHETIC SURFACE FOR CULTURE OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS IN A DEFINED AND XENO-FREE MEDIUM**

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Mesenchymal stem cells (MSCs) are multipotent stem cells with the ability to differentiate into bone cells (osteocytes), cartilage cells (chondrocytes) and fat cells (adipocytes). MSCs are an important tool in regenerative medicine and tissue engineering, the therapeutic potential of these cells is being evaluated for several disorders. *Ex vivo* expansion of these cells requires either bovine serum containing media or coating of the culture vessel with human or animal-derived extracellular matrix (ECM) protein. Growing concerns about introducing human and animal-derived pathogens into the culture necessitate the need for an animal free (defined as xeno-free and human origin components-free) culture environment. Also, media components and coating matrices of biological origin may have batch to batch variability and can be undefined. Self-coating requires additional time, resulting in coated vessels with limited shelf-life. Here, we report BD PureCoat™ Fibronectin peptide surface, a synthetic peptide surface for culture of cell types that require Fibronectin coating. The peptide surface is a pre-coated, synthetic, xeno-free, human origin components free, and room temperature stable surface. In this study human bone marrow derived mesenchymal stem cells were cultured on BD PureCoat Fibronectin peptide surface for 5 passages in MesenCult, a defined and xeno-free media. Cell growth and morphology were comparable to cells grown on an ECM coated surface. During the course of culture cells retained their differentiation capability and were successfully differentiated into osteocytes and adipocytes following multiple passages. Expression of MSC markers was determined by flow cytometry. Immunophenotyping revealed that the MSC population was positive for CD90, CD105, and CD73 markers. Cells did not express CD34, CD11b, CD19, CD45 and HLA-DR and stained negative for these markers. BD PureCoat Fibronectin peptide surface provides a ready to use alternative to Fibronectin coating for cell culture with comparable cell attachment and functionality.
INTRODUCTION: Bone marrow-derived mesenchymal stem cells (MSCs) are widely used for cartilage tissue engineering but they have also limitations of low differentiation capacity and dedifferentiation in vitro. Many studies reported that fetal progenitor/stem cells (FP/SCs) present in various fetal tissues had higher proliferation capacity and differentiation ability than adult stem cells. In this study, we isolated human fetal cartilage-derived progenitor cells (FCPCs) and investigated their characteristics in comparison with MSCs. Materials & Methods: Cartilage tissues of 12-week fetus were obtained after legal abortion with written consent of donors. It was also approved by the Ethics Committee at Inha University Hospital. Cartilage tissue was first washed in PBS containing 1% penicillin/streptomycin. Then, it was minced and treated with 0.1% collagenase type 2 (Worthington Biochemical) in serum free Dulbecco’s Modified Eagle’s Medium (DMEM) for 16 hrs at 37°C under 5% CO2. Isolated cells were plated at 6000 cells/cm2 and cultured in basal medium (α-MEM containing 10% FBS and 1% penicillin/streptomycin). When the cells reached 80% confluence, the medium was changed to a differentiation medium (α-MEM containing 10% FBS, 1% penicillin/streptomycin) at 37°C under 5% CO2. Cells were cultured until passage 10, while proliferation rate of cells were measured by doubling time following formula DT = (T-T0) log2 / (logN-logN0). T-T0 is culture period, N is final cell number, N0 is initial cell number. Differentiation of FCPCs into chondrogenic, adipogenic, and osteogenic lineages were examined by safranin O, Oil-red O and Alizarin red S stains after 3 weeks of differentiation in vitro. FCPCs were also analyzed for the expression of various cell surface markers for MSCs (CD90, CD29, CD44, CD90 and CD105), hematopoietic stem cells (HSCs) (CD34 and CD45), and embryonic stem cells (ESCs) (SSEA4, SSEA1, Oct4, Nanog, and Sox2). In addition sorted by FACS using STRO-1, CD14, CD49C, CD49F, CD49E, CD133, CD166 makers to isolate small size populations (SP). Results & Discussion: We found that cells in basal medium maintained doubling time of less than 3 days and original morphology until passage 10. Differentiation ability of FCPCs was higher than human MSCs for adipogenesis, osteogenesis, and chondrogenesis, which was maintained well until passage 10. FCPCs showed expression of some of MSCs markers and ESCs markers such as CD44, CD90, SSEA4 and Oct4. In the cell sorting experiment, subpopulation of FCPCs with small size populations (SP) were isolated and showed higher expression of CD49F and CD133 than large populations (LP). These results showed that human FCPCs are highly potent cells with unique stem cell characteristics and could be a promising cell source for cartilage tissue engineering.
Detailed Program and Abstracts — Thursday, June 14

**NOVEL SYNTHETIC POLYMERS AND XENO FREE SERUM FREE MEDIA FOR HIGHLY EFFICIENT EXPANSION OF HUMAN MSC**

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The goal of this project was to identify novel polymeric surfaces for the culture and expansion of mesenchymal stem cells (MSCs) in a highly promising xeno-free, serum-free chemically defined medium (ITRI-SFM) developed by ITRI (Taiwan). Due to their proliferation and differentiation, as well as their function in pro-angiogenesis and immune modulation, MSCs have been used in treating a variety of serious diseases, such as critical limb ischemia and graft-versus-host disease. Although MSCs are mostly cultured in serum-containing media, the cell therapy industry has been seeking to use an animal-free, serum-free media to avoid the production challenges caused by the safe sourcing of animal components and batch variation of the serum. Unfortunately, the use of such media with standard culture plastics leads to poor attachment and poor growth of MSCs. As a result, ITRI initiated an international collaboration, with Altrika Ltd. (UK), to develop new synthetic substrate candidates which allows rapid expansion of MSCs grown in ITRI-SFM. Altrika used its in-house high throughput polymer microarray platform to screen large libraries of custom designed synthetic polymers. Over 1200 polymers and polymer blends were screened for their capacity to immobilize MSCs and about 20 "hit" polymers were identified. These initial results were subsequently confirmed by scaling-up the surfaces via coating the "hit" polymers onto glass coverslips. Structure activity relationship studies (SAR) were run and novel focused libraries were prepared. These focused libraries allowed the establishment of a clear relationship between the structure and composition of these polymers and their performances in terms of supporting the growth of a range of MSCs: bone marrow MSCs (BMSC), adipose tissue-derived stem cells (ADSC) and Wharton's jelly stem cells (Wj cells). Finally, flow cytometry studies showed that the cells grew on these selected surfaces and maintained characteristic MSC phenotypes of surface markers (CD105+, CD90+, CD73+) and a gene of a selection marker. We next compared efficiency of establishing stable clones using traditional plasmid-based transfection for establishing bio-engineered sublines of hES cells inducibly overexpressing complex transgenes.

**BIVAIENT MUC1* LIGANDS FORM A NOVEL AND IMPROVED SYSTEM FOR DEFINED, XENO-FREE CULTURE OF HUMAN ES AND IPS CELLS**

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We report that a single growth factor, NM23, enables serial passaging of both human ES and iP cells in the absence of feeder cells, their conditioned media or bFGF in a fully defined xeno-free media on a novel defined, xeno-free surface. Stem cells cultured in this system express a pattern of gene expression indicating that they are in a more "naïve" state than stem cells grown in bFGF-based media. NM23 and MUC1* growth factor receptor cooperate to control stem cell self-replication. By manipulating the multimerization state of NM23, we override the stem cell's inherent programming that turns off pluripotency and trick the cells into continuously replicating as pluripotent stem cells. Dimeric NM23 binds to and dimerizes MUC1* which stimulates growth and promotes pluripotency. Inhibition of the NM23-MUC1* interaction accelerates differentiation and causes a spike in miR-145 expression which signals a cell's exit from pluripotency.
Detailed Program and Abstracts — Thursday, June 14

**Poster Board Number: T-2231**

**HUMAN NEURAL STEM CELL FATE CONTROL BY BIOMIMETIC CUES: FROM SINGLE CELL POSITIONING TO MULTICELLULAR ARRANGEMENTS IN DEFINED IN VITRO CONDITIONS**

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Biomimetic cues are defined as in vitro microenvironmental conditions mimicking cell natural niche. In our hands biomimetic cues used for neural stem cell fate control included creation of biofunctional adhesive domains as well as modulation of oxygen tension conditions, influencing cellular epigenome and stimulation of intracellular pathways by small signalling molecules. Neural stem cells derived from Human Umbilical Cord Blood (HUCB-NSC) were directed either for differentiation or reprogramming in defined in vitro conditions. Bioactive domains obtained by micro-contact printing or micro-spotted technology, with controlled content and geometry served as a template to immobilize neural stem cells to the surface and direct their differentiation. It was shown, that specific, receptor mediated versus electrostatic interactions on the cell membrane/bioactive domain interface were crucial to keep the cells either in neurally committed or non-differentiated stage by fibronectin or poly-l-lysine pattern respectively. Single cell positioning in contrast to multicellular domains further promoted non-differentiated stage of HUCB-NSC Activation of intracellular pathways by signalling molecules (Wnt-3a, CNTF, Jagged, Notch and DKK-1) microspotted with fibronectin as bioactive domains directed differentiation of HUCB-NSC into astrocytic and neuronal lineages, as revealed by immunocytochemical and molecular analysis. Low oxygen tension conditions and epigenetic stimulation with small molecules influencing methylation and acetylation status of the cellular chromatin were essential microenvironmental factors for effective induction of iPS cells from HUCB-NSC by poly-arginine tailed Klf4, Oct4 and Sox2 transcription factors. Our studies revealed that combining biomimetic cues with the proper stimulation of intracellular pathways is decisive for successful reprogramming and differentiation of neural stem cells derived from human umbilical cord blood. Sponsored by grant from Polish Ministry of Science Research and Higher Education No 5978/B/PO1/2010/38 and No 2211/BP01/2010/38

**Poster Board Number: T-2232**

**A ROBUST SYSTEM FOR EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO HEMATOPOIETIC STEM AND PROGENITOR CELLS**

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Major advances in differentiating human pluripotent stem cells (hPSCs) to primitive hematopoietic stem and progenitor cells would greatly accelerate hematology research and clinical development. In collaboration with Woods et al., Primorigen has developed a high-efficiency hematopoietic stem cell (HSC) differentiation system that currently yields a) 10-100 fold more hematopoietic progenitor cells (CD45/43+, CD34+) than the publication record, b) a 40-fold increase in the number of hematopoietic cells with an adult cell surface phenotype (CD45/43+, 34+, 38+, 90+, 45RA+), and c) clonogenic progenitors (CFUs) at equivalent numbers as umbilical cord blood CD34+ cells. The system combines a proprietary small molecule based medium with an optimized protocol to achieve striking improvements over existing high yield methods across multiple hPSC lines. The resulting cells also show both lymphoid and myeloid cell differentiation potential, greatly increasing their value both for pre-clinical hematology research, and ultimately clinical development of HSC-based therapeutics.

**Poster Board Number: T-2233**

**UNIVERSAL BANK OF IMMUNE-MATCHED CLINICAL GRADE PLURIPOTENT STEM CELLS AND THEIR DERIVATIVES**

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Creating a bank of pluripotent immune-matched stem cells is an essential precursor to developing stem cell based therapies that are both broadly applicable and economically viable. In this abstract we describe a universal bank of pluripotent human parthenogenetic stem cells for use in clinical applications. Such a bank could be used to create banks of differentiated cells that prima facie immune-match millions of individuals, making cellular replacement therapy a clinical reality. Human parthenogenetic stem cells (hpSCs) have been proposed as an alternative source for cell replacement therapies and are known to behave similarly to conventional human embryonic stem cells (hESC) in their proliferative and multilineage differentiation potential. One advantage of hpSCs is that their derivation does not involve the destruction of a viable human embryo. hpSCs are derived from unfertilized oocytes that have been chemically activated and isolated from the inner cell mass of the parthenogenetic blastocyst. Importantly, hpSC can be made to be homozygous at the human leukocyte antigen (HLA) loci. This HLA-homozygosity significantly reduces immunogenicity and simplifies immune matching in clinical applications. Although there are often hundreds of HLA types in any particular population, the majority of individuals carry at least one of high frequency. Differentiated cells derived from hESC will not immune-match any individuals in the population, unless they are naturally HLA-homozygous, and therefore their use as a cell source requires immunosuppression therapy alongside the replacement therapy. However a bank of HLA-homozygous stem cells and their derivatives consisting of the highest frequency haplotypes will immune-match millions of individuals and therefore could provide an economical and accessible therapeutic treatment paradigm. Here we describe our process of creating a bank of ethically derived, clinical grade (Good Tissue Practice and current Good Manufacturing Practice), hpSC, including the regulatory approvals necessary to derive new lines in California. Our strategy is to build on and expand our existing collection and create cell lines that can be used for the clinical development of cellular replacement therapies. Our current bank consists of ten hpSC lines suitable for research, along with HLA-homozygous neuronal lines and retinal pigment epithelial (RPE) cells.
Detailed Program and Abstracts — Thursday, June 14

Poster Board Number: T-2234

SCALABLE EXPANSION OF HUMAN EMBRYONIC STEM CELLS IN A MICROCARRIER SUSPENSION CULTURE SYSTEM

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Human embryonic stem (hES) cells offer great potential for cell therapy and regenerative medicine due to their pluripotency and high capacity for self-renewal. The challenge is to produce these cells in large enough numbers for such therapies, whilst retaining their characteristics. Historically, hES cells have been grown using largely undefined media on mouse or human feeders in cell culture flasks/dishes - a labour intensive system with limited scalability. We have assessed a microcarrier suspension system for the culture of hES cells. A number of hES cell lines were grown on gelatin, laminin or Matrigel coated microcarriers in the Levitube, an impeller free vessel for microcarrier and suspension cell cultures. The system supported the growth and serial subculture of all hES cell lines used. Cell yields were increased by up to 25% compared to the same line on inactivated mouse feeders in 6-well plates over a five day period. hES cells were also frozen on the microcarriers and successfully thawed to produce viable cultures. This system, when compared to 2-D culture systems, provides a method for producing the equivalent of 15 to 20 six-well plates in a single 50 mL tube under defined, feeder-free conditions with the key benefits being significant reductions in both handling time and media consumption.

Poster Board Number: T-2235

THE IMPORTANCE OF DIGITAL HOLOGRAPHIC MICROSCOPY FOR AUTOMATED, REAL-TIME MONITORING OF HUMAN ADULT STEM CELL CONFLUENCE IN LARGE-SCALE CULTURES.

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To guarantee robust cell expansion on a large scale, an automated control method becomes essential for obtaining sustainable and useful stem-cell-based products. Given that both stem cell behavior and the differentiation mechanisms are sensitive to cell density, monitoring of cell confluence is mandatory. Actual observation protocols of traditional polystyrene T-flasks, or multitrays, are ineffective for large scale manufacturing. Integrity™ Xpansion™ multiplate bioreactors have been developed to enable noninvasive, real-time observation of stem cell growth at large scales. The specific design of the bioreactor combined with the iLine — a differential digital holographic microscope (DDHM) of the newest generation — enables automatic multiplate cell monitoring. The DDHM technology captures 3-D information, enabling label-free image processing and automatic cell confluence counting. The data presented will highlight the benefits of differential digital holographic microscopy as a reproducible and consistent method to track stem cells confluence during large-scale production.

Poster Board Number: T-2236

COMPARATIVE ANALYSIS OF EX VIVO EXPANDED HUMAN CD34+ HEMATOPOIETIC STEM CELLS ON NANEX™ NANOFIBER PLATES

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Hematopoietic stem cells (HSC) are increasingly used in treating a wide variety of malignant and degenerative diseases with promising results. Cells expressing CD34 (CD34+) in bone marrow (BM), mobilized peripheral blood (PB) and umbilical cord blood (UCB) identify a rare cell population with HSC progenitor characteristics. The number of CD34+ HSC available is invariably limited and for some clinical applications, ex vivo expansion is required to generate the cell numbers needed for successful transplant. The development of ex vivo culture systems that enable efficient expansion and maintenance of CD34+ HSC is a crucial step to harnessing its full potential for use in cell-based therapies. Arterioocyte recently launched an ex vivo culture system, the NANEX™ HSC Expansion Kit. The NANEX™ coating is a chemically-modified polymeric nanofiber mesh that forms a 3D scaffold upon which the cells adhere. This 3D scaffold mimics the BM microenvironment promoting cultures of CD34+ cells on NANEX™ plates to efficiently expand and maintain the CD34+ phenotype. Most of these studies were performed using CD34+ selected cells from UCB. In this study, we independently evaluated Arterioocyte’s NANEX™ HSC Expansion Kit as an ex vivo culture system using cryopreserved CD34+-selected cells from UCB and mobilized PB cultured in parallel. Three samples each of frozen CD34+-selected cells from UCB and mobilized PB were thawed and cultured in parallel for eight days on NANEX™-coated or regular 6-well tissue culture (TC) plates. The cells were maintained in serum-free HSC expansion medium and optimized cytokine cocktail provided in the kit. Cell counts, flow cytometry analyses and colony-forming cell (CFC) assays were done both at initiation and at termination of cell cultures. There was on average, a 124-fold expansion of UCB CD34+ cells and four-fold expansion of mobilized PB CD34+ cells on NANEX™-coated plates after the eight-day culture period. In contrast, UCB CD34+ cells cultured in uncoated TC plates had a 25-fold expansion and mobilized PB CD34+ cells did not proliferate at all. Flow cytometry analyses of cells harvested from NANEX™ plates showed that UCB CD34+ cells maintained a larger percentage of CD34+ cells (average = 24.5 %) compared to mobilized PB CD34+ cells (average: 9.7 %). UCB and mobilized PB CD34+ cells harvested from uncoated TC plates had markedly lower percentages of CD34+ cells (8.5 % and 5.0 %, respectively). There was no significant difference in CFC content (CFC per 500 CD34+ cells plated) between CD34+ cells used to initiate the cultures and the cells harvested after the eight-day culture period, indicating that NANEX™ culture maintains colony-forming cells. This study validates the use of NANEX™ HSC Expansion Kit as an efficient system for the ex vivo expansion of UCB CD34+ cells. Expansion of mobilized PB CD34+ cells was likewise enhanced but not to the same degree. Mobilized PB CD34+ cells are known to be less proliferative than UCB CD34+ cells, and efforts to expand these cells ex vivo have not been as successful to date. It is likely that mobilized PB CD34+ cells might require other growth factors and/or interactions with the microenvironment for their optimal growth. Nonetheless, the results of these initial ex vivo experiments look promising and further studies are warranted.
embryonic fibroblasts (iMEFs). We looked to determine whether hypoxia in iMEFs is permissible for the propagation and pluripotency of human pluripotent cells (hPSCs) in vitro, yet the xenogenic origin of Matrigel® is problematic for iPSC clinical translation. Here we describe human basement membranes (HBMs) derived from allogeneic tissues for iPSC culture and therapeutic delivery. HBMs share a protein composition similar to Matrigel® in terms of laminin, collagen IV, HSPG, and nidogen content as shown by western blotting and MALDI-TOF mass spectrophotometry. Further, HBM can be synthesized into three dimensional electrospun nanofibers and sponges, forming scaffolds that are implantable in vivo which we have characterized in vitro as cell delivery vehicles. For iPSC culture, a wet and dry coating method was formulated to attach the HBMs to tissue culture plastic, with Bradford assays showing equivocal protein coating between HBMs and Matrigel®. In serum-free attachment assays, HBMs, both as films and nanofibers, showed strong stem cell binding kinetics.

Introduction:
Human pluripotent stem cells (hPSCs) such as embryonic (hESCs) and induced pluripotent stem cells (iPSCs) are capable of producing dopaminergic (DA) neurons that could be used to treat patients suffering from Parkinson’s disease. Hypoxic culture of hPSCs is utilized to mimic the in vivo environment surrounding the pre-implantation embryo, where undifferentiated hESCs are isolated. This environment typically ranges from 1.5 - 5.3% O2 between different mammalian species, which is substantially lower than atmospheric oxygen tension (~20%). When cultured in hypoxia, hESCs and hiPSCs have enhanced proliferative capacity and enhanced potential for neuronal differentiation, produced increased yield of target neurons. Most published works culturing hPSCs under hypoxia use costly feeder-free culture systems that are limited by problems associated with cell instability, poor cell attachment and acquirement of chromosomal abnormalities. This work examines the effects of hypoxia on the traditional co-culture system of inactivated mouse embryonic fibroblasts (iMEFs). We looked to determine whether iMEFs are compromised by hypoxia in a way that may affect their ability to produce growth factors required to maintain hPSC self renewal and pluripotency, or whether it is permissible to use iMEFs during hypoxic culture of hiPSCs. Results: We demonstrated that under hypoxia, iMEF viability and metabolic activity is not significantly altered over 7 days when compared to the normoxic control. In addition, there was no significant difference in the consumption or production of media components or metabolites in iMEFs cultured under hypoxia or normoxia. Furthermore, there was no difference in growth rate of colonies cultured in hypoxic versus normoxic culture conditions in hESCs. Cells cultured in hypoxia for over six months still expressed pluripotency markers Tra1-60 and Oct4, indicating that pluripotency of hPSCs are maintained under hypoxic culture utilising iMEFs. To verify that hypoxic conditions were attained, nuclear accumulation of HIF1α was confirmed in iPSC cultured in hypoxia for 6 hours by immunostaining, and transient expression of HIF1A in cells cultured for up to 7 days in hypoxia was demonstrated by western blotting. Conclusions and Future Work: We conclude that hypoxia does not significantly affect viability or metabolic activity of feeder cells, nor is it detrimental to hPSC pluripotency. Our ongoing work addressing DA neuronal differentiation of HIPCs therefore adopts a hypoxic culture system using iMEF feeders. In our current research, we are assessing the effect of overexpression of Lmx1α, Pitx3 or FoxA2 on acquisition of a DA neural phenotype in hypoxic culture conditions. We hypothesise that overexpression of these markers of DA lineage, coupled with the positive effects of hypoxia, will enhance the yield and purity of DA neurons.

Poster Board Number: T-2238

HYPOXIC CULTURE OF HUMAN PLURIPOTENT STEM CELLS IS PERMISSIBLE USING MOUSE EMBRYONIC FIBROBLASTS

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Human pluripotent stem cells (hPSCs) such as embryonic (hESCs) and induced pluripotent stem cells (iPSCs) are capable of producing dopaminergic (DA) neurons that could be used to treat patients suffering from Parkinson’s disease. Hypoxic culture of hPSCs is utilized to mimic the in vivo environment surrounding the pre-implantation embryo, where undifferentiated hESCs are isolated. This environment typically ranges from 1.5 - 5.3% O2 between different mammalian species, which is substantially lower than atmospheric oxygen tension (~20%). When cultured in hypoxia, hESCs and hiPSCs have enhanced proliferative capacity and enhanced potential for neuronal differentiation, producing increased yield of target neurons. Most published works culturing hPSCs under hypoxia use costly feeder-free culture systems that are limited by problems associated with cell instability, poor cell attachment and acquirement of chromosomal abnormalities. This work examines the effects of hypoxia on the traditional co-culture system of inactivated mouse embryonic fibroblasts (iMEFs). We looked to determine whether
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Poster Board Number: T-2240

NON-INVASIVE DETECTION OF MODIFIED MOUSE AND HUMAN HEMATOPOIETIC STEM CELLS DURING IMMUNE CELL DEVELOPMENT WITH A HUMANIZED PET REPORTER GENE AND [18F]-L-FMAU

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Positron Emission Tomography (PET) is a quantitative whole-body clinical imaging modality which utilizes radiolabeled probes to detect specific biological processes in vivo. PET reporter genes (PRGs) non-invasively monitor the location of transplanted cells carrying the gene in vivo by imaging with a reporter specific probe. Current clinical PET reporters are derived from Herpes Simplex Virus Type I thymidine kinase (HSV1-TK) which is immunogenic and has led to the elimination of therapeutic cells. Transgenes of human nucleoside kinases are potentially non-immunogenic alternatives to viral-based PET reporters. We tested whether a human deoxycytidine kinase with three point mutations (hdCK3mut) could monitor therapeutic regeneration of the hematopoietic system in mice transplanted with modified hematopoietic stem cells (HSCs) of mouse (m) and human (h) in xenotransplantation protocols. hdCK3mut provides a broader substrate capacity accommodating thymidine derivatives like [18F]-L-FMAU which was tested as a potential PET reporter probe. In vitro murine bone marrow colony assays determined that hdCK3mut labeled cells retain equivalent colony forming capacity compared to non-transduced bone marrow. In vivo, serial [18F]-L-FMAU PET analysis of hdCK3mut labeled mHSC recipient animals over 32 weeks depicted the dynamics of hematopoietic reconstitution visualizing the successive homing of mHSCs to different hematopoietic tissues in the murine system. hdCK3mut retroviral vector could be detected in all major lymphoid and myeloid/bone marrow cell types within multiple hematopoietic tissues by FACS and IHC. Continuous long-term expression of hdCK3mut reporter in the hematopoietic system during mouse development has been demonstrated in the murine system. hdCK3mut retroviral vector could be detected in all major lymphoid and myeloid/bone marrow cell types within multiple hematopoietic tissues by FACS and IHC. Continuous long-term expression of hdCK3mut reporter in the hematopoietic system during mouse development has been demonstrated in the murine system.

Poster Board Number: T-2241

AN IN VITRO ASSAY FOR CLONOGENIC, HIGH-THROUGHPUT ANALYSIS OF MURINE INTESTINAL STEM CELLS

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Recent advances in the isolation and culture of intestinal epithelial stem cells (IESCs) facilitate testing functional properties of stemness in vitro. To establish ‘stemness’ of putative IESCs that are defined by restricted gene expression signatures, single cells must demonstrate multipotency and self-renewal. Although current in vitro assays support growth of IESCs, they are not amenable to efficient clonogenic analysis at the single cell level. Additionally, limitations of the current assay make IESC-derived enteroid structures (bodies of crypt/villus-like units) difficult to process for downstream molecular analysis. The development of micro-fabricated cell culture arrays and computational image analysis provides the next technological step toward a clonogenic assay for IESCs and other somatic stem cells. Here, we show that single Sox9low-expressing IESCs can be seeded into cell culture microwell arrays and develop into enteroids, exhibiting the same behavior as IESCs in conventional in vitro assays. Single IESCs can be separated into distinct microwells, facilitating day-to-day quantification of clonogenic development. We demonstrate that this microwell array technology enables efficient clonogenic analysis and provides a platform for powerful statistical analysis of the effects of small molecules on IESCs. Support: NIH R01 DK091427, NIH R03 DK089126, NC Biotechnology Center Grant.

Poster Board Number: T-2242

RADIATION EXPOSURE ON MOUSE AND HUMAN INDUCED PLURIPOTENT STEM CELLS ENABLES REMOVAL OF TERATOMA-FORMING CELLS IN THE TRANSPLANTATION OF DIFFERENTIATED CELLS

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There are problems that tumor-like cells appear in transplantation therapy using iPSCs (iPSCs). Contamination and proliferation of undifferentiated cells that do not respond to differentiation induction are thought to cause tumorigenesis. The undifferentiated cells are generally sensitive to radiation exposure. We performed an experiment to test the hypothesis that the irradiation selectively removes undifferentiated iPSCs which heterogeneously coexist in differentiated cell population. Colony forming abilities were investigated by various radiation doses in mouse iPSCs (miPSCs, MEF-Ng-20D-17) and human iPSCs (hiPSCs, 20187). The irradiation inhibited colony formations in miPSCs (8 Gy) and hiPSCs (4 Gy).
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However, it had no effects on the feeder cells (fibroblast cells). Next, miPSCs and hiPSCs were induced differentiation into cardiac muscles, neurocytes and other differentiated cells according to conventional methods. As markers of coexisting undifferentiated cells by flow cytometry analysis, GFP positive (Nanog genes expression) cells were examined for miPSCs and anti SSEA-4 antibody were for hiPSCs. In miPSCs, mean ratios of GFP positive cells were 14% in embryoid bodies and decreased to 4% in 4 weeks differentiation induced cells. In hiPSCs, mean ratios of SSEA-4 positive cells were 22% in 4 weeks differentiation-induced cells and decreased to 4% in 8 weeks cells. We tried to eliminate undifferentiated miPSCs on the culture dishes by irradiation. By a single exposure dose of 8 Gy to the culture dishes, GFP positive cells were decreased to lower than 0.1% without disorder in myocardial beats. As in vivo studies, we injected non-irradiated or irradiated differentiated cells into the testis of SCID mouse to examine teratoma formations. Although non-irradiated cells formed teratomas in all the mice examined (n=13), irradiated cells formed either no or localized teratoma in the testis (n=20). Therefore, it was suggested in the miPSCs that the irradiation before transplantation selectively eliminates residual undifferentiated cells and inhibits post transplantation tumorigenesis. In hiPSCs, the irradiation also inhibits teratoma formations by an appropriate amount of dose since coexistence of undifferentiated cells was observed in the differentiation induced cells.

Poster Board Number: T-2243

A NOVEL METHOD FOR INDUCING A CELL-TYPE-SPECIFIC CRE-LOX RECOMBINATION IN MOUSE ADULT BRAIN STEM CELLS

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One of the major challenges of brain tumour research remains the precise and selective targeting of specific cells in neurogenic regions. These cells, thought to be the origin of most intrinsic brain tumours, reside in the subventricular zone, one of the major neurogenic areas of the brain.

Here we demonstrate a novel approach in which we target the B-Type of SVZ cells, by combining a cell type-specific glutamate aspartate transporter (GLAST)-Cre ERT2 mouse model with the intraventricular injection of a recombination inducer - the active Tamoxifen metabolite. Our new technique is also compared to the previously reported method using Adeno Cre virus. With this novel approach we aim at selectively targeting the SVZ B-type of cells. Mice expressing the CreERT2 fusion protein under control of the Glutamate-aspartate transporter promoter express cre in astrocytes including B-Type SVZ stem cells. Tamoxifen administration induces nuclear transfer of the cre-ERT2 protein in GFAP-expressing cells, where it can mediate loxP-dependent recombination. GLAST-CreERT2 mice were crossed with a LacZ reporter mouse (ROSA26RloxP/loxP) which allows detection of a recombination with the β-galactosidase assay. We administered the metabolically active Tamoxifen derivative by injection into the ventricle of GLAST-CreERT2 x ROSA26RloxP/loxP mice. Identification of the recombined cell types was confirmed by immunohistochemistry or by β-galactosidase assay, and by colocalisation with GFAP, Nestin, Sox2, PDGFRα and Doublecortin. Direct injection of the Tamoxifen metabolite into the ventricles of GLAST-Cre mouse brains induced cre mediated recombination. Recombination occurred in GFAP positive cells in the SVZ, and at higher doses, occasionally also in adjacent differentiated astrocytes. The co-expression of the SVZ stem cell marker GFAP and to a much lesser extent, the progenitor marker Nestin in recombined SVZ cells proves the efficiency of the spatially- and temporally-controlled recombination of GLAST-positi...
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transfer difference would. Therefore, smaller concentration gradient i.e. smaller size of the MB instead of slower mass transfer rate than that of the 6WP would have the prominent role to activate effect of cell-secreted BMP4 on mESC behaviors in the MB.

**Poster Board Number: T-2246**

**IDENTIFYING SHARED HUMAN AND MOUSE STEM CELL PATHWAYS ASSOCIATED WITH LEUKEMIA STEM CELLS: STANDARDIZING COMPARISONS USING THE HSCI STEM CELL COMMONS**

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The weight of evidence indicates that tumor propagation depends on a subpopulation of tumor-initiating cancer stem cells (CSC) endowed with true self-renewal capacity. It is attractive to target pathways to cancer progression through selective action on CSCs. Leukemia stemness signatures have been defined using functionally well defined leukemic stem cells. Although these signatures share a number of significant oncogenic pathways, the signatures only have three genes in common. Stem cell molecular and experimental comparison is thrown into sharp relief by inconsistencies in actual results, data formats, and descriptions among biomedical research discoveries. The Harvard Stem Cell Institute (HSCI) has created an open, community-based approach to data sharing: The Stem Cell Commons (stemcellcommons.hsci.harvard.edu) that captures information in an Investigation-Study-Assay (ISA) format used by over 30 organizations. The common framework means that workflows and methods are consistent, and that experiments can be matched across 148 studies using common experimental descriptions. To further address inconsistency and find key activating signaling pathways in CSCs, we have developed pathway fingerprinting, a method that standardizes gene expression to a fixed set of curated pathways (KEGG, Reactome and Wikipathways). Pathway fingerprinting out-performs gene-based approaches for cross-experiment analysis, permitting comparisons of experimental results independently of technology, species or experimental source and providing a simplification of the cellular functional state. We have pathprinted haematopoetic stem cells in mouse, human and leukemic stem cells in mouse models and human published data. We show that cancer stem cells share pathway activation to recapitulate stem cell ontogeny, sharing immune signaling, hemostasis, and a number of pathways at several levels of functional organization. We welcome participation from the stem cell community and invite researchers to join the Commons to promote discovery in stem cell research by sharing data and tools.

**Poster Board Number: T-2249**

**HETEROGENEOUS EXPRESSION OF PLURIPOTENCY-ASSOCIATED GENE IN MOUSE EMBRYONIC STEM CELLS VISUALIZED BY BIOLUMINESCENCE MICROSCOPY**

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The embryonic stem (ES) cells consist of various cell subsets which express different levels of pluripotency associated genes such as Nanog, Oct4, and Sox2. The heterogeneous expression of the transcription factors associated with pluripotency within ES cells has been shown by the use of RT-PCR, luciferase reporter assay, flow cytometry, and immunohistochemistry. Although these conventional methods provide the end-point data of gene expression, they cannot monitor the temporal changes within individual cells. Therefore, the temporal and spatial analysis of gene expression at the single cellular level is required for studies on self-renewal and differentiation processes of ES cells. To understand the exact profile of gene expression in the process of ES cell differentiation, gene expression of Nanog in mouse ES cells were monitored by using the single cellular bioluminescence imaging system (LV200, Olympus). The time course imaging analysis using luciferase as a reporter revealed that (1) the promoter activity of Nanog gene mostly fluctuated at single cellular level, and the pattern of fluctuation varied among colonies of ES cells in the presence of leukemia inhibitory factor, (2) the promoter activity of Nanog gene decreased in the most of ES cells after bFGF-induced differentiation, whereas higher Nanog expression was sustained in small subpopulation of ES cells, and (3) the promoter activity of Nanog gene recovered heterogeneously among ES cells in response to dual inhibition of mitogen-activated protein kinase signaling pathway using PD184352 and SU5402. This is the first study to demonstrate the temporal and spatial dynamics of Nanog gene expression at the single cellular level using bioluminescence imaging system.

**Poster Board Number: T-2247**

**3D-MODELING OF EMBRYOID BODIES FORMED BY MOUSE PLURIPOTENT STEM CELLS IN DIFFERENT CELL CULTURE SYSTEMS**

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Pluripotent stem cells derived from different cell sources can recapitulate in vitro the early stages of mammalian development. Despite different origin, embryonic stem (ES) and embryonic germ (EG) cells express similar gene sets and display similar differentiation potentials after the in vitro cell culture adaptation. Their malig-
Use of rats for studies in behavior, pharmacology, and disease modeling has been limited because gene targeting technology has been lacking. However, recent discovery of a culture system using small molecules specifically to inhibit spontaneous differentiation pathways of pluripotent stem cells (PSCs) has permitted generation of germine-competent rat PSCs. This stable and reproducible culture system in rat PSCs constitutes a breakthrough for generating not only transgenic rats by introducing exogenous genes into PSCs but also knock-out rats via gene targeting. Various genetically modified rats will soon be available for analyses of gene functions or physiological features as can now be done in mice. In mouse or human, one widely used technique to express a gene of interest stably and ubiquitously is to insert that gene into the Rosa26 locus via gene targeting of PSCs. Rosa26 knock-in mice conditionally expressing a reporter or a toxin gene have contributed to tracing or ablation of specific cell lineages. Here, as a third model, we report identification of a Rosa26 locus in the rat. We found a highly conserved region in rat chromosome 4 that contains not only the a Rosa26 locus but also genes that are neighbors to Rosa26 in mouse. To see if this region, like the Rosa26 locus in mouse or human, actually allowed ubiquitous expression of an inserted gene, we cloned homology arms from genomic DNA of the DA rat and constructed a targeting vector to insert a splice acceptor with tdTomato-IRES-Puro1-PA sequence. After electroporation into rat PSC lines derived from various strains and tissues, on average, about 30% of picked up clones demonstrated correct targeting, judged by PCR using genomic DNA and Southern blotting. As expected, all positive clones ubiquitously expressed tdTomato. We injected rat embryonic stem cells (ESCs) from one of the Rosa26-tdTomato knock-in lines into rat blastocysts. Neonatal chimeric rats expressed tdTomato throughout their bodies and they grew into adults normally and showed high coat color chimerism. Next, we mated 3 male rats exhibiting relatively higher chimerism with wild type females to see if Rosa26-tdTomato knock-in rat ESCs were capable of germline transmission. Offspring of 2 chimeras expressed tdTomato ubiquitously throughout their bodies, indicating successful germline transmission. Expression of tdTomato was detected in all main organs at the neonatal stage and in blood cells at the adult stage. These data provided strong evidence for functional conservation of the Rosa26 locus among rat, mouse, and human. We have successfully generated Rosa26 knock-in rats expressing tdTomato ubiquitously via gene targeting of PSCs. The new tools described here (targeting vectors, knock-in PSCs, rats) should be useful for a variety of research using rats.

**GENЕRATION OF ROSA26-TDTOMATO KNOCK-IN RATS VIA GENE TARGETING OF PLURIPOTENT STEM CELLS**

**Kobayashi, Toshihiro**

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Use of rats for studies in behavior, pharmacology, and disease modeling has been limited because gene targeting technology has been lacking. However, recent discovery of a culture system using small molecules specifically to inhibit spontaneous differentiation pathways of pluripotent stem cells (PSCs) has permitted generation of germine-competent rat PSCs. This stable and reproducible culture system in rat PSCs constitutes a breakthrough for generating not only transgenic rats by introducing exogenous genes into PSCs but also knock-out rats via gene targeting. Various genetically modified rats will soon be available for analyses of gene functions or physiological features as can now be done in mice. In mouse or human, one widely used technique to express a gene of interest stably and ubiquitously is to insert that gene into the Rosa26 locus via gene targeting of PSCs. Rosa26 knock-in mice conditionally expressing a reporter or a toxin gene have contributed to tracing or ablation of specific cell lineages. Here, as a third model, we report identification of a Rosa26 locus in the rat. We found a highly conserved region in rat chromosome 4 that contains not only the a Rosa26 locus but also genes that are neighbors to Rosa26 in mouse. To see if this region, like the Rosa26 locus in mouse or human, actually allowed ubiquitous expression of an inserted gene, we cloned homology arms from genomic DNA of the DA rat and constructed a targeting vector to insert a splice acceptor with tdTomato-IRES-Puro1-PA sequence. After electroporation into rat PSC lines derived from various strains and tissues, on average, about 30% of picked up clones demonstrated correct targeting, judged by PCR using genomic DNA and Southern blotting. As expected, all positive clones ubiquitously expressed tdTomato. We injected rat embryonic stem cells (ESCs) from one of the Rosa26-tdTomato knock-in lines into rat blastocysts. Neonatal chimeric rats expressed tdTomato throughout their bodies and they grew into adults normally and showed high coat color chimerism. Next, we mated 3 male rats exhibiting relatively higher chimerism with wild type females to see if Rosa26-tdTomato knock-in rat ESCs were capable of germline transmission. Offspring of 2 chimeras expressed tdTomato ubiquitously throughout their bodies, indicating successful germline transmission. Expression of tdTomato was detected in all main organs at the neonatal stage and in blood cells at the adult stage. These data provided strong evidence for functional conservation of the Rosa26 locus among rat, mouse, and human. We have successfully generated Rosa26 knock-in rats expressing tdTomato ubiquitously via gene targeting of PSCs. The new tools described here (targeting vectors, knock-in PSCs, rats) should be useful for a variety of research using rats.

**THE EXPRESSION OF MULTICISTRONIC VECTORS LINKED BY THE SELF-PROCESSING 2A PEPTIDES ON PROTEIN EXPRESSION**

**Gao, Steven, Morgan, Hugh D., Jack, Michelle, O’Neill, Chris**

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Multicistronic vectors linked by the self-processing 2A peptides have been successfully used in cellular reprogramming. The function and expression characteristics of these vectors have yet to be well documented in embryonic stem cells. In the present study, we generated vectors containing different combinations of 3 pancreatic transcriptions factors (Pdx1, Nkx2.2 and Ngn3) together with an eGFP reporter, all linked by the self-processing 2A peptides. The multicistronic vectors were transfected into mouse embryonic stem cells and the expression of these vectors was characterised using qPCR and FACS. Similar level of gene expression of a given gene was observed regardless of its position within a 2A multicistronic construct. Expression of a terminal EGFP was 30.6% and 23.8% more efficient when linked by T2A compared with F2A and E2A peptides, respectively. Highest level of expression was achieved when all genes in a multicistronic vector was linked the genes exclusively with T2A peptides. The study provides guidance on successful strategies for the efficient expression of 2A multicistronic constructs in embryonic stem cells.
The osteocalcin contents secreted from the MSCs were measured at 37 ºC for 48 hours (H2O2), then treated by autoclaving (H2O2-Ti) or heat-treated at 37 ºC (Ti-Ap). Osteocalcin contents measured after 3 weeks of culture were as follows: 2987±1237 (300 °C-Ti-Ap), 2430±222 (H2O2-Ti-Ap), 1841±245 (Ti-Ap), 1604±519 (Ti) and 83±133 (TCPs). Cell morphology of rat MSCs strongly correlates with osteoblastic differentiation activity. Correlation coefficient of the living cell adhesion areas and osteocalcin contents of rat MSCs was 0.9589.

Poster Board Number: T-2254

EARLY RESPONSE OF PIG MESENCHYMAL PROGENITOR CELLS TO INTRACELLULAR INCORPORATION OF GADONANOTUBES RESEMBLES ANOIKIS RESISTANCE

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Detachment from the extracellular matrix causes cell death, or anoikis, a process that some neoplastic cells evade during cancer progression to metastasis. We are reporting that mesenchymal progenitor cells (MPCs) respond to the incorporation of gadolinium-loaded carbon nanocapsules, or Gadonanotubes (GNTs), by a non-proliferative, non-apoptotic cell cycle arrest mechanism similar to anoikis resistance, also termed synoikis. In previous studies, we have demonstrated that GNTs can readily labeled MPCs (10^9 Gd³⁺ ions/cell) without significant loss of viability, phenotypic characteristics and differentiation potential. However, GNT-labeled MPCs showed an extended growth lag phase (48 hours) compared to controls (24 hours) and decreased adhesion to collagen and fibronectin. These events were transient, as GNT-labeled MPCs displayed the same properties of control cells after 48 hours. To further characterize the cellular response to GNTs, we investigated possible changes in gene expression by transcriptome analysis. Pig bone marrow-derived MPCs at the log phase were incubated with GNTs for 24 hours. Control and GNT-labeled cells were then re-plated and cultured for another 24 hours, followed by RNA extraction. Microarray (GeneChip Porcine Genome Array; Affymetrix, Santa Clara, CA, USA) data generated from 3 independent experiments were used to compare gene expression changes between control and GNT-labeled cells and to analyze the affected biological pathways (Ingenuity Systems Inc, Redwood City, CA, USA). More than 130 genes were significantly altered, including those involved in cell cycle, DNA damage, and stress response pathways. Data is currently being validated by qPCR, immunocytochemistry, flow cytometry, ELISA, and Western blotting. Flow cytometry studies revealed a significant accumulation of cells at the G2/M phase, indicating that growth arrest is effectively ongoing in GNT-labeled cells. In addition, the expression of DNA replication machinery components, such as MCM2 and 4, CHAF1, CDC45, CKS2, and GINS complex subunit Psf1 were found to be significantly decreased. The G2-M cyclins, B1 and B2, were downregulated, as well as transcription factors and other regulatory proteins that are involved in the control of cell cycle progression and replication, such as EZF1, PCNA, and F-Box protein-5. The cyclin G2, a negative regulator of cell cycle progression, was upregulated. Expression of Angiopoietin-like 4 (ANGPL4) and BNIP3, both implicated in synoikis, were significantly elevated. In order to fully demonstrate that the survival of GNT-labeled cells is assured by a synoikis-like mechanism, we are presently developing oligonucleotide-based assays to knock down the expression of ANGPL4 in GNT-labeled cells. As more investigators, including our group, study the in vivo application of biocompatible mesenchymal stem cells, the osteocalcin contents secreted from the MSCs were measured using a rat osteocalcin ELISA kit according to the manufacturer's instructions. Results and Discussion: Living cell adhesion areas measured after 2 hours culture were as follows: 199±46 (300 °C-Ti-Ap), 220±60 (H2O2-Ti-Ap), 351±97 (Ti-Ap), 352±124 (Ti), and 500 (TCPs).
THE TRANSPLANTED RAT NEURAL STEM CELLS DOWNREGULATE EXPRESSION OF TLR4 IN CEREBRAL ISCHEMIA OF RATS

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[Objective] Neural stem cells (NSCs) are believed to promote the function recovery in cerebral ischemia in rat. Toll-like receptor TLR4 play a critical role in the neuroinflammatory condition. However, it is not clear that how the transplanted NSCs influence the expression of TLR4 during cerebral ischemia. In this study, we detect the transplanted rat NSCs modulate the expression of TLR4 in cerebral ischemia of rats. Methods] The NSCs from the embryonic 12.5~14d cerebral cortex of Wistar rats were isolated, identified by the detection of the self-renewal capacity and multi-differentiation potential, labelled through the red fluorescent dye PKH26. The labelled NSCs were transplanted into the cerebral ischemia in rats. The immunohistochemistry and Western Blot technique were performed to discover the expression of TLR4 at several timepoints after NSCs transplantation.[Results] The results of immunohistochemistry suggested that the expression of TLR4 decrease. The Western Blot analysis also revealed that the expression of TLR4 decrease.[Conclusions] These results indicated that the transplanted rat NSCs can downregulate the expression of TLR4 in cerebral ischemia in rats.

EFFECT OF A NOVEL SYSTEM OF PORCINE IN VITRO MATURATION AND CULTURE ON COLONIZATION OF PUTATIVE PORCINE EMBRYONIC STEM CELLS

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Porcine embryonic stem cells (pESCs) have a great potential in genetic engineering for produce of cloned or gene modified pigs. For derivation of pESCs, the majority source of embryos is in vivo produced blastocysts, because they are expected to be high quality. However, obtainment of in vivo blastocyst is expensive and toilsome. Thus, many researchers are used blastocysts produced from in vitro such as in vitro fertilization (IVF) and parthenogenesis (PA). However, it is difficult to establish pESCs from in vitro produced blastocysts because in most cases there is no obvious inner cell mass (ICM) or they eventually contain only few ICM cells. Therefore, we investigated the relationship in vitro produced blastocyst quality and colonization efficiency of pESCs. In experiment, we performed the IVF and PA with treatment of some chemicals. The control group is produced using M199 media in IVM and porcine zygote medium-3 (PZM3) in IVC. The treatment group is produced using M199 with 2 μM resveratrol (RV) in IVM and PZM5 with 10 ng/ml porcine granulocyte-macrophage colony stimulating factor (pGM-CSF), 2 μM RV and 10 μM β-mercaptoethanol (β-ME) in IVE. Data were analyzed with SPSS 17.0 using Duncan’s multiple range test. In total, 1210 embryos in PA, 612 embryos in IVF evaluated. As results, we observed overall blastocyst quality was increased. The blastocyst formation rates were significantly higher (p < 0.05) in the treatment groups (54.5%) compared to the control group (43.4%) in PA and hatched blastocysts rates in day 6 and 7 were also increased significantly. Total cell numbers of blastocyst were significantly higher (p < 0.05) in the treatment group (55.1) compared to the control group (43.4%) in PA and hatched blastocysts rates in day 6 and 7 were also increased significantly. Total cell numbers of blastocyst were significantly higher (p < 0.05) in the treatment group (55.1) compared to the control group (45.6). In IVF; hatched blastocysts rates in day 7 were increased significantly, too. After seeding porcine blastocyst, the attachment rates were higher in the treatment group (36.2%) compared to the control group (23.4%) in IVF and 32.2% in PA) than the control group (26.6% in IVF and 19.5% in PA). Also, colonization rates and cell line derivation rates were higher in treatment group than control group. Colonization rates of control group were 10.8% in IVF and 2.4% in PA, but treatment group were 17.75% in IVF, and 13.1% in PA. The cell line derivation rates were 4.2% (IVF) and 2.4% (PA) in control group. In treatment group, they were 10.0% (IVF) and 7.2% (PA). In conclusion, the treatment of RV during IVM and RV, β-ME, and pGM-CSF during IVC increased quality of porcine blastocysts produced from in vitro, subsequently increased derivation rates of porcine putative ESCs. This work was supported by a grant from Next-Generation BioGreen 21 program (# P1008121), Rural Development Administration, Republic of Korea.
FEASIBILITY OF AN IN-VITRO MODEL - TO EXPLORE THE INTERACTION BETWEEN MESENCHYMAL STEM CELLS AND -CANCER CELLS: USING MYELOMA CELLS AS A TARGET

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Background: Multiple myeloma (MM) is a clonal plasma cell neoplasm characterized by proliferation of neoplastic plasma cells in bone marrow (BM) and osteolytic bone lesions. The MM bone disease is clinically important and is a result of overt activity of osteoclasts and inactivation of osteoblasts (OBs). OBs are normally derived from BM mesenchymal stem cells (MSC) under physiological bone maintenance and remodeling. To understand the interaction between the myeloma cells (MCs) and MSC is urgently required; however, which is currently hampered by limited cell numbers and difficulty in detection of the BMMSC in-vivo. Here, a human telomerase reverse transcriptase-immortalized cord blood MSC (hTERT-cbMSC) established from one of our labs (Hung CJ, et al. Cytotherapy, 2010), could be an ideal material for the MSC study. We try to use the hTERT-cbMSC and MM cell lines, to mimic the interaction between the MCs and BMMSC in-vivo, and also to explore the mechanisms involving the inactivation of OBs in MM. Method: Primary BMSC from MM patients were isolated for in-vitro culture and a co-culture of hTERT-cbMSC and MM cell lines was also used. Multiple phenotypic and genotypic assays on senescence, proliferation, differentiation ability and functional alternations of these MSCs were performed. Results: Primary BMSC were isolated from newly-diagnosed MM patients (n=9) and non-myeloma control (n=18). The proliferation ability of BMMSC was significantly decreased in the MM patients compared to that in non-myeloma control (0.55 and 0.69, respectively; p=0.047). Similarly, in the co-culture system, while comparing to the control group, the proliferation ability of hTERT-cbMSC was also significantly reduced when it was co-cultured with MM cell lines for one-week (1.125 and 0.947, respectively; p<0.001) and two weeks (1.125 and 0.573, respectively; p<0.001). In addition to the preliminary results from the proliferation assay, the quantification of senescence, differentiation ability and functional alternations of the primary BMMSC and the hTERT-cbMSC from co-culture with MM cell lines will be also presented. Conclusion: The co-culture by using the hTERT-cbMSC and MM cell lines, instead of primary BMSC and MM cells, may be a suitable in-vitro model for further investigation elucidating the interaction between BMMSC and the MCs in MM.

Poster Board Number: T-2259

NEURONAL NETWORKS FROM STEM CELL DERIVED NEURONS AS IN VITRO ALTERNATIVE ASSAY FOR NEUROTOXICITY EVALUATION AND PREDICTION

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Testing of compounds for potential neurotoxic properties is required for all drugs and chemicals in Europe. Due to the complexity of neurotoxic effects especially systemic repeated dose toxicity testing is frequently performed in animals. Here we present a high throughput and high content screening platform based on stem cell derived neuronal networks cultured on microelectrode arrays. These assays allow parallel recording of nine neuronal networks with 28 electrodes each for extended periods of time. Thus, e can demonstrate repeated dose chronic toxicity as well as acute toxic effects with one integrated assay.

Poster Board Number: T-2260

MONITORING OF THE DAMAGE TO FREEZE-THAWED CELLS BY GLYCAN PROFILING USING A LECTIN MICROARRAY

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Cell-based therapies, such as stem cell transplantation, have become a promising strategy for the treatment of many diseases. New discoveries regarding the regenerative potential of stem or progenitor cells for the treatment and prevention of various diseases have transformed experimental research and led to an explosion in clinical investigations. However, the cell-based therapies have the problems of therapeutic efficiency and safety of cells for transplantation. In addition, cells may be modified or changed after each step of cell processing in vitro, i.e. isolation, subculture and freeze-thawing. Therefore, characterization of cell is the most important task for transplantation. Cell surface is covered with various glycoproteins and the cell surface glycans have been assumed to vary among cell types, stages of development and differentiation, and even in malignant transformation processes. In order to analyze the glycan, lectin microarray has been developed. The array is an emerging technology that can be applied to ultrasensitive detection of multiplex lectin-glycan interactions. We reported that lectin microarray analysis was applicable to ES, iPS and other stem cells. In this study, we investigated whether a lectin microarray could be used as a validation tool to monitor cell characteristics after freeze-thawing. Human diploid fibroblast (TIG-1) cells were used in this study as a model system of cellular ageing because they have been well characterized with respect to cellular growth and freeze-thawing. TIG-1 cells at population doubling level (PDL) 24 were assigned to two groups; one was cryopreserved and the other was cultured as control cells (non-frozen). The cryopreserved cells were thawed one week later and re-assigned to two groups; one was cryopreserved and the other was cultivated as the once-frozen thawed cells (Group I). The twice-frozen cells (Group II) were thawed 3 weeks later and subcultivated. We observed cell proliferation and performed lectin microarray analysis of the three groups. In these results, the doubling time of Group II cells was prolonged when compared with that of Control or Group I cells after PDL 50, although the cells of these three groups had similar morphology. However, lectin microarray analysis showed that the signal intensities of Group II cells were significantly higher than those of the other groups at PDL 37 or 38 for Sambucus nigra agglutinin (SNA), Sambucus sieboldiana lectin (SSA), Pokeweed mitogen (PWM) and Urtica dioica agglutinin (UDA). Differences in the cell surface glycan profile after freeze-thawing between Group II cells and the other groups could be detected quickly using the lectin microarray. Our results suggested that a lectin microarray may be an easy and quick method for validation of cellular characteristics. It is hope that cell validation using this system could make it possible to evaluate the condition of various stem cells for transplantation.
**Detailed Program and Abstracts — Thursday, June 14**

**Poster Board Number: T-2261**

**IMPORTANCE OF PHYSIOLOGICAL OXYGEN AND MEDIA CONDITIONS FOR STEM CELL CULTURE**


Stem cells and differentiated cells intended for research and clinical therapy are almost always grown in incubators flushed with CO2 and air to maintain an aerobic environment and physiological pH in the medium. The oxygen levels under such conditions (atmospheric oxygen; atmosOx) are 20% and are 2-8 times higher than the oxygen levels cells encounter in vivo (physiological oxygen; physOx). We, and several others, published a series of studies that showed that culturing differentiated cells at atmospheric oxygen levels significantly skews cellular responses, increases risk of DNA damage and alters the gene expression similar to ‘stress-response expression’. Here we provide evidence to show that physiological oxygen levels are superior for culturing mesenchymal stem cells, iPSCs and other differentiated cells. Using a variety of cellular and biochemical measures of cellular redox status, mitochondrial function, cell surface expression and morphological features, we show that cells cultured at physOx are ‘healthier’, maintain steady growth rate and pluripotency, and are less subjected to culture induced genomic artifacts than cells cultured at atmosOx.

**Poster Board Number: T-2262**

**PHENOTYPIC MODULATION OF PLURIPOTENT STEM CELLS BY MICROFABRICATION MATERIALS**

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Pluripotent stem cells (PSCs; i.e., embryonic and induced pluripotent stem cells (ESCs and iPSCs)) hold a great potential for applications in cell-based therapy, regenerative medicine and drug development/screening due to their unique characteristics, such as unlimited self-renewal without any karyotypical abnormality and differentiation capability into any kind of cells in a body. To control PSC functions, it is necessary to precisely regulate extracellular microenvironments, which have an important role in controlling cellular functions in vivo and in vitro. However, it is still a challenge for current PSC experimental settings to study such environments in vitro at a nano/micro-meter scale. Polymer Micro Electro Mechanical Systems (MEMS) technology using a photoresist as a constructional material has recently afforded a simple three-dimensional (3D) fabrication of microfluidic devices, and is advantageous for creating new cellular microenvironmental cues to control stem cell functions due to its great controllability of soluble and insoluble factors on a nano/micro-meter scale. Even though nano/micro-fabrication and mechanical properties of photoresists have been well-discussed, chemical effects, such as toxicity of antimycolysis salt (an optical polymerization initiator) in photoresists and its derivatives, on cell functions are still unknown. Therefore, as the first step of applying MEMS technology in stem cell research, we investigated the effects of MEMS materials on PSC phenotypes and functions, such as pluripotent status, proliferation, cytotoxicity and cellular heterogeneity. First, two photoresist substrates (i.e., TMMRTM S2000 (antimony) and N-S3000 (non-antimony)) prepared in a way typical for photoresist processing were employed for this study. Photoresist substrates treated with gelatin, Matrigel or Corona treatment were tested for their usability for culturing mouse ES cells (mESCs). These results illustrated that, while mESCs didn’t adhere on the non-coated S2000 and N-S3000 substrates, Matrigel coating provides better mESC adhesion and maintains high expression levels of alkaline phosphatase, which is one of the pluripotent markers. It should be noted that corona treatment and gelatin coating, which are typical approaches for general cell lines, were not sufficient to support mESC adhesion. Second, the capability of photoresists for culturing hPSCs was evaluated. hPSC colonies were cultured on both photoresist substrates. We observed that hPSCs cultured on Matrigel-coated photoresist surfaces in a mTeSR1 defined medium were able to form colonies over the observation period of 7 days. Next, alkaline phosphatase staining was performed to evaluate their pluripotent status. hPSCs cultured on both substrates highly expressed alkaline phosphatase. Then, a flow cytometric analysis for quantitative monitoring of SSEA4 pluripotent marker expression in individual hPSCs was conducted to investigate how cellular heterogeneity was influenced by these substrates. hPSCs on both substrates showed similar heterogeneity compared with a conventional plastic substrate. These results indicate: (1) photoresist in MEMS are usable for culturing PSCs without feeder cells, and (2) the small effects of antimycolysis salt observed during the 7-day culture, a time span sufficient for maintaining PSCs routinely, encourage further investigations on nano/micro-structured photoresist components.

**Poster Board Number: T-2263**

**SUSTAINED LEVELS OF FGF2 REDUCE DIFFERENTIATION OF STEM CELL CULTURES WITH LESS FREQUENT MEDIA CHANGES**

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Cell culture techniques aimed at maintaining a number of different stem cell types in an undifferentiated state involve the frequent addition of FGF2 to the culture media. For human pluripotent stem cells, this is done daily, and for tissue specific stem cells, a typical feeding regime is every 3rd day. Yet even with these laborious feeding schedules, stem cells spontaneously differentiate, indicating that the self-renewal is sub-optimal. We assessed the levels of FGF2 in culture medium with or without added heparin, and found that it is highly unstable, with a 60% reduction after 5 hours and 95% by 24 hours. Hence we postulated that rapid loss of FGF2 could be responsible for stem cell culture differentiation. To address this, we created a cell culture environment with significantly more stable levels of FGF2. We show this new culture method results in higher expression of stem cell markers and decreased differentiation during the expansion phase. Moreover, we reduced the feeding schedule for hESCs to once per 3 days, resulting in significant savings in medium costs and fewer interventions in the cultures, which reduce the risk of contamination. The resulting hESCs were able to differentiate into the three germ lineages as well as cells growth with conventional daily feeding. This new method can be applied to other types of stem cells that respond to FGF2, such as iPSCs or MSCs. We suggest that stabilizing the FGF2 environment mimics stem cell niches that promote maintenance of the stem cell state.
Dedicated Program and Abstracts — Thursday, June 14

**Poster Board Number: T-2264**

**SYNTHETIC PEPTIDES AND POLYETHYLENE GLYCOL SURFACES FOR MICROPATTERNING OF PLURIPOTENT STEM CELLS**

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Techniques for patterning pluripotent stem cells (PSCs) are important for a wide range of fundamental and clinical applications, such as studies on stem cell biology, tissue engineering and drug screening using PSC-derived cells. To use surfaces coated with synthetic peptides derived from active domains of extracellular matrix (ECM) protein is one of the promising ways to make xeno-free and scalable cell adhesive surfaces as an alternative to conventional surfaces coated with ECM extracted from animals (Z. Melkoumian et al., Nat. Biotech., 2010). In this paper, we report a simple surface modification method for micropatterning of mouse PSCs using synthetic peptides immobilized on a surface via an amine-to-sulfhydryl crosslinking having poly(ethylene glycol) (PEG) units as spacer (SMIPPEG12, Thermo Scientific Pierce Protein Research Products). Cell adhesive surface is defined through the patterning of synthetic peptide by means of microcontact printing or microfluidic patterning to provide synthetic peptides to the surface previously coated with the crosslinker to bond them through the reaction of the amino group of the N-terminal of peptide with the N-hydroxysuccinimide ester (NHS) group of the crosslinker. The surface without peptide doesn't have cell adhesiveness due to the PEG units in the crosslinker. Microstructures for microcontact printing or microchannel networks were fabricated onto microchips made of poly(dimethylsiloxane) through conventional soft lithography process (G. M. Whiteside et al., Annu. Rev. Biomed. Eng., 2001). The feasibility of selective cell adhesion of peptide-patterned surfaces for culturing mouse PSCs was evaluated by using a mouse induced pluripotent stem (iPS) cell line (mouse iPS-MEF-Ng-20D-17 cell line). Since the crosslinker intrinsically has PEG units to avoid cell adhesion, the present method is more rapid and easy way to make patterned selective adhesion surface without any extra-process for making anti-cell adhesive surfaces.

**Poster Board Number: T-2265**

**LARGE SCALE MANUFACTURE OF CUSTOM TAL EFFECTOR NUCLEASES FOR GENOMIC EDITING.**

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Specific DNA-binding transcription activator-like effector nucleases (TALENs) are powerful tools for genome engineering with broad applications in a variety of cell types, including stem cells. The TAL binding domain, from Xanthomonas spp., governs the simple code used to assemble the DNA-binding specificity. We have developed large scale manufacture capability to custom-design TAL nuclease Gateway expression vectors that provide gene editing at targeted loci. In association with GENEART, a TALE monomer library has been created to generate rapid assembly of custom TALE proteins. We are working to optimize the TALE nuclease tool by comparing its activity in a variety of single expression vectors and developing a cell-free QC assay to measure cleavage and repair of specific loci. The TALEN expression clones are being assessed in mammalian cell lines and cell based models. It is expected that TAL effector nucleases will be used in precise genome engineering technologies such as generating IPSCl clones, stably modified human embryonic stem cell clones and broader areas of regenerative medicine, research in disease models and the production of therapeutics.

**Poster Board Number: T-2266**

**GENE ACTIVATION USING TRANSCRIPTION ACTIVATOR LIKE EFFECTOR PROTEINS**

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Transcription Activator-Like Effectors (TALEs) are proteins secreted by *Xanthomonas* spp. into plant cells to facilitate bacterial invasion. They accomplish this by binding specific promoter sequences and activating the expression of various signaling pathway genes. These proteins are comprised of constant N and C termini flanking repeat domains of various numbers. In nature, there are Tals with commonly between 12 to 18 repeats each repeat consisting of approximately 34 amino acids. These domains are essentially identical in sequence except for 2 central codons’ termed repeat variable domains (RVDs). The variation in the RVD sequence determines DNA base binding specificity of each repeat and the combination of different RVD containing repeats determines the sequence specificity of the TALE. Since the TALE `code` has been solved recently, it has been shown that by engineering RVDs designer TALEs can be made that bind to essentially any sequence in the genome. Here we describe a set of experiments to examine the size (repeat length) requirement for optimal activity of designer TAL activators in mammalian cell model systems. We assay TAL activators with binding target site lengths of 11, 14, 19, 25 base pairs to determine the optimal functionality in our system. Next we use these configurations to attempt activation of endogenous genes by scanning promoter regions with various TALE activators. Ideally, these types of tools could be used in somatic cells to induce pluripotency or in ESC and iPSC to transiently activate specific signaling pathways to direct differentiation toward desired lineages.

**Poster Board Number: T-2267**

**A NETWORK BIOLOGY PLATFORM TO EVALUATE ENGINEERED MAMMALIAN CELLS**

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Directing state transitions of mammalian cells in vitro, either through directed differentiation or reprogramming, is a novel paradigm that has transformed our capacity to study otherwise inaccessible cell types in development and disease. While engineered cells are often assayed functionally, a more comprehensive and statistically rigorous approach is needed to define the resemblance of the derived cells to in vivo cell types. To address this issue, we developed a computational platform, ClassNet, which uses reconstructed context-dependent transcriptional regulatory networks underlying 44 distinct cell types and tissues to both classify derived cells and ascertain the critical regulatory nodes at which derived cells differ from their in vivo counterparts. By applying the platform to all available direct fate conversions of murine cells, we determine the extent to which target cell types have been achieved, identify the cascade of regulatory events that characterize distinct examples of reprogramming, and find unintended, yet predictable, targets of reprogramming factors.
CHLORAL OPTIMIZATION OF BOTH CULTURE SURFACES AND MEDIA MARKEDLY ENHANCES MSC PROLIFERATION

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Culture media contain serum of inconsistent compositions, and plastic tissue culture dishes are inhomogeneously ionized due to plasma irradiation. So in conventional cultures, adult stem cells easily lose self-renewal activity and multipotency, and quality of transplantable cells varies depending on culture periods, serum batches and lots of dishes. Recently we developed serum-free chemically defined media STK1 and STK2 for human mesenchymal stem cells (MSC), which are now available from DS Pharm Biomedical, Osaka, Japan. In primary and passage cultures, STK1 and STK2, respectively, enhance MSC proliferation and maintain their multipotency at much higher levels than does DMEM supplemented with 10% FBS. In this study, we developed a new culture system consisting of the serum-free media and self-assembled monolayers (SAMs) expressing various functional groups, since conventional dishes were not optimal for MSC under serum-free conditions. SAMs made of alkanethiols with a long methylene chain give well-defined model surfaces for studies on interfacial phenomena and intermolecular interactions. Although numerous serum proteins easily bind to SAM surfaces, serum-free media allow us to examine direct interactions between cell surfaces and culture surfaces. We made mixed SAMs with various ratios of amino, hydroxyl, carboxyl, and methyl groups. SAMs enhanced proliferation of MSC derived from human bone marrow, synovium and dental pulp in STK2 at a certain ratio of functional groups. The combination of the SAMs and STK1 enhanced MSC proliferation 10-fold in primary cultures compared to plastic dishes with STK1. The combination of the SAMs and STK2 also enhanced MSC proliferation in passage cultures 2- and 10-fold compared to plastic dishes with STK2 or 10% FBS, respectively, within 4-5 days. Some SAMs also enhanced MSC proliferation in the presence of 10% FBS at different ratios of functional groups, probably because serum proteins adhered to these SAMs enhanced MSC proliferation. Furthermore, the optimal ratios of functional groups on SAMs differed among cultures of MSC, fibroblasts and osteoblasts, indicating that certain SAMs can selectively enhance MSC proliferation under serum-free conditions. So this new culture system allowed us for the first time to optimize a whole environment of MSC cultures under chemically defined conditions, and is promising in both basic cell biology and clinical cell therapy.

POSTER BOARD NUMBER: T-2269

SINGLE NUCLEUS RNA-SEQ

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Until recently, single cell gene expression could only be evaluated by conventional methods such as reverse transcriptase-PCR (RT-PCR), which is limited to measuring gene expression from a small number of candidate genes, or microarray analysis that suffers from false positive signals, low dynamic range and the inability to discover new transcripts and novel splice isoforms. With improvements to cDNA synthesis from single cells and the development of next generation transcriptome sequencing methods (RNA-Seq) it is possible to sequence cDNA libraries derived from the < 1 picogram of mRNA from a single cell. This has exposed an unprecedented panorama on the regulation of gene expression at the most fundamental level of the single cell. However, in cases where single intact cells are impossible to recover an approach using a single nucleus is attractive. Extending these methods, we have measured and digitally recorded the steady state mRNA signature of a single nucleus from a mouse neural progenitor cell (NPC) line. Although nuclear transcriptomes were found to be largely similar to whole cell controls, notable exceptions were identified. We also observed a strong averaging effect of the pooled controls from 10 and 100 cells or nuclei and a marked decrease in statistical variability between 1 and 10 cells or nuclei. This underscores the importance for single cell or nucleus resolution - as opposed to bulk sample- approaches for defining cellular uniqueness. These advances will enable gene and transcript expression discovery from tissues where whole cells are difficult or impossible to recover.

POSTER BOARD NUMBER: T-2270

GENERATING ARTICULATED EMBRYOID BODY FOR SPATIALLY CONTROLLED DIFFERENTIATION

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Pluripotent stem cells (PSCs) have the potential to indefinitely proliferate and to differentiate into derivatives from three germ layers. And it was shown that the process for generating embryoid body (EB) could be an initialization step of differentiation procedures. Moreover it was shown by previous research that there were several similarities between the procedure of PSCs differentiation and in vivo phenomena. So it is important to develop tools for controlled differentiation of EB spatiotemporally, especially for the field of developmental biology. Here, we show a culture technique to realize exposing EB to spatially different differentiation factors in a microfluidic device. The device consists of upper and lower channels separated from each other by a membrane having a through-hole of 100μm in diameter. All the structures are made of polydimethylsiloxane. First an EB derived from mouse embryonic stem cells (T alpha-1 tubulin-GFP mES cell line. T alpha-1 tubulin is one of markers of early neural differentiation and this cell line express GFP in neural differentiated state) is generated in a well-plate. After EB generation at 24 hours of the culture in the well-plate, the EB is transferred into the upper channel. And the EB is moved and fixed by negative pressure applied from the lower channel. And by injecting different media into the channels, the EB can be exposed to each of the media. In the experiment, we filled the upper and lower channels with FBS 20% medium and neural differentiation medium (ND medium, RHB-A medium, StemCells Inc.) each. And we confirmed that when we actually cultured an EB in the device, the EB became an articulated shape (art-EB), which is like having two spheroids below and above the membrane. On the day 4, the EB was collected from the device and transferred to a collagen-coated dish filled with FBS 20 % medium and the articulated shape could clearly be observed. The extended cells show neural-like shapes in the part exposed to ND medium. Moreover, fluorescence intensity of GFP, which is associated with the expression of T alpha-1 tubulin, was much higher in the neural differentiation part than in the part exposed to FBS 20% medium. This result shows...
that neural differentiation was induced in one spherical part of the art-EB. Another spherical part shows clearly different morphology from neuronal cells and does not express GFP. Here, we developed a microfluidic culture technique to form the art-EB, which can be used for spatially controlled differentiation of EB. And it was shown that neural differentiation in one sphere of the art-EB could successfully be conducted.

**Poster Board Number: T-2271**

**BIO-LUMINESCENT SYSTEM FOR DYNAMIC IMAGING OF CELL AND ANIMAL BEHAVIOR**

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The bioluminescent light technique has been broadly applied to the analysis of promoter activity in cells and the observation of internal structures in the animal body. However, because of their relatively weak luminescence, bioluminescent proteins have been difficult to use for the optical imaging of cultured living cells or in freely moving animals. The advantages of bioluminescence over fluorescence imaging include a low background signal, the ability to observe luminescence without an excitatory stimulus (which can damage cells), and the concomitant preservation of delicate subcellular organelles and structures which can be destroyed by excitation light. Because only living cells that produce ATP support bioluminescent reactions, bioluminescent proteins are useful for identifying living cells, such as in transplantation experiments. This study describes a novel imaging method for single cells and whole mice, by using a new luminescent protein, fFluc, comprising of a modified form of Venus fused with luciferase. This new luminescent protein enabled the visualization of extending neuronal growth cones and small protrusions on microglial cells in culture. Expression of fFluc in transgenic mice allowed the fast time-lapse bioluminescent imaging of freely moving mice in hurryded with a short exposure time. Oral administration of luciferase-containing aagarose or topical application of luciferase-containing Vaseline permitted imaging of the stomach and skin, respectively, of the moving mouse. This new luminescent protein can be used to visualize cell migration, differentiation, and division events in cultured cells, and to clarify metabolic disorders, eating behaviors, and drug infiltration in freely moving, nonanesthetized animals.

**Poster Board Number: T-2272**

**ESTABLISHMENT OF AN ALGORITHM FOR AUTOMATED DETECTION OF IPS/ NON-IPS CELLS UNDER A CULTURE CONDITION BY NONINVASIVE IMAGE ANALYSIS**

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It is expected for iPS cells as tools for drug development and as resources for regenerative medicine. However, such medical applications demand a stable supply of iPS cells in large quantities. Issues to be resolved include clarification of the mechanism by which differentiated cells become initiated as undifferentiated cells, optimization of the iPS cell generation method, and establishment of a safety assessment method for visual inspection of the cell colony formation process. In this experiment, a cell culture observation system (BioStation CT) was used to observe a culture, and an image analysis software (CL-Quant) was used to scan phase-contrast images and automatically detect IPS / non-IPS cells generated by reprogramming of normal somatic cells. In implementing the automatic detection, an algorithm was designed to categorize cells of different origin based on parameters pertaining to morphological characteristics of the cells. For samples consisting of various numbers of colonies, the coefficient of correlation was extremely high between the number of colonies counted by inspection and the number of colonies counted by CL-Quant. Accuracy in iPS / non-iPS identification was next compared. For the cell type used for algorithm design, accuracy was high at an average of 80.00%. For five types of cells of different origin, the average accuracy was 82.29%. This indicates the high robustness of the method in providing accurate iPS/non-iPS identification results. To confirm consistency of the analysis results, accuracy was compared with the results of analyzing cells stained with the TRA1-60 dye (marker specific to iPS cells) and the AP dye. This experiment shows that IPS/non-IPS identification during the establishment stage can be automated, eliminating the need for human intervention, and allowing for non-invasive analysis without the use of fluorescent dyes. This provides several advantages. 1) Reduction of the required time and effort. The iPS cell identification process previously required several hours. The time and effort used can be reduced significantly by automating this process. 2) Non-invasive analysis. Since the process does not damage the cells, the cells can be reused for the subsequent processes. 3) Homogenization. The method allows for consistent selection of good IPS cells without requiring training. 4) Historical management. By maintaining a record of the identification results, quality evaluations can be reanalyzed with ease. It is expected that by applying this method to time-lapse images and obtaining information on time-variable morphological changes in the iPS cells, changes in intra-cell signals affecting the establishment efficiency of iPS cell, sequential gene expressions after the introduction of the reprogramming factors, and other differences in cloned IPS cells can be observed. The method is also considered to become an effective support tool for establishing regenerative medicine.
Introduction: Nanoparticles may serve for both diagnostic and therapeutic purposes. Superparamagnetic or ferromagnetic nanoparticles represent a suitable contrast agent for magnetic resonance imaging (MRI) in vivo and for monitoring transplanted nanoparticle-labeled cells by MRI. Ferromagnetic particles can also be used for guided temperature-controlled thermoablation, with labeled stem cells used as carriers. The aim of our study was to prepare perovskite ferromagnetic particles, optimize their size and encapsulation with respect to their magnetic and biological properties, and test them on rat mesenchymal stem cells and in vivo as a contrast agent for MRI. Materials: Six samples of manganese perovskite nanoparticles (La1-xSrxMnO3), varying in size over a range of 18 - 21 nm and with different La/Sr ratios, were synthesized. The particles were coated by SiO2 to minimize their toxicity and adverse effects on cells; the coating thickness was 20 nm. The particles were added to culture medium with rat mesenchymal stem cells and incubated for 48 hours, then the nanoparticles were washed out and cell viability was evaluated using trypan blue. Cell proliferation was evaluated using the Xcellencing System. Labeled nanoparticles were added to culture medium with rat mesenchymal stem cells and in vivo as a contrast agent for MRI. Results: The viability of cells incubated with the nanoparticles was in the range of 72 - 85%, whereas a control sample reached 92%. Although the coating substantially improved cell viability, the nanoparticles were still slightly toxic. This also resulted in the lower adhesion of a small part of the labeled cells after 48 hours of incubation. Proliferation was slowed down at the time the nanoparticles were added to the media; however, overall proliferation was not changed. The viability of labeled cells exposed to a high frequency magnetic field decreased by 50% compared to unlabeled ones, although the sample temperature did not increase. This implies that the nanoparticles only heated their immediate vicinity in the cytoplasm sufficiently to kill the cells containing the nanoparticles. In contrast, the viability of cells mixed with a nanoparticle suspension decreased to 15-20% after their exposure to a high magnetic field, along with a temperature increase to 43°C, thus producing an effective thermoablation method. The injection of the nanoparticles into the cortex of a rat provided superior MR contrast. Conclusion: Coated perovskite nanoparticles are only slightly toxic and may be improved by further surface functionalization. After their injection, the nanoparticles can be easily tracked by MRI in vivo. Due to their ferromagnetic properties, they can be locally heated by an external high frequency magnetic field. Therefore, the nanoparticles can be used for image-guided thermoablation with stem cells used as carriers.
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bimations, and enable development of robust, reproducible and chemically defined cell culture systems. Using Multilayer Soft Lithography, we have developed a microfluidic chip and an automated instrument that can culture cells on chip for extended period of time and deliver multiple combinations of different factors to cells. Each chip includes thirty-two cell culture microchambers and eight reagent inlets. Reagents can be automatically multiplexed to desired combinations and ratios at various pre-defined time points. Cells can also be harvested from the chip for continued off-chip cultivating, single-cell genomic analysis, and/or functional assays. We demonstrate on-chip trans-differentiation of human BJ fibroblasts to neurons by direct transfection with combinations of synthetic microRNA mimics. The identities of cells were confirmed with immunostaining and gene expression profiling. This non-integrative approach of miRNA administration resulted in trans-differentiation with high efficiency and cell viability. In summary, the microfluidic system reported here provides the potential to study and screen the precise combinatorial effects of multiple factors on cell culture maintenance, reprogramming and differentiation, hence could be a valuable tool for the stem cell research community.

Poster Board Number: T-2276

STREAMLINED ANALYSIS OF HETEROGENEITY IN STEM CELL POPULATIONS USING SINGLE-CELL GENE EXPRESSION PROFILING

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Single-cell gene expression profiling has recently been used to characterize emergent properties in cell populations that drive lineage choice and specificity in reprogrammed cells; resolution of cell fate decisions in very early embryonic development, and for identification of cancer stem cell biomarkers in tumor biopsies. We have developed a simple, modular workflow for streamlined analysis of cell populations down to the single-cell level. The workflow is centered on two key components: a new benchtop system for automated cell isolation and cDNA preparation, and the Biomark HD™ system for highly parallel gene expression analysis. Starting from samples containing only a few hundred cells, the preparation system isolates individual cells into discrete compartments within a microfluidic device. Cells can be inspected after isolation using either brightfield microscopy or fluorescent markers to verify cell number and type. Following cell inspection and verification, the selected individual cells are automatically processed to prepare and output high-quality targeted cDNA for gene expression analysis. The targeted cDNA samples are then loaded and analyzed with up to 96 gene expression assays in parallel on Dynamic Array™ IFCs using the Biomark HD system. This workflow has been used to identify and analyze underlying heterogeneity in a variety of cell types and provides a general method for detailed analysis of stem cell cultures.

Poster Board Number: T-2277

GENERATION OF MATURE DOPAMINE NEURONS BY MANIPULATING EXOGENOUS NURR1 EXPRESSION PATTERN REFLECTING THAT IN THE DEVELOPING BRAIN

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Neural stem/precursor cell (NSCs/NPCs) cultures can be a source of dopamine (DA) neurons for experimental and transplantable purposes. Nurr1, a midbrain developmental gene, is regarded as a potent factor to overcome limited DA neuron differentiation from cultured NPCs. However, forced expression of Nurr1 in NPC cultures generates non-neuronal/immature DA cells. We show here that the level and period of Nurr1 expression critically affects neuronal differentiation and maturation of Nurr1-induced DA cells. Mature DA neurons were generated by manipulating the Nurr1 expression patterns into patterns similar those in the developing midbrain. This research was supported by a grant KRF-2011-0008952 funded by the Ministry of Education, Science and Technology, Republic of Korea.

Education and Outreach

Poster Board Number: T-2278

REPROGRAMMING STEM CELL UNDERGRADUATE EDUCATION

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The emerging discipline of stem cell biology is a scientific field uniquely affected by the political environment and ethical standards. Stem cell biology has radically transformed how we think about cells, development and disease, and is equally transforming the educational priorities of our undergraduate and graduate teaching programs in the biological sciences. Topical texts and undergraduate teaching materials on stem cell biology are scarce or non-existent, in part because the science itself progresses so rapidly. In addition to these challenges, human stem cell biology offers unique, cross-disciplinary learning opportunities in biomedical ethics, law, and public policy. Finally, traditional and didactic methods of science instruction often fall short of producing enduring ways of learning, especially in rapidly changing disciplines of the life sciences. In the fall of 2010, a team of educators and scientists at Stanford, Harvard and local community colleges galvanized by their common interests in science education came together to address these needs. The group began a three-year project to develop the nation’s first formal, multidisciplinary, undergraduate curriculum in stem cell biology targeted at students in technical training programs that fuel the life sciences workforce. Resolving to “teach more by lecturing less” the team employed backwards design methodologies to produce the first of two suites of teaching and learning materials, titled From Embryo to Beating Heart. This paper describes the first eighteen months of our effort to devise a novel pedagogical approach for teaching stem cell research. The content development work resulted in 54 hours of instructional materials in stem cell research for undergraduates preparing for careers in the life sciences. Elements include 1) a set of contextually-driven lectures and accompanying 5-10 page introductory reading assignments; 2) complimentary laboratory protocols for the derivation, maintenance, and differentiation of mouse and human embryonic stem cell lines, including directed
differenatlization of neural and cardiac cell types; 3) learning assessments with pre- and post-tests, embedded, open-ended questions and case studies for group discussion, quizzes, laboratory notebook rubrics, and a multi-week concept mapping exercise; and 4) two major activities for student pairs designed to connect themes and disciplines. The first activity features a set of mini-experiments focused on directed differentiation of embryonic stem cells, the second engages students in a public policy debate on the regulation, law, and ethics of stem cell research. We describe the methodology used to design of the course, and present preliminary data on one of our evaluations, the concept mapping exercise. We then discuss the challenges and successes of our Spring, 2011 launch of the course at Stanford and other sites, paying particular attention to whether the students were able to successfully integrate major concepts into their understanding of stem cell research.

Poster Board Number: T-2279

MODIFIED TECHNIQUE FOR EXTRACTION OF DENTAL PULP STEM CELL

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ABSTRACT Various techniques have been reported in the literature for the isolation of Dental pulp stem cells (DPSCs), those techniques were summarized by Huang et. al. 2006 as: 1-The outgrowth method (Tsukamoto et. al. 1992) 2-The enzymatic digestion using collagenase/dispose/trypsin EDTA (Onishi et. al. 1999, Gronthos et. al. 2000) Since the most widely used technique was the one proposed by Gronthos et. al. 2000, The Purpose of this study is to test the validity of a MODIFIED technique based on Gronthos et. al technique, used for extraction of human dental pulp stem cells. Material and methods Dental pulp stem cells were isolated from human pulp tissue of extracted teeth after patients’ signed a consent form. The pulp content of every 2 teeth were mixed and subjected to enzymatic digestion with Collagenase type I (1mg/ml) and disperse (5,000 Ca seynitic units). Cells were then cultured on different plates using cell culture medium. The morphology of the resultant cell lines was evaluated microscopically through hematoxylin and eosin staining (H&E), as well as for their colony forming ability to confirm the presence of stem cell morphological features. Additionally for characterization of these extracted dental pulp stem cells a Fluorescence-Activated Cell Sorting (FACS) analysis, and Immunocytochemical staining was utilized to evaluate the expression of Vimentin in cellular cytoskeleton. Osteogenic and Adipogenic differentiation was induced and evaluated through cytochemical staining, in addition Real time Polymerase Chain Reaction was used to measure certain osteogenic and adipogenic gene expression in different time points. Results: After 5-10 days in culture, few scattered spindle shaped cells were present, attached to the plastic surface of the culture plate. H&E staining illustrated cells with predominant spindle-shaped appearance and different sizes. The nucleus was central, large, pale with 2 or more nuclei in some cells and prominent multiple nucleoli. In 7 days, cells from the second passage formed several colonies, with more than 50 cells in each colony, as evaluated by crystal violet staining. Images for cells in the fourth passage demonstrated samples positive to Vimentin marker. FACS analysis revealed a high expression of stromal cells-associated markers CD105, CD90, CD73 as well as CD13, CD29, CD44 throughout the samples (>95%) while they were negative for hematopoietic markers CD34, CD45, CD14, CD31, as well as for the MHC class II HLA-DR molecule. Osteogenic induction resulted in marked expression of alkaline phosphatase, osteopontin as well as osteocalcin markers as evaluated by RT-PCR, with the highest fold induction at day 14, and induced cells were positive to ALP staining as early as day 3, however, only little adipogenic differentiation was evident both by RT-PCR evaluation for Adiponecin, PPAR-

INTERNATIONAL HESC REPOSITORIES

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It is well established that scientific advances are dependent on collaborative, international research—as science is not constrained by geography. As a result, researchers, universities, private enterprises, and other interested parties are increasingly facing uncertainty related to the diversity of regulatory schemes that govern human embryonic stem cell (hESC) research at local, state, national and international levels. Nonetheless, even in this uncertain scientific, political and commercial climate, OECD countries, including most of the G8 nations, have committed considerable public investment in hESC research. Public investment in hESC research has increased, in part, because it is a key aspect of life science innovation taking place in what is known as the “knowledge economies” of developed nations. This most recent form of scientific innovation has also quickly turned into a discourse of political controversy around the world. The most debated issues relate to various strategies to obtain hESCs (e.g., left-over embryos from IVF treatment or from embryos created through parthenogenesis or SCNT), in addition to hESC application in medical research (consent and donor information). While standardization is imperative to science, a less publicized obstacle to advancing hESC research is that the field is poorly standardized compared to more established fields of biomedical research (e.g., genomics). Accordingly, basic aspects of classification, culture protocols and specimen handling protocols are not universally standardized for hESCs, which has hindered scientific collaboration nationally and internationally. The science-minded legal community, in collaboration with interdisciplinary stakeholders, have the ability to facilitate the advancement of transnational hESC repositories through standardization. Consistently structured hESC repositories would ensure that appropriately characterized hESCs function in the intended manner when applied in research or clinical settings. Facilitating the creation of transnational hESC repositories would thus increase the movement towards the standardization of the storage, transfer, and laboratory protocols for handling hESCs. The legal community successfully helped develop a blueprint for basic human tissue repositories and is uniquely positioned to help facilitate the creation of standard-ized, transnational hESC repositories, similar to the model UK Stem Cell Bank. And, given that the UK’s hESC repository has emerged as a key benchmark towards standardization of protocols on the national and international level, continuing this momentum can help depoliticize some of the issues related to hESC research by

Ethics and Public Policy

Poster Board Number: T-2280

INTERNATIONAL HESC REPOSITORIES

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It is well established that scientific advances are dependent on collaborative, international research—as science is not constrained by geography. As a result, researchers, universities, private enterprises, and other interested parties are increasingly facing uncertainty related to the diversity of regulatory schemes that govern human embryonic stem cell (hESC) research at local, state, national and international levels. Nonetheless, even in this uncertain scientific, political and commercial climate, OECD countries, including most of the G8 nations, have committed considerable public investment in hESC research. Public investment in hESC research has increased, in part, because it is a key aspect of life science innovation taking place in what is known as the “knowledge economies” of developed nations. This most recent form of scientific innovation has also quickly turned into a discourse of political controversy around the world. The most debated issues relate to various strategies to obtain hESCs (e.g., left-over embryos from IVF treatment or from embryos created through parthenogenesis or SCNT), in addition to hESC application in medical research (consent and donor information). While standardization is imperative to science, a less publicized obstacle to advancing hESC research is that the field is poorly standardized compared to more established fields of biomedical research (e.g., genomics). Accordingly, basic aspects of classification, culture protocols and specimen handling protocols are not universally standardized for hESCs, which has hindered scientific collaboration nationally and internationally. The science-minded legal community, in collaboration with interdisciplinary stakeholders, have the ability to facilitate the advancement of transnational hESC repositories through standardization. Consistently structured hESC repositories would ensure that appropriately characterized hESCs function in the intended manner when applied in research or clinical settings. Facilitating the creation of transnational hESC repositories would thus increase the movement towards the standardization of the storage, transfer, and laboratory protocols for handling hESCs. The legal community successfully helped develop a blueprint for basic human tissue repositories and is uniquely positioned to help facilitate the creation of standard-ized, transnational hESC repositories, similar to the model UK Stem Cell Bank. And, given that the UK’s hESC repository has emerged as a key benchmark towards standardization of protocols on the national and international level, continuing this momentum can help depoliticize some of the issues related to hESC research by
providing a procedural solution that is less conflict ridden. While the law cannot fully mitigate the uncertainty with the complexity of regulatory schemes based on political regimes that ultimately govern public funding mechanisms related to hESCs, the law can effectively create governance and management infrastructure through hESC repositories to enhance quality for both research and clinical practice. Working with the legal community to develop hESC repositories that create technical standardization criteria and enforce key material standards for hESC lines will mediate relationships between laboratories in the field of hESCs—while maintaining both technical and ethical standards for the benefit of the public.

Poster Board Number: T-2281

EMBRYONIC STEM CELL RESEARCH IN SAUDI ARABIA; THE CURRENT PRACTICE AND FUTURE DIRECTIONS

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Recent advances in the field of cloning and stem cell research has raised many complex questions. It is rare that a field of science causes debate and challenge not only among scientists but also among ethicists, religious scholars, governments and politicians. There is no consensus on the morality of human cloning, even within specific religious traditions. In countries in which religion has a strong influence on political decision making, the moral status of the human embryo is at the center of the debate. We will discuss our experience; how Islamic teachings make this very promising research and therapeutic technique, and modality of treatment permissible; and the Islamic perspectives about stem cell therapy, in particular embryonic stem cell therapy. Embryonic stem cells (ESCs) have huge therapeutic potential because they can give rise to every cell type in the body (pluripotency) as compared to stem cells from certain adult tissues which can only differentiate into a limited range of cell types. For this reason scientists stress the importance of embryonic stem cell research. However, this research raises sensitive ethical and religious arguments, which are balanced against possible great benefit of such research for the patients suffering from so far incurable diseases. Serious questions remain about safety. In Saudi Arabia for the last five years stem cell research has been established with launching of several projects. One of these projects is embryonic stem cell therapy for Genetic Metabolic disorders which is one of the most promising modalities for the therapy and prevention of mental and physical handicap in children. In conclusion, it is still unclear which human stem cells, whether embryonic or adult, will be developed and for which conditions. Qualities of the ideal stem cell in a clinical setting are expected to be extensive and far reaching. The ability for stem cells to be expanded in culture without genetic and epigenetic abnormalities, their ability to form functional cell types in vitro and in vivo, and their immuno-compatibility with the patient need to be studied. Given this, the focus of research community, should be on developing human research capacity in both ASCs and ESCs. Each type of research will take time to mature. The ethical debate will need to produce acceptable policy and regulatory compromises so that the regulatory burden can be reduced and investors’ risk aversion can be overcome. If these things happen, the major remaining barrier to realizing the medical benefits of stem-cell research might be the lack of skilled scientists in the field. Our experience in Saudi Arabia will be presented.

Poster Board Number: T-2282

RISK PERCEPTION AND COMMUNICATION IN STEM CELL TRIALS FOR SCI

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In November 2011, the sponsor of the first stem cell trial for spinal cord injuries (SCI) halted its study prematurely due to ‘capital scarcity and uncertain economic conditions’. The abrupt discontinuation of this trial raised immediate questions about the informed consent of participants: whether or not they were informed about the ‘risk’ of the trial stopping early, whether such risk would be ‘acceptable’ in first-in-human trial settings, and how participants perceived the materialization of this risk in the light of their decisions to participate. Risk perception and risk communication play a major role in the translation of stem cell research to clinical trials and treatment. These are important elements in the informed consent process and are relevant to an individual’s decision to participate in a trial. However, both are fraught with difficulties. FDA guidelines on clinical trials require that informed consent documents disclose all reasonable and foreseeable risks, but we know that such documents are not always effective in actually communicating and informing participants about potential risks (Anderson & Ilits, 2008). Further, in the stem cell domain, risk communication is a complex matter; many medical risks are still unknown and perceptions of non-medical risk remain underexplored. Our focus is on the following questions: What risks are relevant to prospective trial participants? How do individuals perceive different risks? And, how can risk communication in stem cell research be optimized? We explore these questions toward the development of a theoretical framework for risk communication in first-in-human trials. Building on the existing literature that has largely concentrated on medical risks, we assess the extent to which non-medical risks, including emotional or social risks, the risk of exclusion from future trials, and the early stopping of a trial should be communicated, and explore best practices to address them. Anderson, E. E., & Ilits, A. S. (2008). Assessing and Improving Research Participants’ Understanding of Risk: Potential Lessons from the Literature on Physician-Patient Risk Communication. Journal of Empirical Research on Human Research Ethics, 3(3), 27-37.

Poster Board Number: T-2283

A TALE OF TWO EGGS: CALIFORNIA POLICY ON CONTRIBUTING OOCYTES TO STEM CELL RESEARCH AND ITS ORIGINS.

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Like the rest of the United States, California has an active, if niche, human oocyte trading industry which is an offshoot of the private fertility sector. Large-scale financial compensation, generally US $3500 and up, in exchange for oocytes is the norm and other than Federal requirements to report their success rates to the Centres for Disease Control, there are very few regulations surrounding fertility treatment. However, eggs that are destined for stem cell research laboratories must be donated voluntarily with only the most minimal reimbursement for expenses. Depending on the location and funding source of the research, stem cell research protocols involving oocyte contribution may be subject to some combination of two institutional review boards, two sets of national guidelines, one
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set of state regulations and two state laws. In the California context, selling eggs to make babies, it appears, is both ethical, acceptable and none of the State’s business. Selling them to make stem cells, however, is ethically indefensible and even exchanging them merits the careful intervention of public regulators. The process by which eggs are extracted from women is identical whatever their ultimate ex-vivo destination: why should it be ethical and acceptable to offer large sums of money for them in one situation and not in another? The situation is further confused by the fact that New York, after careful deliberation, made the opposite policy decision, declaring that oocyte contributors to research should receive equivalent compensation to those who contributed to fertility treatments. A policy that contributed to one of the sole successes in somatic cell nuclear transfer (SCNT) research in recent years. This paper argues that the situation surrounding the contribution of oocytes to stem cell research is both shaped by and reveals larger schisms in political forces surrounding reproduction, research and the delineation between the public and the private in the US. In particular, historical debates between libertarian and communitarian approaches to the regulation of private life have splintered existing policies surrounded assisted reproduction, abortion and gamete donation in ways that appear radically inconsistent. Although the relevant association between these broader issues and SCNT research may not be immediately apparent, these larger forces had a formative impact on the formulation of oocyte donation policy in California, and by extension other venues. We will explore how these policies developed and their contribution to stem cell policy in California.

Poster Board Number: T-2284

OBTAINING ADULT HUMAN STEM CELLS THROUGH CLAIM OR CONTRACT: A LIBERTARIAN PERSPECTIVE

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In the 1990 case of Moore v. Regents of the University of California, the Supreme Court of California held that adult stem cell lines could be derived (and later patented) from “waste” tissues of a patient without requiring the researchers to first obtain consent. This contentious ruling must be seen in light of previous rulings, laws, and presumed governmental duties. When one assumes only a minimal libertarian understanding of physician/researcher-patient interactions and governmental duties, I argue that the position of the court is flawed on the basis of actual research practices for obtaining the stem cells. For the researcher to claim a right to any tissue removed from a patient (and thus formerly owned by that patient), the tissue must be said to have been abandoned by the patient and thus open to homesteading by the researcher and others; otherwise, the researcher will be violating the patient’s ownership rights over that property. Yet when holding any reasonable standard for abandonment of property, the tissue removed during surgery or sampling does not qualify as it must be intentionally preserved and modified before a sufficient amount of time has passed for its ownership by the patient to have wither away. Instead of being able to claim stem cells from removed tissue on the basis that that tissue has been discarded and abandoned, the researcher may only acquire these stem cells from the patient via the other means of property acquisition, a contractual arrangement. In such a contractual arrangement, the patient as original and absolute owner retains all rights not explicitly granted to the researchers. Thus, the right to conduct various types of research leading to patenting of stem cells must be granted by the patient for the researcher to possess such rights. The patient may choose to void any claim to his or her former tissue and grant the researchers absolute rights, but given that many governmental protections of modern society are not assumed in a libertarian framework, it is far more likely that the contract will confer only conditional ownership to the researcher, either specifying certain types of research that are acceptable or unacceptable. Finally, the special features of stem cells—theoretically perpetual renewal and identifiability coupled with the exponential growth of biotechnology in the private and public sectors result in the potential for gross privacy breaches and perhaps unintended harms resulting from future stem cell research, which only fully informed consent about future research can adequately guard against. Inasmuch as the withdrawal of stem cells requires some involvement of a health-care worker and the potential violation of bodily integrity if the tissue removal is unwanted based upon information that could have been provided to the patient, to withhold such information before the procedure would be tantamount to battery. Even a minimal libertarian government has an interest in protecting its constituents against such harms, and thus the licensure of health-care workers will necessarily include the requirement of informed consent before such procedures. The researchers, though not party to the surgery/donation itself, will nevertheless be bound by the conditional ownership granted by the informed consent agreement.

Poster Board Number: T-2285

DATA MINING ON NIH FUNDING DISTRIBUTION TO STEM CELL RESEARCH

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Stem cell research continues to gain momentum in the biomedical research and drug discovery field because of its renewable cell source in nature and potential to enable better treatments of human diseases. This study examines the National Institute of Health (NIH) funding distribution to stem cell research at state level and compare their percentage shares of human non-embryonic stem cell research vs. human embryonic stem cell research from FY2008 to FY2011 (non-ARRA). In 2008 total NIH funding allocated to stem cell research was over $930 million, which accounted for about 4% of the overall NIH spending on extramural funding. By analyzing data on NIH RePORT website, the top five states received stem cell research funding in 2008 were California (CA) at 16.3%, Massachusetts (MA) at 10.9%, New York (NY) at 7.6%, Washington (WA) at 5.7%, and Pennsylvania (PA) at 5%. These five states received close to half (46%; $426 million) of all NIH awards devoted to stem cell research. Compared with the funding distribution in 2010, CA (16.6%), MA (11.2%) and NY (9.2%) showed moderate growth on competing stem cell research support whereas the shares of WA (4.8%) and PA (4.7%) have declined slightly. The emerging efforts from state initiatives and strong institutional supports in CA, MA and NY might have leveraged growing shares of federal dollars. Human stem cell research plays a pivotal role on transforming laboratory based research into life saving therapies successfully. Human stem cells and their derivatives are essential assets for toxicity test, drug screening or cell-based transplantation down the road. Further analysis of the funding subcategories indicated that in 2010 NIH spent $467 million to support human stem cell research - 73% to human non-embryonic stem cell research and the remaining 27% to human embryonic stem cell research. CA, MA, NY, WA and PA each shared 13.2%, 10.3%, 6.9%, 7% and 3.8% respectively of total NIH funding in the area of human non-embryonic stem cell research. Human embryonic stem cell research appeared to be more
prominent in states like CA, MA, and WA. CA was the most highly-funded state and accounted for 28.4% of all NIH funding to human embryonic stem cell research compared with MA at 12.1%, NY at 5.1%, WA at 12.6% and PA at 4.1%. Pie charts and bar graphs will be used in the poster to best illustrate the NIH stem cell funding trends and distributions.

Poster Board Number: T-2286

AN ELSSI APP FOR STEM CELL SCIENCE AND MEDICINE

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In this study, we explore how Internet cloud computing, driven by apps and social media, can play a role in disseminating to relevant stakeholders information about the ethical, legal, and social implications (ELSI) of stem cell science and its clinical translation into regenerative medicine and cell therapy. This pursuit arises not only against the backdrop of cloud computing, apps, and social media growing generally in all scientific, commercial, industrial fields, but specifically in the life sciences, medicine, and health care. Applications of cloud/apps/social media computing and communications technologies in life science/medicine/health care include: use by patients and consumers for obtaining information about diseases and treatments and communications among those suffering from a common ailment; medical students seeking educational tools that they can view on their smart phones; physicians seeking information about new medicines and procedures and efficient means of communicating with their patients; and various government agencies and policymakers engaged in health care and facing the need to communicate with each other as well as their various constituencies. The format of the digital content in these communications includes text narratives, podcasts, videocasts, and various types of graphics. Beyond use for information dissemination and education, apps and social media networks are being extended to information collection in clinical trials. Moreover, in health care settings, mobile apps have become available for remotely collecting observations of patients’ vital signs and wirelessly transmitting these observations to professionals in central locations responsible for monitoring multiple patients simultaneously. These trends present at least two questions in the intersection of ELSI, internet cloud/apps/social media, and life science/medicine/health care: 1) how to assure use of such technology comports with “good” or “best” practices within the tenets of ELSI; and 2) how to use this technology to communicate ELSI-related information to patients, consumers, medical students, physicians, government officials, policy-makers and others in life science/medical/health care centers of activity? We focus on the second of these two questions, particularly as it relates to stem cell science and medicine. We hypothesize that an app can be developed for communicating such information to the various stakeholders. We examine this hypothesis by proposing the design of an app that will be international in scope. Its content will include: various formative documents giving rise to bioethics generally (e.g., the 1979 Belmont Report) and stem cell-related bioethics (e.g., the 1984 Warnock Committee); various documents reflecting the translation of bioethical principles into statutory and regulatory requirements (e.g., the U.S. Federal Policy for the Protection of Human Subjects (the “Common Rule”), the U.K. Human Fertilization and Embryo Act of 1990, and the 2009 NIH Guidelines for Human Stem Cell Research); and the extensive body of relevant writings from bioethicists and legal commentators. We envision that this app would be available on an open source basis.

Poster Board Number: T-2287

THE NIH HUMAN EMBRYONIC STEM CELL ELIGIBILITY WORKING GROUP: THE FIRST TWO YEARS

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In this presentation, two inaugural members of the National Institute of Health’s Human Embryonic Stem Cell Eligibility Working Group look back across the last two years, and discuss the issues the Working Group faced and how they have resolved them. In March 2009, United States President Barack Obama fulfilled a campaign promise by expanding the number of human embryonic stem cell (hesc) lines available for research use with public funds. Hesc lines that fulfilled the administration’s criteria were considered eligible for funding and were entered into a public registry. In order for hesc lines to be eligible for use in research with public funding, they must have been originally created for reproductive purposes, and donated, without financial incentive, by the individuals who sought reproductive treatment, and who provided written consent for their embryos to be used for research. During the public comment stage on the proposed regulations, many scientists expressed concern that existing stem cell lines, including those approved under the Bush administration, would not be eligible for funding under the Obama rules because of slight deviations from the new requirements, deviations that might not be ethically significant. In response, NIH created a Working Group (WG), whose first task is to review applications for embryonic stem lines created before March 2009. The WG’s job is a combination of “history detective” and IRB. It determines whether the lines were derived from embryos originally created for reproductive purposes, and whether the donors gave their embryos for research without financial incentive. It looks backwards to determine the quality of consent given by embryo donors years ago for cell lines being scrutinized today. The WG’s second task is to evaluate lines for which embryos were donated in countries other than U.S. Was the consent process adequate? Were the embryos donated without coercion? How does one apply these important concepts across different cultures? How should the WG take into account factors that do not exist in the U.S., such as legally mandated limits on how long human embryos may be frozen? Are these limits coercive in ways that weaken informed, voluntary consent of the donors? The WG makes recommendations to the NIH Advisory Council to the Director, which makes its own recommendation to the Director, who makes the final decision. Since its inception, the WG has recommended approval of 48 lines and rejected 65 lines. The Director has accepted all of the WG’s recommendations. Lines were recommended for disapproval based on variety of concerns, including lack of clarity in the consent process, and lack of IRB approval. The WG also engaged with a number of novel issues, often leading to new NIH policies. For example, it was determined that when a consent document informed prospective embryo donors that their embryos would be used for research on a specific disease or disease cluster, the lines would be approved only for research in that area, thus “keeping faith” with the donor.
ESTABLISHING AN EVIDENCE-BASE AND ASSERTING LEGITIMACY IN THE CLINICAL TRANSLATION OF STEM CELL SCIENCE: AN EXAMINATION OF INTERNATIONAL GUIDELINES FOR THE INNOVATIVE USE OF AUTOLOGOUS ADULT STEM CELLS.

Lysaght, Tamra
Centre for Biomedical Ethics, National University of Singapore, Singapore, Singapore

Stem cell science is an emergent field of research that offers enormous potential in the treatment of many significant diseases, illnesses and conditions. However, the clinical translation of stem cells raises many regulatory and ethical issues that yet to be fully explored. One such issue concerns the evidence-base that should be required for the clinical use of novel stem cell based-therapies. Many argue that evidence should be gathered in formal clinical trials to first demonstrate the efficacy and safety of an experimental intervention before it is accepted into clinical practice. However, others claim that novel interventions with adult stem cells for autologous use are innovative medical practices that need not be subject to formal clinical trial. The clinical guidelines of two professional organisations - the International Society for Stem Cell Research and the International Cellular Medicine Society - have each adopted these divergent approaches. In this paper, I examine the guidelines of both organisations to identify the tensions between them regarding the establishment of an evidence-base for the clinical use of autologous adult stem cells. I argue that through these guidelines, both organisations are attempting to establish legitimacy for their version of an evidence-base for stem cell medicine and assert epistemic authority over the clinical translation of stem cell science. These claims to authority are made through proscriptions for the type of evidence that should be required, how it should be validated and who should oversee the collection and validation of evidence before novel stem cell applications are accepted into clinical practice. I conclude by reflecting on some of the implications involved in developing a framework for the oversight of innovative stem cell interventions.

NEXT GENERATION SEQUENCING OF INDUCED PLURIPOTENT STEM CELL LINES: ETHICAL AND POLICY CONSIDERATIONS.

Isasi, Rosario
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Stem-cell genomics promises to improve our ability to prevent and cure disease by providing cells for organ transplantation and cell therapies. It will also enable the creation of successful model systems for drug discovery and contribute to the understanding of the processes of human cell differentiation for the treatment of several diseases including cancer. For instance, the use of next-generation sequencing (NGS) technologies in induced pluripotent stem cell (iPSCs) lines is expected to greatly contribute to the development of new testing methods for drug efficacy, toxicity and safety for individuals, thereby expediting progress towards personalized medicine. The challenges posed by stem-cell ‘omics’ efforts are not constrained only to scientific hurdles, the field is also confronted by an array of ethical, social and policy concerns as pertains for example, informed consent, feedback of incidental findings, privacy and the governance of research. While, some of these concerns are not completely new, the old answers might not suffice given the scale of challenges. NGS methods are now routinely applied to iPSCs lines, allowing fine, detailed, genotypic information of the cell lines at high resolution. Given that such iPSCs are (often) derived from living individuals - including paediatric populations - are contemporary mechanisms for protecting the autonomy, privacy and confidentiality of donors sufficient? Are current governance procedures adequate? In an era of stem-cell genomics what is the appropriate role for regulatory bodies? This presentation will provide an overview of some key ethical issues surrounding applying next generation sequencing techniques to stem cell research. Issues surrounding informed consent, privacy and confidentiality, data security, feedback of research results and governance of research will be addressed.
in the stem-cell research field, which can be used as fundamental information to design national scientific and technological policies and institutional research plans.

**Regeneration Mechanisms**

**Poster Board Number: T-2291**

**REGENERATION OF AMPUTATED ZEBRAFISH FIN RAYS FROM DE NOVO OSTEOLASTS**

Singh, Sumeet Pal, Holdway, Jennifer E., Poss, Kenneth D.

Department of Cell Biology and Howard Hughes Medical Institute, Duke University Medical School, Durham, NC, USA

Determining the cellular source of new skeletal elements is critical for understanding appendage regeneration in amphibians and fish. Recent lineage-tracing studies indicated that zebrafish fin ray bone regenerates through the de-differentiation and proliferation of spared osteoblasts, with limited if any contribution from other cell types. Here, we examined the requirement for this mechanism by using genetic ablation techniques to destroy virtually all skeletal osteoblasts in adult zebrafish fins. Animals survived this injury and restored the osteoblast population within two weeks. Moreover, amputated fins depleted of osteoblasts regenerated new fin ray structures at rates indistinguishable from fins possessing a resident osteoblast population. Inducible genetic fate-mapping confirmed that new bone cells do not arise from de-differentiated osteoblasts under these conditions. Our findings demonstrate diversity in the cellular origins of appendage bone, and reveal that de novo osteoblasts can fully support the regeneration of amputated zebrafish fins.

**Poster Board Number: T-2292**

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Detailed Program and Abstracts — Thursday, June 14

**Timing of synapse formation between hESC-NP and their peripheral (hair cell) and central (cochlear nucleus) targets in vitro.**

**Poster Board Number: T-2295**

**EFFECT OF CTX0E03 HUMAN NEURAL STEM CELLS ON THE TIME COURSE OF SKELETAL MUSCLE REPAIR AND GENE EXPRESSION FOLLOWING HIND LIMB ISCHEMIA IN MICE**

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1Stem Cell Discovery, ReNeuron, Guildford, United Kingdom, 2Bristol University, Bristol Royal Infirmary, Bristol, United Kingdom

We have previously reported that intramuscular injection of CTX0E03 human neural stem cells (hNSCs) into a mouse model of hind limb ischemia results in a high and sustained therapeutic improvement in blood flow. Increased neovascularisation was afforded in the hNSC treated muscle without evidence of long term cell survival. We hypothesised therefore that hNSC treatment may exert an effect on early host muscle responses post-ischemia and upstream of the end point effects reported to date. Skeletal muscle demonstrates rapid degenerative and regenerative phases following injury. In particular, responses of the innate immune system including cellular recruitment and release of pro-inflammatory cytokines have been implicated in the success of skeletal muscle regeneration. Briefly, CD1 mouse cells (8 per time point) underwent unilateral hind limb ischemia followed by immediate injection of either, vehicle or CTX0E03 hNSCs (300K) into the ischemic adductor muscle. Ischemic and contralateral adductor muscles were collected, at 30 minutes, 4, 24 and 72 hr and 7 days post ischemic insult and treatment. To determine the effect of hNSC treatment on these early inflammatory host responses in adductor muscle tissue we investigated cell types, cytokines, chemokines and growth factors associated with the key cellular and molecular events of skeletal muscle repair using histology and an OpenArray™ pathways microarray and localized their expression by in situ hybridization.

**Poster Board Number: T-2296**

**SOX2-POSITIVE ADULT MOUSE STEM CELLS GIVE RISE TO ALL EPITHELIAL CELL LINEAGES DURING TOOTH RENEWAL**

Juuri, Emma1, Saito, Kan1, Ahtianen, Laura1, Tummers, Mark1, Seidel, Kerstin1, Kleim, Ophir1, Hochdølger, Konrad1, Theesfeld, Irma1, Michon, Frederic1

1Developmental Biology Program, Institute of Biotechnology, Helsinki, Finland, 2Departments of Orofacial Sciences and Pediatrics, University of California, San Francisco, CA, USA, 3Howard Hughes Medical Institute and Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA

Putative stem cells have been localized in the stellate reticulum of the continuously growing mouse incisor by label-retaining experiments. Specific markers for stem cells that give rise to dental epithelial cell lineages, however, are still unknown. The aim of this study was to find such markers. To achieve this, we defined the genes that are specifically expressed in the incisor cervical loop by microarray and localized their expression by in situ hybridization. Sox2 was specifically expressed in a cell population in the labial cervical loop that is known to give rise to the enamel-producing ameloblasts. By lineage tracing approach, we revealed that in addition to ameloblasts, the Sox2+ epithelial stem cells renew also all other epithelial cell lineages of the incisor. Additionally, we found that the early progeny of Sox2+ stem cells transiently express Sfrp5. The discovery of Sox2 as a marker for epithelial stem cell and Sfrp5 for progenitor populations is an important step towards developing a complete bioengineered tooth. Our study provides valuable new insights into the role of stem cells in tooth development and renewal with a potential impact on cell biology, stem cell research, and medicine.

**Poster Board Number: T-2297**

**IDENTIFICATION OF FUNCTIONAL THYMIC EPITHELIAL PROGENITOR CELLS IN THE ADULT MURINE THYMUS**

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Thymic epithelial cells play a vital role in the instruction of T cell development, differentiation and tolerance induction. Thymic function and naive T cell output is at optimum levels during the early years of life, however, declines sharply with the onset of puberty (thymic atrophy). This can have a profound impact on immune system function, particular in the aged or following cytoablative regimes such as chemotherapy and irradiation in cancer treatments. Potential methods of thymic regeneration from pre-clinical studies include sex steroid ablation and administering growth factors such as keratinocyte growth factor, growth hormone or interleukin-7. Whilst some of these approaches are in early clinical trials, ideal T cell recovery levels have not yet been met and patient responsiveness, particularly in those with severe thymic atrophy, cannot be guaranteed. Therefore, an alternative approach would be to activate resident thymic epithelial progenitor cells to aid in the regeneration of the thymus in states of dysfunction. Using flow cytometric analysis, a relatively quiescent thymic epithelial progenitor was deduced based on long-term BrdU label-retention over a 6 month chase. Functional studies using reaggregate thymic organ cultures found that these putative progenitors were the only population capable of forming a thymic graft when placed under the kidney capsule of recipient mice, contributing to both medullary and corti-
We recently identified a novel population of LIF-dependant neural stem cells (NSCs) in the adult mouse brain, termed adult-derived primitive NSCs (AdpNSCs). AdpNSCs comprise a rare population of GFAP-negative cells that are similar to embryonic primitive NSCs, as they give rise to LIF-dependent neurospheres in culture. AdpNSCs can be passaged in vitro to self-renew or give rise to definitive (d) NSCs, which are GFAP-positive and EGF- and FGF-dependent. AdpNSCs were shown to express low levels of Oct4 and contribute to chimeric blastocystcs, both markers of pluripotency not exhibited by adult dNSCs. Unlike dNSCs, AdpNSCs depend on Oct4 expression in the adult brain. Transgenic mice with a floxed Oct4 gene that is excised when exposed to Cre recombinase expressed under the Sox1 promoter were used to assess the requirement for Oct4 in AdpNSCs. Most interesting, the forebrain of adult mice homozygous for the deleted floxed Oct4 allele no longer gave rise to any LIF-dependent neurospheres, while EGF- and FGF-dependent neurospheres were not affected by loss of Oct4. This indicates a requirement for Oct4 expression in AdpNSCs, and provides a key model for an AdpNSC-null mouse. Next, we sought to determine whether AdpNSCs are required for repopulation of dNSCs following ablation of dNSCs with AraC infusion. We ablated dNSCs and downstream neural progenitors in the excised Oct4 transgenic and control mice using a 14-day AraC infusion. After the 14-day AraC infusion and a 14-day recovery after AraC, we observed that the EGF- and FGF-dependent neurospheres did not return in mice lacking Oct4 expression whereas control mice had repopulated dNSCs to 40% baseline conditions at this time point. This suggests that AdpNSCs are essential for repopulation of dNSCs. We are continuing this experiment with longer survival times to further confirm this observation. Furthermore, we took advantage of the Histone2B-GFP transgenic line to perform label retention studies. We observed that after 1-month chase, AdpNSCs retained the same degree of labeling, whereas over half the labeled dNSCs had diluted out their label. This indicates that AdpNSCs are a quiescent cell population in the adult mouse brain. Together, these experiments indicate that AdpNSCs are a quiescent population upstream of dNSCs in the adult mouse brain, capable of proliferation after removal of the dNSC population to reconstitute the neural lineage.

**Poster Board Number: T-2298**

**QUIESCENT PRIMITIVE NEURAL STEM CELLS IN THE ADULT MOUSE BRAIN REPOPULATE ABLATED DEFINITIVE NEURAL STEM CELLS**

**Leeder, Rachel**, Sachewsky, Nadia, Xu, Wenjun, Morshed, Cindi M., van der Kooy, Derek

*University of Toronto, Toronto, ON, Canada*

We recently identified a novel population of LIF-dependant neural stem cells (NSCs) in the adult mouse brain, termed adult-derived primitive NSCs (AdpNSCs). AdpNSCs comprise a rare population of GFAP-negative cells that are similar to embryonic primitive NSCs, as they give rise to LIF-dependent neurospheres in culture. AdpNSCs can be passaged in vitro to self-renew or give rise to definitive (d) NSCs, which are GFAP-positive and EGF- and FGF-dependent. AdpNSCs were shown to express low levels of Oct4 and contribute to chimeric blastocysts, both markers of pluripotency not exhibited by adult dNSCs. Unlike dNSCs, AdpNSCs depend on Oct4 expression in the adult brain. Transgenic mice with a floxed Oct4 gene that is excised when exposed to Cre recombinase expressed under the Sox1 promoter were used to assess the requirement for Oct4 in AdpNSCs. Most interesting, the forebrain of adult mice homozygous for the deleted floxed Oct4 allele no longer gave rise to any LIF-dependent neurospheres, while EGF- and FGF-dependent neurospheres were not affected by loss of Oct4. This indicates a requirement for Oct4 expression in AdpNSCs, and provides a key model for an AdpNSC-null mouse. Next, we sought to determine whether AdpNSCs are required for repopulation of dNSCs following ablation of dNSCs with AraC infusion. We ablated dNSCs and downstream neural progenitors in the excised Oct4 transgenic and control mice using a 14-day AraC infusion. After the 14-day AraC infusion and a 14-day recovery after AraC, we observed that the EGF- and FGF-dependent neurospheres did not return in mice lacking Oct4 expression whereas control mice had repopulated dNSCs to 40% baseline conditions at this time point. This suggests that AdpNSCs are essential for repopulation of dNSCs. We are continuing this experiment with longer survival times to further confirm this observation. Furthermore, we took advantage of the Histone2B-GFP transgenic line to perform label retention studies. We observed that after 1-month chase, AdpNSCs retained the same degree of labeling, whereas over half the labeled dNSCs had diluted out their label. This indicates that AdpNSCs are a quiescent cell population in the adult mouse brain. Together, these experiments indicate that AdpNSCs are a quiescent population upstream of dNSCs in the adult mouse brain, capable of proliferation after removal of the dNSC population to reconstitute the neural lineage.

**Poster Board Number: T-2299**

**ENDOVENOUS ADMINISTRATION OF MESENCHYMAL STROMAL CELLS IMPROVES KIDNEY FUNCTION IN MICE WITH TYPE 2 DIABETES MELLITUS INDUCED BY HIGH FAT DIET FEEDING.**

**Ezquer, Marcelo E., Ezquer, Fernando E., Ricca, Micaela, Conget, Paulette A.**

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Diabetes mellitus (DM) is one of the current main threats to public health. According to its etiology, DM is classified as: i) Type 1 diabetes mellitus (T1DM), due to the autoimmune destruction of pancreatic β-cells, which leads to an insulin deficiency and ii) Type 2 diabetes mellitus (T2DM), a metabolic disorder whose principal characteristic is the presence of insulin resistance, together with an impaired insulin production. The most detrimental complication of DM is diabetic nephropathy (DN), a clinical syndrome comprised of kidney damage and increased risk for cardiovascular disease. DN associates with functional and histological changes that include: albuminuria, mesangial expansion, glomerulosclerosis and tubulointerstitial fibrosis. To date there is no cure to DN; patient’s management comprises the use of drugs to control hyperglycemia and blood pressure. If required, hemodialysis is prescribed; unfortunately, those treatments only help to slow DN progression. Multipotent mesenchymal stromal cells also referred as mesenchymal stem cells (MSCs) are one of the most promising tool to manage DN progression, not only because they can be safely transplanted in human patients but also due to their proved renoprotective potential. The later, has been attributed to the capacity of MSCs to: i) reduce oxidative stress, ii) modulate chronic inflammation and macrophage infiltration, iii) avoid/revert fibrosis, iv) secrete trophic factors, and v) differentiate into pericytes, mesangial and tubular cells. Recently, we have shown that the intravenous administration of MSCs into mice with DN derived from T1DM, prevents kidney damage. At present, our research aim was to evaluate whether the MSCs renoprotective effect depends on DM etiology (type 1 vs type 2). The complex etiology of T2DM makes it difficult to perfectly mimic this disorder in experimental models; however C57BL/6 mice fed with high-fat diet containing 60% kcal. saturated fat (HFD) develop renal functional and histopathological abnormalities similar to those observed in patients with obesity, metabolic syndrome or T2DM onset. Here, C57BL6 mice were exclusively fed with HFD and 33 weeks later, separated in two groups. One received two times 0.5x10^6 MSCGFP that have been constitutively express GFP (MSC-treated). The other group received vehicle (untreated). Both groups continued to eat HFD all along the study period (50 weeks). Renal failure did not progress in MSC-treated T2DM mice, while in untreated T2DM mice albuminuria gradually increased. These changes were correlated with morphological alterations and glomerulosclerosis index. Two and 17 weeks post-MSC administration, biodistribution was evaluated by flow cytometry. We found donor cells in the kidney of mice with T2DM but not in normal mice. The observed renoprotective effect of MSCs was not related to a reversion in the metabolic syndrome since MSC-treated mice kept hyperglycemic, hyperinsulinemic, insulin resistant and hypercholesterolemic along the study period. Although donor cells were found in the kidney of treated mice, they scarcity suggest that MSCs systemic administration improve kidney function not by functional complementation from direct differentiation parenchyma. Probably is the consequence of microenvironment changes. Additional experiments are in course.
to clarify the mechanisms associated to the observed therapeutic effect. Supported by FONDECYT 1120133 to ME

Poster Board Number: T-2300

OLFACTORY DERIVED NEURAL PROGENITOR CELLS TRANSPLANTATION IN A RAT INTRACREREBRAL HEMORRHAGE MODEL

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Background: Olfactory epithelium is easily accessible neural tissue and harbors abundant neural progenitor cells which produce new neurons every day in response to various environmental stimuli. We tried to investigate neuronal regeneration effect of olfactory derived neural progenitor cells (ONPC) transplantation in intracerebral hemorrhage model. Methods: We harvested ONPCs from human nasal olfactory mucosa culture from DMEM/HAM F12 media, followed by DMEM/HAM F12 supplemented with insulin, transferrin, selenium (ITS-X 1%), EGF, and FGF2 for four days. We transplanted 0.3 million ONPCs in a collagenase induced intracerebral hemorrhage model, and functional outcome were measured every week until fifth week. In vitro and in vivo cell marker studies were performed to evaluate cellular phenotype of ONPCs. Results: In vitro cell marker studies before transplantation showed that ONPC are combined with doublecortin, microtubule associated protein (MAP2), and GABA, but not with GFAP or NeuN, suggesting immature neuronal progenitor cell morphology. Transplantation of ONPCs attenuated functional deficit after intracerebral hemorrhage model after five weeks, and in vivo cell marker study five weeks after transplantation showed decrement of immature neuronal markers. Discussion: Olfactory epithelium derived ONPC attenuated neurological deficit after intracranial hemorrhage presumably by differentiation of neuronal lineage.

Poster Board Number: T-2301

A TWO-STEP DIRECTED DIFFERENTIATION OF RAT EMBRYONIC STEM CELLS USING FGF2 AND LAMININ AND SONIC HEDGEHOG RESULTS IN MOTOR NEURONS WITH AN EXPRESSION PROFILE SIMILAR TO MOTOR NEURONS DERIVED FROM PRIMARY NPCS DERIVED FROM THE ADULT CERVICAL RAT SPINAL CORD

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New to biomedical research, rat embryonic stem cells (ESCs) differentiate to every adult cell type offering the potential as a component of a multi-rodent model to study differences amongst tissues. We have previously derived rat ESCs from various strains. To create an in vitro model of stimulating central nervous system motor neuron neogenesis, we first differentiated rat ESCs to neural progenitor (NPC)-like cells. Neural progenitors have the capacity to differentiate into neurons and nonneural support cells. NPC-like cells were produced from rat ESCs through administration of basic fibroblast growth factor (FGF2) and laminin on poly-L-ornithine and laminin coated or matrigel coated cell culture plates. Subsequently, we differentiated the NPC-like cells and adult rat cervical spinal cord derived primary NPCs to motor neurons with sonic hedgehog (Shh). Differentiation was accomplished through two ways. One involved expanding NPC-like cells in monolayer culture. The other progressed through neurospheres. Motor neuron cultures were analyzed for expression of Nestin, Pax6, Tubb3, Irx3, Nkx2.2, H89, ISL1 and SM-I-32. This protocol was also applied to primary NPCs isolated from the lumbar region of the adult rat spinal cord. Stimulating in vivo motor neuron regeneration after spinal cord injury remains elusive, and, therefore, the development of an in vitro model to test compounds that promote motor neuron regeneration in the presence of oligodendrocytes, Schwann or Olfactory bulb ensheathing cells may be informative.

Poster Board Number: T-2302

SONIC HEDGEHOG MEDIATES ENDOGENOUS-EXOGENOUS NEURAL STEM CELL SYNERGY AND NEUROPROTECTION IN THE PARKINSONIAN RAT

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Neuroprotective and neurorescue effects after neural stem/precur- sor cell (NPC) transplantation have been reported, but the mecha- nisms underlying such phenomena are not well understood. Our recent findings in a rat Parkinson’s disease (PD) model indicate that transplantation of NPCs before a 6-hydroxydopamine (6-OHDA) insult can result in nigrostriatal protection which is associated with endogenous NPC proliferation, migration and neurogenesis suggesting a ‘synergy’ between transplanted (exogenous) and endogenous NPC actions (Madhavan et al, 2009; J. Comp. Neurol; Madhavan et al, Neuropharmacology, 2010). In addition, the transplanted NPCs expressed certain growth factors (including glial derived neurotrophic factor (GDNF), and sonic hedgehog (SHH)) providing a potential molecular basis for the observed phenomenon. We have investigated mechanisms underlying the observed NPC-mediated neuroprotection and neurogenesis by examining roles of (a) host endogenous NPCs and (b) abovementioned graft-expressed factors. With respect to the endogenous NPCs, we have established that the host neural precursors not only respond to the transplanted NPCs but in fact contribute to dopamine system neuroprotection (Madhavan et al, European Journal of Neuroscience, 2012). The current study focuses on the grafted NPCs and their ‘chaperone effects’, and in particular examines the role of GDNF and SHH using RNA interference. Specifically, NPCs in which either GDNF, SHH, or both had been silenced were transplanted into host rats to determine whether or not they contribute to the observed NPC-mediated neuroprotection and endogenous response to transplantation. Histological and behavioral analyses indicate that GDNF silencing significantly reduced NPC-mediated neuroprotec- tion but not neurogenesis. On the other hand, knock-down of SHH, or a combination of GDNF and SHH caused a profound decrease in both graft-mediated neuroprotection and endogenous NPC activa- tion (proliferation, migration and neurogenesis), suggesting that SHH was a key molecule contributing to NPC mediated therapeutic effects. Overall, the studies help determine some of the micro-environmental signals fundamental to neural precursor based neuroprotection, and contribute towards the development of novel stem cell based therapies for PD.
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Poster Board Number: T-2303

THREE CHEMOKINES, SDF1A, MCP-1 AND HEPATOCYTE GROWTH FACTOR ATTRACT TRANSPLANTED NEURAL STEM CELLS DURING NEUROINFLAMMATION

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Neural stem/precursor cells (NSCs) possess powerful immune-regulatory and neurotrophic properties that underlie their therapeutic effects after transplantation in animal models of multiple sclerosis (MS). Since MS is a multifocal disease, NSCs need to migrate into the multiple lesions in order to exert their therapeutic effects. Therefore, cell migration is a crucial element in regenerative processes in MS, dictating the route of delivery, when cell transplantation is employed. We have previously shown that inflammation triggers migration of multipotential NSCs into white matter tracts of experimental autoimmune encephalomyelitis (EAE) rodents, a widely used model of MS. Here we investigated the molecular basis of this attraction. First, inflammation-triggered NSC migration into white matter tracts was dependent on a motile NSC phenotype, induced by epidermal growth factor (EGF). Specifically, migration of FGF2-expanded NSCs was observed in only 10% of EAE mice, as compared to 47.5% of EAE mice transplanted with FGF2+EGF-expanded NSCs. A similar effect was found in an in vitro migration assay. Then, we examined tissue factors that attract transplanted NSC migration during EAE. Three chemokines, Stromal Derived Factor-1a (CXCL12), Monocyte Chemoattractant Protein-1 (CCL2) and Hepatocyte Growth Factor, were induced in tissue microglia and astrocytes during EAE. Blocking of each of their specific receptors (CXCR4, CCR2 and c-Met, respectively) on NSCs prior to transplantation, caused partial inhibition of cell migration in the corpus callosum of EAE mice. Blocking all three receptors caused a profound 80% inhibition of transplanted NSC migration. These findings were replicated in two different EAE models. Thus, three glial derived chemokines and their receptors account for most of the tissue-derived attraction of transplanted NPCs into inflamed white matter tracts during EAE. These findings identify inflammatory signals that are involved in putting regenerative processes in motion, and suggest potential therapeutic targets for regenerative medicine in multiple sclerosis.

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REGENERATION OF THE DENTAL PULP VIA AMPLIFICATION OF AN ENDOGENOUS WNT SIGNAL

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Adult tissues contain stem cells, which are activated by injury and contribute to the repair of damaged tissues. Dental pulp is one such tissue, but the extent of stem cell activation occurs following injury is unknown, and whether a strategy could be devised that induces dentin regeneration remains untested. Using Axin2-mediated reporter mice, we identified odontoblasts as being responsive to an endogenous Wnt signal that is produced in the dental pulp itself. When the tooth is injured, the dental pulp responds by up regulating Wnt target genes. We tested the consequences of amplifying the endogenous Wnt signal. To do this we employed Axin2 reporter mice, where loss of both alleles of the negative Wnt regulator Axin2 leads to an enhanced Wnt responsiveness that nonetheless retains its ligand-dependency. In control Axin2 mice the injury site was filled with an inflammatory infiltrate that gradually resolved. At no time during the post-surgical period did we detect evidence of a repair/regenerative response. On post-surgery day 7 the injury site in Axin2 mice was filled with undifferentiated fibroblastic cells and some blood vessels. On post-surgery day 10 the appearance of the injury site had not changed appreciably. In Axin2 mice, however, exhibited a dramatically different response: on post-surgery day 7 the injury site was filled with fibroblastic cells and blood vessels and cells lining the injury site had achieved an osteoblast-like morphology. On post-surgery day 10 the injury site in Axin2 mice was filled with a newly formed mineralized tissue. This tissue was localized exclusively at the area of irritation, and had irregular, reduced dentinal tubule architecture. The mineralized tissue stained positive to collagen type I, and thus constituted tertiary or reparative dentin. These data demonstrate that enhancing the endogenous Wnt signal results in a superior regenerative response from dental pulp stem cells. We then employed a biochemical strategy that mimicked the transient elevation in endogenous Wnt signaling observed in Axin2 mice, by delivering to the injured pulp a liposomal formulation of Wnt3a protein that maintains the biological activity of the hydrophobic protein. The regenerative response elicited by this treatment strongly suggests that by amplifying the body’s natural response to injury we can induce a regenerative response in the dental pulp.

Poster Board Number: T-2305

TWO-PHOTON MICROSCOPY TO CAPTURE LIVE CELL BEHAVIOR IN THE HAIR FOLLICLE STEM CELL NICHE

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Stem cells and niche components are responsible for the timely orchestration of the regeneration process that leads to highly organized tissues. Despite recent progress in our understanding of stem cell biology, the dynamic interaction between stem cells and the niche is not well understood. A current challenge in the field is having access to a well-defined stem cell niche in which the orderly development of stem cells can be observed, characterized and manipulated in vivo. To learn which dynamic behaviors occur during mammalian regeneration, we took advantage of the unique accessibility of the skin hair follicle and set up a novel imaging approach to study a physiological regeneration process non-invasively by two-photon microscopy. By these means, we have studied the behavior of the epithelial stem cells and their progeny during physiological hair regeneration and how the mesenchymal niche influences their behavior. Consistent with earlier studies, stem cells are quiescent during initial stages of hair regeneration, whereas the progeny are more prone to divide. Moreover, stem cell progeny cell divisions are spatially and temporally coordinated within follicles. In addition to cell divisions, coordinated cell movements within the progeny allow hair follicle rapid expansion. Finally, we demonstrate the requirement for the mesenchymal niche for hair regeneration through targeted cell ablation and long-term tracking of live hair follicles. Thus, we have established an in vivo approach that has led to the discovery of unexpected mechanisms of growth regulation, and enabled us to precisely investigate functional requirements of stem cell niche components during the process of physiological regeneration.
SOX2 MARKS EPITHELIAL COMPETENCE TO GENERATE TEETH

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Tooth renewal takes place by budding from the epithelium of the predecessor tooth. Mammalian molars develop in succession from a posterior extension of the epithelial dental lamina associated with the previously formed molar. In tooth replacement each individual replacement tooth is initiated as an extension of the dental lamina from the lingual side of the predecessor. Tooth replacement capacity varies between different species from one root in mammals to lifelong replacement in reptiles and fish. For the successional generation of new teeth to be possible there has to be stem cells in the epithelium that retain the competence for renewal. So far the localization and the molecular identity of these cells in mammals have been uncharacterized. Understanding the mechanisms of tooth renewal requires identification of the competent stem cells. Transcription factor Sox2 has been shown to regulate stem cell maintenance as well as iP5 cell induction. We have identified Sox2 as a marker to identify the competent cells in the dental epithelium. We have localized Sox2 expression in tooth replacement of different species such as the ferret (Mustela putorius furo), where Sox2 positive cells are seen in the lingual epithelium giving rise to the permanent teeth. In addition we have localized Sox2 in the dental epithelium of mutant mice where overactivation of Wnt signaling leads to continuous tooth renewal. In the successional development of molars Sox2 expression marks the budding epithelium. Our data suggest that the Sox2 positive dental epithelial cells associated with tooth replacement and posterior molar addition may represent stem cells for successional tooth renewal.

PROSTAGLANDIN E2 PROMOTES POST-INFARCTION CARDIOMYOCYTE REPLACEMENT BY ENDOGENOUS SCA-1 STEM CELLS

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In mammals, adult cardiomyocytes are capable of regenerating themselves following injury or during normal lifespan. Furthermore, the adult stem cells possessing cardiomyocyte differentiation ability have been identified. Nevertheless, it remains unknown how these stem cells are stimulated to repair the heart after injury. In our study, we used adult cardiomyocyte-specific fate-mapping system (a-MHCMerCreMer crossbreeding Z/EG) to examine the time course of stem cell-driven cardiomyocyte replenishment after myocardial infarction (MI) in mice. We found that formation of new cardiomyocytes took place at the border zone within 7 days and saturated on day 10 post-MI. Further analyses indicated that administration of anti-inflammatory drugs, indomethacin and celecoxib, inhibiting cyclooxygenase (COX)-2 diminished cardiomyocyte regeneration after infarction. Moreover, such inhibition was only effective when the drug was administered within 5 days post-MI, suggesting an early inflammatory signal is required to activate endogenous stem cell-dependent cardiomyocyte replenishment. In addition, treating the mice with the COX-2 downstream product prostaglandin E2 (PGE2) not only improved efficiency of stem cell-driven cardiomyocyte replenishment but also rescued indomethacin-mediated inhibition of cell regeneration. Administration of PGi2, another COX-2 product, had no such effects. Quantitative PCR analysis of stem cell marker expression revealed that Sca-1 level changed in response to PGE2, in which its level elevated after treatment on day 3 post-MI. Immunostaining showed presence of the Sca-1+ cells co-expressing mature cardiomyocyte marker cardiac troponin T (cTnT) at the injured site or border zone of the MI heart. Flow cytometric analysis indicated that the Sca-1+ cells expressed Cre and became GFP+ on day 3 post-MI. The number of Sca-1+/cTnT+ cells and the percentage of Sca-1+/GFP+ cells were found to increase upon PGE2 treatment on day 3 after MI. In addition, these cells were not generated as a consequence of cell fusion. To examine if PGE2 directly acted on the Sca-1+ cells, the cells were sorted and quantitative PCR was performed to analyze expression of the PGE2 receptors EP1/EP4 on day 3 after MI. We observed that PGE2 not only induced expression of the EP2 and EP4 receptors but also the level of cardiac progenitor cell marker Nkx-2.5 in the Sca-1+ cells. Moreover, PGE2 treatment stabilized β-catenine protein level in these cells. In vitro culturing, PGE2 treatment promoted cTnT expression of the Sca-1+ cells. These findings suggest that PGE2 may directly act on the Sca-1+ cells via EP2/4 receptors to modulate β-catenine pathway for cardiomyocyte differentiation. Taken together, we demonstrate that the early inflammatory modulator PGE2 promotes cardiomyocyte differentiation from cardiac Sca-1+ cells, and this may be translated for clinical application.

LEUKEMIA INHIBITORY FACTOR ADMINISTRATION ENHANCES ENDOGENOUS CARDIOMYOCYTE REGENERATION AFTER MYOCARDIAL INFARCTION BY ACTIVATING CARDIAC SIDE POPULATION CELLS: A GENETIC FATE-MAPPING STUDY

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Cardiac stem or precursor cells have the potential to regenerate cardiomyocytes, but their contribution to regeneration and their role in the efficacy of cardioprotective drugs remain controversial. We have previously reported the ability of cardiac side population (SP) cells to differentiate into cardiomyocytes after being transplanted. In this study, using a genetic fate-mapping model, we show that leukemia inhibitory factor (LIF) influences SP cell proliferation and stimulates endogenous cardiomyocyte replenishment after myocardial infarction (MI). We generated MerCreMer-LacZ mice, in which more than 99.9% of the cardiomyocytes in the left ventricular field were positive for 5-bromo-4-chloro-3-indolyl-β-D-galactoside (Xgal) immediately after tamoxifen injection. Thus, every Xgal-negative cardiomyocyte was derived from a stem or precursor cell after tamoxifen administration. During normal aging spanning 1 year, the number of Xgal-negative cardiomyocytes in all the mice did not change significantly: 9.8 ± 3.8 and 9.8 ± 5.2 cells per section at 2 weeks and 1 year respectively. However, at 3 months after MI, the MI mice had more Xgal-negative cells than the control mice (57.0 ± 12.0 and 3.0 ± 2.6 cells per section respectively; P < 0.01). Xgal-negative cardiomyocytes significantly co-localized with vessels within the MI area and were smaller than Xgal-positive cells (median: 51 vs. 151 μm2). The part of SP cells were shown to be label-retaining cells (LRCs), that differentiated into Xgal-negative cardiomyocytes after MI. To determine which factors induced stem-cell derived
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**THE ORIGIN OF DIVIDING CELLS FOR HEAD REGENERATION IN THE HEMICHORDATE, PHTYCODERA FLAVA**

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When the body of the marine acorn worm, P. flava, is transected, both pieces regrow the lost parts. The posterior segment grows a new head at its anterior site. The wound heals in about 2 days and a blastema is evident at the anterior site by 3 days. By 5 or 6 days the blastema takes the shape of a nascent head and in about two weeks grows to full size. The source of the cells and the nature of the biological processes that form the new head in P. flava have not been studied extensively. In hydra and planaria, animals know for extensive regenerative capacity, there is a population of stem cells which are activated and mobilized to form the regenerate. In another classical model of regeneration, limb regeneration in vertebrate amphibians, the blastema cells seem to be recruited from the differentiated tissue, possibly from adult tissue stem cells or from dedifferentiation of tissue cells in the various tissues of the transected limb stub. Using BrdU immunofluorescence cytometry to mark dividing cells, we examined the source of cells that produce the regenerate in P. flava. We transected the worms just posterior to the gill basket, let regeneration proceed for various times and then labeled with BrdU for 6 hours. Before cutting the animal, there is a considerable population of cells along the gut epithelium, especially in the liver region of the animal, that incorporate BrdU. This population of dividing gut cells does not seem to change in number or character during regeneration. There are some BrdU labeled cells scattered in other regions of the animal’s body. Some of these occur in the epidermal epithelium and along the basement membrane under this epithelium. It is this population of cells associated with the epidermal epithelium that increases with regeneration. By day 3 after the cut, there are abundant BrdU labeled cells associated with the epidermis within a few millimeters of the cut, both in the epithelium itself and under the basement membrane of the epithelium. This population of cells is more abundant nearer the site of the cut and in the dorsal epithelium compared to the ventral epithelium. By day 3 there is a clear blastema and a large proportion of the cells in the blastema incorporate BrdU during the labeling period. Labeling of cells under the basement membrane, in the epithelium near the regenerating blastema and in the regenerate itself continues for about two weeks as the new head is differentiated. During all these processes, there is no indication of remodelling of the original tissue behind the regenerating blastema. These data suggest that there is a population of cells associated with the worm epidermis that is activated to divide and mobilized to form the blastema and the regenerate Supported by the Pacific Biosciences Research Center Biomedical Fund (UHF#12-209-04).

Poster Board Number: T-2310

**HEDGEHOG SIGNALING PATHWAY ACTIVATION IN THE HEMATOPOIETIC NICHE STROMAL CELLS**

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The Hedgehog (Hh) signaling pathway plays key regulatory roles during development, adult tissue homeostasis and cancer. Recent studies further identified the importance of Hh signaling during regenerative proliferation of stem cells in response to tissue injury. However, the mechanistic basis of pathway activities within certain specific cell types in their native microenvironment remains largely unknown. Hematopoietic stem cells (HSCs) require stromal support for their maintenance at steady state and during injury-induced regeneration. Several developmental conserved pathways are implicated in the maintenance of hematopoietic cells and their cell fate decisions. Importantly, interactions between the hematopoietic cells and their microenvironment in terms of specific regulatory pathways are still poorly characterized. Here we investigate the role of Hh signaling pathway in hematopoietic stem/progenitor cell niches. Current evidence suggests a model where multiple types of stromal cells are responsible for the maintenance of hematopoietic cells. Using genetic reporters for Hh pathway activation in conjunction with surface markers that can distinguish different subsets of skeletal progenitor cells, we found activation of Hh pathway in specific lineages of cells isolated from postnatal bone tissue. In addition, activation of the Hh pathway diverted cell fate decisions in the stem/progenitor cells during their differentiation process. Since these subsets of stromal cells exhibit differential ability to support hematopoietic stem and progenitor cells, our results implicated important roles of Hh pathway in regulating hematopoietic niches. We will also present our investigations on Hh pathway activities during normal tissue maintenance and injury induced hematopoietic regeneration.

Poster Board Number: T-2311

**EMBRYONIC TRANSCRIPTION FACTOR UPREGULATION DURING NORMAL LACTATION AND BREAST ONCOGENESIS**

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Mammary stem cells (MaSCs) play important physiological roles during pregnancy and lactation in the female mammary gland. In the resting breast, MaSCs are believed to be in a quiescent state and are represented in scarce numbers. However, during...
pregnancy and lactation these cells become activated in response to hormonally-driven cues, and differentiate into two distinct populations, the basal myoepithelial and the luminal cells. Recent evidence suggests that MaSCs are primary targets of malignant transformation giving rise to aggressive triple negative breast carcinomas. The molecular determinants that drive aberrant self-renewal in these cells are largely unknown. Progress has been limited because of our lack of understanding of normal MaSC biology and the absence of cell culture model systems to propagate and characterize these cells. To discover early determinants of transformation in MaSCs, we used human breastmilk as a novel, non-invasive, and patient-specific source of MaSCs in the activated mammary gland. A multipotent MaSC population was identified in breastmilk, expressing the pluripotency transcription factors (TFs) Oct4, Sox2 and Nanog, known to maintain self-renewal in human embryonic stem cells (hESCs). Upon expansion in feeder cultures, milk-derived MaSCs exhibited the encapsulated ES-like colony morphology. These colonies could be passaged in secondary feeder cultures suggesting clonogenicity and self-renewal. In vitro differentiation assays demonstrated the potential of these cells to give rise to cell lineages from all three germ layers. Propagation of milk-derived MaSCs in spheroid assays resulted in a dramatic up-regulation of TFs known to form an integral part of the complex self-renewal circuitry of hESCs. In contrast, these TFs were not expressed in the resting epithelium and in cultures derived from resting mammoplasties. In accordance with this model, forced ectopic expression of Oct4 in cells from the resting epithelium resulted in aberrant expansion of MaSCs possessing multi-lineage potential and displaying tumor initiating features. Oct4 and targets of Oct4 were examined by IHC and IF across breast cancers and were found enriched in breast cancers associated with pregnancy and lactation. These findings suggest that disruption of controlled expression of these TFs during pregnancy and lactation, failure to silence them during involution, and/or their aberrant upregulation in the resting breast can be at the origin and progression of aggressive breast carcinomas. Our data suggest that milk-derived MaSCs can be used as a non-invasive, personalized source of MaSCs to study molecular determinants of breast cancer.

Poster Board Number: T-2312

BREASTMILK IS A NOVEL SOURCE OF STEM CELLS FROM THE LACTATING BREAST WITH MULTI-LINEAGE DIFFERENTIATION POTENTIAL

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The mammary gland is a dynamic organ that undergoes repeated cycles of proliferation, differentiation and apoptosis in response to hormonal signals initiated during pregnancy and lactation. This unique ability relates to the presence of stem cells in a specialised niche of the mammary gland. Whilst these cells have mostly been studied in mice and in the context of the resting gland, unique insights may be gained by the study of the lactating gland in which these cells are activated and exist in larger numbers, with emphasis on the human gland, for which our knowledge is very limited. We non-invasively accessed stem cells from the human lactating mammary gland via freshly expressed breastmilk. We performed an ex vivo analysis of the cellular populations present in breastmilk using phenotypic and functional stem cell and differentiation markers for various lineages. This analysis resulted in the identification of candidate stem cell and progenitor populations in breastmilk. Importantly, a cell population was identified expressing the pluripotency-associated embryonic stem cell markers Oct-4, Sox-2, Nanog, SSEA-4, Tra-1-60 and Tra-1-81. We then localised the identified cell populations in the human lactating mammary gland, using paraffin-embedded sections of normal human lactating tissues. Culturing breastmilk cells in embryonic stem cell and differentiation media, in feeder-free 2D conditions, using feeders, and in 3D conditions, revealed the potential for both self-renewal/ clonogenicity and differentiation into cell types originating from all three germ layers. The data obtained from our ex vivo, in situ and in vitro analyses were synthesised into a new cellular hierarchy proposed for the human mammary gland. Moreover, for the first time it is shown that the human lactating gland harbours a stem cell population with embryonic-like stem cell features, which can be easily accessed via breastmilk. Future work is focusing on further characterisation of these cells for applications in regenerative medicine and breast cancer research, as well as for elucidating their role(s) for the breastfed infant.

Poster Board Number: T-2313

HUMAN BONE MARROW STROMAL CELLS SHOW RESISTANCE TO HIGH CALCIUM ENVIRONMENT IN VITRO

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Endosteal niche micro-environment within bone marrow is known to have extremely high concentration of calcium, which reaches as high as 40mM locally near resorbing osteoclasts. Because human bone marrow stromal cells (hBMSCs) reside in the environment of high calcium concentration, we expected that hBMSCs might have more resistance to high calcium concentration. We examined the effect of various concentrations of calcium on the survival of hBMSCs compared to human dermal fibroblasts (HDF) as a control. Although both of the cells showed decline in the cell number in high calcium media, we found the viability of hBMSCs is significantly higher than that of HDFs cultured in the media containing from 3 to 30mM concentration of calcium by MIT assay. When proportion of apoptotic cells was measured by FACS analysis of propidium iodide incorporated fraction of cells, proportion of the apoptotic cells increased as the calcium concentration elevated which indicated that the declined cell number in the high calcium media was due to cell apoptosis. While high calcium media severely induced growth arrest of HDFs, this tendency was weakly shown in BMSCs and rather, growth was accelerated by 3–5 mM calcium concentration in BMSCs. These results indicate that hBMSCs are resistant to high calcium concentration and may explain their survival mechanism in endosteal niche environment.

Poster Board Number: T-2314

BANK OF NORMAL AND SPECIFIC MUTATION-CARRYING HUMAN EMBRYONIC STEM CELL LINES

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The Assisted Conception Unit and Centre for Preimplantation Genetic Diagnosis (PGD) at Guy’s Hospital in London has the largest and most successful PGD program in the UK, performing around 200 cycles each year. We are currently performing PGD for more than 60 conditions in our unit. Clinically unsuitable embryos, if donated for research by consented couples, were used to derive specific mutation-carrying human embryonic stem (hES) cell lines. The success rate for derivation using fresh post-PGD embryos now
sits at between 40-50%, which is a significant improvement on our original 20%, and higher than most quoted efficiencies in the literature. We now have derived multiple hES cell lines including these with mutations linked to cystic fibrosis, Huntington’s disease, Von Hippel-Lindau syndrome, Wiskott-Aldrich syndrome, myotonic dystrophy type 1, neurofibromatosis type 1, spinal muscular dystrophy and beta thalassemia (www.kcl.ac.uk/medicine/research/divisions/wh/groups/medicine/hescell.aspx). These specific mutation-carrying hES cells can be differentiated into any cell type that displays pathology of the disease and used in drug discovery for screening of new compounds and testing of unacceptable side effects. We also have a number of research and clinical grade normal hES cell lines and our goal is to make these biological tools accessible worldwide to everyone, from researchers to commercial end users.

**Poster Board Number: T-2315**

**RECOVERY INCREASED BY SIMPLE IMPROVEMENT OF THE CONVENTIONAL CRYOPRESERVATION METHOD FOR THE HUMAN ES AND IPS CELLS**

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Human embryonic stem cells (hESCs) and human induced pluripotent cells (hiPSCs) have great potential as a source of cells for regenerative medicine and also for other research applications, such as developmental biology, toxicology and drug discovery. Recently, a number of hESCs and hiPSCs have been established in the world. Although most of the procedures share many of the principles of routine cell culture, a number of significant steps exist in the case of hESCs and hiPSCs, such as the cryopreservation. Both hESCs and hiPSCs have been well-known to be sensitive to the cryopreservation. At this moment, vitrification method has been considered to be preferable for the local storage of hESCs and hiPSCs. However, vitrification method requires practiced hand because cells can be terribly damaged by failure of rapid-warming. Further, if the low temperature is not strictly maintained, cell viability is rapidly decreased. Therefore, when vitrified cell transported, it needs LN2 dry-shippers with careful documentation for the regulatory requirements, resulting in the high costs involved. On the other hand, the conventional slow-freezing method using dimethylsulfoxide (DMSO) yield poor results for hESCs and hiPSCs, such as low levels of recovery or spontaneously differentiation, although it has been long trusted as a stable cryopreservation for a wide variety of cells including mouse ES cells. Therefore, we improved the conventional slow-freezing cryopreservation using 10% DMSO by simply methods. This method requires neither specific equipment nor reagents and results in the high levels of growing cell numbers and undifferentiation states. Because the conventional slow-freezing cryopreservation is quite familiar, this simple method would gain widespread acceptance.

**Poster Board Number: T-2316**

**THE HISTONE DEMETHYLASE UTX REGULATE CELL ADEHSIN DURING DIFFERENITATION OF HUMAN EMBRYONAL CARCINOMA STEM CELLS INTO NEURON**

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JmjC domain-containing UTX and Jmjd3 are a histone H3K27 demethylase and demethylate trimethylated lys27 on H3. H3K27me3 is a chromatin modification that is repressed gene expression and is key factor for cell fate regulation. To determine the global gene expression changes after neural differentiation with reduction of similar enzyme either of UTX or Jmjd3, we employed a DNA microarray analyses to analyze the differences between the gene expression patterns of among embryonic carcinoma cells transfected by lentiviral vectors with amiUTX (artificial miRNA targeting the UTX), amiJmjd3 (artificial miRNA targeting the Jmjd3), or amiNC (normal control), respectively. After neural differentiation (for 4 weeks), differentiated cells were confirmed by immunocytochemistry. Both of amiNC and amiUTX transfected cells were differentiated into neuronal cells with typical neuronal markers such as β-tubulin type III. We observed that cells transfected amiUTX increases NCAM-mediated adhesion during neural differentiation. Both of UTX and Jmjd3 are a histone H3K27me3 demethylase, however they regulate different target genes during neural differentiation. Reduction of UTX gene expression promotes cell adhesion and differentiation human embryonal carcinoma stem cells into neuron.
Finding the Home of Human Trophoblast Progenitors

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Little is known about the progenitor cells that give rise to human trophoblast populations. Using immunohistochemistry on first trimester placental villi, we investigated where the niches for these trophoblast progenitors are located based on known mouse trophoblast stem cell markers. We found that staining for TCFAP2C (also known as AP-2γ) is concentrated at the base of cytotrophoblast cell columns. This correlates well with the staining pattern for Ki-67. Interestingly, there was unexplained widespread staining by anti-phosphorylated histone H3 in villous and extravillous trophoblast. GATA3 is expressed only in extravillous trophoblast and not in villous cytotrophoblast. Estrogen-related receptor beta (ERRβ) is expressed in most cells within the placenta. We provide further supporting evidence that there is a niche at the base of trophoblast cell columns where a progenitor population resides and where the cells are TCFAP2C, GATA3 and ERRβ positive.

Effect of Cyclophosphamide on Colony-Forming Progenitor Cells of Stromal Fibroblasts

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Hemopoiesis occurs in organs that provide the conditions for hemopoietic and stromal cell interaction. These conditions are formed by specific microenvironment that represents the system of cellular and humoral factors being essential for driving, maintaining and differentiating of stem hemopoietic cell and its descendants. Stromal fibroblasts are responsible for specific microenvironment transfer. Only these cells following reverse transplantation in vivo are known to form hemopoietic loci typical of the initial organ (Fridenstein A.J. et al., 1974). Multiple investigations revealed that properties and state of microenvironment could considerably change under different effects (irradiation, mechanical injuries, hemorrhages, and chemical factor action), that could be judged by the variation in the number of progenitor cells of stromal fibroblasts in bone marrow, and by the dynamics of their ability to form colonies. The aim of this work was the analysis of the alteration in the number of colony-forming progenitor cells of stromal fibroblasts in bone marrow and their clonogenic potential in response to the effect of cyclophosphamide, the preparation that is applied in clinical practice in therapy of many diseases, and particularly in anti-tumor therapy. There was used the method of cloning the stromal progenitor cells of bone marrow from male mice of CBA strain using monolayer cultures with a-MEM medium and 20% calf serum under specific gaseous environment and temperature; following the feeder adhesion the irradiated hemopoietic cells of bone marrow from guinea pigs were added. Mice were intraperitoneally pre-injected with cyclophosphamide in doses of 200 mg/kg or 275 mg/kg, after which bone marrow cells from experimental animals were explanted in cultures at different terms from 1 to 13 days since injection of preparation. Following fixation and staining after 10-12-d-cultivation the number of colonies was calculated in cultures when colonies comprised no less than 50 cells. Under the effect of cyclophosphamide the content of stromal clonogenic cells (CFU-F) in red bone marrow undergoes significant variation. After 12 h since injection of preparation their number reduces to 4% of the control value with dose of 200 mg/kg and up to 5% with dose of 275 mg/kg. In a day the amount of CFU-F increases by 12-fold with dose of 200 mg/kg and by 5-fold with dose of 275 mg/kg, CFU-F content is subjected to a secondary decrease on the 2nd day following the cyclophosphamide injection (up to 12% of control values with dose of 200 mg/kg and up to 3% with dose of 275 mg/kg). Only 7-9 days after the agent introduction the amount of CFU-F content in bone marrow approaches relatively constant level. As a whole, the dynamics of variation in CFU-F content in bone marrow after the cyclophosphamide injection is similar to the variation pattern in the number of these cells after the irradiation with low doses. However the cytostatic possesses more attenuated action on CFU-F as compared with irradiation, as cell regeneration proceeds faster after the cyclophosphamide injection. This work was supported by RFBR grant 11-04-96037r_ural_a and administrative body of Perm Region.

Registration of Human Embryonic Stem Cell Lines in Korea

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In an effort to increase the credibility of human embryonic stem cell lines established in Korea, an obligatory registration was introduced into the Bioethics and Safety Act 2008 and started from January 1, 2010. A total of 79 domestic human embryonic stem cell (hESC) lines were submitted to Korea Centers for Disease Control and Prevention (KCDC) for the registration until 2011. The submitted lines were characterized in the respects of DNA fingerprint, chromosome stability, expression of stem cell markers, and contamination of mycoplasma by KCDC. The characterization data and ethical aspects such as informed consents for donation of surplus embryos were reviewed by an advisory review board for stem cell registry. 59 hESC lines were registered until 2011. This registration has completed an oversight system for the embryo research by registering the products of licensed embryo research projects. The information about hESC lines is available at Korea Stem Cell Registry (kscr.nih.go.kr).
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Poster Board Number: T-2322

CHARACTERISTICS OF MULTIPOTENT STEM CELLS DERIVED FROM THE EPIBLAST AND ECTODERM OF POSTIMPLANTATION MOUSE EMBRYOS

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Among the self-renewing cells that express molecular markers of pluripotency, the epiblast stem cells (EpiSCs) have been derived from epiblast of postimplantation mouse embryo at stages prior to and during germ layer formation. It is not known at which advanced developmental stage that EpiSCs-like cells can still be derived from the epiblast or the ectoderm of the embryo, and if the characteristics of EpiSCs derived from embryos of different developmental stage may reflect those of the tissue of origin. In this study, epiblast or ectoderm from a wide range of developmental stages defined by stringent morphological criteria were tested in an identical culture condition for the ability to generate EpiSCs. Over 40 EpiSCs lines were derived from epiblast at two pre-gastrula stages: cavity and pre-streak, and at several gastrulation stages: early-streak, mid-streak, mid to late-streak, late-streak and at stages as advanced as from ectoderm of early bud and as neural plate stage. EpiSCs derived from all stages display similar morphology and expressed the pluripotency markers Oct4, Sox2 and Nanog. Transcriptome analysis revealed that all these lines show gene expression profiles comparable to established EpiSCs but different from those of embryonic stem cells. EpiSCs lines derived from similarly staged embryos are transcriptionally more closely related to each other than with those from other developmental stages. The lineage potential of the EpiSCs was tested by teratoma formation assay and in vitro differentiation as embryoid bodies (EBs). EpiSCs lines produced teratomas containing derivatives of all three germ layers, with a preponderance of neural cells and a minor representation of endoderm and mesoderm derived tissues. When grown as EBs in serum-containing medium, EpiSCs from later stages of development down-regulate pluripotency markers earlier than EpiSCs derived from cavity stage embryo and expeditiously express the differentiation markers Sox1, Brachyury, and Mixl1. EpiSCs from advanced embryo therefore are more poised for undertaking germ layer differentiation. Ongoing analysis comparing the transcriptome of EpiSCs and dissected epiblasts of corresponding stages will reveal the relationship between EpiSCs and the epiblast from which they were derived and shed further light on the mechanisms of pluripotency maintenance and lineage commitment.

Poster Board Number: T-2323

GENETIC AND EPIGENETIC INSTABILITIES OF SEX CHROMOSOMES IN PLURIPOTENT STEM CELLS

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Our aim is to study the genetic and epigenetic mechanism that regulate X and Y chromosome in human and mouse pluripotent stem cells. In mammalian females, X chromosome inactivation is a process in which one of the two X chromosomes is silenced, following Xist expression. Unlike somatic cells, mouse embryonic stem cells (mESCs) do not express Xist, and harbor two active X chromosomes. Mouse induced pluripotent stem cells (miPSCs) also show two active X chromosome, implying that X is reactivating during the reprogramming process. However, analysis of X inactivation in human pluripotent stem cells, mainly based on Xist expression, was not conclusive. Here, we studied X-inactivation in human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) by meta-analysis of the expression of the entire set of genes on the X chromosome in female pluripotent cell lines. Thus, we could divide the human cell lines into three categories: lines with no X-inactivation, lines with full X-inactivation, and lines with partial X-inactivation. The partial inactivation of the X chromosome always involved the middle of the chromosome, surrounding the Xist transcription site. The status of XCI in some of the cell lines was validated by either allelic specific expression or DNA methylation analysis. Based on our analysis we propose a model for the dynamics of XCI in pluripotent cells. In this model, pluripotent stem cells show three interchangeable states of XCI. Thus, while mouse pluripotent cells show a stable state of two active X chromosomes, the variations in human XCI might be created during the reprogramming process, and/or result from epigenetic changes in culture. We next decided to examine genetic changes in sex chromosomes. We have recently developed a tool based on gene expression arrays that enable a reliable analysis of aneuploidy in autosomes. However, evaluating the stability of sex chromosomes poses difficulties due to the small size of the Y chromosome and the variability in X inactivation. We have thus adjusted the methodology to rely on the expression of only few genes, and then analyzed the stability of X and Y chromosomes in pluripotent stem cells. Surprisingly, whereas Y chromosome loss was identified in 15-30% of mouse male cell lines, no evidence for this phenomenon was found in human pluripotent cells. Furthermore, by comparing aberrant and normal cell lines from the two species, we identified candidate genes which may contribute selective advantage to the aberrant cell lines. Finally, we use these genes to study the connection between the different processes of genetic and epigenetic stability.

Poster Board Number: T-2324

COMBINATION OF FEEDER CELLS GENERATES PROPER ENVIRONMENTAL NICHE FOR MOUSE HEMATOPOIETIC STEM CELLS

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A variety of in vitro culture conditions have now been described that permit expansion of hematopoietic stem cells (HSC). Yet, so far it has not been possible to expand HSCs ex vivo without losing their stemness properties. The ability of stem cells to self-renew and to differentiate into different types of mature cells depends on both their intrinsic genetic programs and external control from the special microenvironment or niche where they reside. In adult bone marrow, niche of hematopoietic stem cells is composed of many different cell type, including osteoblasts, adipocytes, stromal cells, and vascular endothelium cells. Osteoblasts are thought to provide factors that are crucial for the maintenance of the quiescent status of HSC, whereas BM stromal stem cell/fibroblasts produce a large variety cytokines that promote proliferation. In order to study how these signals are integrated and whether the balance between quiescent, self-renewal and differentiation can be achieved in long-term culture, we developed a special feeder system for HSC culture by mixing various amounts of genetically modified stromal cells with mesenchymal stem cells (MSC) before they were induced to form bone. We found that the system using stromal cells expressing BMP antagonist, noggin together with MSC, could reproducibly generate a pattern of thin osteoblast strips surround by stromal cells on the tissue culture disc. Compared to undifferentiated MSC, this feeder system (NSMB) produced higher level of Jagged-1, angiopoietin-1, wnt-3a, various extra cellular matrix proteins, as
well as BMP2, 4 and TGF-β. When lin-sca1+c-kit+ mouse HSC were cultured on NSMB they generated both cobble stone area and a large amount of differentiated cells. After one month in culture, cells cultured on NSMB generated 4 times higher number of LTC-IC than those grown on MSC. Interestingly, LSK HSC cultured on NSMB produced significant higher number of colony forming unit granulocyte, erythrocyte, macrophage and megakaryocyte (CFU-GEMM) than other feeders. Our results suggested that this feeder system might be able to promote cells proliferation as well as HSCs maintenance.

**Poster Board Number:** T-2325

THE ASYMMETRIC DIVISION OF DAMAGED PROTEINS IS STEM CELL-TYPE DEPENDENT

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An asymmetric division of damaged proteins during mitosis has been linked to the protection of one cell from aging in yeast and bacteria. Recent evidence suggests that stem cells may employ a similar mechanism; however, there is no in vivo evidence suggesting this occurs in healthy adult stem cells. We report that stem cells in the larval (neuroblast, NB) as well as adult (female germline, GSC and intestinal stem cell, ISC) Drosophila asymmetrically segregate damaged proteins, such as the difficult to degrade and age-associated 2,4-hydroxynonenal (HNE), and we propose that the stem cell niche is involved in this segregation. Surprisingly, we found that the ISC protects itself by segregating HNE to a differentiating progeny while the NB and GSCs retain HNE during asymmetric division compared to their progeny. The ISC has a high level of delta, the Notch ligand, and the differentiation of its daughter enteroblast, which expresses high levels of Notch, depends on the interaction between the stem cell and enteroblast. In a temperature sensitive Notch mutant (NTS1), clusters of delta-positive cells can arise when Notch signaling is lost between the ISC and enteroblast and only proliferation of ISC/enteroblast-like cells is seen. We found that in these clusters of poorly differentiated delta-positive cells, there was a variation in HNE intensities between cells. This suggests that the mechanism of HNE segregation remains intact when differentiation is disrupted. DE-Cadherin, involved in anchoring stem cells to their niche, colocalizes with HNE within the asymmetrically dividing cells of each Drosophila stem cell model. Furthermore, mechanical disruption of the stem cell niche and mutation of the extracellular domain of DE-cadherin abolishes HNE asymmetry in neuroblasts. Even when stem cell fate is disrupted in NTS1 intestinal stem cells, HNE remains colocalized with DE-Cadherin within cells of delta-positive cell clusters. We propose that the anchorage of damaged proteins to DE-cadherin is one mechanism that allows stem cells to segregate damaged proteins. Furthermore, we predict that this segregation will always be to the cell that has the shortest functional lifespan, as the divisions in all three stem cell populations were asymmetric with respect to cell fate, lifespan, mitotic activity and HNE, but in each case it was the cell with the shortest functional lifespan, regardless of mitotic activity or cell fate, that received the majority of damaged proteins during division.

**Poster Board Number:** T-2326

AMINO ACID DEPLETION CONTRIBUTES TO THE IMMUNE PRIVILEGE OF MOUSE EMBRYONIC STEM CELLS AND TISSUES DIFFERENTIATED FROM THEM

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We have shown previously that tissues derived from mouse embryonic stem cells (mESC) display a fragile form of immune privilege that secures their indefinite survival across a minor histocompatibility barrier. This form of immune privilege depends on the polarisation of infiltrating T cells towards an induced regulatory phenotype (iT₃). Given increasing evidence that depletion of essential amino acids (EAA) plays an important role in conferring on otherwise immunogenic tissues protection from immune responses, we investigated whether amino acid catabolism might determine the immunological properties of ESC. We have shown that a number of candidate enzymes responsible for depleting EAAs are expressed at elevated levels by mESC and are responsible for the active depletion of lysine, threonine and valine from the culture medium. Furthermore, medium conditioned by mESC inhibited T cell proliferation and biased the polarisation of CD4+ T cells towards a FoxP3+ iT₃ phenotype in vitro, similar to control medium in which lysine, threonine and valine were limiting. In order to investigate whether depletion of any one of these EAAs might confer immune privilege, we generated mESC deficient in the enzymatic function of cytosolic branched chain aminotransferase (Bcat-1), an enzyme responsible for the catabolism of branched chain amino acids including valine. Our results show that expression of Bcat-1 alone is not sufficient to confer immune privilege in vitro and in vivo, but likely works collaboratively with other enzymes to create a protective microenvironment. Indeed, we were able to show redundantly at the level of the cytokine milieu by demonstrating the active secretion of TGF-β by mESC, the neutralisation of which partially inhibited iT₃ induction. Our data therefore suggest that mESC employ multiple mechanisms to establish a form of acquired immune privilege, including the depletion of EAAs.

**Poster Board Number:** T-2327

OLIGODENDROCYTE PRECURSOR CELL DIFFERENTIATION INDUCING FACTORS AS A REGENERATIVE THERAPY FOR MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is an autoimmune disease characterized by demyelination of axons and subsequent neuronal dysfunction. MS is primarily mediated by T-lymphocytes, which triggers inflammatory processes targeting myelin-producing oligodendrocytes (OLs), causing lesions in the myelin sheath, eventually leading to impaired neuronal conduction. Modulating the pathological immune response is the primary focus of current therapeutic approaches for the treatment of MS. While these treatments are moderately effective in the early stages of MS, none have proven to modify the course of primary progressive or relapsing–remitting MS. In the central nervous system, a widespread proliferating population of nerve/glial antigen-2 (NG2), platelet-derived growth
factor receptor (PDGFRα) positive adult stem cells, termed NG2-glia or oligodendrocyte precursor cells (OPCs), are the major source of newly formed mature OLs required for remyelination. Remission in MS is largely dependent upon OPCs migrating to sites of injury, and subsequently differentiating to a mature cell fate capable of repair. However, even with immune suppression, progressive phases of MS are associated with inhibited differentiation of OPCs, wherein OPCs migrate to lesions in the spinal cord, but either fail to differentiate or form immature OLs that do not produce myelin. As such, the identification of drug-like small molecules that selectively induce differentiation of OPCs into mature myelin producing OLs at sites of demyelinated lesions may provide an effective alternative strategy of enhancing remyelination as a treatment for MS. We developed a High Throughput-High Content Imaging based screening platform with the expression of myelin basic protein (MBP) as a readout, to rapidly identify molecules that induce the differentiation of rat primary OPCs into functional myelinating OLs. This platform was used to screen large libraries of drug-like small molecules, natural products and other pharmacologically active agents, and led to the identification of several classes of novel compounds that induce robust differentiation of OPCs in vitro. Amongst these classes of effective compounds was BA404 (EC50 ~350 nM), which showed excellent brain exposure properties and in vivo safety profile. BA404 showed promising preliminary in vivo efficacy in pre-clinical animal models for MS. Evidence derived from in vitro and in vivo T-lymphocyte assays with BA404 indicates that the observed efficacy of this compound results from an enhancement of remyelination rather than immune suppression. Detailed in vivo characterization as well as mechanism of action studies with BA404 are currently in progress. Identified molecules from other classes, possessing suitable drug-like properties are also being evaluated using in vitro and in vivo models for MS and could serve as potential candidates for a regenerative therapy for MS. In addition, pharmacological and mechanistic studies using these novel regulators of OPC differentiation could serve to elucidate new pathways and targets regulating the remyelination process. These molecules that harness endogenous adult stem cells (OPCs) to effectively regenerate the myelin sheath in vivo, could have a significant impact on the development of clinical combination regimens with existing immuno-modulatory drugs for the treatment of MS and other demyelination related diseases.

**Poster Board Number: T-2328**

**CHARACTERIZATION OF RAT CARTILAGE-DERIVED PROGENITOR CELLS ISOLATED AT DIFFERENT DEVELOPMENTAL STAGES.**

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Introduction: Fetal cartilage-derived progenitor cells (FCPCs) are a promising cell source for cartilage tissue engineering because they have high abilities for both cell proliferation and chondrogenesis. However, FCPCs have limitations that they are not clearly identified and characterized yet particularly depending on the developmental stages. This study focused on comparing FCPCs at gestation ages of 14 (mesenchymal cell condensation stage), 16 (chondrocyte maturation and hypertrophy stage) and 20 (separation of cartilage growth regions, vascular invasion, and initiation of both cortical and trabecular bone stage) days in terms of their stemness and chondrogenic potential. Materials and methods: The cells isolated from rat fetal cartilage in gestation ages 14, 16, and 20 days were compared with adult rat MSCs and chondrocytes.
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PROCESS AND REGULATION OF STEM CELLS

Pluripotent stem cells are key players in developmental biology, and are also an urgent topic for potential medical applications. Some of the basic questions concerning such systems-level understanding include: What characteristics in a cellular state distinguish multipotent stem cells from differentiated cells? How are developmental processes robust to molecular noise in spite of their complexity? Following the progress in the analysis of the dynamics of gene expression and the structure of gene regulatory networks, the time is ripe to answer the above questions to unveil nature of differentiation from stem cells. In this study, using a dynamical system modeling cell approach, we performed simulations of the developmental process using all possible topologies of gene regulatory networks with a few genes, and screened those that could generate cell type diversity through cell-cell interactions. We found that stem cells that both proliferated and differentiated always exhibited oscillatory expression dynamics, and the differentiation frequency of such stem cells was regulated, resulting in a robust number distribution. Moreover, we uncovered the common regulatory motifs for stem cell differentiation, in which a combination of regulatory motifs that generated oscillatory expression dynamics and stabilized distinct cellular states played an essential role. Based on the result of computer simulations, we propose a hypothesis for the mechanism of stem cell differentiation and its regulation to maintain robust developmental processes, in which the expression levels of some genes in multipotent stem cells exhibit temporal oscillation, and itinerate over several sub-states. As development progresses, each of these quasi-stable sub-states is modified and stabilized, leading to differentiated cell types. This temporal oscillation in expressions introduces variety in state changes and maintains regulated differentiation from stem cells, resulting in a robust number distribution of cells of different types. Importantly, this hypothesis can explain the roles and mechanism of the recently observed dynamic heterogeneity [1-4] and oscillatory behavior [5] in cellular states of stem cells, and it can predict the regulatory motifs responsible for the dynamic differentiation process. Based on the comparison between experimental observations and the results of computer simulations, we make several predictions on a network structure and cellular state to provide stemness. These discussions promote a system-level understanding of multicellular development and provide a basis for clinical application of stem cells. [1] Toyooka Y, et al. (2008) Identification and characterization of subpopulations in undifferentiated ES cell culture. Development 135(5): 909-918. [2] Chambers I, et al. (2007) Nanog safeguards pluripotency and mediates germline development. Nature 450(7173): 1230-1234. [3] Hayashi K, et al. (2008) Dynamic equilibrium and heterogeneity of mouse pluripotent stem cells with distinct functional and epigenetic states. Cell Stem Cell 3: 391-401. [4] Chang HH, et al. (2008) Transcriptome-wide noise controls lineage choice in mammalian progenitor cells. Nature 453: 544-548. [5] Kobayashi T, et al. (2009) The cyclic gene Hes1 contributes to diverse differentiation responses of embryonic stem cells. Genes and Dev 23(16): 1870-1875.

Poster Board Number: T-2331

TEMPORAL OSCILLATION IN PROTEIN EXPRESSIONS GENERATES ROBUST DIFFERENTIATION DYNAMICS OF STEM CELLS

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A systems-level understanding of cell differentiation from multipotent stem cells to committed cell types is essential to the advance in developmental biology, and is also an urgent topic for potential medical applications. Some of the basic questions concerning such systems-level understanding include: What characteristics in a cellular state distinguish multi-potent stem cells from differentiated cells? How are developmental processes robust to molecular noise in spite of their complexity? Following the progress in the analysis of the dynamics of gene expression and the structure of gene regulatory networks, the time is ripe to answer the above questions to unveil nature of differentiation from stem cells. In this study, using a dynamical system modeling cell approach, we performed simulations of the developmental process using all possible topologies of gene regulatory networks with a few genes, and screened those that could generate cell type diversity through cell-cell interactions. We found that stem cells that both proliferated and differentiated always exhibited oscillatory expression dynamics, and the differentiation frequency of such stem cells was regulated, resulting in a robust number distribution. Moreover, we uncovered the common regulatory motifs for stem cell differentiation, in which a combination of regulatory motifs that generated oscillatory expression dynamics and stabilized distinct cellular states played an essential role. Based on the result of computer simulations, we propose a hypothesis for the mechanism of stem cell differentiation and its regulation to maintain robust developmental processes, in which the expression levels of some genes in multipotent stem cells exhibit temporal oscillation, and itinerate over several sub-states. As development progresses, each of these quasi-stable sub-states is modified and stabilized, leading to differentiated cell types. This temporal oscillation in expressions introduces variety in state changes and maintains regulated differentiation from stem cells, resulting in a robust number distribution of cells of different types. Importantly, this hypothesis can explain the roles and mechanism of the recently observed dynamic heterogeneity [1-4] and oscillatory behavior [5] in cellular states of stem cells, and it can predict the regulatory motifs responsible for the dynamic differentiation process. Based on the comparison between experimental observations and the results of computer simulations, we make several predictions on a network structure and cellular state to provide stemness. These discussions promote a system-level understanding of multicellular development and provide a basis for clinical application of stem cells. [1] Toyooka Y, et al. (2008) Identification and characterization of subpopulations in undifferentiated ES cell culture. Development 135(5): 909-918. [2] Chambers I, et al. (2007) Nanog safeguards pluripotency and mediates germline development. Nature 450(7173): 1230-1234. [3] Hayashi K, et al. (2008) Dynamic equilibrium and heterogeneity of mouse pluripotent stem cells with distinct functional and epigenetic states. Cell Stem Cell 3: 391-401. [4] Chang HH, et al. (2008) Transcriptome-wide noise controls lineage choice in mammalian progenitor cells. Nature 453: 544-548. [5] Kobayashi T, et al. (2009) The cyclic gene Hes1 contributes to diverse differentiation responses of embryonic stem cells. Genes and Dev 23(16): 1870-1875.

Poster Board Number: T-2332

GENES INVOLVED IN PROLIFERATION OF PLANARIAN PLURIPOTENT STEM CELLS

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Planarians have remarkable regenerative ability. Planarians can regenerate their whole functional body from a tiny fragment within one week. This regenerative ability of planarians absolutely depends on a unique population of pluripotent stem cells called neoblasts. Neoblasts are the only cell population possessing ability to proliferate and to differentiate into all types of cells in planarians. Therefore, neoblasts play an important role in regeneration after artificial amputation or fission and tissue homeostasis. Fission is the means by which asexual reproduction normally occurs in planarians: it consists of division into two fragments dependent on body growth. Fission occurs in the pre- or post- pharyngeal region when planarians grow to a sufficient size (about 8-9 mm) in rich nutrient conditions after feeding. After feeding, transient acceleration of neoblast proliferation is observed. This temporary increase of proliferation of the neoblasts is called a mitotic burst, and a mitotic burst is also induced during regeneration. After this mitotic burst, neoblast proliferation returns to the normal rate in one week but the molecular mechanism which causes the increase and restoration of neoblast proliferation still remains unclear. In order to understand the mechanism that controls the acceleration of neoblast proliferation after feeding and amputation, we tested some candidate neoblast-specific genes obtained by the HiCEP method. One of the genes, Djp2x-A (a Dugesia japonica ionotropic ATP receptor, a P2X homologue), was expressed in about half of the neoblasts. Intriguingly, RNAi of Djp2x-A showed that RNAi planarians enhanced their fission frequency at a threshold body size in the nutrient-rich condition. We found that expression of Djp2x-A was negatively correlated with mitotic burst after feeding, suggesting that Djp2x-A is involved in restoring the neoblast proliferation state after feeding. Indeed, we revealed that mitotic burst after feeding was increased by RNAi of Djp2x-A. A similar result was obtained in Plac8 (Placenta specific gene8 homolog ; a candidate that was also obtained by the HiCEP method) knock-down planarians. RNAi of Plac8 enhanced fission frequency at a threshold body size, like RNAi of Djp2x-A. In addition to this, we could observe similar results in expression dynamics of Plac8 after feeding with that of Djp2x-A, suggesting that Plac8 has similar function to Djp2x-A in modulating neoblast proliferation after feeding. In planarians regenerating after artificial amputation, the expressions of Djp2x-A and Plac8 disappeared from the neoblasts located in the vicinity of the wound where mitotic burst of
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the neoblasts was induced. These observations and results indicate that multiple factors regulate the rate of stem cell proliferation in both homeostasis and regeneration in planarians. Here we show the function of DpFXA and Placo8 in proliferation of the neoblasts and discuss the relation between these genes and growth and regeneration.

Poster Board Number: T-2333
IDENTIFICATION OF CRITICAL MiRNAs INVOLVED IN HUMAN MSC MOTILITY AND CELL GROWTH BY RNA-SEQ TECHNOLOGY
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Mesenchymal stem cells (MSCs) found in bone marrow (BM-MSCs) are an attractive source in regenerative medicine. MSCs can also be obtained from other postnatal, perinatal or fetal tissues, yet stem cells isolated from different origins express distinct biological activities, such as differentiation abilities. We recently showed that MSCs from Whatort's jelly of umbilical cord (WJ-MSCs) are more primitive and similar to embryonic stem cells, while BM-MSCs were more efficient in osteogenic and adipogenic differentiation. On the other hand, WJ-MSCs expressed less genes involved in immune response (such as CXCL12 and HLADR) and have a higher immunosuppressive effect over all organotypic macrophages, indicating WJ-MSCs being a better choice in allogeneic transplantation (Hsieh et al., 2010).

In this following study we explored other biological differences between these 2 MSCs and applied RNA-seq technology for understanding the underlying mechanisms. We found that BM-MSCs possess higher motility than WJ-MSCs, while WJ-MSCs proliferated better. These differences may be the outcome of higher NF-kB activity in WJ-MSCs than in BM-MSCs. To understand the molecular mechanisms of these phenotypes, we compared the transcriptome profiles between BM- and WJ-MSCs using miRNA-seq. We identified hundreds of miRNAs differentially expressed in BM-MSCs and WJ-MSCs, and miR-146a contributes in MSC motility and proliferation ability through targeting CXCL12 and SIKE1, respectively. Knockdown miR-146a down-regulated NF-kB activity, and NF-kB activity can be partially recovered by double knockdown SIKE1 in WJ-MSCs. Novel miRNAs which have never been deposited in databases were also found from our RNA-seq data and could be verified by RT-PCR and RNA-IP with AGO2 antibody. Our results provide the different biological properties of two kinds of MSCs from different sources, as well as the underlying regulatory miRNAs and genetic networks, thereby providing a better basis for cell-based therapy and the following mechanistic studies on MSCs biology.

Poster Board Number: T-2334
CARDIAC MITOCHONDRIAL INTEGRITY REGULATED BY CR6 INTERACTING FACTOR 1
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Background and Objectives: The major cause of metabolic syndrome and diabetes is reduced cellular performances in fuel metabolism, but the underlying pathways and mechanisms are not completely understood. Dysregulation of energy homeostasis can lead to metabolic disturbances and it predispose diabetes, cardiovascular disease, aging and cancer. CR6-interacting factor (CRIF1) contacting coiled-coil domain that is required for both genomic stability and mitochondrial integrity. We performed this study to determine the role of CRIF1 on the mice hearts. Methods and results: CRIF1-deficient mouse was embryonic lethal, we made heart specific CRIF1-deficient mouse using Cre-loxP system. We made thoracotomy and directly injected adeno-Cre virus into the heart of CRIF1 loxp mice. Betagal virus was used as a control. Serial echocardiography showed decreased left ventricular ejection fraction and fractional shortening in the CRIF1-deficient mice at four and seven weeks later compared to wild type mice (p<0.05). H&E showed increased myocardial inflammation in the CRIF1-deficient mice. TUNEL staining and LC3 staining showed increased apoptosis and autophagy in CRIF1-deficient mice compared with wild type(p<0.01). Electrophorecopy revealed that the mitochondria in CRIF1-deficient cardiomyocytes showed abnormal morphogenesis. For example, the cells showed excessively fragmented mitochondria and intracriatal swelling, thinning of myocardial fiber. The stability of mitochondrial complexes in CRIF1-deficient cells showed marked derangements. Conclusion: CRIF1 is required for maintenance of normal mitochondrial function and modulate apoptosis and autophagy in heart.

Poster Board Number: T-2335
PLACENTA-DERIVED MULTIPOTENT CELLS (PDMCs) INDUCE FUNCTIONAL CD4⁺CD25⁺FOXP3⁺ T REGULATORY LYMPHOCYTES
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Recent advances in stem cell research have brought new hope for regenerative medicine through cell therapy. However, immunological rejection is a major concern unless transplantation is autologous, which would severely limit prevalent use of stem cell products. Interestingly, one population of adult stem cells (ASCs), the mesenchymal stem cells (MSCs), has been found to possess immunosuppressive properties which allow for allogenic use. However, these ASCs are rare, with numbers decreasing with age. Placenta-derived multipotent cells (PDMCs) share many properties with adult MSCs, including multilineage differentiation and immunomodulatory capacity. Additionally, these fetal-stage progenitors possess embryonic stem cell surface markers and are exponentially more proliferative than adult MSCs, making PDMCs ideal for clinical applications requiring large cell volumes. Since T regulatory cells (Tregs) are one of the most important immunomodulatory effectors of the immune system, we explored whether the suppressive effects of PDMCs are mediated by this population of lymphocytes. We found that in mixed-lymphocyte reactions in which PDMCs are added as 3rd party cells, leukocyte cell division and proliferation can be suppressed. Co-culture of PDMCs with stimulated allogeneic peripheral blood leukocytes (PBL) also resulted in a shift away from a Th1 axis to a Th2 axis. This was correlated with an increase in IL-10 secretion as well as numbers of CD4⁺CD25⁺FOXP3⁺ Tregs. The numbers of Tregs increased over time as a fraction of total lymphocytes, and this can be sustained out to 8 days. These PDMC-induced CD4⁺CD25⁺FOXP3⁺ Tregs are Foxp3⁺, non-dividing, non-proliferating lymphocytes, and functionally are able to suppress stimulated lymphocyte proliferation in a dose-dependent fashion. These findings along with multilineage differentiation capacity, and the ease of isolation/expansion may render PDMCs as an attractive source for cell therapy.
Poster Board Number: T-2336

SALL1 HAS DUAL FUNCTIONS IN THE KIDNEY DEVELOPMENT.

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Objectives;
The kidney develops through the reciprocal interaction between the ureteric bud and the metanephric mesenchyme. The metanephric mesenchyme surrounding the ureteric bud, called the cap mesenchyme, is characterized by the elevated expression of the transcriptional factor, Six2 (Sine oculis homeobox homolog 2). The cap mesenchyme represents the self-renewing progenitors, which eventually differentiate into the nephron, the basic structural and functional unit of the kidney. In the mouse embryonic kidney, both the cap mesenchyme and differentiating cells express the transcriptional factor Sall1 (Sal-like 1). Sall1 is a protein which has multiple double-zinc finger motifs. In Sall1-null mice, the ureteric bud failed to invade the metanephric mesenchyme, resulting in kidney agenesis. In cultured cells, Sall1 binds to co-repressor proteins, nucleosome remodeling and deacetylase corepressor complex (NuRD), and represses gene expression. However, the precise mechanism on how Sall1 regulates kidney development has not been clarified. Here we investigated the role of Sall1 in the mouse embryonic kidney. Methods; Results; To examine the requirement for Sall1 in the cap mesenchyme, we generated Sall1 floxed mice and intercrossed these mice to Six2-GFP-Cre mice. Six2-GFP-Cre:Sall1flox/flox mouse embryos showed reduced kidney size. The embryonic kidneys also showed decreased Six2 expression and poor maintenance of the mesenchyme structure. These results indicate that Sall1 plays a critical role in the maintenance of nephron progenitors in the cap mesenchyme. To reveal the details of Sall1-mediated regulatory mechanism, we generated a Flag-tagged Sall1 knock-in mouse line. Co-immunoprecipitation analysis using Sall1-Flag mice kidneys showed that Sall1 was bound not only to the NuRD complex components, HDAC2 and MIZ8, but also to the transcriptional coactivating proteins, CBP and p300. As Sall1 binds to these two types of transcriptional co-regulators which have opposite roles, Sall1 could function both as a repressor and as an activator in the kidney development. Conclusions;Sall1 is essential for the maintenance of the cap mesenchyme in the developing kidney. Our results also showed that Sall1 binds to an activator and a repressor complex. As Sall1 is expressed not only in the cap mesenchyme but also in differentiating cells, Sall1 could function differently in these two populations, thereby maintaining the immature nephron progenitors.

Poster Board Number: T-2337

PRINCIPLES & POLYCENTRICISM: DESIGNING CROSS-BORDER REGULATORY SYSTEMS FOR BIOTECHNOLOGY - THE CASE STUDY OF STEM CELL RESEARCH.

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The problem of how to regulate emerging biotechnologies is a permanent issue on the modern political agenda. The rate of scientific advancement in fields such as, synthetic biology, assisted reproduction, enhancement, genomics and stem cell research, is such that there is renewed interest in governance mechanisms. This paper critically assesses the regulatory fit between principles-based, polycentric regulatory regimes (one strand of the new governance movement within contemporary regulatory theory) and biotechnology, using stem cell research as a case study. (Although each area of biotechnology presents unique issues, I contend that there are regulatory concerns that cut across the entire field.) I ultimately advance an argument in favour of this regulatory approach to biotechnology, considering along the way, issues of legality and the interface between ethics and regulatory design. Biotechnology - specifically, stem cell research - is an international, often collaborative sector; scientific work travels and translates across borders. Equally importantly, the ethical issues provoked by stem cell research are universal. Although national governments may compete to capitalize on scientific innovation, a number of international research initiatives have arisen in the field of stem cell research. This paper looks specifically at the international dimension of regulation. I argue that given the competing interests of parties (governmental and non-governmental institutions), the inescapable ethical challenges this research presents, and the rate of scientific progress, a polycentric, principles-based regime is the most appropriate and sustainable form of regulation in this field. The great attraction principles based regulation (PBR) holds for the field of stem cell research - indeed all biotechnologies - is its flexibility it affords both the regulator and regulatee. Given the speed of scientific advance - which by far outstrips the speed of the legislative, litigation, or political process - flexibility is key to effective, on-going regulation of current research activity that is also ethically defensible. PBR as an adaptive mechanism will, I submit, go some way towards addressing the regulatory ‘lag’ that often occurs in traditional, command and control systems of regulating biotechnologies. Underlying international collaborations in stem cell research are a myriad motivations and tensions. For regulators, balancing these interests whilst maintaining authoritative credibility is a challenge. I argue that a polycentric, principles-based regime is structurally secure form of regulation as it allows for the degree of pluralism often demanded in this field; principles afford flexibility, polycentricism disperses the task of regulation to the appropriate regulatory organization(s). Moreover, part of the strength of the argument in favour of international, polycentric, PBR is that the process of developing useable principles across regulators facilitates dialogue between different interest groups and regions/nations - which is arguably in itself, desirable.

Embryonic Stem Cell Pluripotency

Poster Board Number: T-3001

A HIGH QUALITY SERUM-FREE, FEEDER-FREE MEDIUM FOR HUMAN EMBRYONIC STEM CELLS (HESC) AND INDUCED PLURIPOTENT STEM CELLS (IPSC)

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The conventional method to culture human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) has been on irradiated murine embryonic fibroblasts (iMEFs) as feeder supported cultures or in iMEF conditioned medium. However, feeder supported cultures are time consuming, labor intensive and exhibit variability in cell performance. In addition, the morphology and cell growth in a feeder supported cultures are different. In order for researchers to enable effective pluripotent stem cell cultures, it is essential to have feeder free culture conditions with a culture medium that readily promotes adaptation of cells, promotes proliferation and maintains the pluripotent state of stem cells (PSCs) with
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Consistency and reproducibility. Here, we report a new PSC feeder free medium that supports the expansion of hESCs and iPSCs with an ideal cellular morphology of compact colonies and high nuclear to cytoplasmic ratio. This feeder free culture medium has been further validated with different iPSC lines and shown to support robust proliferation for several passages with normal karyotype. This medium further supported the generation of iPSCs with the non-integrating CytoTune™-iPS Sendai Reprogramming Kit under serum-free, feeder-free conditions on a GelTrex™ coated matrix. The hESCs and iPSCs cultured or derived in this medium express normal pluripotency markers and differentiated to ectoderm, endoderm and mesodermal lineages via embryoid body generation. This culture medium enables feeder free cultures with ease and robustness.

Poster Board Number: T-3002

**X CHROMOSOME INACTIVATION IN HUMAN EMBRYONIC STEM CELLS: ALL OR NOTHING!**

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Introduction: Human embryonic stem cells (hESC) are often considered as an in-vitro model for human development and disease modelling, as well as a promising cell source for regenerative medicine. X inactivation, necessitating thorough characterization and monitoring of genetic and epigenetic (in)stability in hESC to ensure their quality. X chromosome inactivation (XCI), an example of epigenetic gene regulation, ensures dosage compensation of the X chromosome in female cells. After completion of XCI, the two X chromosomes in female cells can be distinguished by differential transcription of the XIST gene, DNA methylation, and epigenetic chromatin modifications. In this study we aimed to investigate the XCI pattern through analysis of DNA methylation in undifferentiated female VUB hESC lines at different time points during long-term culture. Materials and methods: hESC were cultured on inactivated mouse embryonic fibroblasts in standard SR-medium with bFGF addition, in 5% CO2 and atmospheric O2 concentrations. Undifferentiated hESC were collected at day 4 to 6 after passaging, using collagenase. DNA was extracted by phenol-chloroform. To determine if X-inactivation was present, whether it was random or skewed and to identify the parental origin of the inactivated X (whenever DNA samples of the donors are available e.g. lines derived from PGD embryos), we applied methylation-sensitive restriction by HpaII and PCR for the polyadenylation site located in the XIST gene, DNA methylation, and epigenetic chromatin modifications. We examined the RNA expression of 17 hESC lines derived at the VUB, sixteen female lines and one male line as control. This male hESC line showed one PCR fragment for control DNA and absence of any fragment after restriction, proving the presence of an unmethylated locus on the X chromosome. Of the 16 female hESC lines, 4 lines (25%) displayed 2 active X chromosomes, even at later passages (up to P50). The remaining 12 lines all displayed XCI, however, with different patterns. Only two lines (13%) displayed the expected random XCI pattern at different passages, while nine lines (56%) showed a completely skewed XCI pattern. One line (6%) was not informative and although this line clearly shows XCI, we are not able to determine a random or skewed pattern. The lines with skewed XCI displayed this pattern already at early passages, e.g. P3 or P10. We were able to identify the active X chromosome as derived from the female donor in 4 lines, while in 4 other lines the active X was derived from the male donor. For one line, no DNA from the donors was available (VUB07). Discussion: It has already been shown that XCI in hESC is highly variable. This data set, however, is the first to show the extent of preferential inactivation of a specific X chromosome in hESC. In fact, only 13% of the hESC lines in this study displayed a random XCI pattern, while most lines had either no or skewed XCI. The presence and correct patterning of XCI in undifferentiated and certainly in differentiated hESC might be crucial for their applicability as in-vitro model for human development and even more as cell source for regenerative medicine. Therefore, in-depth studies on the effects of skewed XCI patterns on differentiation capacity and efficiency should be performed. Moreover, it would be of great interest to explore whether XCI skewing is a random event or whether it originates due to a selective advantage.

Poster Board Number: T-3003

**ELABELA IN HUMAN EMBRYONIC STEM CELL PLURIPOTENCY AND DIFFERENTIATION.**

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Based on expression profiling data we identified a novel gene, ELABELA (ELA), which is highly expressed in human embryonic stem cells (hESCs). To investigate the function of this gene we manipulated its expression levels in hESCs and derived over-expressing induced pluripotent stem cells (iPSCs) lines. Heterozygous ELA knock-out clones express lower levels of pluripotency markers than control hESCs and many markers of meso-endoderm are up-regulated. Cultures of iPSCs with elevated levels of ELA express higher levels of the pluripotency genes than controls, or ELA knock-out lines, and demonstrate increased proliferation. Although embryoid bodies generated from the ELA over-expressing iPSCs express genes representative of each cell lineage, extraembryonic markers are detected at a higher level than those in control iPSCs. These data suggest ELA plays a role in the regulation of pluripotency and differentiation in hESCs and in the balance between induction of definitive and extraembryonic endoderm.

Poster Board Number: T-3004

**PROTEIN KINASE C INHIBITOR PROMOTES CONDENSED ORGANIZATION OF COLONIES FROM ENZYMATIC-DISSOCIATED SINGLE HUMAN EMBRYONIC STEM CELLS**

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Human pluripotent stem cells (hPSCs) self-renew indefinitely as highly organized pluripotent colonies. Unlike mouse pluripotent stem cell colonies, human colonies form a uniform, flat epithelium-like monolayer. Interestingly, it has been reported that colony morphology is closely correlated with the maintenance of pluripotency. However, the molecular mechanisms that underlie human pluripotent colony formation and organization are poorly understood. In this study, using real-time imaging tools, we examine in vitro colony formation by enzymatically dissociated single hESCs under feeder-free conditions. We showed that colony formation consists of 4 stages: attachment, migration, colony formation (aggregation), and colony organization. Moreover, we found that blocking PKC signaling with isoenzyme-specific PKC inhibitors results in enhanced cell-cell interactions and plays an integral role in promoting the survival of hESCs in culture. Using various visualization methods, we identified conditions required for colony formation, and we suggested a promising new target for modulating hESC colony formation and organization. These results are likely to be useful for engineering hPSCs to further study the mechanisms involved in pluripotency.
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Poster Board Number: T-3005
ESSENTIAL ROLES FOR CULTURE CONDITIONS IN GENOMIC INSTABILITY AND CELL PHENOTYPE CHANGES OF HUMAN PLURIPOTENT STEM CELLS IN SHORT- AND MID-TERM IN VITRO CULTURE
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Human pluripotent stem cells (PSC) can self-renew in culture and have the potential to differentiate into all adult cell types. Therefore, PSC might provide in vitro models for human development and genetic disease and a source of cells for regenerative medicine. PSC can be obtained either from discarded embryos, i.e. embryonic stem (ES) cells, or by reprogramming differentiated cells into induced pluripotent stem (iPS) cells. However, recent evidence suggests that PSC might present genetic abnormalities that arise during cell culture or cell reprogramming and that are a serious concern for their use in in vitro or clinical applications. Here, we report that alterations of features of hESC could be induced even in short-term or mid-term in vitro culture, but firmly associated to the culture condition. In this report, three hESC lines (HD291, HD129 and H5306) at early passage were cultured in KO-SR, KO-DMEM and bFGF medium using one of two different passaging methods: (i) “single cell” passaging combined with ROCK-kinase inhibition or (ii) mechanical passaging (“cut and paste”). During the culture, the genome integrity and transcriptome of the cell lines was monitored by karyotyping, Affymetrix SNP6 DNA microarrays and mRNA sequencing. Morphology, cell proliferation, colonogenicity and expression of pluripotency markers were regularly monitored. Our data showed that HD291 cell line acquired quickly the copy number variation (CNV) changes under the “single cell” passaging, while the change of CNV in mechanical passaging was much less rapid. The same observation was also obtained for chromosome aberrations: HD291 cell line acquired a trisomy of chromosome 12 at passage 30 when using single cell passaging. The cells that were mechanically passaged showed a stable karyotype in this time frame. The genome alterations observed under single cell passaging were associated with cell phenotype changes such as an increased cell population growth rate, increased pluripotency marker expression, acquired colonogenicity etc., while no significant change was observed in the culture under mechanical passaging, even after 45 passages. Data acquisition for HD129 and H5306 is underway. Our data document for the first time the dynamic of the genome alterations observed under single cell passaging, but firmly associated to the culture condition. The aneuploid hESC lines represent an excellent model to study human chromosomal abnormalities especially in the early stages of development.

Poster Board Number: T-3006
WNT-DEPENDENT AND FGF/TGFB-INDEPENDENT HUMAN PLURIPOTENT STEM CELL RENEWAL
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Decision of self-renewal or differentiation in stem cells in vitro culture and in vivo is controlled by extrinsic factors such as signalizing molecules. The extrinsic factors regulating human pluripotent stem cell (iPS cells or ES cells) self-renewal and early differentiation events seem to differ from mouse pluripotent stem cell and to date are incompletely understood, but activation of bFGF and TGFβ/activin/Nodal signaling form the cornerstone of most systems for human ES cell propagation. The Wnt/β-catenin signaling pathway plays an important role in mouse ES cell self-renewal in LIF-independent culture but it is dispensable in LIF-dependent culture. In human pluripotent stem cells, the role of Wnt/β-catenin signaling is still poorly understood and controversial because of the dichotomous behavior of Wnt/β-catenin signaling in proliferation and differentiation. While investigating small molecule chemical compounds that could segregate the dual role of Wnt signaling, we have identified a compound that could modulate Wnt/β-catenin signaling pathway and support Wnt-induced human ES cell self-renewal without affecting differentiation. Utilizing Wnt and the compound, we have developed a novel and simple chemically defined xenofree culture system that allows for long-term expansion of human pluripotent stem cells without FGF or TGFβ supplementation. Cells in this culture can self-renew even with FGFR or ALK inhibitors. These culture conditions do not include xenobiotic supplements, serum, serum replacement or albumin. Using this culture system, we have shown that several human pluripotent cell lines maintained pluripotency (>20 passages) and a normal karyotype, and still retained the ability to differentiate into derivatives of all three germ layers. This Wnt-dependent and bFGF/TGFB-independent culture system would provide a platform for complete replacement of growth factors with chemical compounds.

Poster Board Number: T-3007
THE IN VITRO SURVIVAL OF HUMAN MONOSOMIES AND TRISOMIES AS EMBRYONIC STEM CELLS
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Chromosomal aneuploidies are responsible for severe human genetic diseases. Aiming at creating models for such disorders, we have generated human embryonic stem cell (hESC) lines from pre-implantation genetic screened (PGS) embryos. The overall analysis of more than 400 aneuploid PGS embryos showed similar occurrence of monosomy or trisomy in any specific chromosome. However, the generation of hESCs from these embryos revealed a clear bias against monosomy in autosomes, while only specific trisomies showed a high chance of survival as hESC lines, enabling us to present another categorization of human aneuploidies. The spectrum of aneuploidies in these hESC lines reflects the range of common embryonic chromosomal aberrations and significantly differs from the spectrum of aneuploid hESC lines generated by cell adaptation in culture. The aneuploid hESC lines represent an excellent model to study human chromosomal abnormalities especially in the early stages of development.
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**Poster Board Number: T-3008**

**POTENTIAL ROLE FOR HYPOXIA INDUCIBLE FACTOR IN HPSC DIFFERENTIATION**


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Human embryonic stem cells (hESCs) originate from the hypoxic microenvironment of the blastocyst inner cell mass. Hypoxia inducible factors (HIFs) constitute a family of labile transcription factors that regulate gene expression in response to hypoxia. HIFs are stabilized in low oxygen and are degraded by prolyl hydroxylation and subsequent proteolysis with increased intracellular oxygen levels. Recently, it was shown that HIF2a increases OCT 4 transcription to promote self-renewal in human pluripotent stem cells. Also, the loss of HIF1a, HIF2a and HIFa (ARNT) impair placentation, heart development, and endochondrial bone formation in knockout mice. These opposing roles for HIF activity generate a paradox in which HIFs seem to promote pluripotency but could also promote differentiation in distinct cellular contexts. To investigate this seeming paradox, we examined the role of HIF1a-mediated gene transactivation during early lineage non-specific differentiation under normoxic and hypoxic conditions. HIF1a was required for the induction of embryonic trigrimer layer and trophoblast gene expression in both conditions. In contrast, ectopic HIF1a expression alone was insufficient to initiate spontaneous differentiation. Therefore, HIFs appear to have a dual role in hESC physiology: they prevent differentiation of self-renewing hESCs but can also promote hESC differentiation after its induction.

**Poster Board Number: T-3009**

**COREGULATORS OF PRDM14 IN HUMAN EMBRYONIC STEM CELLS**

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PRDM14, a PR domain-containing transcriptional regulator, was identified to be one of the major determinants of the human embryonic stem cells (ESC) identity in a genome wide RNAi screen. PRDM14 was shown to regulate Oct4 activity in the human ESC and therefore safeguards the pluripotency of the human ESC. On the other hand, previous studies have highlighted potential repressive role of PRDM14 in human ESC as the ectopic expression of PRDM14 represses the upregulation of lineage genes in embryoid bodies. To fully understand how PRDM14 can potentially play activating and repressive role in the human ESC, we perform biochemical assays to identified potential PRDM14 interactors. We found that PRDM14 interacts with the core pluripotency factor Nanog in the human ESC and its binding to active genes are regulated by Nanog binding activity.

**Poster Board Number: T-3010**

**INVESTIGATING THE EFFECTS OF EXTRACELLULAR MATRIX MOLECULES ON THE REGULATION OF HESC PLURIPOTENCY.**

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Human embryonic stem cells are pluripotent cells that have indefinite replicative potential and ability to differentiate into derivatives of all three germ layers. HESCs are conventionally grown on mitotically inactivated mouse embryonic fibroblasts and some alternative feeder types of human origin have been used to culture hESCs. However, there are also supportive culture systems for hESCs lacking feeder cells, suggesting that extracellular matrix-stem cell interactions might contribute to the regulation of self-renewal or differentiation. Using defined feeder-free systems, we investigated the effects of receptor binding by blocking the integrin engagement to fibronectin and vitronectin. We have shown that hESCs express all the machinery for integrin signalling and blocking αvβ5 and β1 integrins led to a slight decrease in pluripotency marker expression. Moreover, selective chemical inhibition of Src which is a receptor-independent signalling intermediate molecule, also resulted in a decrease in pluripotency marker expression. Thus this study further illuminates the role that ECM interactions play in the hESCs phenotype which has until recently been a neglected area of hESCs biology.

**Poster Board Number: T-3011**

**MICRORNAS REGULATE P21WAF1/CIP1 PROTEIN EXPRESSION AND THE DNA DAMAGE RESPONSE IN HUMAN EMBRYONIC STEM CELLS (HESCS).**

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Introduction: Studies of hESCs commonly describe the non-functional p53-p21 axis of the G1/S checkpoint pathway with subsequent relevance for DNA damage response (DDR). This is likely due to the absence of p21 protein in hESCs, which was repeatedly described, but never explained. Recently, microRNAs (miRNAs) were identified as crucial post-transcriptional regulators of gene expression and were directly linked with physiology of hESCs. Curiously, whether miRNAs play a role in the DDR and regulation of p53-p21 pathway in hESCs has not been studied thus far. Results: To address this question we used UVC irradiation (3 J/m2) as a DNA damaging agent in undifferentiated hESCs. The expression of miRNAs was analyzed at three different time points after UVC irradiation (0, 4, 8, and 16 hrs) using miRNA microarrays (LNA, EMBLI). We identified 100 miRNAs (17.9%) whose expression increased (>1.5-fold) upon UVC irradiation (data were verified by qRT-PCR). Interestingly, 22% (22/100) of miRNAs upregulated upon UVC irradiation in hESCs are putative regulators of p21 mRNA as predicted by TargetScan (www.targetscan.org). Observed abundance of up-regulated miRNAs that are predicted to target p21 suggest their role in its regulation. We next tested the expression of p21 protein and other p53-downstream targets in UVC treated hESCs. Classical p53-downstream
genes Bax, GADD45 and EGR1 were activated in hESCs upon DNA damage, but p21 protein was undetectable before or after UVC irradiation. Importantly, p21 mRNA was up-regulated in hESCs upon UVC (27-fold increase) indicating a major post-transcriptional regulation of p21 mRNA. Therefore, to directly address involvement of miRNA-mediated p21 metabolism, we co-transfected hESCs with shRNAs against key players in miRNA biogenesis, proteins Aragonaute2 (Ago2) and Dicer1 (Dic1). Results show that p21 protein is clearly detectable in hESCs with silenced Ago2 and Dic1 proteins while key pluripotency regulators Oct4, Nanog and Sox2 remained unaffected. Finally, to identify individual miRNAs involved in p21 regulation, we used miRNA microarrays to compare hESCs with their differentiated counterparts that show normal p21 expression (hESC-derived neural precursor cells). This approach revealed that several miRNAs upregulated after DNA damage in hESCs are also expressed specifically in this cell type. In particular we identified members of the miRNA family miR-302 (miR-302s) that were also previously linked to the maintenance of stemness in hESCs. To test if miR-302s function as post-transcriptional regulators of p21 protein expression, we co-transfected undifferentiated hESCs with silenced miRNA pathway with miR-302s miRNA mimics. Importantly, mature miRNAs of the miR-302 family repeatedly rescued by about 30% the effect of global inhibition of miRNA synthesis on the level of p21 protein (p<0.05). We thus conclude that members of the miRNA family miR-302 are specific regulators of p21 protein in undifferentiated hESCs. Conclusion: The described mechanism explains the lack of p21 protein in hESCs before and after the DDR and elucidates the role of miRNAs in regulation of one of the most important molecular pathways governing the G1/S transition and cell cycle checkpoint in undifferentiated hESCs. Supported by IGA MZCR NT11218-6/2010, MSM0021622430 and by European Regional Development Fund (FNUSA-ICRC, No. CZ.1.05/1.1.00/02.0123)

Poster Board Number: T-3012

OVERCROWDING AND STARVATION: CULTURE DENSITY AND DNA DAMAGE IN HUMAN EMBRYONIC STEM CELLS.

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Worldwide research has uncovered genomic instability during long-term culture in human Embryonic Stem Cells (hESCs), casting doubt on the safety of these cells in cell therapy and the reproducibility of experimental results. An improved understanding of the effect of culture conditions on hESCs might contribute to the preservation of genomic integrity, and thus safeguard their scientific and clinical value. In high-density cultures, fewer nutrients are present per cell, possibly leading to a shortage of substrates for nucleotide synthesis. A lack of nucleotides could then lead to stalled replication forks, causing an increase in double-stranded DNA breaks (DSBs), and to intra-and inter-chromosomal rearrangements as a result of mis-repair. To test this hypothesis, we plated 3 different hESC lines (VUB07, VUB14 and VUB31) in 4 different densities increasing from 2 ± 0.7 colonies per cm² to semi-confluency. We measured a larger decrease in aspartate and glutamine (both essential for nucleotide synthesis) concentration in media of dense cultures (preliminary data), and a more than five-fold increase of lactate concentration up to 14.8 ± 2.2 mmol/L, which is described to have negative effects on the hESCs metabolism. We have also found a significant increase in the mean number of γH2AX foci per cell, the earliest marker for DSBs, and a 3-fold relative increase in the fraction of cells with more than 30 foci in the dense condition compared to the least dense. In addition, we demonstrated a 110% increase in DNA fragmentation as shown by a single cell gel electrophoresis (COMET) assay, and preliminary data suggests an increase of more than 50% of the incidence of segmental chromosomal aberrations as shown by FISH. Our preliminary data suggest a correlation between culture density and the occurrence of DNA breaks and segmental aberrations in hESC cultures, although we still have to perform functional experiments to determine which factors are decisive for the observed effects. Nevertheless, bearing in mind that we find these substantial differences after 1 passage of 5 days, the long-term effect of culture density could have a strong impact on the genetic stability of hESC cultures.

Poster Board Number: T-3012

THE ROLE OF ABCG2 MULTIDROG TRANSPORTER DURING STRESS CONDITIONS IN HUMAN EMBRYONIC STEM CELL LINE

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The ABCG2 multidrug transporter is known to function as an important mechanism limiting cellular accumulation of various compounds. ABCG2 is widely distributed in normal tissues, moreover, it has also been indicated that ABCG2 confers the SP cell phenotype both in human and mouse progenitors and tumor cells. These findings indicate that ABCG2 expression is a conserved feature of tissue stem cells. Earlier we have shown a heterogeneous expression of ABCG2 in various pluripotent human embryonic stem cell (hESC) lines. Although the role of ABCG2 protein in evolution of multidrug resistant phenotype is well studied, the data about the substrates and function of this protein under normal physiological conditions is still unresolved. In this study we investigated ABCG2 expression in hESC cultures during different stress conditions. We examined the response of these cells to toxic effects caused by mitoxantrone or UV light exposure, measuring cell death and ABCG2 expression by flow cytometry. When the cells were exposed to these treatments causing partial cell death, after 72 hours we could not detect an induction of the overall ABCG2 expression but most of the surviving cells were those expressing ABCG2. When we studied the effects of moderate oxidative stress conditions by using low concentration of H2O2 (150 µM), cell death was negligible and after 72 hours ABCG2 expression was unchanged. However, at the early time points (3-6 hours) of H2O2 treatment the internalization of ABCG2 protein from the plasma membrane was observed by immunostaining and confocal microscopy. This internalization effect was reversible after 24 hours. In light of these results we suggest that a dynamic balance of ABCG2 expression at the population level has an advantage to promptly respond to changes in the cellular environment. Such an actively maintained heterogeneity of ABCG2 expression might be evolutionary favorable to protect valuable sanctuaries such as embryonic stem cells.
LAMININ-521 PROVIDES AN EASY, BIOLOGICALLY RELEVANT, AND RELIABLE METHOD TO CULTURE HUMAN PLURIPOTENT STEM CELLS COST-EFFECTIVELY

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Human pluripotent stem cells (PSCs) are promising cell sources in the clinical development of regenerative medicine for disorders such as spinal cord injuries, type I diabetes, cardiovascular- and Parkinson’s disease. One of the major hinders for creating stem cell-based regenerative therapy has been the many technical problems associated with the basic culturing of PSCs in a manner acceptable for regulatory authorities. Culturing PSCs such as embryonic stem cells (ESCs) has been considered difficult, labor-intensive and highly variable depending on subjective measures. Current coating methods usually involve undefined feeders or mouse tumor protein extract BD Matrigel™ Matrix on which PSCs grow as heterogeneous colonies with spontaneous differentiation contaminating the cultures. There are a few defined and animal protein-free substrate alternatives such as Synthemax™ surface and CELLstart™ matrix, which together with media such as TeSR™2 medium generates a culture system devoid of animal proteins and with defined components.

A possible solution to these problems is the use of proteins in the extracellular matrix that have a major role in vivo cell niches. Even though there are many different proteins in the basement membrane, the laminins are the only ones expressed tissue-specifically. Laminins are large heterotrimeric glycoproteins situated in the basement membrane in the extracellular matrix surrounding all tissues and organs in the body. They are important in cellular processes such as proliferation, migration and differentiation. There are 16 different isoforms and data indicate that different cell types prefer different laminins. Recently, it has been shown that when culturing human PSCs on human recombinant laminin-511, the cells grow as a homogenous monolayer, which makes experimental data more reproducible. In addition, the human PSCs on laminin-511 grow with many available xeno-free media such as NutriStem™ XF/FF, RegES™ and TeSR™2. Most commonly though human PSC cultures are still passaged by cutting the colonies into pieces. The plating of human ESCs as single cells after passaging requires the addition of an inhibitor of apoptosis such as ROCK. The human ESCs on laminin-511 grow with many available xeno-free media such as NutriStem™ XF/FF, RegES™ and TeSR™2.

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Here we show that upon cultivation of human ESCs on laminin-512 cells proliferate significantly faster compared to cultures on either Synthemax™ surface or BD Matrigel™ Matrix. They can be passaged and re-plated as single-cells using enzymatic detachment of cells with similar survival without addition of ROCK inhibitor compared to control. The human ESCs on laminin-512 even survive low single-cell plating density and grow as a homogenous monolayer without almost any spontaneous differentiation. One possible explanation to the effect laminin-521 has on cultivated human PSCs is that it is one of the first proteins expressed already by the 4-cell stage embryo and, thus, supports pluripotency in human ESCs by activating important cell signaling pathways. In conclusion, laminin-521 allows for simple enzymatic single-cell passaging, without the need for extensive training, and with a much higher expansion rate than other alternatives, saving both time and money by e.g. reduced medium costs. Laminin-521 is a biologically and regulatory superior cell culture substrate that can help make stem cell therapy a future reality.

CHARACTERIZATION OF XCI IN A HESC LINE DERIVED UNDER PHYSIOLOGICAL LEVELS OF OXYGEN

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Murine embryonic stem cells (mESC) have been successfully used as a model for understanding molecular events in embryo development, particularly the initiation of X chromosome inactivation (XCI). Nevertheless, many different lines of female human ESC (hESC) have shown some degree of XCI even when in their undifferentiated state. Silva et al. (PNAS, 105(12): 4820-5, 2008) classified female hESC lines into three different categories: class I - lines that exhibit two active X chromosomes (resembling female mESCs) and are capable of inactivating one X chromosome upon differentiation; class II - lines that show a consistent number of XIST RNA positive cells even when in their undifferentiated state, suggestive that XCI has already been completed; and class III - lines that show some degree of XCI but had lost the ability of expressing XIST even when differentiated. The great majority of the hESC lines derived to date fall into class II and Class III categories, and many authors suggested that this could be attributed to culture conditions, especially the way these cells are handled and the level of stress to which they are submitted. However, in a recent work, Lengner et al. (Cell, 141(5): 872-83, 2010) showed that the use of physiological levels of oxygen (5% O2) during the derivation and maintenance of hESC is critical in preserving the pre-XCI state of hESCs. Here we describe the establishment of a new line of hESC in 5% O2, and the characterization of its state of XCI. This line, named BR5, was derived from a surplus embryo generated for reproduction purposes in an IVF clinic, and was donated for research under informed consent. The whole blastocyst was plated on a MEF cell layer, and since then was kept under hypoxia conditions. After few passages, the cells were transferred to matrigel and mTeSR1. The undifferentiated cells expressed pluripotency markers, and were able to differentiate into cell types that are representative of the three embryonic germ layers both in vitro and in vivo. The status of XCI in these cells was verified by XIST RNA-FISH, and by real time RT-PCR for XIST and some X-linked genes that are submitted to inactivation. Our results demonstrate that approximately 20% of both undifferentiated and differentiated cells express XIST, as evidenced by RNA-FISH and real-time RT-PCR. Additionally, accumulation of H3K27me3 (histone H3 lysine 27 trimethylation, a hallmark of XCI) was observed in nearly 100% of both undifferentiated and differentiated cells. Therefore, the BR-5 cells are apparently in the transition of class II to class III. Our data indicates that the establishment and maintenance of hESCs in 5% O2 is not sufficient to keep the cells in a pre-XCI state.
cell-based therapy because of their self-renewal and pluripotent properties. For hESC expansion, many researchers have followed conventional technique using feeder cells despite feeder free culture technique. Classical studies demonstrated that gap junctional intercellular communication (GJIC) is thought to play an important role in hESC survival and differentiation. Indeed, it has been reported that Connexin 43 (Cx43, one of the major gap junction protein) are crucial in maintaining the pluripotency of hESCs. However, the role of GJIC between hESC and feeder cells is unclear and has not been reported. In this study, we examined whether the direct interaction through Cx43 between hESCs and feeder cells influences on the maintenance of hESC stemness. Until 20 passages, the hESCs cultured on Cx43 down-regulated feeder cells sustained normal morphology, proliferation and stemness showing pluripotent markers (AP activity, Oct4, Sox2, Nanog expression). These results demonstrate the direct interaction through Cx43 between hESCs and feeder cells would not be an important factor to maintain cell growth and stemness of hESC. Keywords: hESC expansion, Feeder cells, Gap junction, Connexin 43, hESC stemness

Poster Board Number: T-3017

SMALL MOLECULE-ASSISTED; LINE-INDEPENDENT MAINTENANCE OF HUMAN PLURIPOTENT STEM CELLS IN DEFINED CONDITIONS

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Human pluripotent stem cells (hPSCs) are conventionally grown in a mouse feeder cell-dependent manner. Chemically defined culture conditions are, however, desirable not only for potential medically oriented applications but also for investigating mechanisms of self-renewal and differentiation. In light of the rather high complexity and cost of existing defined hPSC culture systems, we have systematically evaluated over 20 potential media ingredients. Only components that reproducibly gave beneficial effects were ultimately combined to yield a simple and cost-effective formulation termed FTDA. This xeno-free medium is based on mimicking self-renewal factor activities present in mouse embryonic fibroblast-conditioned medium, with all factors applied at minimal dosages. Additionally, selected small molecules served to specifically suppress typical types of spontaneous differentiation seen in hPSC cultures. FTDA medium was suitable for the generation of human induced pluripotent stem cells and enabled robust long-term maintenance of diverse hPSC lines including hard-to-grow ones. Comparisons with existing defined media suggested reduced spontaneous differentiation rates in FTDA. Our results hence imply that using supportive factors at minimal concentrations may still promote robust self-renewal and preserve pluripotency of hPSCs. Moreover, by modifying the growth factor composition but maintaining its basal ingredients, directed differentiation along all three germ layers could selectively be initiated. Thus, FTDA not only presents a simple and cost-effective alternative for robust large-scale expansion of hPSCs but also a versatile media platform for studying mechanisms of self-renewal and differentiation.

Poster Board Number: T-3018

RE-ESTABLISHMENT OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATIONS DURING REPROGRAMMING TO PLURIPOTENCY AND DIFFERENTIATION

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Gap junctional intercellular communication (GJIC) has been described in embryonic stem cells (ESCs) and various somatic cells. GJIC has been implicated in the regulation of cell proliferation, self-renewal, and differentiation. Recently, a new type of pluripotent stem cells was generated by direct reprogramming of somatic cells. Here, for the first time, we show that during reprogramming events GJIC is dramatically reduced and re-established again upon reaching complete reprogramming. The opposite process of cell differentiation from the pluripotent state leads to the disruption of GJIC between pluripotent and differentiated cell subsets. However, GJIC is subsequently re-established de novo within each differentiated cell type, forming “communication compartments” within a histotype. Our results provide the important evidence that the presence of functional gap junctions is an additional physiological characteristic of somatic cell reprogramming to the pluripotent state and differentiation to the specific cell type.

Poster Board Number: T-3019

TRANSCRIPTIONAL REGULATION MEDIATED BY THE DNA REPAIR COMPLEX XPC-RAD23B-CTN2 IN MURINE EMBRYONIC STEM CELLS

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Embryonic stem cell (ESC) self-renewal and pluripotency are transcriptionally controlled by the “core” transcription factors Oct4, Sox2 and Nanog together with cofactors, chromatin regulators, non-coding RNAs and other terminal effectors of developmental signalling pathways. Identifying components of these complex circuitries and understanding their interplay provides the knowledge base to promote the efficacious deployment of ESCs and improves methods for induction and differentiation of pluripotent stem cells. We recently identified a new stem cell transcriptional coactivator (SCC) required for synergistic activation of the Nanog promoter by Oct4 and Sox2. Biochemical characterization of SCC revealed it to be the nucleotide excision repair complex Xpc-Rad23b-Ctn2, although the DNA-repair activity is not required for its transcription function. SCC interacts directly with Oct4 and Sox2 on their target genes, and its depletion compromises both ESC pluripotency and somatic cell reprogramming. To further dissect the transcriptional network orchestrated by the SCC complex in ESCs, we used chromatin immunoprecipitation followed by high-throughput sequencing to map regions bound by Rad23b in the mouse genome. Rad23b peaks are enriched around the transcription start site (TSS) of active and inactive genes, suggesting a role for SCC in both activation and repression of transcription. Around 60% of the high-confidence Rad23b binding sites co-localize with Oct4/Sox2-occupied regions, and these are predominantly at distal enhancer elements of active genes. Sequence analysis of Rad23b peaks confirmed that Oct4/
Sox2 recognition motifs are the most significantly enriched at these sites. Interestingly, the majority of genes targeted by Rad23b at core promoter proximal sites (±500bp around the TSS) are also marked by distal peaks, suggesting that SCC might participate in the crossstalk between distal enhancers and the basal transcription machinery. Intriguingly, a significant proportion of Rad23b binding sites are also co-occupied by Mediator and cohesin, two components thought to mediate gene activation by DNA-looping. A gene ontology classification of Rad23b targets revealed a weak over-representation of transcription regulators among the active genes, but a strong bias towards Polycomb-repressed developmental genes, regardless of Oct4/Sox2 association, hinting at Oct4/Sox2-independent functions for SCC/Rad23b. Indeed, overlap of Rad23b binding sites with other key ESC transcription factors (Stat3, c-Myc, n-Myc, Klf4, Zfx, E2f1, Tcfcp211, Essrb) showed variable degrees of co-occupancy. Thus our initial genome-wide analysis may have uncovered previously unrecognized and potentially widespread roles for SCC in coordinating ESC-specific transcriptional programs, both at repressed and active gene loci, while also laying the basis for a potential novel connection between DNA-repair and pluripotency.

**Poster Board Number: T-3020**

**DEFINING THE ROLE OF REST IN REGULATING HUMAN EMBRYONIC STEM CELL FATE**

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Human embryonic stem cells (hESCs) have the unique ability to undergo self-renewal and are pluripotent, which enables them to differentiate into all three germ layers. Harnessing these properties could result in significant therapeutic outcomes for repair or cell replacement in many disease settings. However, many hurdles remain prior to use of these cells in cell-based therapies including lack of understanding of what signals regulate pluripotency and how to maintain stability of hESCs. OCT4, NANOG and SOX2 are transcription factors that regulate multi-gene networks involved in control of cell fate decisions important for maintaining self-renewal and stability of hESCs. RE1-Silencing Transcription Factor (REST) is one of the direct targets of this network which is a well known transcriptional repressor. REST acts by binding to RE1 sites in greater than 1900 genes in the genome. The main role of REST is to act as a transcriptional repressor in non-neuronal cell types to repress neuronal differentiation. This repression has been shown in some but not all mouse embryonic stem cell studies, however, its role in hESCs is not clear. To examine the role of REST in hESCs, we have performed lentiviral mediated knockdown of REST (REST KD) in three different cell lines and we are currently evaluating the effects of loss of REST on hESC self-renewal, differentiation, genetic stability and survival. REST KD hESCs seem to exhibit enhanced self-renewal and survival capacity compared to controls. We have also found that three out of four hESC lines with REST KD showed genetic instability, whereas control hESC lines were karyotypically normal. To determine the gene networks involved in this instability, we are currently examining candidate genes differentially expressed upon REST KD by performing qPCR of individual REST targets. We are also evaluating the expression of lineage markers in embryoid bodies from REST KD and control cells in order to examine the effects of REST on differentiation. Upon REST KD hESCs exhibit a biased towards endodermal lineage. To examine the role of REST in maintaining genomic stability, we are analyzing H2AX accumulation after DNA damage of hESCs. In summary, our work has shown that REST is a regulator of hESC genetic stability and elucidating the targets mis-regulated upon REST KD could shed light on the gene networks regulating instability in pluripotent stem cell cultures and improve our ability to stably expand and differentiate hESCs for use in regenerative medicine.

**Poster Board Number: T-3021**

**INHIBITION OF P53 SUPPRESSES SELF-RENEWAL OF MURINE EMBRYONIC STEM CELLS**

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Embryonic stem (ES) cells have the remarkable capacity to divide indefinitely while retaining their wide range differentiation potential, and they represent a promising source for cell transplantation therapies. Recent studies have reported the roles of p53 in suppressing the self-renewal of ES cells after DNA damage and block the reprogramming of somatic cells into induced pluripotent stem cells (iPSCs). However, to date no evidence has been presented to support the function of p53 in unrestricted ES cells. In this study, we investigated the effect of pifithrin (PFT)-α, an inhibitor of p53 transactivation, on self-renewal of ES cells. Our results revealed that treatment of ES cells with PFT-α resulted in the inhibition of ES cell propagation in a dose-dependent manner, as indicated by a marked reduction in the cell number and colony size. Furthermore, PFT-α caused a cell cycle arrest and a significant reduction in DNA synthesis. In addition, inhibition of p53 reduced the expression levels of cyclin D1 and Nanog. These findings indicate that p53 pathway in ES cells rather than acting as an inactive gene, is required for ES cell proliferation and self-renewal.

**Poster Board Number: T-3022**

**STEM-CELL SPECIFIC REGULATION OF RNA PROCESSING IN MOUSE AND HUMAN EMBRYONIC STEM CELLS**

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Embryonic stem cells (ESCs) are characterized by their unusual cell cycle and in particular abbreviated G1 phase. As ribosomal biogenesis is typically tied to checkpoints regulating G1 transition, we investigated how ribosomal biogenesis is regulated in pluripotent and differentiated mouse and human ESC. We find that as expected, transcription of ribosomal DNA is higher in ESC compared to differentiated ESC. However, processing of primary 47S rRNA precursor to cytoplasmic 28S RNA was significantly less efficient under stem cell conditions in both mouse and human ESC. Relative to their differentiated counterparts, we find that rRNA processing appears to be delayed at an intermediate step between 32S to 28S. Mechanisms contributing to delayed processing were investigated including nuclear export, limiting RNA processing proteins, and others. We present further evidence that developmental regulation of rRNA processing is not common event in most tissues during development and propose that this process represents a novel, evolutionarily conserved, stem-cell specific event contributing to the pluripotency and self-renewal of ESC.
INDUCED PLURIPOTENT STEM CELLS DERIVED FROM ADULT MOUSE TESTIS DIFFERENTIATED INTO MESODERM, ENDODERM AND ECTODERM

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Induced pluripotent stem cells are cells that were originally from adult tissues, but have been enforced to produce proteins that are thought to be essential for the pluripotency of human embryonic stem cells. By making cells express these embryonic stem cell proteins, adult cells can be created that look and act nearly identical to hESCs. The ability to reprogram a somatic cell and restore its pluripotency has been one of the most significant fields that created new opportunities for treating many of the human diseases. Most of the research that has been done on reprogramming somatic cells was done with the use of viral vectors that deliver certain transcription factors (Oct4, Sox2, c-myc, Klf4, and nanog) which initiate the pluripotency of a somatic cell. Using viral vectors has showed some disadvantages such as this viral genome may integrate the reprogramming factors into the host genomes and may increase the risk of tumor formation. Throughout our research we have used spermatogonial stem cells which are extracted from mice’s testicles to convert them into induced pluripotent stem cells. Spermatogonial stem cells had been reprogrammed without the use of any viral vectors; these cells were reprogrammed by the addition of the appropriate growth factor and embryonic stem cells medium. The resulted induced pluripotent stem cells were then differentiated to the three germ layers and organ lineage which are the endoderm (islet like cells), mesoderm (cardiac cells) and ectoderm (epidermal cells). Our results have shown totally reprogrammed embryonic stem cells which were then differentiated into islet like cells, cardiac cells and epidermal cells.

ROLE OF KLF5 IN THE MAINTENANCE OF SUBPOPULATIONS OF MOUSE ES CELLS

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ES (Embryonic Stem) cells are derived from the blastocyst and have the potential to give rise to derivatives of each germ layer. Induced pluripotent stem (iPS) cells can be derived from lineage-restricted cells, such as fibroblasts, by forced expression of specific transcription factors. Although recent studies indicate that Krüppel-like factors (Klf5) are essential for both maintenance of ES cell self-renewal and reprogramming of somatic cells into a pluripotent state, the molecular mechanism of these processes remains unknown. Thus, understanding the molecular mechanism of ES cell self-renewal by Klf5 would be important for the efficient generation of patient-specific pluripotent stem cells and for the development of regenerative medicine. Although we have showed that Klf5 is important for the self-renewal of mouse ESCs and blastocyst development, the molecular mechanism underlying these functions remains unknown. Recent studies indicate that mouse ES cells are heterogeneous and consist of Rex1 positive and negative subpopulations even in the presence of LIF. To understand the mechanism of self-renewal of mouse ES cells controlled by Klf5, we addressed the role of Klf5 in the maintenance of Rex1 positive and negative cells in mouse ES cells. By using Rex1-GFP knock-in ES cells (OCR9G), we have generated Klf5 KO and Klf5-overexpressing ES cells. We also generated ES cells which overexpress other Klf member such as Klf2, Klf4 and Klf10 to investigate the overlapped and non-overlapped functions between Klf family members. These studies will shed light on the molecular mechanism of mouse ESC self renewal controlled by Klf family members.
SCREENING FOR CRITICAL EPIGENETIC FACTORS INVOLVED WITH THE REGULATION OF PLURIPOTENCY AND DIFFERENTIATION USING A HOMOZYGOUS MUTANT MOUSE EMBRYONIC STEM CELL BANK

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Pluripotent cells have a unique and characteristic epigenetic signature that reflects their broad developmental potential. This epigenetic state dynamically changes during differentiation. Dramatic changes of epigenetic state also occur during reprogramming of somatic cells into induced pluripotent stem cells (iPSCs). Despite increasing evidence that epigenetic regulation is key to the maintenance of the stem cell state, detailed mechanisms associated with this are still unknown. Therefore, elucidation of the functions of epigenetic regulators (such as chromatin modifiers, transcription factors and non-coding RNAs) will help better understand the pluripotent states of embryonic stem cells (ESCs) as well as the induction of iPSCs from somatic cells. We recently reported a method to rapidly generate homozygous mutant mouse ESCs from heterozygous mutant ESCs using the conditional knockout of the Bloom’s syndrome gene (Blm). This method takes advantage of the high rate of loss of heterozygosity associated with Blm-deficient condition. We have so far generated ~200 homozygous mutant ESC lines from heterozygous ESC lines. Utilizing these homozygous mutants, we are currently screening for novel epigenetic factors involved in the regulation of pluripotency and differentiation. We are also screening for unknown functions of known epigenetic factors (such as polycomb complex, DNA and histone methyltransferases, chromatin-remodelers, MBD proteins and miRNA processing factors) in the differentiation and maintenance of pluripotency. In order to identify such factors, we compared the epigenetic state between wild type ESCs and each homozygous mutants by the following methods: (1) digest the genome of each mutant by methylation-sensitive restriction enzymes, (2) carry out immunostaining of various histone modifications, (3) quantify the expression level of various retroelements (such as IAPs, MLV, Etn-MusD, ERVK, LINEs and SINES), (4) examine the response against inhibitors of known epigenetic modifiers. We also conducted phenotypic analysis upon differentiation induction to screen for mutant ESCs that are prone or resistant to differentiation. We could so far identify some candidate genes that might play a critical role in epigenetic regulation of pluripotency and differentiation. We plan to examine expression levels of cell lineage markers in embryoid body formation to gain insight into the role of the candidate genes in cell lineage commitment. We also plan to conduct molecular and genome-wide analysis of these candidates in order to elucidate the mechanisms of these epigenetic regulations. Our approach allows the phenotype comparison amongst many homozygous mutants under the same experimental conditions, and to identify critical epigenetic regulators involved in the regulation of pluripotency and differentiation.

ANALYSIS AND CHARACTERIZATION OF NANOG-INTERACTING PROTEINS IN MOUSE EMBRYONIC STEM CELLS

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Embryonic Stem (ES) cells are maintained in an undifferentiated state by a gene regulatory network centred on the triumvirate of transcription factors Nanog, Oct4 and Sox2. Genome-wide chromatin immunoprecipitation studies indicate that in many cases target genes are bound by closely localised binding sites for each of these proteins, as well as additional members of the extended pluripotency transcription factor network. However, the biochemical basis of the interactions between these proteins is largely unknown, as are the mechanisms by which these interactions control ES cell identity. We recently presented an optimised method for identification of protein-protein interactions and used this to characterise an extended Oct4-centred ES cell interactome (1). Interestingly, Nanog is not present in the Oct4 interactome but it is a member of the extended protein network generated by combining the interaction data of Oct4, Esrrb, Sal1, Dax1 and Tcfcp2l1. We have used a similar approach to identify an extended Nanog interactome that includes more than 60 putative partners. Validation of interactions was obtained by co-immunoprecipitation of Nanog with putative partners. In specific cases, we show that transcription factor interactions occur independent of DNA binding. Moreover, mutational analyses have been used to pinpoint the sites of interaction on Nanog. Mutation of tryptophan residues within the Nanog tryptophan repeat (WR) abolishes some interactions. In addition, the WR (but not the W0 derivative in which all tryptophan residues are mutated to alanine residues) is sufficient to mediate Nanog binding to some partners. These data show that the tryptophans present in the WR are necessary and sufficient to mediate interaction between Nanog and such interacting partners. Likewise, amino acid residues on partner proteins required for the interaction with Nanog have been identified. Our data shed light on the biochemical nature of the interaction between Nanog and its partner proteins that are crucial in maintaining optimal mouse ES cell self-renewal efficiency. (1) Van den Berg, D., Snoek, T., Mullin, N.P., Yates, A., Bezstarostki, K., Demmers, J., Chambers, I. and Poot, R.A. (2010) An Oct4-centred protein interaction network in embryonic stem cells. Cell Stem Cell, 6, 369-381.

INHIBITION OF TGF-B SIGNALING COULD SUBSTITUTE THE INHIBITION OF MULTIFUNCTIONAL GSK3 IN NAIVE MOUSE EMBRYONIC STEM CELLS

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The first mouse embryonic stem (ES) cell lines generated on undefined culture conditions including serum and mouse embryonic fibroblasts (MEF) as feeder layers from blastocysts of strain 129. The feeder cells and serum were later substituted by LIF and BMP4, respectively and in this manner feeder- and serum-free culture conditions for long-term cultivation of mouse ES cells were established. However this defined media is not enough to derive ES cells from...
mouse strains other than strain 129. Although the precise reason for this problem has yet to be identified, but with usage of dia- pause embryos or some small molecule (SM) inhibitors the deriva- tion of authentic mouse ES cells from recalcitrant strains have been practicable. Especially the application of SM inhibitors of ERK1/2 (PD0325901) and GSK3 (CHIR99021), known as 2i, has provided the possibility of derivation and propagation of ES cells in a chemically defined medium from different mouse strains. However, an impres- sive disadvantageous of this condition is the usage of CHIR; as it is shown that this chemical or other GSK3 inhibitors induce chromo- somal instability. We have recently shown that the inhibition of TGF-β signaling pathway by SM inhibitors of TGF-β type I activin receptor-like kinase (ALK) 4, 5, 7 inhibitors (such as SB431542 or SB) in combination with PD0325901 (PD), will result in highly efficient and reproducible derivation of mouse ES cells from NMR1, BALB/c, C57BL/6, DBA/2 and FVB/N that previously considered as refractory or non-permissive strains under conventional condition of mouse ES cell cultures. This 100% efficiency of derivation encouraged us to evaluate the derivation and maintenance of mouse ES cells under chemically defined media supplemented with PD+SB as an alternative way to gain pluri potency instead of the suppression of multifunctional GSK3 molecule. Our results showed that PD+SB could support the generation and long term cultivation of mouse ES cells from different mouse strains. Derived lines showed the typi- cal morphology of ES cells and expression of pluri potency markers. Also they showed the ability of differentiation by EB formation (spontaneously differentiation), direct differentiation or chimera formation into derivatives of three embryonic germ layers. Interest- ingly the results of qRT-PCR showed that PD+SB not only support the pluri potency but also give rise to better expression of some pluri potency markers such as stella and extremely low or complete lack of expression of some early lineage differentiation genes such as Brachyury, Lefty1 and Lefty2 in comparison to 2i. Especially, PD+SB maintain the entirety of chromosomal stability contrary to 2i. Consequently, it seems that PD+SB could be introduced as suit- able substitute for 2i in derivation and cultivation of rodent ES cells in chemically defined conditions.

Poster Board Number: T-3029

INVOLVEMENT OF ETS-RELATED TRANSCRIPTION FACTORS ETV4 AND ETV5 IN PLURIPOTENCY AND PROLIFERATION OF MOUSE EMBRYONIC STEM CELLS

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Pluripotent embryonic stem (ES) cells maintain an undifferentiated state in the presence of LIF. Several transcription factors including Oct3/4 are involved in the self-renewal of ES cells. Recent high-throughput, genome-wide analyses revealed that Ets-related transcription factors ETV4 and ETV5 are downstream molecules of Oct3/4 in ES cells. In the present study, we investigated roles and functions of ETV4 and ETV5 in ES cells. ETV4 and ETV5 are specifically expressed in undifferentiated ES cells, and suppression of Oct3/4 resulted in down-regulation of ETV4 and ETV5. Reporter and electrophoresis mobility shift assays identified a binding site for Oct3/4 in the enhancer region of the ETV5 gene, suggesting that ETV5 is one of the direct downstream target genes of Oct3/4 in ES cells. ETV4 and ETV5 have similar molecular structures and exhibit overlapping expression, indicating that ETV4 and ETV5 have redundant activities. Therefore, next we compared and examined phenotypes between wild-type (WT) and ETV4/- double knockout (dKO) ES cells. WT ES cells form compact colony, which is the typical feature of undifferentiated ES cells; whereas, cell shapes of dKO ES cells were flat like epithelial cells. Of note, expression levels of self-renewal marker genes (Oct3/4, Nanog and Dax1) were similar between these two cell lines, suggesting that ETV4 and ETV5 are dispensable for the self-renewal of ES cells. We also examined differentiation ability of dKO ES cells with the system of embryoid bodies (EBs) in suspension culture which mimics early embryogenesis in vitro. dKO ES cells did not form EBs but grew as adherent cells. Interestingly, these cells showed enhanced expression of extra-embryonic endoderm (ExEn) marker genes including Gata4, Gata6, Sox17 and HNF3β and reduced expression of other lineage marker genes (T and Fgf5), suggesting that dKO ES cells are prone to differentiate into ExEn and defective in pluripotency. Since ETV4 and ETV5 are involved in proliferation of cancer cells, we compared growth of these ES cells. Direct cell counting and WST-1 assay demonstrated that proliferation of dKO ES cells was significantly decreased when compared to that of WT ES cells, and cyclin D1 expression level was downregulated in dKO ES cells. Taken together, these results indicate that ETV4 and ETV5 are involved in pluri potency and proliferation of mouse ES cells.

Poster Board Number: T-3030

E-CADHERIN AND, IN ITS ABSENCE, N-CADHERIN PROMOTES NANOG EXPRESSION IN MOUSE EMBRYONIC STEM CELLS VIA STAT3 PHOSPHORYLATION

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We have recently shown that loss of E-cadherin in mouse embry- onic stem (mES) cells results in significant alterations to both the transcriptome and hierarchy of pluripotency-associated signalling pathways. Here we show that E-cadherin promotes Klf4 and Nanog transcript expression in mES cells via STAT3 phosphorylation and that β-catenin, and its binding region in E-cadherin, is required for this function. To further investigate the role of E-cadherin in LIF-dependent pluripotency, E-cadherin null (Ecad-/-) mES cells were cultured in LIF/BMP supplemented medium. Under these condi- tions, Ecad-/- mES cells exhibited restoration of cell-cell contact and STAT3 phosphorylation and upregulated Klf4, Nanog and N-cad- herin. Abrogation of N-cadherin using an inhibitory peptide caused loss of pSTAT3, Klf4 and Nanog in these cells, demonstrating that N-cadherin supports LIF-dependent pluripotency in this context. We therefore identify a novel molecular mechanism linking E- and N-cadherin to the core circuitry of pluripotency. This mechanism may explain the recently documented role of E-cadherin in efficient induced pluripotent stem cell reprogramming.

Poster Board Number: T-3031

EPIGENETIC REGULATION OF GERMLINE GENES IN PLURIPOTENT CELLS

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While there has been extensive characterization of the transcriptional program underlying pluripotency in embryonic stem (ES) cells, much less is known about the pluripotency transcription network in vivo. To address this, we transcriptionally profiled two pluripotency-associated cell populations present in the mouse embryo: the inner cell mass (ICM) of the blastocyst, the cells from
which ESCs are derived, and primordial germ cells (PGCs), the embryonic germline. This analysis reveals strikingly similar transcriptional profiles between PGCs, ICM, and ESCs. We noticed that many germline genes were also expressed in ESCs, albeit at lower levels, and validated the data by qRT-PCR and IF. Two important questions arise from these observations: 1) How is the expression of germline genes regulated in ESCs? We hypothesize that these genes are regulated epigenetically. We developed culture conditions in which the expression of germline genes is induced in ESCs, while maintaining the undifferentiated state. We are investigating the various possible epigenetic mechanisms that may regulate induction of germline genes in ESCs. Intriguingly, DNA de-methylation correlates well with germline gene induction. We are carrying out further studies on the mechanisms that regulate the expression of germline genes in ESCs. It has been proposed that during ESC derivation, under some culture conditions, ESCs may pass through a germ cell stage. Our data suggest that during ESC cell propagation a state permissive for germline gene induction is maintained, and can be modulated by culture conditions. In particular, we find that the "2i" medium, which maintains mouse ESCs in a naive ground state, can respond robustly to conditions that induce germline genes.

However, epiblast stem cells (EpiSCs) and embryonic fibroblasts (MEFs) appear to not be capable of inducing germline genes in response to the same culture manipulations. We are studying this differential response further. In addition, we are investigating the effect that manipulations that lead to germline gene induction have on mouse iPS cell generation. We have found that these manipulations highly increase the efficiency of iPS cell generation, though it remains to be determined if germline genes have a functional role in the process. Our most recent results will be presented.

Poster Board Number: T-3032

REGULATION OF STEM CELL PLURIPOTENCY BY BET PROTEINS

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The pluripotent state of embryonic stem cells (ESC) is maintained by high-level of expression of stem cell-specific transcription factors including Oct-3/4, Sox2, Klf4 and c-Myc (OSKM). ESC possess an unusual "open" chromatin conformation that allows readily access to the genome by the transcription machinery thus resulting in elevated plasticity. Transcription factors and chromatin remodeling complexes are key determinants of ESCs identity. The Bromodomain (BRD)- and extraterminal domain (BET) family of proteins regulates chromatin dynamics through modulating acetylation-mediated protein-protein and protein-chromatin interactions in control of gene transcription. We sought to investigate the role of BETs in governing ESCs self-renewal and pluripotency. Both, small-molecule chemical inhibition and downregulation of BET by RNA interference effectively reduced the expression of OSKM genes in human and mouse ESC and the number of undifferentiated alkaline phosphatase-positive colonies. Furthermore, chromatin immunoprecipitation (ChIP) experiments of compound-treated ESC revealed displacement of BrD-containing proteins from stem cell genes promoters, with concomitant reduction of active histone marks and RNA polymerase recruitment, thus revealing a role for BET proteins in the stem cell genes transcriptional network. Changes in cell morphology and cell cycle arrest accompanied BRD inhibition. RNA sequencing analysis of compound-treated ESCs revealed differentially expressed genes of the PDGF, TGFβ and Wnt signaling pathways. Epithelial to mesenchymal transition (EMT) markers and neuroectodermal lineage commitment increased following compound treatment, without any overt effect on cell viability or apoptosis. In addition, using an ESC-based reprogramming system, we found that BET inhibition impairs somatic reprogramming of MEFs to iP5. All together, these findings unravel a key role for BET proteins in the maintenance of the embryonic stem cell state.
COMPREHENSIVE ANALYSIS OF GENOMIC IMPRINTING IN PARTHENOGENETIC AND ANDROGENETIC MOUSE EMBRYONIC STEM CELLS

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To unbiasedly survey the mouse genome for imprinting genes in the early embryo, we profiled RNA transcriptome in both parthenogenetic and androgenetic mouse embryonic stem cell (mESC) lines to identify parent-specific transcripts. We uncovered a novel set of protein-coding and non-coding RNAs that show parent-of-origin expression, indicating that these genes are imprinted in mESCs. SIGNIFICANTLY, we identified many paternally expressed genes involved in differentiation and development, including an entire Hox cluster. Furthermore, small RNA-Seq demonstrated some miRNAs are also regulated by genomic imprinting, such as miR-675 which is imbedded within the maternally expressed H19 gene. Because imprinting control regions contain differential methylation regions that are regulated by DNA methylation, we further tested whether these imprinted genes are deregulated in demethylated mESCs deficient of all known DNA methyltransferases. We found that mESCs null of DNA methylation show deregulation in a large subset of predicted imprinting genes. Interestingly, for the list of known imprinting genes, many of them are either not expressed or do not show mono-allelic expression in mESCs, suggesting that mESCs have a unique repertoire of expressed imprinting genes. Together, our study provides a comprehensive analysis of imprinting genes in mouse embryonic stem cells and validates the essential role for DNA methylation in genomic imprinting.

THE POWER OF CELL AUTONOMOUS GROWTH IN THE ABSENCE OF ERK-SIGNALLING IS ACQUIRED JUST BEFORE IMPLANTATION

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One of the defining features of naïve pluripotent mouse embryonic stem cells (ESC) is their ability to thrive in the absence of Erk signalling. Dual inhibition of Gsk3 and Mekk (2i) with the optional use of leukaemia inhibitory factor (LIF) has been proposed to “capture” cells in a naive ground state directly from late preimplantation epiblast. Nevertheless, the process of ESC derivation remains a black box. Even in defined conditions, intact inner cell masses (ICM) provide themselves with unknown paracrine signals as well as intercellular interactions. Also, this micro-niche might “carry” cells, providing them with unknown paracrine signals as well as intercellular interactions. Furthermore, small RNA-Seq demonstrated some miRNAs involved in differentiation and development, including an entire Hox cluster. Nevertheless, the process of ESC derivation remains a black box. Even in defined conditions, intact inner cell masses (ICM) provide themselves with unknown paracrine signals as well as intercellular interactions. Also, this micro-niche might “carry” cells, providing them with unknown paracrine signals as well as intercellular interactions. Further experiments will be carried out to clarify the role of LIF in this context. In conclusion, our results suggest that the founder cells of the embryo proper become responsive to 2i plus LIF only about 18h before blastocyst implantation. This supports the hypothesis that naïve pluripotency is a transient state, which can be directly captured in vitro.

ESTABLISHMENT OF GERMLINE-COMPETENT ES CELLS FROM NZB/BINJ MICE

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As mouse embryonic stem (ES) cells can differentiate into almost any type of cell in the body, including germ lineage, ES cells have been routinely used to generate gene-targeted mice. A wide range of mouse strains have been characterized in detail and widely used in biomedical research according to phenotypic requirements. Nevertheless, germ-line-competent ES cells have only been established from a few strains. Therefore, we aim to establish ES cells derived from various mouse strains. Here, we attempted to establish germ-line-competent ES cells from NZB mice with 4 different culture media. First, we performed a comparative investigation of effects of leukemia inhibitory factor (LIF) at high concentrations. In previously study, we reported that although addition of 1,000 IU/ml LIF was effective in derivation of C57BL/6 ES cells, 5,000 IU/ml LIF was supported the effective establishment of germ-line-competent ES cells from BALB/c mice. However, in NZB mice, increasing the concentration of LIF in medium was not effective because established ES cells showed somatic pluripotency but are incapable of contributing to the germ lineage. Second, we investigated the effects of 2 signal inhibitors (2i). 2i is a composed of a glycogen synthase kinase 3 inhibitor (CHIR99021) and a mitogen-activated protein kinase kinase inhibitor (PD0325901), and it is already reported that 2i is effective in establishment of germ-line-competent ES cells from refractory strains (i.e. NOD mice) and species (i.e. rats). Unexpectedly, when we used ES cell culture medium containing 1,000 IU/ml LIF and 2i, established NZB ES cells contributed to the somatic lineage in vivo, but not to germine lineage. In contrast, when we used ES cell culture medium containing 5,000 IU/ml LIF and 2i, established NZB ES cells showed both somatic and germ-line competency. Our findings suggest that factors required for commitment to germine lineage are independent of those for somatic lineage, and that both high concentration of LIF and 2i are determinants for authentic pluripotency in NZB ES cells. Moreover, to our knowledge, this is the first report of germine-competent NZB ES cells. Because NZB mice display a number of autoimmune abnormalities including hemolytic anemia, elevated levels of im-
GROWTH AND MAINTENANCE OF EMBRYONIC STEM CELLS IN 3-D CULTURE

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Biocompatible and biodegradable scaffolds have promising applications in tissue engineering and regenerative medicine. They may provide a suitable microenvironment or niche that could stimulate and promote stem cell proliferation, differentiation, and integration in situ. The goal of this research is to develop 3-D culturing systems that mimic in vivo growth, differentiation, and development for tissue regeneration and repair. In this report, we describe thiol-functionalized dextran (Dex-SH) hydrogel scaffold formation via Michael type addition using poly (ethylene glycol) tetra-acrylate (PEG-4-Acr). When Dex-SH and PEG-4-Acr are mixed under aerobic conditions, they self-assemble into a scaffold, which is compatible with embryonic stem cell (ESC) growth. The scaffold was degraded over a period of time in an ESC concentration dependent manner. Scaffold seeded with 2X10^6 cells/ml degraded slowly compared with the scaffold seeded with 4X10^6 cells/ml. Seeded ESCs show prolonged growth and remain viable while maintaining self-renewal state. Scaffold cultured ESCs expressed high levels of the pluripotency markers Oct-4, Sox2, and Nanog, in comparison to control ESCs plated by traditional 2-D culture. The self-assembling scaffold provided the necessary support for the growth of ESCs and maintained undifferentiated for several weeks withoutpassagings. The results showed that 3-D ESCs grown in the hydrogel remained pluripotent after 22 days, as judged by their potential for differentiation into osteogenic, myogenic and neural cell types which expressed cell-specific markers, including collagen type 1, myogenin, and nestin, respectively. These results suggest that 3-D growth of ESCs in a self-assembled scaffold provides an in vitro niche that promotes proliferation while maintaining the self-renewal and pluripotency of ESCs.

Poster Board Number: T-3038

TET1 PROTEIN DEGRADATION SETS THE STAGE FOR THE TRANSITION OF NAÏVE-TO-PRIMED PLURIPOTENCY

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Recent studies have indicated that Tet-family proteins (Tet1/2/3) catalyze the conversion of 5-methylcytosine to 5-hydroxymethylcytosine which can eventually lead to DNA demethylation. Among the Tet proteins, Tet1 in particular has been proposed to be important for the self-renewal of mouse embryonic stem cells (mESCs). On the other hand, the full pluripotency of Tet1-knockout mESCs was confirmed by tetraploid complementation assay and therefore, Tet1 seems to be dispensable for maintaining pluripotency during development. As Tet2, a close Tet1-homolog, is also expressed in mESCs, a single loss of Tet1 may have been compensated by residual Tet2-activities in this case. As a result, the precise roles of Tet-proteins in the pluripotency and self-renewal of mESCs remain elusive. Furthermore, the role of Tet proteins in human pluripotent stem cells (hPSCs) is totally unexplored. We first analyzed Tet1 expression profile during the early differentiation of mESCs. As reported previously, Tet1 was abundantly expressed in the undifferentiated mESCs both at the transcript as well as protein levels. Curiously, when these mESCs were induced to differentiate toward an epiblast-like cell (EpiLC) state using bFGF and ActivinA, we started to find discordance between the transcript and the protein level. While Tet1 mRNA level was transiently up-regulated during the process, protein level went significantly down. We attributed this discordance to a ubiquitin-proteasome-mediated protein degradation pathway, as the proteasome inhibitor MG-132 reversed this protein down-regulation. As MG-132 treatment had little effect on Tet1 protein level at the naive undifferentiated state, we assumed that Tet1 protein degradation might be a developmentally regulated process programmed to occur during the transition from the inner cell mass to the epiblast stage. We then asked whether TET1 instability is also observed in hPSC known to be phenotypically akin to the primed EpiLC. Although TET1 protein is hardly detected in hPSCs despite of the relatively abundant transcript level, treatment with MG-132 dramatically increased TET1 protein level within the cell nuclei of various hPSC clones, supporting our assumption that a primed PSC cannot stabilize nuclear TET1 protein as much as a naive PSC. To examine this possibility further, we sought to assess whether TET1 protein stabilization in hPSCs induces a conversion to a naive pluripotent state. WNT/β-catenin signaling pathway has divergent roles in naive and primed pluripotency. It operates for self-renewal to mESCs but induces mesendodermal differentiation to hPSCs. Naïve mESCs canself-renew at the ground state (in the presence of MEK inhibitor and GSK3β inhibitor; 2i) and therefore with potent WNT/β-catenin signaling, but primed hPSC fail to do so and swiftly differentiate even with a single inhibition (MEK or GSK3β). We found that hPSCs over-expressing a stabilized nuclear form of TET1 exhibit differentiation resistance against the 2i condition over 10 passages and kept self-renewing. This observation indicates that TET protein activities determine whether a stem cell self-renew or differentiate upon a certain extracellular stimulus. Altogether, our results are suggestive for a primary role for Tet protein degradation in initiating an early event during mammalian development, the naïve-to-primed transition.

Poster Board Number: T-3039

NCOA3 BRIDGES NUCLEAR RECEPTORS, EPIGENETIC REGULATORS, AND GSK3 TO REGULATE NANOG AND PLURIPOTENCY

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Nuclear receptors, including Esrrb, Dax1 and Nr5a2, have been shown to be involved in pluripotency maintenance. Yet, the role of their co-activators in embryonic stem cells (ESCs) remains unexplored. Here, we demonstrated that Ncoa3, but not Ncoa1 or Ncoa2, is essential for pluripotency maintenance. Knockdown of Ncoa3 not only compromises the expression of pluripotency markers, but also impairs in vitro and in vivo differentiation potential of ESCS. Ncoa3 binds to the Nanog promoter, and recruits the histone acetyltransferase CBP and the histone arginine methyltransferase CARM1 to activate Nanog expression. Moreover, GSK3 signaling...
down-regulates Ncoa3 protein level to suppress Nanog expression. Thus, Ncoa3 not only contributes to self-renewal by activating Nanog, but also facilitates ESC differentiation as a break point to disrupt the core transcriptional circuitry of pluripotency.

**Poster Board Number: T-3040**

**MMTR FUNCTIONS AS A MOLECULAR RHEOSTAT IN FATE DECISION OF THE PLURIPOTENT ES CELLS BY MEANS OF ITS MULTI-FACETED TRANSCRIPTIONAL REGULATORY ROLES**

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Dnm1-associated protein 1 (DMP1) was originally identified a DNMT1-interacting molecule and was implicated in gene regulation through modification of chromatin. Recent studies have also revealed that DMP1 is a core component of the ATP-dependent chromatin-remodeling complexes NuA4/Tip60 HAT and Swr1/SRCAP. MAT1-mediated transcriptional repressor (MMTR) was independently isolated and characterized from mouse embryonic stem (ES) cells as a novel clone and found it to be identical to DMP1. MMTR is not only a key component of RNA Pol II-mediated gene expression that interacts with HDAC1 and modulates of TFIIH kinase activity via MAT1, but also involved in the regulation of cell cycle progression as an intrinsic negative regulator of CAF that regulates mitotic cell cycle progression. It is noted that TFIIH-mediated transcriptional repression activity of MMTR requires intact MMTR whereas CAF-mediated cell cycle regulatory activity of MMTR requires only MAT1-interacting C-terminal half region of MMTR. Thus, MMTR is suggested to play roles in transcription, cellular response to DNA damage, and cell cycle control by interactions with proteins in different contexts. Here we have sought to dissect the functional roles of MMTR in ES cell self-renewal and pluripotency.

We find that the proper expression level of MMTR is crucial for maintenance of ES cell identity. Comparing to wild type cells, ES cell lines with MMTR knockdown or overexpression maintained self-renewal activity with the reduced cell proliferation rate. Embryoid bodies of both these cell lines can undergo the initial steps of differentiation, but seem to be severely compromised in the ability to become committed to a lineage. Indeed, both cell lines could not maintain pluripotency in teratoma assays. Furthermore, we find that N-terminal half-mediated MMTR function is more important than TFIIH-mediated transcriptional repression activity or CAF-mediated cell cycle regulatory activity for maintenance of ES cell identity. Finally, we find that perturbation of the proper MMTR expression level leads to global genetic imbalance at early differentiation stage causing deregulation of pleiotropic biological functions and MMTR exerts both transcriptional activating and repressing function in target gene expression as a component of different protein complexes, including the NuA4/Tip60 HAT complex. Thus, MMTR functions as a molecular rheostat in fate decision of the pluripotent ES cells by means of its multi-faceted transcriptional regulatory roles.

**Poster Board Number: T-3042**

**GENERATION OF EMBRYONIC STEM CELL LINES FROM SOMATIC CELL NUCLEAR TRANSFER BOVINE EMBRYOS IN KOREAN BEEF CATTLE, HANWOO**

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Autologous embryonic stem cells derived from somatic cell nuclear transfer embryos (SCNT-ESC) are still valuable in animals for many research applications such as conservation of endangered species and production of transgenic animals. Moreover, SCNT-ESC studies in large animal species like cattle can be a pre-clinical model for patient-specific stem cell therapy in human. In the present study, we established and maintained the SCNT-ESC lines of traditional Korean beef cattle species called HanWoo (Bos taurus coreanae), which is one of the most important livestock in Korean peninsula. Reconstructed embryos by SCNT were cultured in vitro for 7 days following electric fusion and activation to obtain blastocyst stage embryos. Each SCNT blastocyst were placed individually on the feeder layer with 3i stem cell culture medium consisting of an equal volume of DMEM/F12 glutamax and Neurobasal medium with 1% (v/v) N2 and 2% (v/v) B27 supplements, plus three inhibitors (30), 0.8 mM PD184352 (MEK1/2 inhibitor), 2 mM SU5402 (FGF receptor inhibitor) and 3 mM CHIR99021 (GSK3 inhibitor). Primary colonies formed after 2-3 days of culture and the cell colonies were moved onto new fresh feeder layer after 8-9 days of incubation. The intact colonies were routinely passed every 5-6 days. Eight bovine SCNT-ESC lines have been established from eight different
SCNT bovine blastocysts. The cells show ESC-like colonies with a distinct boundary and are positive to alkaline phosphatase staining. Immunofluorescence and RT-PCR analyses also confirm their characteristic of pluripotency as Oct4, Sox2, Nanog and stage-specific embryonic antigen-4 is positive. All bovine SCNT-ESC lines are being maintained in culture over 50 passages for more than 270 days. The cells show normal karyotypes consisting of 60 chromosomess (58XX) at passage 50. Embryoid bodies (EBs) were formed by suspension culture to analyze in vitro differentiation capability. The marker genes representing the differentiation into three germ layers such as ectodermal: beta-3-Tubulin, Nestin and Vimentin, endodermal: Somatostatin and Gata6, mesodermal: Connexin40 and BMP4 are expressed in EBs derived from bovine SCNT-ESC. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST; Grant number 2011-0027807) and Technology Development Program for Agriculture and Forestry, Ministry for Agriculture, Forestry and Fisheries (MAFF; Grant numbers 109020-3 and 111160-4), Korea.

**Embyronic Stem Cell Clinical Application**

**Poster Board Number: T-3043**

**A NOVEL XENO-FREE DEFINED CONDITION FOR CULTURE OF HUMAN EMBRYONIC STEM CELLS AND HUMAN INDUCED PLURIPOTENT STEM CELLS WITH NOVEL HUMAN FEEDER LAYERS**

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Pluripotent human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) have the capacity to differentiate into all of the somatic cell types and therefore hold great promise for regenerative medicine. One key issue that needs to be addressed in guiding pluripotent stem cell technology from “bench” towards “bedside” is developing defined cell culture systems for making large banks of cells in cell therapy compliant manner. Here, we have developed a xeno-free culture system for hESCs/iPSCs and derived a xeno-free cell line of hESC. To completely avoid exposure of hESCs to culture system with animal products, we developed a xeno-free cultivation system for new hESC lines (named as SEES1-4) based on human mesenchymal stromal (HMS) feeders with xeno-free medium. HMS cells were isolated from human dermal tissue samples collected from juvenile donors undergoing surgical procedures of polydactyly with parental written consent of donors and approved by the Institutional Review Board of the NCCHD. To derive and expand HMS feeder layers in xeno-free conditions, we used StemPro MSC SFM Xeno-Free (XF) medium supplemented with StemPro LipoMax Defined XF Lipid Supplement on culture dishes coated with xeno-free matrix Cellstart. We developed a xeno-free culture conditions with the medium for hESCs/iPSCs that consisted of a KnockOut (KO)-DMEM base supplemented with 15% KO-Serum Replacement XF, amino acids, vitamin C, and several growth factors. All components of the medium were synthetic, recombinant, or of human origin. Three hESC lines (SEES1-3) and 6 hiPSC lines have been maintained and tested their pluripotency over 30 passages in the xeno-free medium on the HMS feeder layer. The cell lines have been maintained by mechanical passaging at early passages and by recombinant enzyme such as TrypLE Select. In addition, the hESCs and hiPSCs were frozen using a serum-free cryoprotectant. The cell lines exhibit normal hESC characteristics including pluripotency markers, multiple differentiation characteristics as determined by embryoid body analysis and teratoma formation, and are karyotypically normal. Finally, we derived a new hESC line (SEES4) completely in the described xeno-free condition using laser-mediated ablation. We describe a comprehensive characterization of SEES cell lines for retaining hESC-specific features, including an unlimited and undifferentiated proliferation capacity, marker gene expression, stable karyotype, and three germ layers differentiation in vitro and in vivo. In addition, we observed the expression of nonhuman N-glycolyneuraminic acid (Neu5GC) by HPLC analysis, which was xenooantigenic components of contamination in hESCs cultures, was not detected in xeno-free hESC line. In conclusion, this culture system proved stable maintenance of self-renewal and pluripotency of hiPSCs and newly established hESCs. It will promote the progression toward clinical-grade hESC culture that is critical for developing cell therapy/regenerative medicine paradigms.

**Poster Board Number: T-3044**

**HLA AND ABO GENOTYPES OF EIGHT TAIWANESE HUMAN ES CELL LINES**

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Human embryonic stem cells (hESCs) provide a potentially unlimited source for various cell therapies because they can propagate indefinitely in vitro and they are pluripotent, which means they can differentiate into all different kinds of tissues of a man. However, the first obstacle encountered in the human ES cell regenerative medicine applications is the histocompatibility problem. Detailed characterization of the blood-histotype of the human ES cells is thus vital to the construction of a human ES cell bank. Here we perform the high-resolution sequence based typing (SBT) of HLA-A, B, C, -DQ and -DR loci and acquire complete typing results of eight hESC lines in Taiwan. In our survey, there are several hESC lines that are predicted serologically homozgyous at single or double HLA loci: one is homozygous for HLA-A; three are homozygous for HLA-C; one is homozygous for both HLA-C and HLA-DQ loci and one is homozygous for both HLA-A and HLA-C loci. Three of these hESC lines are A blood type (A/A or A/O), three are type O and two are type B (B/B). These documented histotypes of human embryonic stem cell lines as a whole not only provide a detailed reference database for their use in cell transplantation, but also helps to identify their drug testing potentials of the banked human ES cells.
**Poster Board Number: T-3045**

**BCL-XL OVER-EXPRESSION CONFER A SELECTIVE GROWTH ADVANTAGE TO hESCs**

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During long-term culture hESCs often accumulate chromosomal abnormalities. These tend not to be random and commonly reflect those observed in embryonal carcinoma cell lines. This raises concerns for their use in clinical application and calls for routine karyotype analysis. While standard techniques are able to identify large-scale abnormalities (> ~5MB) molecular analysis is required for detection of small-scale copy number variants (CNVs). A large-scale screen of an ethnically diverse pool of early and late passage hESC lines identified a gain of a region of chromosome 20 amplified in >20% of karyotypically normal hESC lines, predominantly in late passage. This region (20q11.21) has also been found to be over-represented in several cancer tissues. The minimal amplicon identified through the screen contains 13 genes, 3 of which are expressed in hESCs: BCL2L1, ID2 and HM13. By performing competition assays we observe that cells expressing elevated levels of Bcl-xL (the dominant isoform of BCL2L1 expressed in hESCs) have a growth advantage over control cells. Shortly after single-cell seeding control cells appear to go into crisis, displaying high levels of apoptosis. Over-expression of Bcl-xL provides protection against this stress-induced cell death and other stresses placed upon cells through general culture. Bcl-xL over-expressing cells are also more resistant to chemical insults when applying agents that activate apoptosis through either DNA damage or cell cycle arrest. This implies that Bcl-xL expressing cells are more robust in evading cellular protective measures, which maybe undesirable for their use in clinical application. The results suggest that amplification of this region should be screened in addition to standard karyotype analysis.

**Poster Board Number: T-3046**

**ALS DISEASE MODELING USING MOUSE AND HUMAN EMBRYONIC STEM CELLS**

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, which is characterized by the loss of upper and lower motor neurons in the brain and spinal cord. To date there is only limited insight in the disease mechanisms and only one drug, Riluzole, is currently FDA approved as a treatment for ALS. Drug discovery has been hampered by the limited amount of animal models and the inability to culture affected cells from patients. Here, we are using mouse and human embryonic stem cells (ESCs) to model ALS. Co-culture of ESC derived motor neurons and primary mouse glia of different genotypes show a non-cell autonomous effect of glia on the survival of motor neurons. This effect is motor neuron specific and is mediated through secreted diffusible factor(s). Using our in vitro co-culture system we have identified compounds that inhibit this neurotoxic effect, most of which are targeting inflammatory pathways. Exploring these pathways will lead to a better understanding of disease mechanisms and these compounds could potentially be used as a new ALS therapeutic in the future. We have carried out clinical trials in mice to confirm the protective effect on motor neurons of our most promising candidates. Together, our results propose a model in which ALS is a complex non-cell autonomous disease in which inflammation, glutamate excitotoxicity and oxidative toxicity are involved.

**Poster Board Number: T-3047**

**ENABLEMENT OF ALLOGENEIC STEM CELL THERAPIES VIA RADIATION-FREE CONDITIONING INDUCED MIXED CHIMERISM**

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Current strategies to combat graft rejection of allotransplants are based on lifelong immunosuppressive regimes, which lead to generalised immunodeficiency and high levels of morbidity, including opportunistic infections and malignancy. A significant problem that will need to be overcome for the successful application of stem cell derived therapeutics. A logical solution to prolonged global immunosuppression is to induce graft-specific tolerance, allowing long-term acceptance of the graft, whilst maintaining immunocompetence. This can be routinely achieved through hematopoietic stem cell (HSC) transplants (HSCT), where graft-matched HSCs re-educate the host’s immune system to be tolerant to both host and donor tissue. However current regimes to condition the bone marrow (BM) to receive HSCT involve highly toxic doses of irradiation. Furthermore, successful T cell chimerism, the key to long-term graft acceptance, is dependent on a functional thymus. Paradoxically the thymus undergoes profound atrophy with age, significantly reducing T cell output. Thus, we have been developing a radiation-free protocol to induce tolerance to fully MHC-mismatched allografts and extending this to the aged setting. We show that conditioning with low doses of the anti-neoplastic drug busulfan, in combination with T cell-depleting antibodies and transient immunosuppression, can generate robust levels of mixed chimerism and long-term acceptance of skin grafts. Our laboratory has previously shown that endocrine-based strategies can reverse age-dependent thymic atrophy and enhance immune recovery following HSCT in both mice and humans. Combining a reduced-intensity conditioning regime with strategies for boosting thymopoiesis may aid in the successful translation of this technology to the clinic, to enable long term, drug-free acceptance of stem cell grafts.

**Poster Board Number: T-3048**

**TAU PATHOLOGY FOCUSED DRUG DISCOVERY USING IPSCS IN ALZHEIMER’S DISEASE**

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Alzheimer’s disease (AD) is the most common neurodegenerative disease and the 6th leading cause of death in the U.S. The histopathological hallmarks of the disease are extracellular amyloid-beta protein (Aβ) plaques and intracellular aggregates of tau protein, with the 2 acting in concert to destroy neurons, leading to a decline in memory function. Multi-transgenic mouse models are commonly used to mimic human disease and while these models have helped our understanding of AD, they have been less successful in identifying new treatments. To address this issue, we at iPierian use human induced pluripotent stem cells (iPSCs) from patients and healthy volunteers to model AD with the goal to identify disease-modifying drugs. In particular, we are interested in the reduction of pathological hyperphosphorylation of tau protein. Due to the already near maximal Aβ burden at the time of AD diagnosis, stop-
ping the spread of tau pathology may be a more successful strategy when compared to Aβ reducing therapies. We collected skin fibroblasts from over 50 AD patients and controls, including familial presenilin mutants (PSEN1 and PSEN2) and generated iPSC lines from 18 individuals by retroviral transduction. Upon directed neuronal differentiation of these iPSCs, we observed the stage-specific appearance of genes known to regulate neuronal development and cortical maturation. The emergence of cortical layer specific genes marked the onset of neuronal maturation, and together with a typical neuronal morphology and patch-clamp electrophysiology, indicated a maturation stage suitable for disease modeling. We first characterized the secretion of the amyloid precursor cleavage products Aβ40 and 42. Normally, the Aβ40/A42 ratio is 10:1, but in familial AD cases (PSEN1 and PSEN2) this ratio is skewed in favor of Aβ42 (4:1). Elevated Aβ42/A40 ratio was confirmed in supernatants from our neuronal cultures. We then tested the hypothesis that an elevated A42 load would cause additional pathologies, in particular hyperphosphorylation of tau protein. We were excited to observe that iPSC derived cortical neurons faithfully recapitulated the disease phenotype by exhibiting increased levels (~40%) of phosphorylation at two disease specific tau epitopes when compared to healthy controls. Furthermore this differential was reduced by γ- and β-secretase inhibitors, demonstrating Aβ dependency of tau phosphorylation in these cultures. Currently, we are using this system to validate existing compounds and to identify novel modulators of tau pathology that reduce downstream functional deficits of neuronal health. Functional assays under investigation at iPierian include an assessment of synaptic health, electrophysiological parameters, as well as neuronal survival and process length. Importantly, in addition to studying neurons in isolation, iPSC technology allows us to apply a ‘systems approach’ to AD, combining patient derived neurons with patient derived astrocytes and microglia. We have successfully established neuron-glial cocultures and are using these culture systems to study cell non-autonomous pathologies of AD. For example, the identification of secreted factors from non-neuronal cells, including pro-inflammatory cytokines, allows us to pursue multiple avenues for therapeutic intervention. In summary, we have established a novel multi-cell type in vitro model system of Alzheimer's disease which we are using to explore new treatment options and to better existing ones.

**Poster Board Number: T-3049**

**GENERATION OF FLUORESCENCE-TAGGED HUMAN EMBRYONIC STEM CELLS BY PIGGYBAC TRANSPONSON SYSTEM**

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Genetic modification of human embryonic stem cells (hESCs) is an important tool for gene function research, cell purification and cell tracing after transplantation therapy. To establish transgenic hESCs with fluorescence reporter, we use the piggybac transposon system to generate permanent and reversible gene modification on a Taiwan hESC, TW1. In this transposon system, the piggybac transposase was optimized by mammalian codon usage to increase protein expression efficiency in hESCs. In order to get higher genome integration efficiency, piggybac transposase was driven by CAG promoter. The fluorescence reporter gene was driven by the PGK promoter. The stable transgenic TW1 cells were purified with fluorescence-activated cell sorting and selected with G418 for two weeks. After genome integration, the ratio of GFP-positive cells was about 70%. To eliminate the remaining GFP-negative cells, cells were sorted again and the GFP-positive cell content became over 90%. After FACS purification, we culture these cells for at least five passages without G418-selection. More than 93% of cells were still GFP-positive and the pluripotent marker was persistently in these cells. These transgenic cells were potent to differentiate into rosettes-like neural precursor cells without fluorescence-attenuation. Based on these approaches, researchers can trace this transplanted cell in vivo by the GFP fluorescence. Other neuron specific reporter will be applied with this transposon-mediated transgenic system for gene function research, harvesting ES-derived specific neuron lineages and further regeneration researches.
**A NOVEL METHOD OF INDUCING GABAERGIC NEURONS THROUGH PDGFRα+ CELLS DERIVED FROM MOUSE EMBRYONIC STEM CELLS**

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**Background:** Successful derivations of specific neuronal and glial cells from embryonic stem cells (ESC) have enormous potential for cell therapies and regenerative medicine. However, the low efficiency, the complexity of induction methods, and the need for purification all represent major obstacles. Takushima et al. induced PDGFRα+ cells from mESC through neuroepithelial cells, and showed that the PDGFRα+ cells contain embryonic mesenchymal stem cells (MSC) (Cell, 2007). In our current study, we found that PDGFRα+ cells induced by the same method can also serve as a useful source from which to differentiate into GABAergic (γ-aminobutyric-acid) neuronal cells. Methods: We differentiated mouse ESC (mESC) on collagen IV-coated plates in αMEM containing 10% MSC-defined FBS with limited exposure to retinoic acid during Days 2-5, and PDGFRα+ cells were sorted on Day 9. PDGFRα+ cells were maintained and proliferated in αMEM containing 10% MSC-defined FBS and ROCK inhibitor (Y-27632). To direct further differentiation, PDGFRα+ cells were cultured for up to 12 days in αMEM supplemented with different combinations of factors: B27, FBS, VEGF, NaH3, bFGF, PDGF-BB, TSA, TGF, Hepa-rin, Thrombin, dexamethasone, β-mercaptoethanol, and Y27632. Results: After 6 days of induction with B27 (or FBS), VEGF, NaN3, bFGF, and Y27632, we detected remarkable expression of GABAergic neuronal markers, such as Dlx2, GAD67, and Pax2 by real time RT-PCR, which reached levels comparable to that of the embryonic brain. We also observed almost 100% GABA- and GAD67- positive cells based on immunostaining. Moreover, the differentiation ability of PDGFRα+ cells was maintained even after more than 10 passages and cryopreservation. Conclusions: This study presents a simple and highly efficient method of inducing GABAergic neurons in only 6 days. Together with its ease of maintenance in vitro, PDGFRα+ cells derived from mESC thus prove to be a useful source from which to induce GABAergic neurons.

**CORRELATING EFFECTS OF GEL MICROSTRUCTURAL FEATURES WITH SPECIFIC DIFFERENTIATION PATTERNING OF MOUSE EMBRYONIC STEM CELLS**

**Task, Keith, D’Amore, Antonio, Singh, Satish, Jaramillo, Maria, Kumta, Prashant, Banerjee, Ipsita**

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Embryonic stem cells (ESCs) have the potential to be used in many applications due to their ability to differentiate into cells of any of the three germ layers (ectoderm, endoderm, and mesoderm). There are several different routes by which ESCs can be guided to differentiate into specific germ layers, including genetic manipulation, chemical cues, and mechanical stimuli. With the latter, it has been observed that substrate stiffness plays a significant role in governing phenotype specific differentiation. In our previous study we investigated the effect of mechanical stiffness of fibrin on ESC differentiation. Our results indicated that while ectoderm and mesoderm germ layers responded weakly to the change in fibrin substrate stiffness in the chosen range (2 - 250KPa), endoderm markers were strongly responsive, with softer substrates up-regulating endoderm specific markers. Although cells respond differently to substrates of varying stiffness, it was also observed that gels with the same stiffness but fabricated under different conditions lead to different differentiation patterns. Moreover, this macroscopic property is perhaps not what the cell truly experiences; modification of fabrication conditions changes the gel microstructure, which in turn changes the stiffness, and these micro-characteristics directly interact with the cells. However, it is not clear what specific microstructural features are the most influential in inducing cellular differentiation, and how they affect ESC behavior. In the current work, we investigate the effect of microstructural features of fibrin gels on the differentiation of mouse ESCs. Twelve different fibrin gels were fabricated by varying the fibrinogen concentration and fibrinogen to thrombin crosslinking ratio. The fibrin gels were used to induce ESC differentiation employing both 2D and 3D cultures. After the differentiation protocol the ESCs were analyzed for phenotypic commitment by performing qRT-PCR for the specific germ layer markers. Each of the 12 different fibrin gels was imaged with scanning electron microscopy. Microstructural features of each of these gels were quantified using an image analysis tool for the characterization of fibrous scaffolds. Specific features which were characterized include fiber diameter, node density, and pore size, and comparison of these attributes along with principal component analysis led to a subset of features which vary most across gel conditions. These gel microstructural features were then correlated with the ESC differentiation patterning using regression analysis. The gels are heterogeneous in nature, and therefore a Monte Carlo sampling technique on the microstructural features was used for regression to account for the variability of the system. Stepwise backward elimination regression was used to reduce the correlation model to include only the most influential parameters. These results are also compared to the correlation of gel stiffness, determined by both by rheological measurements and atomic force microscopy, to phenotype commitment. This analysis reveals the sensitivity of cellular phenotype commitment and differentiation patterning to each of the microstructural features. Moreover, such information can be used to help guide the design of scaffolds with specific properties for tissue engineering applications.

**POPULATION BASED MODEL OF HUMAN EMBRYONIC STEM CELL DIFFERENTIATION DURING ENDODERM INDUCTION**

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Understanding the dynamics of differentiating embryonic stem cells (ESC) is essential in the efficient derivation of mature, functional cell types from pluripotent progenitors. Although the theory of differentiation to primary germ layers is known, a mechanistic understanding of the process is still lacking. In this project we are using a population based modeling approach to understand the mechanism of endoderm induction in human ESC. Endoderm induction is performed under two different conditions: Activin A and Activin A supplemented with FGF2 and BMP4. The differentiating cell population is analyzed each day for proliferation, apoptosis, and protein expression. Flow cytometry is used to track the dynamics of expression of two proteins which are indicative of endoderm: Sox17 and CXCR4. In order to elucidate the mechanism of differentiation, a stochastic population based mathematical model was developed based on an earlier model reported for the hematopoietic stem cell system. The simulation is initiated with a population of un-
differentiated cells, wherefrom it evolves in time by assigning each cell a propensity to proliferate, apoptose and differentiate using certain user defined rules. The propensity of each cell to differentiate is tracked with time, and upon reaching a certain threshold the cell is considered committed to a particular lineage. Because it is likely that a parameter hyper-space will better describe the experimental data than a single parameter combination, an ensemble parameter estimation was performed by randomly generating values from the hyper-space of the sensitive parameters and taking those parameter sets which give errors less than a tolerance threshold. It is observed that the simulated dynamics are quite sensitive to the mechanism of differentiation prescribed to the model. A comparison of the agreement of experimental data with model prediction for several competing mechanisms allows identification of the most probable mechanism of endoderm specification of hESCs. Twelve different plausible mechanisms which might describe the observed dynamics were developed, and this ensemble method was performed on each of these mechanisms. For each case, the model ensembles of proliferation, apoptosis and differentiation dynamics are compared with respective experimental data to determine the most probable mechanism. This analysis led to the identification of one mechanism which adequately describes the observed dynamics. The results indicate that HEp2 commitment to endoderm occurs through an intermediate mesendoderm germ layer which further differentiates into mesoderm and endoderm, and that the proliferation of specific phenotypes is favored over others. Furthermore, our model suggests that CXCR4 is expressed in the mesendoderm and endoderm germ layers, but is not expressed in mesoderm. Comparisons between the two induction conditions indicate that supplementing FGF2 and BMP4 to Activin A enhances the kinetics of differentiation. The model is validated against a totally different set of test data for two different genes which was not used during model training, and shows excellent agreement with the experimental data. The population based model used in this study represents a valid platform for identifying mechanisms governing dynamic biological systems, and can be extended to any system of adult stem cells or to later stages of ESC differentiation to more mature phenotypes.

Poster Board Number: T-3055

DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO CD34+ HEMATOPOIETIC PROGENITOR CELLS USING STEMDIFF™ HEMATOPOIETIC KIT

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Human pluripotent stem cells (hPSCs), including embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs), have two defining functional characteristics: self-renewal divisions to generate pluripotent progeny, and differentiation capacity to any somatic lineage. Here we report the development of the STEMdiff™ Cardiomyocyte Kit, a defined, serum-free system for the efficient differentiation of beating cardiomyocytes from hiPSCs. The system utilizes an embryoid body (EB)-based method of differentiation, wherein EBs are cultured with a specific series of factors designed to mimic in vivo developmental cues driving cardiac specification. Using the hiPSC lines W8T4D1 (provided by Dr. William Stanford, Toronto, Canada) and A13700 (Life Technologies), we harvested cells from mTeSR™ cultures, and used AggreWell™400 microwell-textured plates to form EBs of 1,000 cells each in STEMdiff™ Cardiomyocyte Basal Medium with a unique Supplement A. STEMdiff™ Cardiomyocyte Basal Medium is a novel serum-free and animal component-free medium, which is used to maintain EBs in this protocol with a series of unique supplements (A through E) used sequentially at defined timepoints from day 0 through day 16. The EBs were cultured within the AggreWell™400 plates for the entire duration of the protocol, and on specific days the culture media was changed carefully to minimize disruption of the EBs in the microwells. Due to differences in individual cell lines’ responses to inductive signals, Supplement B, was first titrated on each cell line to determine the optimum amount for maximal cardiac induction, and that amount was then used for subsequent experiments. Using optimized amounts of Supplement B on day 1 and indicated amount of Supplements C through E added on days 4, 8, and 12 respectively for each hiPSC line, beating EBs were observed as early as day 12 of differentiation. By day 16 synchronous beating was ob-
served in 87% ± 9% of WCS-4D1 EBs (n=7) and 92% ± 5% of A13700 EBs (n=5; values represent mean ± standard deviation). All EBs were also harvested from the AggreWell™400, dissociated into single cell suspensions using Collagenase II, and analyzed by flow cytometry for expression of the intracellular cardiomyocyte marker cardiac troponin T (cTnT). Under these conditions, 23 ± 11% (n=5) and 26 ± 14% (n=3) of WCS-4D1 and A13700 derived cells, respectively, expressed cTnT. Moreover, EBs could be dissociated and resulting cells plated onto Matrigel™ coated dishes where they could be maintained as a beating monolayer for at least 60 days. Cardiomyocytes replated onto Matrigel™ were also fixed and analyzed by immunocytochemistry for cTnT expression, and displayed a characteristic striated staining pattern. Overall, these results demonstrate that the STEMDiff™ Cardiomyocyte Kit can be used to efficiently and reproducibly form beating cardiomyocytes from hiPSCs maintained in mTeSR™1 defined and feeder-independent culture conditions.

Poster Board Number: T-3057
GENERATION AND FUNCTIONAL CHARACTERIZATION OF HEPATOCYTES FROM HUMAN PARTHENOGENETIC STEM CELLS
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Hepatocellular transplantation offers an attractive alternative to orthotopic liver transplant, and in vitro cultured pluripotent stem cells could become a great source of hepatocytes. Human parthenogenetic stem cells (hpSC) possess all advantages of embryonic stem cells yet their derivation does not require viable embryo destruction, and does not pose ethical concerns. In addition, hpSC homozygous for HLA antigens can be obtained relatively easily, providing a great histocompatibility advantage during implantation. Previously, we described a three-dimensional (3D) culture system allowing for generation of definitive endoderm at unprecedented homogeneity. We demonstrated the usefulness of this system producing high purity populations of hepatocyte-like cells. Here we optimize, streamline and evaluate our system as a potential source of hepatocytes for cell-based liver therapy. In order to enable the adequate testing, and possible future therapeutic use, we first optimized our culture conditions using larger custom-built culture devices with adequately bigger porous membranes, which allowed to generate several millions of highly pure hepatocytes in a single batch. Careful analysis using flow cytometry, immunostaining and PCR confirmed the desired phenotype of the obtained cells, as evidenced by enzymatic activity of CYP450, ICG uptake, glycogen storing, and demonstrated secretion of hepatic proteins, such as AFP, ALB, AAT1, Plasminogen, Factor IX. To gain further insight into the functional capabilities of the generated hepatocytes we tested them using two animal models. First, we intrasplenically injected the hpSC-derived hepatocytes into SCID mice with acetaminophen-induced acute liver failure. We observed that our CFSE-labeled cells migrated to the liver, integrated into the liver parenchyma, and acquired hepatic characteristics. Immunohistochemical analysis revealed the presence of the engrafted human, albumin-positive cells. Detectable levels of human liver-specific proteins (hAFP, hAAT1, hALB) in the serum of injected mice were observed at day 30 post transplantation. At day 60, hAFP became undetectable, while relatively high levels of both hAAT1 and hALB were found in the blood. This indicates further differentiation to fully mature hepatocytes in their appropriate endogenous environment of the host liver. In addition, we have treated juvenile Gunn rats with a single intrasplenic injection with hpSC-derived hepatocytes. Over-all, the inoculation of tested cells revealed stable clinical remission of both acute liver failure and chronic metabolic disease in experimental animals. Our work demonstrates that pure hepatocytes can be produced from hpSC in reasonable numbers at acceptable cost. Personalized immuno-matching, as well as the ability to undergo terminal maturation in vivo make these cells an attractive candidate for cell-based transplantation in regenerative therapy.

Poster Board Number: T-3058
LINEAGE PRIMING AND CONTROL OF ENDODERM DIFFERENTIATION IN HUMAN EMBRYONIC STEM CELLS
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Following prolonged culture in vitro human embryonic stem cells become more robust and easier to maintain, displaying higher cloning efficiencies and reduced population doubling times. This phenomenon termed ‘culture-adaptation’ is also closely associated with cytogenetic changes including partial or whole chromosome duplications and deletions. Interestingly these karyotypic abnormalities are similar to those observed in human embryonal carcinoma cells suggesting that these cytogenetic aberrations may provide a selective advantage over their ‘normal’ diploid counterparts. Recent evidence suggests that ‘culture-adapted’ human embryonic stem cells may also be lineage biased when induced to spontaneously differentiate via the formation of embryoid bodies. The ‘culture-adapted’ cells appeared to be deficient in their ability to differentiate towards an endodermal lineage. To investigate this further we used a late passage, karyotypically abnormal human embryonic stem cell line, which displayed characteristics of ‘culture-adaptation’ and compared it with its early passage, diploid counterpart. Using whole population and small-scale differentiation protocols we found that ‘culture-adapted’ cells have a reduced capacity to differentiate towards a definitive endoderm lineage when compared with their early passage counterpart. This is determined by marked decreases in transcript levels of definitive endoderm markers such as Sox17 and FoxA2. We also observed reduced levels of endoderm markers by immunofluorescence and flow cytometry, with the ‘culture-adapted’ cells displaying up to 70% reduction in Sox17, FoxA2 and CXC4R4 levels when compared with lower passage, ‘normal’ cells. We are investigating the mechanisms underlying this lineage bias to gain insight into the early lineage decisions of differentiating human embryonic stem cells.

Poster Board Number: T-3059
REGULATION PATTERN OF REST IN HUMAN INDUCED PLURIPOTENT STEM CELLS UNDERGOING NEURAL DIFFERENTIATION
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Introduction: The neuronal repressor REST (RE1-silencing transcription factor) is expressed at high levels in embryonic stem cells (ESCs) with a critical role in self-renewal and pluripotency signaling network of these cells. REST is an essential element for brain devel-
Detailed Program and Abstracts — Thursday, June 14

.operation and also neuronal differentiation of ESC in vitro. Although REST is a well-known regulatory element in embryonic stem cells, but according to our knowledge, it has not been evaluated in neuronal differentiation of human induced pluripotent stem cells (hiPSCs) and till now. Aims: In this study, we analyzed the expression pattern of REST during the stepwise differentiation of human ESCs and human iPSCs to neural precursor cells (NPCs) and then to mature neural cells (MN). Material and Methods: The quantitative expression of REST in each step was evaluated by real-time RT PCR and the presence of REST was showed by immunofluorescent assay. The epigenetic regulation of this pattern was performed by Chromatin Immunoprecipitation (ChIP) assay. Results: q-RT PCR analysis showed that the expression level of REST decreased significantly during differentiation of human pluripotent stem cells (hPSCs) to neural precursor cells, and remained stable until neuronal differentiation. Immunofluorescent data revealed the both nuclear and cytoplasmic presence of REST in hiPSCs as well as NPCs, while in the mature neurons it was only detectable in the cytoplasm. The data from the study of selected epigenetic marks was in accordance with the gene expression results. Conclusion: The pattern of REST expression during hPSCs neural differentiation in our findings was in accordance with one of the previously reported articles. This study opens a new window for further experiments in this field for gaining an efficient differentiation of neuronal cells.

Poster Board Number: T-3060

HIGH QUALITY RNA ISOLATION AND TRANSCRIPTOME PROFILING OF HUMAN CELLS PURIFIED BY FACS USING INTRACELLULAR MARKERS WITHOUT REPORTERS

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Stem cell differentiation and somatic cell reprogramming typically give rise to highly heterogeneous cell populations, hampering molecular analysis of specific subpopulations. We optimized a method to obtain high-quality, global gene expression profiles of primary human and hESC-derived cells purified by fluorescence-activated cell sorting (FACS) using cytoplasmic markers. A molecular signature was obtained from primary human pancreatic beta-cells FACS-purified from other endocrine cell types. We compared the beta-cell transcriptome to that of insulin-positive cells derived from genetically unmodified hESCs. Differences in signaling and metabolic pathways suggested directed differentiation culture modifications that generated insulin-positive hESC progeny whose gene expression more closely resembled mature human beta cells. This strategy holds broad potential for comparing pure cell populations from directed differentiation cultures and native human tissue without the use of reporters.

Poster Board Number: T-3061

EFFICIENT DERIVATION OF VASCULAR SMOOTH MUSCLE CELLS FROM HUMAN EMBRYONIC STEM CELLS

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Developing disease-specific cellular models is critical for understanding the molecular mechanism of the disease and is expected to have a major impact on the development of new therapies and regenerative medicine. Several genetic syndromes (such as Marfan and Lafayette-Dietz Syndromes), congenital vascular diseases and hereditary influences are closely associated with vascular smooth muscle (vSMCs) dysfunction in the thoracic aorta (Atherosclerosis, hypertension, aortic aneurysm). A major challenge in vascular disease drug development is the identification of model systems that accurately recapitulate normal and diseased vascular physiology in vitro. Human Embryonic Stem Cells (ES) are pluripotent and can differentiate into all the different cell types of the body. Additionally, ES cells are capable of propagating themselves indefinitely. Because of their plasticity and unlimited capacity to proliferate, ES cells represent a potentially renewable source of vSMCs. We are now in the process of deriving hESC lines carrying the chromosomal mutations responsible for Marfan and Lafayette-Dietz Syndromes. The availability of disease-affected stem cells is the first step towards a vascular disease model. However, the critical next step is to differentiate stem cells into vSMCs, the affected cell type. Currently, vSMC derivation from human embryonic stem cells (hESCs) usually requires serum, the generation of 3D embryoids bodies which does not provide a pure population of vSMCs and is not suitable for drug development. Therefore, we performed a high-throughput screening to determine optimal differentiation conditions and establish a protocol to efficiently generate vSMCs in vitro. Using our well-established stem cell differentiation discovery platform (including robotics and high-content analysis), we screened a targeted library of known regulators of cell differentiation pathways to identify a simple and rapid process to produce vSMCs with high efficiency. Here, we describe a chemically defined monolayer system in which hESCs are initially induced to form cardiovascular progenitors from the mesoderm lineage (Isl1+, Nkx2.5+, SSEA1+). This almost pure, intermediate population is further differentiated toward SMCs (80% SM-MHC+, α-SMA+, Calponin+, Caldesmon and Smoothelin B), which displayed contractile ability in response to a vasoconstrictor (carbachol). This differentiation approach and our ability to produce large amounts of vSMCs from disease-specific hESCs will have broad applications in modelling vascular diseases and is therefore a significant contribution towards a better understanding of disease mechanisms, and it will ultimately to assist in drug discovery and the development of effective treatments.

Poster Board Number: T-3062

REGULATION OF TRANSCRIPTIONAL FACTOR GATA4 BY MIR-200C IN HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESCs) can self-renew and differentiate into almost all types of cells of the body. In vitro, hESCs can differentiate into embryoid bodies (EBs) that contains three germ layers ectoderm, mesoderm, and endoderm. MicroRNAs (miRNAs) are small non-coding RNAs that govern key cellular processes. miR-200 family plays crucial roles in epithelial-mesenchymal transition (EMT) in cancer cells. EMT process is important for hESC differentiation. However, no studies have been performed to investigate the functional roles of human miR-200 family in hESC renewal and differentiation. In this study, we found that miR-200c expression is most abundant in undifferentiated hESCs among the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429). Furthermore, miR-200c is enriched in undifferentiated hESCs and is downregulated in EBs. The knockdown of miR-200c in hESCs caused cell differentiation and induced GATA binding protein 4 (GATA4) expression. Interestingly, overexpression of miR-200c decreased the efficiency of EB formation and repressed the markers of...
all three lineages (ectoderm, endoderm, and mesoderm). We also found that knockdown of GATA4 in hESCs also decreased EB formation. Of note, miR-200c can directly and specifically target the 3'-untranslated regions of the GATA4 in a luciferase assay. Furthermore, miR-200c-mediated GATA4 expression played an important role in hESC proliferation. Knockdown of miR-200c inhibited cell proliferation which can be partially rescued by the blockage of GATA4. On the other hand, the overexpression of miR-200c increased the expansion of hESCs that can be blocked by the overexpression of GATA4. Taken together, miR-200c-mediated GATA4 regulation coordinates hESC proliferation and differentiation. In undifferentiated state, the miR-200c expression promotes hESC renewal and blocks hESC differentiation. During EB formation, the decreased expression of miR-200c is responsible for GATA4 upregulation and promotes the commitment to EBs. This findings uncover a new mechanism of the undifferentiated and differentiated state of hESCs with a focus on the hESC to EB transition.

Poster Board Number: T-3063

MODELING HUMAN GERM LAYER FORMATION USING HUMAN PLURIPOTENT STEM CELLS

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Human embryonic and induced pluripotent stem cells potentially serve as very powerful tools for studying human development. The early events of development have been well studied in model organisms such as sea urchin and mouse, but analogous events in human are not yet well described at the molecular level, due to both technical and ethical issues. Human embryoid bodies (hEBs) are cell aggregates generated from hPSCs. Human EBs have the potential to differentiate into all three germ layers and have been suggested as a possible model for human development. We have characterized the early stages of hEB differentiation and screened for various markers of germ layer formation. We have devised a method for labeling and purifying a population that appears to mimic the first epithelial-to-mesenchymal transition in the human embryo and that shows characteristics of the early mesoderm. We have isolated and characterized this population in order to uncover novel germ layer determinants and understand the molecular basis for germ layer formation. We are currently examining the potential of this mesendodermal population to not only model human development but also to develop novel methods for the generation of high purity mesendodermal cell types from hPSCs.

Poster Board Number: T-3064

THE GENERATION OF LUNG PROGENITORS FROM HUMAN ES CELLS

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Directed differentiation of human embryonic stem (hES) cells and induced pluripotent stem (hiPS) cells into midgut and hindgut endoderm derivatives has been successfully established. The generation of anterior foregut lineages (i.e. trachea, lung and pharyngeal organs) proved more challenging, however. Previously, we showed that dual inhibition of transforming growth factor (TGF)-β and bone morphogenetic protein (BMP) signaling in definitive endoderm resulted in a highly enriched population of anterior forget endoderm (AFE) cells. By mimicking in vivo sequential lineage specification, we ventralized the AFE and specified a fraction of the cells into FOXA2+Nkx2.1+PAHX- early lung progenitors. Here, we further pursued the differentiation of cells of the respiratory system. By modifying the anteriorization protocol, we increased the efficiency of FOXA2+NKX2.1+PAHX- cell generation at day 15 from 37% to approximately 60%. Our data suggest that at this early point in differentiation, AFE cells are prespecified to a subsequent lung, as opposed to pharyngeal fate ten days later. We next devised conditions that specify proximal vs. distal fates. By day 23, cultures from both proximal and distal conditions consisted of highly structured dense epithelial colonies. 70–100% of the cells were FOXA2+NKX2.1+. Under proximal conditions, 90–100% of the cells were SOX2+. Cells at the periphery and discrete cells within the colony expressed p63, a marker of airway basal cells. Continued culture with addition of maturation components yielded clusters of CC10+ Clara cells, mcin5AC+ goblet cells or mcin2+ cells at d35 within these colonies. Mucin5AC+ cells formed tubular structures. Linear structures composed of acetylated-α tubulin+ ciliated cells occurred at the edge of the colonies. Cultures in matrigel yielded spherical structures lined by p63+ cells that are consistent with tracheospheres obtained from adult tracheal basal cells. Day 35 cultures from ‘distal’ conditions were also able to differentiate into CC10+, mcin5AC+ or mcin2+ airway lineages although distal alveolar epithelial (AE) markers such as SPB and mcin 1 could be detected in the cultures, distal conditions need to be further optimized to block the proximal fates and enrich type I and type II alveolar epithelial cells. Importantly, after seeding onto slices of decellularized lung matrices, the cells lined up along the matrix. Further differentiation and spatial organization of the cells in this model is currently investigated. We conclude that we achieved, for the first time, highly efficient differentiation of hES cells into most lineages of respiratory cells with minimal contamination from other lineages. Importantly, this strategy does not require enrichment of cells committed to a respiratory fate based on reporter gene expression. This work is a major step towards regenerative medicine for lung disease using decellularized human lung matrices seeded with hiPS-derived respiratory cell populations.

Poster Board Number: T-3065

DIRECTED DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO HOMOGENEOUS MULTIPOTENT NEURAL PROGENITORS

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Numerous protocols have been published on directing pluripotent or multipotent stem cells to the neuroectodermal lineages. Most of these reports demonstrate the propensity of the cells toward a single neural lineage, either primarily neuronal or glial, but not both. We sought to capture a homogeneous population of multipotent NPCs derived from human ES/iPS cells. As a function of homogeneity, the majority of the starting NPC population should in response to appropriate inductive signals, be directed towards specific cell lineages. For example, in response to neuronal inductive signals, a majority of NPCs should be directed to βIII-tubulin positive neurons. Similarly in response to glial inductive signals, the same starting multipotent neural progenitors should now give rise to mostly glial cells. Using a cocktail of three small molecules that inhibited TGFβ, BMP and GSK3β signaling pathways, human ES/iPS cells could be efficiently differentiated in 7 days to multipotent NPCs that expressed 70% Pax6 positive cells. The protocol was able...
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to accommodate variable initial cell densities and was applicable for both ES and iPS cells cultured on either feeder or feeder-free based culture systems. To demonstrate multipotency, NPCs were differentiated into either predominantly neurons or oligodendrocytes. Under neuronal differentiation conditions, approximately 90% of NPCs expressed neuronal markers, βIII-tubulin and MAP2 after 9 days. Alternatively NPCs could be directed toward primarily oligodendroglial lineages (>50% PDGFRa and GalC) after 14 days of oligodendrocyte differentiation. These results suggest that we have captured a homogeneous population of multipotent NPCs. Homogenous multipotent NPCs are an effective tool in which to apply existing knowledge to pattern neural cells toward anterior, posterior and mid-brain fates for region specific differentiation.

Poster Board Number: T-3066
EFFICIENT MYOGENIC COMMITMENT OF HESC-DERIVED CELLS ON THE BIOMIMETIC SUBSTRATE REPLICAING MYOBLAST TOPOGRAPHY

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Recent development in stem cell technology has demonstrated human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) as possible cells for cell-based therapy and studies of particular differentiation program. However, spontaneous and heterogeneous differentiation of these cells may limit the potential use. Here, we describe the efficient generation of mesenchymal stem cell population from both hESCs and iPSCs having non-tumorigenic potential and their commitment into myoblast. In skeletal development, increased cell-cell contact plays critical initial steps for myogenic commitment. We demonstrate that iPSCs and hESCs-derived cells can undergo efficient myogenic commitment by topographical cues present in their environment. We have created substrates from biomimetic materials that can replicate the micro- and nanoscale topography of fully differentiated skeletal myoblast. When hESCs and iPSCs-derived mesenchymal cells were cultured on biomimetic pattern, mesenchymal cells followed the underlying myoblast pattern. Furthermore, gene expression and cell fusion index showed enhanced myogenic commitment on these substrates. These results demonstrate that myogenic potential of hESCs and iPSCs-derived cells are highly dependent on the micro- and nanoscale topographical cues provide by skeletal myoblast

Poster Board Number: T-3067
NOVEL COMBINATORIAL APPROACHES TO DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TO HEPATIC-LIKE CELLS.

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Hepatocytes derived from human embryonic stem cells (hESCs) have significant potential to provide an unlimited source of cells for evaluation of drug metabolism and toxicity, and for regenerative medicine. To realise this potential an efficient and robust protocol that approximates natural hepatocyte development is required. This project aims to systematically specify and optimise such a protocol using novel combinations of small molecules and growth factors. hESC are directed towards hepatocytes in a stage-specific and time-dependent manner. HESC cultured in monolayers are first exposed to Activin A and varying combinations of small molecules to initiate and direct differentiation towards definitive endoderm. To initiate gene expression leading to hepatoblast formation cells are treated with a combination of growth factors that are released during embryogenesis, supplemented with additional factors that further enhance this process. Combinations of cytokines and growth factors, including OSM and HGF, together with glucocorticoids and insulin, are used at specific time points and concentrations to prime the cells to form hepatocyte-like cells. Finally a range of defined culture conditions are used in order to create niche conditions stimulating further hepatic maturation. We have found that inclusion of certain small molecules at various time points of differentiation has a significantly beneficial impact on generation of hepatic-like cells. The identity of these cells has been confirmed by the analysis of hepatocyte-specific markers including Albumin and ASGR1 using flow cytometry, quantitative RT-PCR and immunocytochemistry and ELISA. Results from this work will be presented to highlight the differences between standard and small molecule-enhanced protocols for differentiation and maturation of HESC derived hepatocytes.

Poster Board Number: T-3068
RAPID AND EASY MONOLAYER NEURAL DIFFERENTIATION OF HUMAN ES CELLS

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Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) have the potential to differentiate into various cell types including neural cells. Recently, hESC- and hiPSC-derived neural cells have been expected to be applied not only to regenerative medicine, but also to studying pathophysiology of neurological diseases and human neural development as an in vitro model. However, previously established neural differentiation methods for human pluripotent stem cells, including our own method, take long time to derive neural cells in vitro. Moreover, because of its length of time, it has been difficult to analyze functional properties of hES cell-derived neural cells by transient overexpression or knockdown of specific target genes. Thus, in the present study, we developed a rapid and highly efficient monolayer neural differentiation system, with high transfection efficiency. Because our neural induction method needs to be simple and easy to handle, we optimized culture medium, culture period, and differentiation substrates for neural induction, and established a monolayer culture system which requires only several-times of culture medium change during wholeperiod of the neural induction. Finally, by using our monolayer neural induction method, hESCs could rapidly differentiate into SOX1-positive neural progenitors only in 6 days, and into βIII-tubulin-positive post-mitotic neurons only in 16 days. In addition, by transfecting a siRNA against a neural specific gene SOX1 at the beginning of neural induction, we confirmed high knockdown efficiency at day 3 and day 6 after transfection. Currently, by using this system, we are screening candidate genes, which may be involved in human neural development, identified based on global gene expression analysis of several lines of hESCs and hiPSCs.
HUMAN EMBRYONIC STEM CELL DIFFERENTIATION IS CHARACTERIZED BY PROMOTER SWITCHING IN GENES CODING FOR TRANSCRIPTION FACTORS

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Transcription initiation is a key component of gene regulation. Insight into promoter regulation and choice of transcription start sites (TSS) in differentiation using cap analysis gene expression (CAGE) gives high resolution alternate initiation patterns during developmental events and cellular reprogramming. Developmental TSS switching implies promoter utilization changes or ‘switches’ from one to another during differentiation which provides us with insight into: a) lineage specific regulatory events, b) alterations in the form of the final transcript, c) changes in TF utilization and binding, and thus associated changes in regulatory networks directing cell differentiation. Unprecedented resolution of exact TSS choice provides precise locations for upstream regions and thus yields high-fidelity models of transcription factor (TF) binding events using motif prediction. Further, a genome-wide, multi-expression state TF binding prediction can be performed across promoters in-silico complementing ChIP-Seq approaches, which may be used for independent validation. We have developed SwitchEngine, a statistical algorithm for detecting TSS switching in CAGE time-course data. It robustly detects switching events that have been manually validated. In this study we present a 41-day time-course experiment in which h9 embryonic stem cells were differentiated to melanocytes. SwitchEngine detected statistically significant promoter switching events in 439 genes in the most differentiated state as compared to the original embryonic stem cells (p-value < 0.05). These show a significant enrichment for transcription factors and other regulatory elements when analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Benjamini-adjusted p-value < 0.001). Of all genes in which switching was detected, 22.5% of them belong to the “regulation of transcription” GO category, implying that TSS switching plays an important part in TF’s role in driving differentiation.

SMALL MOLECULE-BASED SELECTIVE ENRICHMENT OF CARDIOMYOCYTES FROM HUMAN ESC AND IPS CELLS

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Purpose: Pluripotent stem cell (PSC)-derived cardiomyocytes (CMs) have a great potential for cardiac replacement therapies, in vitro tissue engineering and more reliable safety pharmacology assays. These applications require generation of highly purified, well characterized CMs depleted of other cell types. We and others have previously described genetic methods for lineage enrichment resulting in essentially pure CMs. These methods, however, rely on the genetic manipulation of PSC and the expression of reporter genes which has a number of limitations, particular for therapeutic applications. CM-enrichment based on a lineage-restricted surface marker has also been published, but cell recovery and vitality subsequent to cell sorting procedures such as MACS or FACS is usually low. To overcome above issues we have investigated small molecule-based strategies for CM enrichment from human PSCs. Methods: Human ESCs and iPSCs were differentiated by 2 established, independent methods both requiring ESC aggregation into embryo bodies (EBs). A small-throughput 6well-based screening platform was established to test small-molecule candidates, optimize compound concentration(s) and timing of supplementation. Differentiation efficiency and CM purity was analyzed by flow cytometry, immunofluorescence analysis and qRT-PCR; electrophysiological assessment was further applied for CM characterization. Furthermore, general proliferative and/or toxic influence of applied small molecules on undifferentiated human PSC was assessed by cell cycle analysis and proliferation assay. Results: We have identified a class of molecules enabling efficient enrichment of CMs from other lineages. Protocol optimization allowed enrichment of at least 80% CM purity. Besides generally supporting CMs enrichment, gene expression and electrophysiological analysis further suggested a potential selection of CMs with an atrial/pacemaker-like phenotype in response to small molecule treatment. The method is robust, was successfully applied to several human PSC lines and was compatible with 2 independent differentiation protocols. Currently, potential mechanisms underlying the observed selectivity are under investigation. Conclusion: To our knowledge this is the first report of small molecule-based enrichment of CMs from human ESC and iPSC cells. The adaptation of the method to larger scale CMs production as a next step might provide sufficient cells for their envisioned applications. The technology might not only be utilized for CMs purification and production. Ongoing investigations of the underlying mechanisms will also shed new light on early steps of human cardiomyocyte differentiation, cell survival, proliferation and cell physiology.

STEPWISE INTERVENTION FOR PROMOTED HEMATOPOIETIC DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

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Hematopoietic systems in mouse models have been well characterized based on the defined cell surface markers present on fetal and adult hematopoietic stem cells (HSCs) and their blood derivatives. In humans, by contrast, hematopoietic ontogeny and the subsequent hierarchy have not been determined. Human pluripotent stem cells (ES cells, ESCs and iP cells, iPSCs) are embryo-type cells and a promising cell source for studying the ontogeny of blood cells within a differentiation system. We previously established an in vitro co-culture method using C3H10T1/2 mesenchymal stromal cells, whereby vascular endothelial growth factor (VEGF) promotes the appearance of CD34+ hematopoietic progenitor cells (HPCs) from human ESCs or iPSCs (Takayama et al., Blood, 2008; Takayama et al., J Exp Med, 2010). Here we demonstrate that optimized in vitro hematopoietic differentiation system can be divided into specific developmental stages that include CD56+-CD232+ mesodermal progenitors during the early-phase (days 0-4),
CD34+CD56+CD90+CD105+CD43-KDR- hemangioblasts during the mid-phase (days 5-7), and CD34+ hematopoietic and KDR+ endothelial cells during the late-phase (days 8-10). Using these markers as indicators, we attempted interventions in each phase and found novel factors towards efficient hematopoiesis. During mid-phase, activation of basic fibroblast growth factor (bFGF, 10 ng/ml) under 1% O2 significantly increased numbers of CD34+ cells by 5-fold, as compared to cells without bFGF under 21% O2. Administration of a MYC inhibitor (50 μM) to cells during the early-phase, and tumor growth factor beta (TGF-beta) receptor inhibitor (SB431542, 10 μM) during mid-phase, also stimulated generation of CD34+CD43+ HPCs (1.5-fold and 3-fold in increase respectively). Notably, although this protocol resulted in a prominent yield of mesodermal progenitors during the early phase and of HPCs during the late phase (which were 50% and 10% of all derivatives from human iPSCs, respectively), this signaling manipulation had the opposite effect at other stages. For example, bFGF or TGFbeta receptor inhibition significantly depressed HPCs during the early-phase. Our co-culture system did not require factors known to affect ex vivo human CD34+ HSC / HPC expansion from cord blood cells, which contain stem cell factor, thrombopoietin, FMS-like tyrosine kinase 3 ligand, erythropoietin, interleukin (IL)-3 and IL-6. Comparing between with and without MYC inhibitor in early phase, MYC inhibitor gave rise to higher potential on the creation of hematopoietic cells in late-phase. Our novel culture protocol implicates new players in the stepwise development from a pluripotent state to blood cell generation. These players appear to be governed by circumstances resembling a developmental niche with lower oxygen conditions.

Poster Board Number: T-3072

EPIGENETIC REGULATION OF STEM CELL FATE: ELUCIDATING THE ROLE OF HISTONE MODIFICATIONS AND DNA METHYLATION DURING THE GENERATION OF Pancreatic Lineages FROM HUMAN EMBRYONIC STEM CELLS

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Embryonic stem (ES) cells represent a potential novel source of insulin producing β-like cells for treatment of type 1 diabetes mellitus. When ES cells are differentiated, key genes controlling cellular fate are up- or down-regulated on the transcriptional level via different epigenetic mechanisms, including DNA methylation, miRNA silencing and chromatin remodelling by histone modifications. Understanding these mechanisms is pivotal for the development of novel differentiation protocols to generate β-cells from human ES cells. In this project we focus on the role of DNA methylation as well as histone methylation and acetylation in the differentiation of human ES cells towards pancreatic progenitors. This will be done by lentiviral-mediated knock down/overexpression studies using short hairpin RNA (shRNA) libraries against a broad spectrum of DNA methyltransferases, histone methyltransferases/demethylases and histone acetyltransferases/deacetylases. Using a quantitative RT-PCR (qPCR) array, we observed that the expression of several epigenetic modifiers is changed during the generation of definitive endoderm from ES cells, indicating a differentiation induced change in the epigenetic landscape. The expression pattern and function of these enzymes will be mapped using methylated DNA immunoprecipitation (MeDIP), chromatin immunoprecipitation with sequencing (ChIP-Seq), qPCR and western blotting. During the knock down/overexpression studies, the cellular fate of the ES cells will be monitored using qPCR and quantitative immunocytochemistry (qICC). With this knowledge we hope to elucidate some of the epigenetic mechanisms regulating the cellular fate of ES cells, and use this to improve the strategies used to guide the differentiation of human ES cells towards pancreatic progenitors.

Poster Board Number: T-3073

OLIGODENDROCYTE AND MOTONEURON PROGENITORS DERIVED FROM HESC PROMOTE ASTROGLOIogenesis FOLLOWING COMPLETE SPINAL CORD TRANSECTION THROUGH NOTCH AND JAK/STAT SIGNALING

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Spinal cord injury (SCI) results in neural loss and consequently motor and sensory deficit below the injury. Neuronal progenitors derived from hESC and iPSC cells neural induces locomotor improvement following their transplantation into the animal models of SCI, but little is known about the effects and the underlying mechanism of these grafted cells on local tissue and endogenous neural stem cells. Recently, we have reported the regenerative effects and significant improvement of locomotor function in complete transection rat model of SCI following transplantation of oligodendrocyte progenitors cells (OPC) and motoneuron progenitors (MP) derived from hESC. In the present study, we further analyzed the underlying cell and tissue mechanisms of functional recovery after cell transplantation of OPC and MP hypothesized that beneficial effect is mediated by regenerative signalling pathways activated in the host tissue by transplanted cells. Here we show that transplantation of hESC-derived OPC and MP promote astrogliosis, thorough activation of Jagged1-dependent Notch and Jak/Stat signalling supporting axonal survival.

Poster Board Number: T-3074

THREE-DIMENSIONAL SCAFFOLDING FOR LINEAGE SPECIFICATION OF HUMAN EMBRYONIC STEM CELLS

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Generation of pancreatic insulin-producing cells from human embryonic stem cells (hESCs) has arisen as one of the attractive cell-based therapies for diabetes treatment. Previously, we have demonstrated that a three-dimensional (3D) scaffold culture system can advance insulin secreting β-cell differentiation of mouse embryonic stem cells. In this study, we revealed that 3D scaffolding can significantly promote hESCs pancreatic lineages as well. A fine porous fibrillar network structure of collagen scaffold fabricated for pancreatic differentiation of hESCs was observed using SEM graphic analysis. The definitive endoderm (DE) differentiation is the first step and also the most critical step of hESC pancreatic differentiation. We observed a 5.5 fold increase in DE differentiation efficiency when hESCs were exposed to physiological cues provided by a 3D scaffold designed for hESC DE differentiation. Interestingly, blending distinct ECMs to the scaffold shows remarkable impact on hESC lineage specification. It was found that the expression of Sox17, Foxa2, and CXCR4, three DE marker genes increased 79
folds when hESCs were differentiated in ECM-blended scaffolds. Immunofluorescence staining revealed a high level expression of DE marker proteins in these differentiated cells within 3D scaffolds. No significant cell apoptosis was observed under 3D environments. In particular, SEM imaging revealed abundant cell-cell and cell-matrix interactions in these cells within scaffolds. These results suggested that niches offered by a 3D scaffold play key roles in regulating pancreatic lineage specification of hESCs. Further efforts along this direction will lead to the development of an efficient hESC differentiation technology for producing mature β cells for cell-based diabetes therapy.

Poster Board Number: T-3075

ENHANCING THE DIFFERENTIATION EFFICIENCY OF HUMAN EMBRYONIC STEM CELLS TOWARDS CARDIOMYOCYTES BY ISL1 PROTEIN TRANSDUCTION

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Human embryonic stem cells (hESCs) have the potential to provide an unlimited source of cardiomyocytes that offers a precious tool for drug discovery and regenerative medicine. However, this application is limited due to insufficient efficiency of differentiation protocols. Several studies have shown that by over expression of cardiac transcription factors such as ISL1 the differentiation efficiency can be improved but genetically manipulation of hESCs is difficult and differentiated cells are not safe enough to be applied in cell therapy. To overcome these problems, we investigated the effect of direct introduction of ISL1 recombinant protein, as an alternative of genetic manipulation, in to the cells. Here we present an efficient protocol for differentiation of hESCs in suspension by direct introduction of a LIM homeodomain transcription factor, ISL1 (ISL1) recombinant protein into the cells. To this end, ISL1 ORF was cloned into the pENTR-D/TOPO Gateway entry vector. The ISL1 sequence from pENTER-D-TOPO/ISL1 entry clone was transferred into the pDest17 Gateway and transformed to E. coli strain BL21 (DE3) by heat shock method. The sequence of ISL1 was verified by DNA sequencing. Recombinant fusion protein expression was then induced by the addition of Isopropyl-d-thiogalactopyranoside (IPTG). The expressed His6-TAT- ISL1 fusion proteins (rISL1) were purified by immobilized metal affinity chromatography (IMAC). The purified protein was analyzed by SDS-PAGE and Matrix-assisted laser desorption/ionization-tandem time of flight mass spectrometry (MALDI TOF/TOF MS). Interaction of the rISL1 protein with DNA was confirmed using gel shift assay, then the penetration of the protein into the hESCs was checked using immunostaining with both anti ISL1 and TAT antibodies. After protein expression and testing its functionality, the recombinant protein was applied for differentiation into cardiac cells. To find the best time of protein addition during differentiation, qRT-PCR was performed in every day of the first 15 days after differentiation induction. Differentiation was performed by addition of Activin A 100ng/ml for one day followed by 4 days treatment by BMP4 10ng/ml in RPMI medium containing 2% B27. Our data showed that ISL1 is expressed during day 2-8 after differentiation initiation. After that to find the optimum concentra-
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Poster Board Number: T-3079

THE USE OF HUMAN EMBRYONIC STEM CELLS TO ELUCIDATE THE ROLE OF CD4 IN MACROPHAGE DIFFERENTIATION AND FUNCTION


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We are utilizing a novel system we have recently developed involving the derivation of functional human monocytes/macrophages from human embryonic stem cells (hESC) to closely examine the role of the CD4 molecule in monocyte/macrophage development and function. CD4 has an important role in the human immune system and in the pathogenesis of HIV. One of the primary targets and an important and poorly understood reservoir for HIV in an infected individual are monocytes/macrophages. Other than allowing HIV infection of these cells, the role of the CD4 receptor in the function and development of monocyte/macrophages is not known. We have recently determined that ligation of CD4 on monocytes/macrophages modulates gene and cytokine protein expression as well as macrophage differentiation. We are currently interested in further examining the roles and functions of the CD4 molecule on the monocyte/macrophage cell subset. As a part of our analysis, we would like to examine the effects of the ablation of CD4 expression on macrophage biology. Interestingly, mouse macrophages do not express CD4 receptor at any stage of their differentiation, hence genetic ablation of this receptor in a murine model would not yield any information in this regard. Thus, we will utilize a hESC-based system that we can first genetically manipulate and then examine monocyte/macrophage differentiation and function from a cell type that is among the earliest in human hematopoietic development. To accomplish this goal, we constructed lentiviral vectors expressing a GFP reporter gene and either a siRNA specific for CD4 molecule, or a control siRNA with a scrambled sequence. After initial testing for the ability to downregulate CD4 expression on cell lines and primary macrophages, we introduced these vectors into H1 hESC. The transduced GFP+ cells were sorted out and propagated on MEFs for more than ten passages, establishing the lack of toxicity of transgenic siRNAs in hESC. Next, we differentiated these transgenic hESC into macrophages using a two-step procedure. As expected, we observed reduced expression of CD4 receptor on the resulting macrophages, suggesting that this protein may not play a major role in macrophage development, at least in an in vitro system. We are currently investigating if CD4 knockdown influences the function of these transgenic macrophages and if these cells are susceptible to HIV infection. In summary, it is anticipated that these studies will shed light on the potential roles and importance of CD4 in monocyte/macrophage development and/or function. Further, the use of embryonic stem cells in this research, due to their high plasticity, self-renewal ability, and ability to be genetically manipulated, may allow the development of immune replacement therapeutic strategies to be developed for HIV and other similar diseases.

Poster Board Number: T-3078

HISTONE H3K27ME3 DEMETHYLASES MODULATE DEFINITIVE ENDODERM DIFFERENTIATION FROM HUMAN ES CELLS BY MODULATING THE WNT SIGNALING PATHWAY

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In human ESCs, poised genes, including those that encoding developmental transcriptional factors, exhibit bivalent mark with histone H3 lysine27 trimethylation (H3K27me3) and histone H3 lysine4 trimethylation (H3K4me3). Loss of H3K27me3 mark is believed to be crucial for the rapid expression of cell type-specific regulators during differentiation. However, how loss of this mark contributes to specific cell lineage differentiation is currently unknown. Here, we show that during definitive endoderm differentiation, the H3K27me3-specific demethylases KDM6A and KDM6B is increased with both are enriched in purified definitive endoderm cells. Knockdown of KDM6A/KDM6B does not affect human ESC maintenance but impaired their capacity of definitive endoderm differentiation. Further studies establish that KDM6A/KDM6B contributes to definitive endoderm differentiation through regulating the WNT signaling pathway. Knock-down of KDM6A/KDM6B results in decreased expression of WNT genes that impaired the response to endoderm differentiation signals. Importantly, treatment with growth factors and chemicals regulating WNT signal can rescue the KDM6A/B knockdown effect of endoderm differentiation. Collectively, these results suggest that the H3K27me3 demethylases are required for robust endoderm differentiation from human ESCs, and this requirement involves the modulation of the WNT signaling pathway.

man iPSC lines from control subjects as well as from HHT patients. Human iPSCs were generated by retroviral overexpression of the four Yamanka factors. Undifferentiated iPSCs on matrigel/mTESR medium expressed a set of typical markers of PSCs (OCT3/4, Nanog, TRA-1-81) and were able to differentiate into derivatives of the three germ layers in vitro. A prerequisite for the applications mentioned above is the successful derivation of the desired cell type, in particularly pericytes/vSMCs. We developed an efficient protocol for derivation of pericytes from hPSCs grown as colonies on matrigel/mTESR medium. To induce mesodermal differentiation embryoid bodies (EBs) were generated in low attachment plates in the presence of serum and VEGF and subsequently plated on gelatin in the presence of VEGF and the ALK4/ALK5/ALK7 inhibitor SB-431542. SB significantly increased the total yield of cells in the differentiating culture. Characterization of differentiating EBs at day 12 resulted in high percentages of CD146+ mesenchymal cells (>50% for multiple hPSC lines tested). Additional characterization revealed expression of known markers of MSCs (CD73, CD105) and perivascular cells (NG2, PDGFRβ). Pericytes were partially positive for smooth muscle actin but lacked expression of contractile smooth muscle cell proteins (smooth muscle myosin heavy chain and calponin). Culture of CD146+ cells on collagen IV-coated plates in MSC culture media (MEM-10FBS) resulted in the generation of a homogeneous pericyte population which could be expanded for 15-20 passages. Interestingly, supplementation with FBS and FGF2 significantly potentiated cell growth, while TGF-β inhibited cells proliferation. TGF-β mediated growth inhibition could be reversed by the addition of SB. Pericytes could be readily differentiated into osteocytes and adipocytes. Furthermore pericytes were able to stabilize in vitro networks of human umbilical vein endothelial cells (HUVECs). Assessment of the in vivo potential of PSC-derived pericytes is currently ongoing.
Detailed Program and Abstracts — Thursday, June 14

**Poster Board Number: T-3080**

**IDENTIFICATION OF SMALL MOLECULE KINASE INHIBITORS CAPABLE OF MODULATING THE MESENENDOERM DIFFERENTIATION PATHWAY IN HUMAN ES CELLS.**

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Human pluripotent stem cells provide a powerful platform for modeling human diseases and development. Herein, we describe a screen of kinase inhibitors aimed at identifying pathways involved in early germ layer specification. Starting from a master cryopreserved stock of H9 human embryonic stem cells, we first established conditions capable of reproducibly inducing 40-50% mesendoderm formation as judged by the nuclear co-localization of SOX17 and EOMES protein. Once the baseline differentiation conditions were established, we performed a primary screen of 301 compounds with known kinase inhibition profiles. The kinase inhibitor library was assembled to provide broad coverage of the known kinome. Approximately 100 compounds in the library were considered “highly selective” (inhibiting 1-10 kinases), 100 compounds “moderately selective” (inhibiting 10-25 kinases), and 100 “promiscuous” (inhibiting >25 kinases). During the primary screen, individual wells of differentiating H9 cells were treated on day 1 and day 2 with 5uM of test compound or a DMSO vehicle control. Following 3 days of differentiation, the cells were fixed, stained with antibodies against SOX17 or EOMES and analyzed using immunofluorescence-based high-content imaging. 60 compounds were found to be cytotoxic, 20 compounds reduced both SOX17 and EOMES expression without overt cytotoxicity, 14 compounds increased both SOX17 and EOMES expression, and 2 compounds increased EOMES without a concomitant increase in SOX17 expression. Current efforts have focused on the compounds that increased expression of both SOX17 and EOMES. The 14 compounds identified in the primary screen were subject to secondary screening consisting dose response treatment (10uM, 5 uM and 1uM) with each compound. From this group, 5 compounds were found to reproducibly induce SOX17 in the secondary screen. Two of the compounds increased the endoderm differentiation significantly (~95% of cell population are Sox17 positive at Day 3), and targeted the same kinase. Experiments are underway to examine the signaling pathways modulated by the compounds, examine their effects in additional independent human ES and iPSC cell lines, and to determine if any combination of small molecules can efficiently drive mesendoderm differentiation in the absence of other protein growth factors. If successful, these compounds may prove to not only increase the efficiency of differentiation to specific cell types, but also to provide a first tool to mechanistically separate endoderm and mesoderm differentiation pathways.

**Poster Board Number: T-3081**

**THE ROLE OF MHC CLASS I IN THE IMMUNOGENIC PROPERTIES OF HUMAN PLURIPOTENT STEM CELLS AND THEIR DIFFERENTIATED DERIVATIVES**

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Human pluripotent stem cells (hPSCs) are a promising source for cell replacement therapy because they can replicate indefinitely and generate any cell type in the body. However, in order to use these cells clinically we need to determine whether it will be necessary to immune match them to the recipient. The Major Histocompatibility Complex (MHC) plays an important role in graft rejection by allowing immune cells to distinguish “self” from “non-self.” Recent studies have shown hPSCs have low expression of MHC proteins and may be “immune privileged.” However, it is not known whether differentiated cell types derived from hPSCs retain low expression of MHC or how germ layer-specific cell types will respond to human immune cells. We have demonstrated by gene expression analysis that hPSCs show increased expression of MHC class I upon differentiation in vitro into cells from each of the three embryonic germ layers including neurons, cardiomyocytes and hepatocytes. In addition, microarray data collected from fetal and adult tissue show a similar trend, with MHC class I expression gradually increasing during in vivo development. As part of our ethnic diversity iPSC project, we have derived iPSCs from individuals with different MHC haplotypes and are using them to investigate in vitro immunogenicity of hPSCs and their germ layer-specific differentiated derivatives at various stages in their development. In our first studies we are using two iPSC lines with very different haplotypes: a Yoruba haplotype (HLA A*23:03/ A*30:02; HLA B*57:03/57:03; HLA C*18:01/18:01) and a Caucasian haplotype (HLA A*01:01/ A*02:01; HLA B*08:01/37:01; HLA C*06:02/07:01). Data collected in these studies will be used to answer critical questions regarding the immune stimulating capabilities of cell types derived from hPSCs for future transplant studies.

**Poster Board Number: T-3082**

**INDUCTION OF HUMAN ES CELL-DERIVED HEPATIC CELLS BY NOVEL METHOD USING LOW MOLECULE**

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Human embryonic stem cells (hES cells) are expected to be applied for regenerative therapy, because they have ability of infinite proliferation and pluripotency. Surely many methods have been proposed to differentiate hepatic cells from hES cells, but most of them have poor efficiency for differentiation, expensive protocols using growth factors and are far from safety in the case of cell processing using virus transfection. To solve these problems, we tried in this paper to establish the new original method using low molecule and to differentiate hepatocytes directly from hES cell without embryoid formation. The phenotypic changes of hES cell-derived hepatic cells were analyzed with RT-PCR, immunostaining and real-time RT-PCR in addition to morphologic and functional analyses. These results showed superiority of this method in simplicity, efficiency and economy, which guarantees to create one possible source of hepatic cell for cell transplantation.
Posters

**T-3084**

**SPECIFIC miRNAs EXPRESSION IN ERYTHROPOIETIC DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS**

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Objective: microRNAs (miRNAs) are small non-coding RNAs that regulate gene expression at the post-transcriptional level through mRNA degradation or translation inhibition. However, it is not known whether miRNAs participate in hematopoietic or in particular erythropoietic processes. The aim of this study is to investigate erythroid lineage-specific miRNAs expression regulated during *in vitro* erythropoiesis using Human embryonic stem cells (hESCs) and human umbilical cord blood CD34+ cells. Materials and methods: CD34+ hematopoietic stem/progenitor cells were produced from hESCs *in vitro* and subsequently induced to differentiate into erythroid cells by culture on OP9 feeder cells followed by erythropoietic differentiation culture using specialized conditioned culture media. The expression profiles of four lineage-specific miRNAs, miR-142-3p, miR-142-5p, miR-146a and miR-451 during erythropoiesis were analyzed by quantitative PCR of extracted miRNAs. Results: Expression levels of these miRNAs were dynamically changed during differentiation of hESCs to CD34+ hematopoietic stem/progenitor cells and subsequent differentiation of CD34+ cells into erythrocytes. Conclusion: This result indicates that miR-142-3p, miR-142-5p, miR-146a and miR-451 express specifically during erythropoietic induction of hESCs. This suggests that these four miRNAs might involve in regulating erythropoiesis.

**T-3085**

**DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO MEDIUM SPINY NEURONS OF THE STRIATUM**

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The objective of this work is to develop a high purity human stem cell based platform for Huntington's Disease (HD) mechanistic research and drug discovery. HD is a neurodegenerative genetic disorder that results primarily in the loss of medium spiny projection neurons (MSN) of the striatum. Current animal models do not sufficiently recapitulate the complex cascade of neurodegenerative events in the human. Current cellular models are complicated by primary extraction and purification methods or are confounded by contaminant progenitor populations. The need exists for high purity MSN and lateral ganglionic eminence progenitors (LGP) at multiple developmental stages for transplant studies as well as drug discovery and predictive toxicology assays. Genetically abnormal blastocysts donated by participants were thawed and an hESC line was derived and maintained in animal-free conditions and characterized. hUC-HD1 is a karyotypically normal HD line carrying 44 CAG repeats. In addition, a genetically normal hESC line and an iPSC line carrying 180 CAG repeats were differentiated side-by-side. Undifferentiated hESC and iPSC were grown on matrigel-coated flasks to subconfluence in a feeder-free system. Cultures were then transitioned from conditioned medium to DMEM-F12 rich medium for neural induction. Cells were grown in suspension and passaged routinely in differentiation medium supplemented with growth factors until day 60, when they were replated and growth factors were withheld for final maturation to MSN by day 66. Stage-specific immunocytochemistry was performed at day 14 (GSH-2), day 25, day 42 and 45 (FoxP1) and day 66 (DARPP-32) to optimize growth conditions. Cultures were additionally matured to day 135 and electrophysiology was performed, demonstrating neuronal firing patterns and functional synaptic connections. During recording of spontaneous post-synaptic currents, NBQX/APV and GABA-Zine were used to block glutamatergic and GABA-ergic events, showing that the cells received both excitatory and inhibitory inputs. The early stage LGP will be used for studies of striatal development and the MSN will be used for transplant studies, drug discovery and predictive toxicology.

**T-3086**

**POSSIBLE USE OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES AS AGING MODEL FOR HUMAN CARDIAC CELLS**

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Human embryonic stem cells (hESCs) have abilities to self-renew and differentiate into all types of cells in human body including cardiac cells. These features of hESCs make them considered as an ideal model for developmental biology, human development and drug screening. Aging of cardiomyocytes (CMs) is natural phenomenon in human cardiac cell, however, aging could not be studied using human cells. In this study, we analyzed aging phenomenon in hESC-derived cardiac cells and investigate the possibility of using hESC-derived CMs as aging model. We induced differentiation into CMs from hESC cultures using direct differentiation and we classified hESC-derived CMs into early (day 12), middle (day 18) and late (day 24)-stage, based on their functionality. We observed morphology of hESC-derived CMs and counted beating rate. In addition, we performed senescence-associated (SA) β-gal staining and counted positively-stained hESC-derived CMs. We performed immunostaining in early stage of hESC-derived CMs and confirmed the cardiac features of differentiated CMs. Middle and late-stage hESC-derived CMs showed increase of pigmented cells and demonstrated slower beating late due to in vitro culture period. Human ESC-derived CMs at day 12 showed small population of stained cells. And, CMs at day 18 and -24 showed larger population of stained cells and the positively-stained cells were abundant in day 24 hESC-derived CMs. In this study, we demonstrated the *in vitro* stage-dependent aging phenomenon in hESC-derived CMs. Furthermore, we insist the possible usage of hESC-derived CMs for aging study of human cells (2011-0007944 and A111539).

**T-3087**

**MAINTENANCE OF THE FUNCTIONAL RETINAL PIGMENT EPITHELIAL CELLS DERIVED FROM HUMAN EMBRYONIC STEM CELLS**

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Maintenance of the functional retinal pigment epithelial (RPE) cells is important issue for cell replacement therapy of retinal degenerative disease because it is related to functional recovery. We regenerated functional RPEs as well as mature neurons from the MSN will be used for transplant studies, drug discovery and predictive toxicology.
EXAMINATION OF CELL SIGNALING PATHWAYS DURING EARLY ENDODERM SPECIFICATION FROM HUMAN EMBRYONIC STEM CELLS UTILIZING FLUORESCENCE CELL BARCODING AND INTRACELLULAR FLOW CYTOMETRY

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The ability of human embryonic stem cells (hESCs) to differentiate into various cell lineages allows for the study of developmental biology and has applications in the fields of regenerative medicine and cellular therapy. To optimize differentiation protocols and to answer biological questions, it is important to understand the signaling pathways that are involved in lineage specification. We applied a multiparametric flow cytometric screening approach to analyze cell signaling during endoderm differentiation from hESCs. H9 hESCs were differentiated to early endoderm in the presence of low serum and Activin A. Our differentiation method was verified using multicolor flow cytometry to monitor the downregulation of the pluripotency marker Nanog and the upregulation of endoderm markers Sox17 and FoxA2. We screened cells at different time points of endoderm differentiation by flow cytometry using over 80 antibodies specific to proteins of many known signaling pathways. We utilized fluorescent cell barcoding to increase throughput and minimize experimental variability, which enabled multiple differentiation time-points to be analyzed in a single sample. Data from the screen suggest a complex signal transduction network for endoderm specification of pluripotent stem cells in culture. We observed changes in the phosphorylation states of Rb and S6, which are implicated in cell proliferation. In addition, we detected changes in multiple proteins involved in SMAD and integrin signaling pathways, which have been implicated in self-renewal and differentiation. We also observed changes in markers involved in B-cell signaling, suggesting this signaling pathway may play an additional role during stem cell differentiation. Further analysis of our data set may reveal additional signaling pathways that are relevant to endoderm specification. This screening methodology can be readily applied to various stem cell populations and their derivatives to explore cell signaling events such as self-renewal, reprogramming, and lineage specification.

IDENTIFICATION AND PROSPECTIVE ISOLATION OF SOMITE-LIKE CELLS FROM MURINE AND HUMAN PLURIPOTENT STEM CELLS

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Due to their intrinsic developmental potential and proliferative ability, human embryonic stem cells (hESCs) hold remarkable promise for their use in cell-based therapies as well as in an vitro model for early human development. Although significant advancements in the derivation of cells with specific neural (CNS, neural crest), endoderm (liver, pancreas) and lateral mesoderm (blood, endothelium) fates have been made, little has been done on the refinement of current techniques for the differentiation of hESCs into derivatives of paraxial mesoderm, such as skeletal muscle. We have set up cul-

NEW BMP INHIBITOR DORSOMORPHIN ROBUSTLY ENHANCES THE DIFFERENTIATION EFFICIENCY OF HUMAN EMBRYONIC STEM CELLS INTO RETINAL PIGMENT EPITHELIUM

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Functional retinal pigment epithelium (RPE) can be generated from human embryonic stem cells (hESC). These cells hold great promise as a potentially unlimited source of material for the treatment of retinal disorders such as age-related macular degeneration. Current differentiation methods rely on spontaneous differentiation or the use of expensive recombinant growth factors such as noggin. Dorsomorphin (DM) is a highly potent small molecule that targets several TGFβ type receptors and interferes with both BMP and activin/nodal type signalling, resulting in high efficiency neural conversion. Being a small molecule it is less expensive, more stable and better able to penetrate large cell masses than the proteinaceous equivalent. In this study we have investigated the ability of early DM treatment to direct hESC-RPE differentiation. Cells from the HESC line Shef6 and Shef3 were differentiated in a monolayer on feeders or feeder-free in the presence of 1µM DM. Pax6 expression was measured after 15 days to determine neural conversion in DM treated vs. control wells. After a 50 day differentiation protocol, the RPE yield was assessed by quantitative PCR and by comparing the increase in surface pigmentation over time. A significant increase in pax6 expression in the presence of DM was detected at day 15 by QPCR and immunocytochemistry. This was coupled with a five fold average increase in pigmented surface area relative to untreated controls. Intriguingly the presence of mitotically inactivated mef feeders or feeder conditioned media appears to act synergistically with DM to enhance both neural conversion and RPE differentiation in both Shef3 and Shef6, a phenomenon that requires further investigation. Efficient and simple protocols for generating large quantities of RPE will be important for progression in research and development and for clinical use. RPE can be reliably derived from a number of HESC and IPSC lines but efficiency varies greatly, with some lines unable to differentiate into RPE at all. In this protocol we demonstrate enhanced RPE yields in feeder-free conditions without the need to make embryoid bodies or use expensive amounts of recombinant proteins. Dorsomorphin provides a simple, cost-effective, one-step method for enhancing neuroectodermal differentiation and enhancing hESC-RPE differentiation efficiency.
specific progenitor cell-binding peptides by selection of a phage display is a powerful method for identifying affinity reagents markers both on hPS cells and various progenitor cell types. Phage of deriving well-defined therapeutic replacement cell populations ability of normal and diseased hPS cells for basic research, a major reprogramming technology has dramatically increased the avail-
dermomyotome, the portion of the somites that gives rise to muscles. Based on these findings, we speculated that this novel cell population, named Somite-Like Progenitor (SLP) cells, could represent an in vitro equivalent of the earliest muscle progenitors emerging in the embryo. Confirmation was attained through the SLP cells’ ability to differentiate into myogenin+ mature cells in vitro under defined conditions. Significantly, we were also able to isolate and subsequently re-expand SLP cells utilizing selective FACS sorting strategies based on a unique combination of surface markers that included CD29. Furthermore, we have observed the appearance of cell clusters with the same unique morphology of SLP cells in differentiating mouse ESC (mESCs). The mESC-derived cell population also exhibited skeletal muscle potential in vitro and had an almost identical gene expression profile as the hESC-derived SLPs. Isolation of mESC-derived SLP will help setting up in vivo strategies to unveil the full potential of these cells. The developmental progression from undifferentiated ESC toward the specification of muscle cells is largely unexplored in humans. The generation of hESC derived somite-like cells, not only will give new insights into human skeletal muscle development, but will also ac-
celerate the move towards the use of hESC-derived skeletal muscle cells for therapeutic use in a variety of muscle atrophy and wasting conditions.

Poster Board Number: T-3092

Identification of Surface Markers on Human Pluripotent Stem Cells and Their Differentiated Progeny

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The self-renewal and differentiation potential of human pluripotent stem (hPS) cells offers a virtually unlimited source of therapeutic replacement cells to treat a variety of degenerative diseases including, arthritis, osteoporosis, heart disease, Parkinson’s, Alzheimer’s and macular degeneration. The ability to reprogram adult cells to induced pluripotent stem (iPS) cells has enabled the creation of patient-specific hPS cells as a source of cells for disease modeling, drug discovery, and eventually cell replacement therapies. While, reprogramming technology has dramatically increased the avail-
bility of normal and diseased hPS cells for basic research, a major bottleneck is the critical unmet need for more efficient methods of deriving well-defined therapeutic replacement cell populations free of residual hPS cells. A current limitation is the lack of surface markers both on hPS cells and various progenitor cell types. Phage display is a powerful method for identifying affinity reagents against cell surface target molecules. However, identification of specific progenitor cell-binding peptides by selection of a phage display library against differentiating hPS cells is difficult because cellular heterogeneity limits the availability of any one particular progenitor target cell type. To circumvent this problem, we selected phage display libraries against clonally pure human embryonic pro-
genitor (hEP) cell lines from the ACTcellerate™ cell line collection, which consists of over 140 scalable lines. A key advantage of this strategy is that it enables peptide selection against large numbers of a single clonally pure cell type which might otherwise be repre-
sented by only a few cells in a mixed population of differentiating hPS cells. Using this approach, we have successfully identified cell-targeting peptides with high specificity and selectivity for progeni-
tors of cartilage and bone. Specificity of the targeting peptides was demonstrated in peptide phage competition experiments with ex-
cess free peptide. Selectivity was demonstrated using immunocy-
tochemistry to assess peptide phage binding against the target cell line compared to other hEP lines and undifferentiated hPS cells. We are currently selecting peptides against additional progenitor cell types (i.e. adipose, smooth muscle) and testing the use of labeled peptides in FACS-based cell separations to enrich mixed cell popu-
lations for specific progenitor cell types such as cartilage and bone. In an effort to develop methods of removing residual pluripotent stem cells, we have identified a novel surface antigen, termed C7, on hPS cells against which we are preparing antibody and peptide affinity reagents. In a model experiment, osteogenic progenitor cells were mixed 1:1 with hPS cells and enriched to >99% purity in a single round of hPS removal by C7-based FACS. Our data suggest that phage display can be used to identify progenitor and hPS cell targeting peptides that will be useful for the production of well-
defined cell populations free of potentially tumor forming hPS cells.

Poster Board Number: T-3093

Bioprocess Strategies for Minimising Heterogeneity During Expansion of Murine Embryonic Stem Cells (mESCs)

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Pluripotent embryonic stem cells (ESCs) represent a potentially un-
limited supply of cells for therapeutic use, developmental studies and toxicity testing. Since their discovery, our understanding of the nature of embryonic stem cell cultures has progressed. Research groups have now identified subpopulations within the pluripotent population that preferentially differentiate into specific lineages. Many differentiation protocols are currently long and inefficient, often resulting in low purity of the target cell type. This may be due, in part, to heterogeneous starting populations. For successful translation of ESC-derived cell therapies to the clinic it is necessary to develop robust and highly efficient differentiation processes. We hypothesised that purer end populations could be achieved with homogenous starting populations. We investigated two ap-
proaches to achieve this. Firstly, we investigated heterogeneity at the cellular level by establishing 40 single cell-derived clonal lines from wild-type mixed populations. Embryoid body differentiation highlighted clonal line developmental bias through comparative analysis of germ layer markers. These data support the notion that a specific clonal line could be chosen for a specific differentiation. Our second approach was to control microenvironmental factors at the expansion stage. We chose to investigate oxygen tension. Mouse early embryo development occurs under hypoxic condi-
tions which suggests that traditional culture conditions may be sub-optimal. Firstly the effects of hypoxia on self-renewal and plu-
ripotency were characterised. We then investigated the hypothesis
that maintenance in hypoxic conditions has an effect on differentiation in traditional normoxic conditions. Our studies suggest two separate approaches for minimising the innate heterogeneity within pluripotent ESC cultures that may improve differentiation efficiency.

**Poster Board Number: T-3094**

**ADAMTS-9 IS INVOLVED IN MESODERM DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS**

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A Disintegrin-like And Metalloproteinase with Thrombospondin (TSP)-Type I sequence motifs 9 (ADAMTS9) is widely expressed in mouse and human fetal tissues. ADAMTS9 null mice cannot survive beyond E7.5 and its haploinsufficiency is associated with cardiac and aortic anomalies. We hypothesized that ADAMTS-9 was important during early embryogenesis. Human embryonic stem cell (hESC) was used as a model for studying ADAMTS9 expression and its role during early differentiation. Our results indicated that ADAMTS9 immunoreactivity was detected in cells located at the boundary of hESC colonies in undifferentiated state. Its mRNA and protein expression increased time-dependently in the first 24 days of embryoid body (EB) formation, The expression pattern was similar to that of mesoderm and endoderm markers. The positive correlation of ADAMTS9 with ESC differentiation was also found in the mouse system, in which ADAMTS9 expression was increased time-dependently during mouse EB formation and down-regulated during reprogramming from somatic cells into induced pluripotent cells. During hESC differentiation ADAMTS9 was co-localized with several specific mesoderm and endoderm markers. Transient ADAMTS9 knockdown by siRNA in hESC significantly decreased the expression level of mesoderm marker, REN. Induction of differentiation of hESCs towards the mesoderm lineages dramatically increased ADAMTS9 expression of the differentiated colonies. In the differentiated mesodermal cells, ADAMTS9 was co-expressed with vascular endothelial markers, VEGF and CD31, but not with pericyte markers, alpha muscle actin. Lentiviral vector encoding ADAMTS9 shRNA was used for long term knockdown of ADAMTS9. Knockdown of ADAMTS9 significantly reduced the expression of certain mesoderm markers, REN, Pdgfra and CD34. In conclusion, ADAMTS9 was induced during mesoderm differentiation and its knockdown led to down-regulation of mesoderm markers. The roles of ADAMTS9 during hESC differentiation and early embryo development warrant further investigation. Acknowledgement: Seed Funding Scheme to Support Research Projects on Human Embryonic Stem Cells (ESCs) and Induced Pluripotent Stem Cells (iPSCs), Stem Cell & Regenerative Medicine Consortium (S2RMC), Li Ka Shing Faculty of Medicine, The University of Hong Kong

**Poster Board Number: T-3096**

**BMP 10, A HEART-SPECIFIC CYTOKINE, INDUCES DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS WITH A MUCH HIGHER POTENCY THAN MEMBERS OF THE BMP7 SUBGROUP**

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Bone morphogenetic proteins (BMPs) are a large family of biologically active cytokines. BMP ligands are present within a broad range of animals where they are highly conserved. In humans they are known to have pleiotropic functions during all stages of life. With respect to human pluripotent stem cells, it has been shown that BMP2, 4, 6 and 7, either as homodimers or heterodimers, can induce differentiation into embryonic (mesoderm, endoderm) and extraembryonic (trophoblast) lineages. In differentiation protocols applying more stringent cell culture conditions, these BMPs have also been successfully used to differentiate human pluripotent stem cells into vascular progenitor cells, chondrocytes and primordial germ cells. However, these ligands only represent a minority of the BMP family, which consists of more than 20 members and can be divided into several subgroups, depending on sequential and structural homology. In addition, it has been shown for certain human adult stem cells and progenitor cells, that one and the same cell type can respond differently to distinct ligands, even if they belong to the same subgroup (e.g. BMP5, 6 and 7). This prompted us to investigate the effect of distinct members of the BMP family - that have to our best knowledge not been tested yet - in terms of their effect on human pluripotent stem cells. For this purpose, we chose BMP5, BMP13 (GDF6) and BMP10, all representing members of distinct subgroups, with BMP5 belonging to the BMP7 subgroup, as well as the well-researched BMP7 as a reference. Furthermore, we successfully reprogrammed human choriogenic villi cells to induced pluripotent stem cells (hiPSCs). In our in vitro experiments we tested these hiPSCs - as well as human embryonic stem cells (hESCs lines H1 and H9) - as a reference, with equal concentrations of these human recombinant BMPs in chemically defined medium (without addition of any other exogenous cytokines or inhibition of autocrine signaling pathways). We observed that hESCs as well as hiPSCs respond identically to all the four BMPs tested, namely they first differentiated to mesendoderm cells and, predominantly to early trophoblast cells (indicated by the expression of primitive streak markers T and MIXL1 and early trophoblast markers CDX2 and HAND1), followed by cells belonging to the definite mesoderm/endoderm and trophoblast lineage (indicated by cell fusion markers and as latest, secretion of hCG as marker of multinuclear syncytiotrophoblast cells). However, as we found out on mRNA (microarray, qPCR) and protein level (Western Blot, immunocytochemistry, ELISA), the kinetics and potency of these ligands differ immensely. Interestingly, we discovered that BMP10, whose expression in vivo is restricted to specific parts of the heart for a short time frame during heart development, induces differentiation with by far the highest kinetics/potency, and BMP13 being the weakest. Detailed comparative kinetic studies of earliest treatment responses - when differentiation of the cells had not occurred, revealed a significantly higher level of phosphorylated SMAD1/5 for BMP10 at any time point investigated, whereby signaling via non-SMAD-frame during heart development, induces differentiation with by far the highest kinetics/potency, and BMP13 being the weakest. Detailed comparative kinetic studies of earliest treatment responses - when differentiation of the cells had not occurred, revealed a significantly higher level of phosphorylated SMAD1/5 for BMP10 at any time point investigated, whereby signaling via non-SMAD-pathways (such as MAPKs and Akt) appears to play only a minor role. In summary, our studies have unveiled additional cytokines that could be applicable for lineage specific differentiation of hiPSCs/hESCs to derive donor cell types useful for cellular regenerative therapies in the future.

**Poster Board Number: T-3097**

**AN IMMOBILIZED CHIMERIC FORM OF CELL ADHESION MOLECULE STIMULATES NEURAL CONVERSION OF MOUSE EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS**

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The unique property of ES/iPS cells represents a promising source to overcome many diseases by providing an unlimited supply of
many cell types, including neurons. The formation of appropriate neural circuits depends on a complex interplay between extracellular guiding cues and intracellular signaling. N-cadherin is one of the best-studied cell adhesion molecules (CAMs) involved in the formation of axon and dendrite (collectively termed neurite). However, little is known about N-cadherin influences on differentiation of ES/iPS cells into neural or glial progenitors remains to be clarified. To elucidate the role of this CAM, we introduced immobilized chimeric form of N-cadherin (N-cad-Fc) using two differentiation induction protocols: serum-free neurosphere (SFN) and hanging-drop-based embryoid-body (HDEB) strategy. In SFN and HDEB methods, the differentiated ES/iPS cells responded to immobilized N-cad-Fc chimera by extending longer neurites than controls. The differentiated cells on N-cad-Fc also showed almost similar effect in absence of any external neural stimulatory factors. The response was significantly reduced by pre-treating differentiated cells with neutralizing N-cadherin antibody or inhibitors to the fibroblast growth factor (FGF). Time lapse imaging also showed that N-cadherin substratum is capable of directing a neurite outgrowth response. In addition, the differentiation induction in adherent monolayer protocol using N-cadherin substratum showed its potentiality to remove undifferentiated or poorly differentiated populations from differentiated neural cells. The neural and glial subtypes were confirmed under transcript and protein level using regional specific differentiation markers. These data showed that the applicability of substrate-associated adhesion molecules to stimulate the healthy neurons from ES and iPS cells and point to the potential of using cadherin-based chimeras to promote nerve regeneration.

**Poster Board Number:** T-3098

**ISOLATION AND CHARACTERIZATION OF A2B5-POSITIVE CELLS FROM HUMAN EMBRYONIC STEM CELLS**

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Currently, oligodendrocyte differentiation from human embryonic stem cells (hESCs) takes a long time. Therefore, we have developed a protocol for producing highly purified cells that exhibit OPC characteristics from hESCs in a short period of time. Embryonic bodies derived from hESCs were differentiated into neural precursor cells (NPCs) through simultaneous inhibition of BMP and Activin/Nodal signals with small molecules. NPCs expanded in N2B27 medium supplemented with bFGF and EGF for one week included lots of A2B5 immunoreactive cells which can subsequently give rise to cells with OPC characteristics. To get enriched A2B5-positive populations and to exclude unwanted cell population, we took advantage of the magnetic activated cell sorting (MACS) technique with A2B5 specific antibody. We confirmed that it is possible to get highly pure population of A2B5-positive cells (98%) after MACS by Fluorescence-activated cell sorting (FACS) and cell counting. After MACS, sorted A2B5-positive cells were expanded in N2B27 media supplemented with PDGF-AA, IGF-1 and bFGF for 10 days to consequently generate OPC-like cells expressing PDGF-Rand NG2 (70–80%). Majority of these cells had bipolar or tripolar morphology and expressed the transcripts of PDGFR, NG2, Olig1/2, Sox families, Ap2a and Snail which were associated with OPC differentiation. Interestingly, we found that the treatment of neuroregulin (NRG) facilitated the proliferation and survival of PDGFR/NG2-positive cells and up-regulated genes of OPCs (NG2, Sox10, Olig1/2). As a result, 70–80% of total cells were OPC-like cells (A2B5+/NG2+/PDGFR+) after ~4 weeks since the beginning of differentiation. When we transplanted hESC-derived OPCs into traumatically injured spinal cord model, OPCs transplanted group improved hindlimb performance in both legs after 3 weeks after transplantation. We observed strong MBP-immunoreactivity in the cell-injected site of injured spinal cord and transplanted group significantly increased the amount of myelinated axons compared to control group. These findings suggest that OPCs could be fast and efficiently generated from hESCs by A2B5-mediated cell sorting and have potential benefits for diseases such as spinal cord injury. This research was supported by the Ministry of Education, Science, and Technology (MEST) (SC1110); National Research Foundation, MEST (2010-0020353), the Stem Cell Research Center of the 21st Century Frontier Research Program funded by the Ministry of Education, Science and Technology, Republic of Korea and from Korean Health Technology R&D Project, Ministry for Health, Welfare & Family Affairs (A100694).

**Poster Board Number:** T-3099

**ANALYSIS OF EARLY REGULATORY FACTORS DURING NEURAL CREST CELL DIFFERENTIATION FROM MOUSE EMBRYONIC STEM CELLS.**

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The neural crest (NC) is a transient multipotent stem cell population. They arise at the progenitor domain, neural plate border (NB) between the neural and non-neural ectoderm. They migrate through the embryo and give rise to a vast range of cells. Abnormal development or loss of the NC cells causes neurocristopathy. Studying the mechanism of NC differentiation allows us to understand the pathogenic mechanisms and to develop effective therapies of neurocristopathy. Previous studies using model animals and cultured cells showed that several transcription factors such as Slug, Twist, play important roles in NC differentiation. However, the regulatory mechanisms during the early step of NC differentiation are not fully understood. This is partly because the experimental models and methods still remain undeveloped to analyze the early NC differentiation accurately. We previously established the NC cell induction system from mouse ES cells in serum-free monolayer culture. This induction system is able to differentiate the mES cells into NC cells which express typical NC marker genes (e.g., slug, Ap2a, Ap2b, c-kit, Twist, Sox9) and differentiate into NC derivatives (e.g., peripheral neurons, oligodendrocytes, smooth muscle cells, and chondrocytes). This induction system enabled us to follow up the process of cell status change during NC cell differentiation. In this study, we attempted to identify the regulatory factors in early NC differentiation by the use of this induction system. For this purpose, we performed global gene expression analysis in mES cells just after the onset of NC differentiation. As results, we identified 53 genes whose expression increased from the onset of NC differentiation. Among these candidates, early transcriptional regulatory genes including Msx, Dlx, Idfamilies, Ap2a, and Snail, which were involved in NC differentiation and NB specification were included. On the other hand, cytoskeletal and extracellular structure genes

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POSTER BOARD NUMBER: T-3100

THE ROLE OF LHX2 IN HUMAN NEURAL FATE DETERMINATION

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The mechanistic basis of early neural development in human is still largely unknown. Human embryonic stem cells (hESCs) are pluripotent stem cells capable of giving rise to all the cell types of body thus provide us an excellent opportunity to explore the genetic and epigenetic mechanisms underscoring early neural development. LIM homeobox 2 (LHX2) is a transcription factor that was found highly expressed in the hESC-derived early neural progenitors. As demonstrated by murine models, LHX2 can serve as a selector that plays both active and suppressive roles in cortex formation. However, the role of LHX2 in human neural development is rarely understood. Here, we demonstrated that LHX2 plays a critical role in early neural differentiation of hESCs. Our results revealed that LHX2 was highly expressed proceeded PAX6 and SOX1 and was co-expressed with many neural and post-mitotic neuronal genes. Furthermore, conditional overexpression of LHX2 promoted hESC differentiated toward neural fate whereas disruption of LHX2 by shLHX2 knocked down compromised the neural differentiation of hESC both in vitro and in vivo. We further confirmed that LHX2 targeted to PAX6 enhancer and cis-regulated PAX6 promoter by ChIP and reporter analysis. Collectively, our results demonstrate that LHX2 is a key factor for neural fate determination during early pluripotent to neural fate transition.

Poster Board Number: T-3101

ELUCIDATING MOLECULAR FUNCTION DOWNSTREAM OF THE HOMEBOX PROTEIN HEX USING MOUSE EMBRYONIC STEM CELL MODELS FOR DISTINCT ENDODERMAL PROGENITOR POPULATIONS

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Immediately following the induction of endoderm, regional identity is initially established in the anterior with the expression of the homeodomain Hex. The anterior endoderm is both an important patterning centre and the precursor population for the liver and pancreas. In mammalian development, endoderm formation occurs in two phases and the fate of these populations is different. In the blastocyst, ICM cells generate the primitive endoderm (PrE) which will give rise to the extra-embryonic parietal and visceral endoderm. Hex is initially expressed throughout the PrE and then becomes restricted to the anterior visceral endoderm (AVE), an important embryonic signalling centre. During gastrulation, a second wave of definitive endoderm (DE) differentiation occurs, generating the foregut. One of the earliest specification events in this lineage is also the specification of anterior fate by Hex, this time the anterior definitive endoderm (ADE). We have developed an embryonic stem (ES) cell model to generate either embryonic or extra-embryonic endoderm under defined conditions while monitoring anterior identity. We have used this system, alongside Hex mutant ES cells, to work out the network downstream of anterior induction in both visceral and definitive endoderm. Gene expression analysis on Hex positive populations in ES cell differentiation has been used to characterize key downstream targets and we are beginning functional analysis on these factors. The overall phenotype exhibited in the Hex null ES cells was also assessed by micro-array. This data indicates that Hex null endoderm expresses higher levels of posterior markers, consistent with posterior and non-anterior visceral identity, and consistent with the role of Hex as a key regulator of anterior gene expression. Our data also suggests that one of the key distinguishing characteristics between endodermal populations may be positional markers, rather than their future contribution. Thus, the potency of visceral and definitive endoderm for downstream differentiation may depend on their respective regional identity, rather than their prospective embryonic fate.

Poster Board Number: T-3102

INSULIN-LIKE GROWTH FACTOR 2 ENHANCES CHONDROGENIC DIFFERENTIATION OF PARTHENOGENETIC MURINE EMBRYONIC STEM CELLS UNDER THE THREE DIMENSIONAL CULTURING ENVIRONMENT

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The generation of cartilaginous tissues using pluripotent stem cells can be one of the solutions for cartilage regeneration and skeletal tissue repair, as the mass production of cartilaginous cells is required for the cure of cartilaginous defects and diseases. Parthenogenetic ESCs (PESCs) can be a useful stem cell source. The defects in full-term development of this cell type enable researchers to avoid the ethical concerns. Moreover, in female patients, if the PESCs are derived from her own oocytes, the cells will have that patient’s genetic information. Here we presented data demonstrating that PESCs can be differentiated into chondrogenic cells by the induction medium containing multiple factors such as ascorbic acid, dexamethasone, bone morphogenetic protein-2 (Bmp-2), and transforming growth factor-beta (Tgf-β) and this can be promoted by exogenous insulin-like growth factor 2 (Igf2), which gene is silenced in PESCs. Before chondrogenic cell induction, floating aggregates called embryoid bodies (EBs) were formed from the PESCs by suspension culture in the medium containing serum and retinoic acid for 5 days. Then, various concentration of Igf2 supplementation (0, 1, 10 or 100 ng/ml) from the formation of EBs to chondrogenic induction by 3D culture system was investigated. After the induction, gene expressions of chondrocyte specific markers of Chordin-like 1, Collagen-2, Aggrekan, Decorin, MMP13 and PAX-1 were analyzed by real time PCR. As a result, supplementation of 100 ng/ml Igf2 showed the highest capability of chondrogenic induc-
tation than other groups. The expression levels of all genes were 7 to 35 fold higher in 100 ng/ml Igf2 treated group than Igf2-free control. The results show that supplementation of Igf2 of optimal concentration improves the efficiency of chondrogenic differentiation of murine PESCs under 3D culturing environment. Our finding provides insights into the processes of cartilaginous tissue repair and the imprinting mechanisms active in monogenic stem cells. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST; Grant number 2011-0027807) and Technology Development Program for Agriculture and Forestry, Ministry for Agriculture, Forestry and Fisheries (MAFF; Grant numbers 109020-3 and 111160-4), Korea.

Poster Board Number: T-3103

THYROID HORMONE PROMOTES DOPAMINERGIC NEURONS DIFFERENTIATION FROM MOUSE EMBRYONIC NEURAL STEM CELLS THROUGH A CALCIUM WAVE DEPENDENT SIGNALING PATHWAY

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Dopaminergic neurons have been implicated in the regulation of motor behavior. Here, we found that hypothyroidism during pregnancy leads to significant decreasing of tyrosine hydroxylase (TH) activity and dopamine neuron loss in the midbrain of the offspring. Results from the open field test carried out when the offspring was 2 month old revealed a significant decrease of locomotor activity of the offspring suffering hypothyroidism during the embryonic phase. Followed, we cultured embryonic neural stem cells (eNSCs) from mouse mesencephalon and treated the cells with a physiological level of T3. We found that T3 treatment significant increase TH gene expression. T3 treatment also increased the number of TH-positive cells after withdrawing the growth factor EGF and bFGF in the culture medium. Further more, we detected the expression of TRPC family members in eNSCs after T3 treatment and found that TRPC1 mRNA and protein levels were remarkably upregulated. In addition, a calcium wave was induced by T3 treatment in cultured eNSCs, and it was significant blocked by SKF96365, an inhibitor of the TRP receptor family. The TRPC1 specific siRNA, which eliminated the TRPC1 expression, was also found to decrease the calcium wave induced by T3. We also found that both SKF96365 and the TRPC1 targeting siRNA eliminated T3-induced upregulation of TH activity and the promotive effects on dopaminergic neurons differentiation. These results suggested that T3 induced dopaminergic neurons differentiation of eNSCs through a calcium wave dependent manner. Taken together, our results reveal the effects of T3 in the dopaminergic neurons generation and explore the underlying mechanisms. This study emphasis the critical effects of T3 in the brain dopamine system development.

Poster Board Number: T-3104

HOXA3 IS ESSENTIAL FOR THE DEVELOPMENT OF THYMIC EPITHELIAL PROGENITORS FROM MURINE EMBRYONIC STEM CELLS

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Hoxa3, a member of the Hox family of transcription factors, has been proposed to be the earliest regulator for thymus organogenesis. Mice homozygous for Hoxa3 deletion (Hoxa3-/-) are athymic. Because Hoxa3 is expressed in the pharyngeal pouch endoderm and surrounding neural crest-derived mesenthexyme, the absence of a thymus in Hoxa3-/- mice could result from defects in either or both of these tissues, or result from a defect in the third pharyngeal pouch formation. We have reported that murine embryonic stem cells (mESCs) can be induced to generate thymic epithelial progenitors (TEPs) in vitro. To determine whether Hoxa3 plays a role in the development of thymic epithelium, we established mESC lines from Hoxa3-/- and the wild-type (Hoxa3+/+) mice. We then induced the differentiation of Hoxa3-/- mESCs and Hoxa3+/+ mESCs into TEPs in vitro. Although EpCam1-/- cells were generated from both Hoxa3-/- and Hoxa3+/+ mESCs, Hoxa3+/+ mESC-EpCAM1+ cells co-expressed Keratin 5 and Keratin 8, a phenotype of TEPs, whereas Hoxa3-/- mESC-EpCAM1-/- cells did not. In addition, Hoxa3+/+ mESC-EpCAM1+ cells expressed TEP-related genes Pax1, Pax9, and Plet1 at significantly higher levels than Hoxa3+/+ mESC-EpCAM1+ cells. Furthermore, when transplanted under the kidney capsule of syngeneic mice, Hoxa3+/+ mESC-EpCAM1+ cells developed into cortical TECs and medullary TECs, reconstituted normal thymic architecture and support T cell development, whereas Hoxa3-/- mESC-EpCAM1-/- cells did not. These data suggest that TEPs can be generated from Hoxa3+/+ mESCs but not from Hoxa3-/- mESCs. Therefore, Hoxa3 is required for the development of TEPs from mESCs.

Poster Board Number: T-3105

GATA4 COOPERATES WITH NODAL/ACTIVIN SIGNALING DURING DEFINITIVE ENDODERM SPECIFICATION OF MOUSE EMBRYONIC STEM CELLS

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Specification of definitive endoderm (DE) during gastrulation is a prerequisite for the formation of organs including liver, pancreas, lung, thymus, thyroid and gut. DE and mesoderm originate during gastrulation when epiblast cells are recruited to the primitive streak (PS). The PS can be subdivided into posterior, middle, and anterior regions with distinct developmental potential. Definitive endoderm forms from the anterior PS, while distinct subpopulations of mesoderm are induced in each of the different PS regions. These differences in potential are correlated with a gradient of Nodal signaling within the primitive streak. Differentiation of mouse embryonic stem (ES) cells in the presence of Activin A, a nodal receptor agonist, efficiently recapitulates this early lineage commitment: high levels of Activin induce endoderm specification and low levels of Activin induce mesoderm commitment. This co-regulation by Nodal signaling and the close proximity of mesoderm and DE within the PS led to the proposal of a bipotential population known as mesendoderm, now largely supported by experimentation in vitro and in the mouse embryo. Nodal/Activin signaling directs the expression of a conserved network of transcription factors that function at different stages in the induction and specification of DE. This network includes Mix-like homeobox genes, Eomesoderm, FoxA2, Sox17 and Gata4/SF6. This network is thought to be involved in segregation of the endoderm and mesoderm lineages, commitment of cells to DE fate, and regionalization of nascent endoderm. To define the role of Gata4 in mouse DE development we generated a mouse ES cell line in which Gata4 is placed under doxycycline-inducible control. We found that in serum-free conditions, Gata4 expression alone favors a primitive endoderm fate, and is not able to induce a DE population. However, expression of Gata4

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under optimal conditions known to favor DE specification (Activin at 75ng/ml) led to a significant increase in the percentage of DE precursors, defined by c-Kit and Cxcr4 expression. More importantly, with Activin concentrations as low as 1 or 5 ng/ml, which is not sufficient to induce DE, Gata4 expression resulted in a large expansion in the DE population. With low Activin, Gata4 directed an increase in the expression of DE specific markers with a concomitant reduction in mesoderm specific markers. This DE population generated by Gata4+low Activin has an equivalent potential for differentiation toward hepatoblast fate, when compared to DE precursors obtained by high Activin concentration. In summary, we show that Gata4 cooperates with Nodal/Activin signaling to specify definitive endoderm and to promote definitive endoderm over mesoderm fate by inducing and reinforcing the expression of endoderm genes, while repressing expression of mesoderm genes. Our results provide the first evidence of a role of Gata4 in the segregation of DE from mesendoderm precursors.

Poster Board Number: T-3106

**UNIQUE 3-O-SULFATED HEPARAN SULFATE STRUCTURE CONTRIBUTES TO THE DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS**

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Recently, we demonstrated that heparan sulfate (HS) chains play important roles in the maintenance and differentiation of mouse embryonic stem cells (mESCs) by regulating extrinsic signaling. Sulfated HS structures are modified by various sulfotransferases during development. However, the significance of specific HS structures during development remains unclear. Here, we show that unique 3-O-sulfated HS structures (3-O-HS) synthesized by HS 3-O-sulfotransferases (3OSTs) increase during differentiation of mESCs following LIF withdrawal. This finding suggested that unique 3-O-HS contributes to primary mechanism of differentiation in mESCs. We performed 3OST-overexpression to up-regulate the unique 3-O-HS. Overexpression of the unique 3-O-HS in mESCs induced spontaneous differentiation into primitive endoderm and primitive ectoderm in the presence of LIF and serum. To examine the requirement for unique 3-O-HS for the differentiation of mESCs, we performed stable knockdown (KD) of 3OST mRNA using RNAi. In the stable 3OST KD, the expression of Gata6 (primitive endoderm marker) did not increase even by LIF withdrawal. Then, we investigated in vitro differentiation into embryo bodies (EBs), which comprise three germ layers: endoderm, mesoderm, and ectoderm. In EBs derived from stable 3OST KD cells, the expression of Fgf5 (primitive ectoderm marker), Sox17 (endoderm marker), Goosecoid (mesoderm marker), and Pax6 (ectoderm marker) was decreased by down-regulation of the unique 3-O-HS. In this study, we demonstrated that the unique 3-O-HS structure was necessary for induction of differentiation of mESCs into primitive endoderm and EBs.
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by the GLUT1-specific siRNA. In experiments to examine related signaling pathways, GlcN increased O-linked N-acetylgalactosamine (O-GlcNAc) and O-GlcNAc transferase (OGT), but did not elicit any changes in O-GlcNAcase (OGA) expression. GlcN-induced increase of O-GlcNAc and OGT were inhibited by GLUT1-specific siRNA. Moreover, GlcN induced phosphorylation of specificity protein 1 (Sp1) transcription factor, which was blocked by OGT inhibitor ST045849 (20 μM) in nuclear fraction. GlcN also increased calmodulin (CaM) expression which was blocked by Sp1-specific siRNA. In addition, GlcN induced binding of CaM to plectin and then plectin-integrin β4 complex disassembly of HSds, which was reversed by OGT- and Sp1-specific siRNA. On the other hand, GlcN increased Ca2+ influx and phosphorylation of the protein kinase C (PKC), which were inhibited by GLUT1-specific siRNA or EGTA plus 8-AM. Furthermore, GlcN increased phosphorylation of integrin β4 serine sites which was reversed by PKC inhibitor bisindolylmaleimide. In addition, GlcN decreased cell-cell junction regulatory proteins such as ZO-1 and claudin, E-cadherin, and occludin, which were blocked by CaM-specific siRNA or bisindolylmaleimide. Finally, GlcN-induced increase of cell migration was inhibited by each signal pathway-related specific siRNA or inhibitors. In conclusion, GlcN regulated dissociation of integrin β4 and plectin through Sp1-dependent-CaM or PKC phosphorylation and thereby stimulating to the mouse ESC migration.

Poster Board Number: T-3109
CONTEXT DEPENDENT ROLE OF OCT4 IN GOVERNING PLURIPOTENCY AND DIFFERENTIATION
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Oct4 is a critical regulator of pluripotency and is dispensable in mouse preimplantation embryo development and embryonic stem cells (ESC) maintenance. In Oct4 knockout embryos, the inner cell mass cannot be maintained and differentiates into trophectoderm. Likewise, ESC undergo rapid differentiation upon the loss of Oct4. However, Oct4 is not restricted only to naive pluripotent cells. Rather its expression persists in the postimplantation epiblast and during early differentiation in the gastrulating embryo. While considerable efforts have been dedicated in elucidating factors that are essential for cell fate specification and commitment, the effects of disrupting the tightly regulated pluripotent network during differentiation in response to different environmental signals is still poorly understood. Here, using conditional knockout approaches, we show that Oct4 is essential in maintaining different states of pluripotency (naive and primed) in the pre- and postimplantation embryos.

Poster Board Number: T-3110
CONTINUOUS LIVE IMAGING OF SINGLE MOUSE EMBRYONIC STEM CELL CYCLE PROGRESSION USING FUCCI AND E-CADHERIN PROTEINS
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With the emergence of embryonic stem cell-based therapy as an attractive treatment for various diseases, there is a parallel need to analyze cell cycle restructur in differentiation process to assess their therapeutic effectiveness. A comprehensive understanding of the cell biology of embryonic stem cells requires direct observation of single ES cell cycle division in the absence of heterogeneous niche. Here, we combined bio-imaging and cadherin-based ECM engineering approaches to visualize mES cells in a single level. By continuous direct long-term single-cell observation of Fucci mES cells, it was possible to track dynamic cell cycle progression from dividing mES up to endoderm lineage-stage cells. With this unique combination, we were able to understand individual cell cycle phase, speed and direction of cell commitment in vitro. Abbreviation: mES-mouse embryonic stem cells; ECM-extracellular matrix; Fucci-fluorescent ubiquitination-based cell cycle indicator.

Poster Board Number: T-3111
TEMPORALLY-REGULATED MECHANOTRANSDUCTION CONTROLS STEM CELL CARDIOMYOGENESIS
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As cells migrate and differentiate throughout development, they secrete and assemble extracellular matrix (ECM), giving rise to time-dependent, tissue-specific stiffness, i.e. cardiac muscle originates from soft mesoderm, ~<500 Pa (Pa; a unit of stiffness), and undergoes ~10-fold myocardial stiffening from HH stages 18-36 in the chick embryo. When mimicked in vitro, myocardial stiffening enhanced cardiac specific gene expression and myofilbril organization in immature pre-cardiac mesoderm. While active mechanotransduction causes maturation, the specific proteins responsible for responding to time-dependent stiffness involved remain unknown. Here we examined expression and organization of well-known mechanosensitive proteins, e.g. focal adhesion kinase (FAK) and vinculin, in mouse embryonic stem cells (mESCs), mESC-derived cardiomyocytes, pre-cardiac progenitors, and isolated mature cardiomyocytes plated on matrices with either dynamic or static cardiac tissue-specific stiffness. Ratiometric western blot analysis shows higher expression of activated focal adhesion proteins when progenitor and mESC-derived cardiomyocytes were plated on hydrogels of cardiac tissue-specific stiffness compared to static, soft matrices, indicating that mechanically driven maturation is at least partially achieved via active mechanosensing. Beating and force generation were also inhibited in cells on matrices that deviated from tissue stiffness, indicating that active forces required for cell maturation and viability are affected by mechanical properties of the matrix on which the cells exert tension. Identifying mechanosensitive pathways that are active in cardiomyogenesis can lead to a better understanding of stem cell differentiation and development.

Poster Board Number: T-3112
UNDERLYING MECHANISMS OF THE IN VITRO INDUCTION OF HEMATOPOIETIC STEM CELL-LIKE CELLS FROM MOUSE EMBRYONIC STEM CELLS BY LIM-HOMEBOX TRANSCRIPTION FACTOR, LHX2
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We have recently reported that hematopoietic stem cell (HSC)-like cells were effectively induced from mouse embryonic stem cells (ESCs) by an enforced expression of LIM-homeobox transcription factor, Lhx2 (Blood 117:3748-58, 2011). When Lhx2 was retrovirally-transduced in mesodermal cells of differentiating mouse ESCs
on OP9 stromal cells, HSC-like cells were induced and robustly expanded. The Lhx2-induced HSC-like cells displayed self-renewing capacity in *vivo* and long-term hematopoietic repopulation activity in *vivo* when transplanted into lethally irradiated congenic recipient mice. The HSC-like cells were also induced from mouse induced pluripotent stem cells. Here we analyzed mechanisms of the *in vitro* induction of HSC-like cells by Lhx2. For this purpose, we newly established a mouse ESC line carrying Dox-inducible Lhx2. When the ESCs were induced to differentiate on OP9 cells and Lhx2 expression was turned on at the mesodermal stage, differentiation of lineage-restricted mature hematopoietic cells from c-Kit+ immature hematopoietic cells was severely impaired. The c-Kit+ cell populations acquired self-renewal ability and were expanded on OP9 cells. This self-renewal ability strictly depended on the presence of Lhx2, since the c-Kit+ cells were immediately differentiated into mature hematopoietic cells after Lhx2 expression was turned off. These data suggest a possibility that Lhx2 would promote the self-renewal of immature hematopoietic cells. To confirm this possibility, we carried out the Lhx2 transduction experiment into mouse adult bone marrow HSCs. Lhx2 indeed promoted the self-renewal of bone marrow HSCs *in vitro*. Next, molecular functions of Lhx2 were investigated. It has been reported that LIM-homeobox transcription factors indirectly affect the amount of LIM-only protein (Lmo). Consistent with this previous report, we found that in the presence of Lhx2, the amount of Lmo2 protein, a critical regulator of hematopoietic differentiation, was reduced. To determine whether the amount of Lmo2 is limiting factor during the induction of HSC-like cells by Lhx2, Lmo2 was co-transduced with Lhx2 in the ESC-derived mesodermal cells. As a result, induction of HSC-like cells by Lhx2 was partially inhibited by increasing the amount of Lmo2. Taken together, we propose that the induction of ESC-derived HSC-like cells by Lhx2 is caused by the promotion of self-renewal of resident immature hematopoietic cells, and the amount of Lmo2 is critically involved in this process.

*Poster Board Number: T-3113*

**HEPATIC DIFFERENTIATION OF ES CELLS IN A HOLLOW FIBER-TYPE BIOREACTOR AND APPLICATION TO A BIOARTIFICIAL LIVER**

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Introduction: We have tried to develop a bioartificial liver (BAL) using cultured hepatocytes and focused on pluripotent stem cells, such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, as a potential cell source of it. These cells must be induced to differentiate into the target cells, hepatocytes, because they are in an undifferentiated state. In this study, we used hollow fiber (HF)/organoid culture method, in which cultured cells form a cellular aggregate called an “organoid” in the lumen of the HF. We have confirmed the hepatic differentiation of mouse pluripotent stem cells using the HF/organoid culture method. To put the BAL using cultured hepatocytes into practical use, it is necessary to develop a high-performance artificial liver device and to demonstrate its therapeutic effect. In the present study, we evaluated the performance of an ES cells-immobilized BAL module based on HF/organoid culture by in vitro and ex vivo experiment. Materials and Methods: [1] Fabrication of the BAL module and hepatic differentiation of mouse ES cells We developed a BAL module (culture volume; 2.97 cm3) with a one-layer textile HF sheet. The HF sheet was comprised of 5 cm, 130 cellulose triacetate HFs for plasma separation. 6.2×106 mouse ES cells were immobilized in the BAL module and the module was cultured under the perfusion condition for 30 days. To induce hepatic differentiation, differentiation-promoting agents were added to the culture medium (1 mM sodium butyrate on days 9-15; 10-7 M dexamethasone; 10 ng/mL oncostatin M; and insulin-transferrin-selenium (10 mg/mL insulin, 5.5 mg/mL transferrin, 6.7 ng/mL selenium acid) on days 15-30). [2] Functional evaluation of the BAL module using liver failure model animals Male Wistar rats weighting 200 to 300 g were used for the animal experiments. The liver failure was induced by a method combining 70% partial hepatectomy with hepatic ischemia for 20 min (spontaneous recovery rate; 60%). After the induction of liver failure, the rats were connected with the ES-module or control-module (without cells) via jugular vein and carotid artery, and 1 hour extracorporeal circulation at 1 ml/min was initiated. We measured changes in the blood biochemistry level during extracorporeal circulation. In case the rat recovered, we also evaluated liver regeneration a week after operation. Result: Mouse ES cells immobilized inside HF's proliferated in culture and formed cylindrical organoids that grew in the longitudinal direction of the HFs. The liver specific functions were detected by about 2 weeks of culture. The maximum ammonia removal rate and albumin secretion rate per ES cell-immobilized module reached 60% and same level of that of primary mouse hepatocyte-immobilized module, respectively. In the animal experiments, all of the 3 rats applied the control-module eventually died. On the other hand, the rat applied ES-module, although the number of application has only been one case, recovered after operation and the blood biochemistry levels a week after become equivalent to the healthy level and liver regeneration was also observed. Conclusion: The hollow fiber type BAL module containing differentiating ES cells expressed liver specific functions. In functional evaluation of the BAL module by ex vivo experiments, although it needs further researches, this module showed the potential for supporting liver functions and inducing liver regeneration of the liver failure rats.

*Poster Board Number: T-3114*

**SMALL MOLECULE SIGNALLING PATHWAY INHIBITOR SCREENING REVEALS MULTIPLE PATHWAYS CONTRIBUTE TO DOPAMINERGIC NEUROGENESIS**

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Identification of key signalling events during neural differentiation of mouse ES cells (mESCs) has identified the role that some tyrosine kinases play in directing lineage specification. Many of the best defined of current neural differentiation paradigms give rise to either non-desired neural phenotypes, or to non-neuronal cells. The undesired cell types may generate unwanted effects if the neurons are to be used for either toxicity, drug screening or transplantation studies. In this study we use homologous knock-in ES cell reporter lines expressing luciferase or eGFP under the control of the transcription factors; Lmx1a, Nurr1 & Pitx3, to investigate the impact of tyrosine kinase inhibitor libraries on the development of dopaminergic neurons. Initial screening used a simple adherent monolayer ES cell differentiation protocol to investigate the effects of 130 kinase inhibitors on Lmx1a-luc expression. From the initial screening, 10 classes of kinase inhibitors were found to consistently influence expression of Lmx1a. In particular, inhibitors of the epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) receptor and...
DNA-dependent protein kinase (DNA-PK) signalling, significantly increased Lmx1a expression (2- to 4- and 2.5-fold change respectively over vehicle, one-way ANOVA, p<0.0001). In addition, protein kinase C (PKC) isoform inhibitors generally decreased Lmx1a expression. To confirm small molecule treatment conferred midbrain dopaminergic potential, Pitx3-eGFP cultures were incubated with combinations of EGF receptor, VEGF receptor & PKC inhibitors. The expression of eGFP was then monitored during neuronal development. Under these conditions the attenuation of EGF & VEGF signalling alone was sufficient to promote Pitx3 expression (6.5 ±1.2% & 7.0 ±1.2% vs control 3.5 ±0.4%). In addition, to show small molecule compounds also increased midbrain dopaminergic precursors, Nurr1-luc cultures were exposed to candidate inhibitors. However, unlike Lmx1a, candidates could not modulate expression of Nurr1. This work shows that small molecule screening reveals select pathways that stimulate or inhibit transcription factors associated with the development of dopaminergic neurons without the use of exogenous patterning factors. However, the timing of inhibitor addition & potential for signalling pathway cross-talk may confound our interpretation of this data; both factors are currently under investigation.

Poster Board Number: T-3115

MICRORNA125B-LIN28 PATHWAY ACTS AS A RHEOSTAT TO CONTROL THE BALANCE BETWEEN SELF-RENEWAL AND MESENODERM COMMITMENT OF EMBRYONIC STEM CELLS

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Embryonic stem cells (ESCs), derived from the inner cell mass of blastocysts, are self-renewing and pluripotent cells with the ability to differentiate into all derivatives of the three primary germ layers, i.e. ectoderm, endoderm, and mesoderm. However, the factors and signaling processes regulating self-renewal and differentiation are not yet fully understood. MicroRNAs (miRNAs), small noncoding RNAs that regulate gene expression at the posttranscriptional level, are important regulators of cell fate decisions. Although thousands of miRNAs and their targets have been identified, their specific roles in regulating the balance between self-renewal and specific lineage commitment of ESCs are largely unidentified. Here we report a novel rheostat function of miR-125b/Lin28 axis in the regulation of mouse embryonic stem cell (mESC) self-renewal and lineage specification. With a MicroRNA Array screen, we identified a number of miRNAs significantly changed during ESC differentiation, among which miR-125b was enriched in undifferentiated ESCs but more significantly downregulated than most other miRNAs during early differentiation. The abundantly expressed miR-125b in undifferentiated mESCs was dramatically downregulated to a level hardly detected during differentiation day 3 to 5, with a concomitant upregulation of Lin28. Ectopically expressing miR-125b did not alter characteristics of undifferentiated mESCs, whereas it sustained the cells in the undifferentiated status, impaired the endoderm and mesoderm development and inhibited cardiomyocyte formation, while the expression of ectodermal genes and the neuronal differentiation remained unchanged. We further demonstrated that miR-125b targeted the 3’-untranslated region of Lin28 and reduced the abundance of Lin28 at both mRNA and protein levels. Moreover, phenotypes of miR-125b overexpressing cells were mimicked by downregulation of Lin28 and rescued by re-introduction of Lin28. In addition, the impaired cardiogenesis in miR-125b introduced cells was greatly recovered when mimicking of endoderm environment by cultivation with the condition medium from a visceral endoderm-like cell line END-2. These results demonstrate that the downregulation of miR-125b is required for the initiation of ESC differentiation and miR-125b acts as a rheostat that controls the balance between self-renewal and lineage-specific commitment of ESCs through directly targeting Lin28. These findings extend our knowledge in understanding of the regulatory mechanism of ESC self-renewal and differentiation as well as the tightly control of cell lineage decisions in ESCs.

Poster Board Number: T-3116

APPLICATION OF STEM CELL BASED SCREENING TECHNOLOGY TO COSMETIC DEVELOPMENT

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Background and Purpose: The appearance of human skin is dramatically transformed as we age. The signs of skin aging include pigmentation, wrinkles, sagging skin, dry skin and gray hair. These signs are considered to be induced when the maintenance of tissue homeostasis is disrupted. Recent studies have shown that stem cells exist in skin tissues such as epidermis, dermis and subcutaneous tissues, and may play an important role in the maintenance of tissues. We have pursued the development of novel cosmetic technologies targeting skin stem cells (Biochem Biophys Res Commun. 396, 837-842. 2010). In this study, we discussed the pathogenic mechanism of pigmentation and gray hair and the screening technology for materials to improve them, focusing on melanocyte stem cells located in the bulge area in hair follicles. Specifically, we established an in vitro stem cell differentiation induction system for melanocytes and conducted a search for materials that regulate the differentiation of melanocytes using the system. Methods: In this study, ES cell (C57BL/6-derived) differentiation induction system for melanocytes established by Yamane et al. was used (Dev Dyn.216, 450-458. 1999). First, ES cells were seeded on the ST2 feeder layer and were induced to differentiate into melanocytes using a culture medium (a-MEM) in which FBS (fetal bovine serum), DEX (dexamethasone), BFGF (basic fibroblast growth factor), CT (cholera toxin) and EDN3 (endothelin-3) were added. Then, a variety of materials was added in the differentiation induction system. And the effects of each material on the differentiation into melanocytes were examined by analyzing the gene expressions of differentiation markers Results: As a result of the screening using the differentiation induction system, we discovered various materials which regulate the differentiation of ES cells into melanocytes. For example, barley (gramineous grain) extract notably promoted the differentiation into melanocytes. We also found a material that altered ES cell fate and promoted the ES cells to differentiate into nerve cells and retinal pigment cells. We will further investigate these materials and analyze the regulatory mechanism of stem cell differentiation. Discussion: The materials discovered in the screening system are considered to be versatile. For example, barely extract, which promoted the differentiation into melanocytes, may be useful for the prevention of gray hair and vitiligo which is caused by loss of melanocytes. On the other hand, Alaria Praelonga (seaweed) extract notably suppressed the differentiation into melanocytes. Additionally, Alaria Praelonga extract, which suppressed the differentiation into melanocytes, may be useful for the treatment of pigmentation, dullness and hyperpigmentation of the skin. Furthermore, the materials, which promoted the differentiation into nerve cells and retinal pigment cells, can be applied to the treatment of neurological diseases such as Parkinson's disease and retinal pigmentary dystrophy. It is expected that the stem cell-based screening technology is a useful means of providing a number of information on abilities of screened materials. Currently, the identification of active
ingredients, analysis of regulatory mechanism of differentiation and consideration of the clinical efficacy of the materials in which we found a regulatory effect of stem cell differentiation are being conducted.

Poster Board Number: T-3117

MODULATING CELL FATE AND LINEAGE COMMITMENT VIA MRNAS ENCODING CELL TYPE-SPECIFIC TRANSCRIPTION FACTORS

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The ability to manipulate cell fate offers tremendous potential as a tool for understanding lineage commitment and differentiation during development, and as a tool for in vitro disease modeling or direct therapeutic applications. Currently, two main strategies are employed in vitro to direct cell fate: (1) directed differentiation, in which pluripotent stem cells are guided through a series of steps that mimic development in vivo, and (2) direct lineage reprogramming or transdifferentiation, in which one fully differentiated cell type is converted into another. Multiple examples of direct lineage reprogramming through forced expression of cell-type specific transcription factors have now been reported, including those of the skeletal muscle, neuronal, cardiac, and pancreatic lineages. Current methods primarily rely on viral-mediated gene delivery, which presents risks associated with genomic integration and insertional mutagenesis that limit translational applications. Furthermore, these methods do not allow for stoichiometric and temporal control over factor delivery. Here, we present the introduction of transcription factors via transfection of non-integrating mRNAs for direct modulation of cell fate. Using the master myogenic transcription factor, MyoD, we present proof-of-concept on the utility of non-integrative mRNA-based methods for the transdifferentiation of mouse and human fibroblasts into skeletal muscle. We show that (1) MyoD mRNA is efficiently translated into protein following transfection (2) MyoD protein is functional in activating a MyoD-sensitive luciferase reporter, and (3) repeated delivery of MyoD mRNA to fibroblasts induces expression of the muscle-specific markers, MF-20, Myogenin, Desmin, as well as the formation of multinucleated myotubes.

Poster Board Number: T-3118

CELLULAR IDENTITIES ARISING DURING MESODERMAL DIFFERENTIATION

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Differentiation of multi- or pluri-potent cells results in the generation of heterogeneity of cell identities in a poorly understood manner. Understanding the nature in which the heterogeneity emerges at the cellular level during differentiation is essential to allowing an understanding of the mechanisms that control this process. This is partly because measurements on heterogeneous populations cannot confirm correlations at the individual cell level, but more importantly because the generation of heterogeneity is the primary output of the differentiation process. The generation of diversity can be considered as the passage of cells (and their descendants) through an N-dimensional descriptive space (i.e. phase space) where each dimension represents some numerical descriptor of the cell state. We are using combinatorial oligonucleotide fluorescent in situ hybridisation (FISH) to quantitatively measure transcript abundancies for up to 7 genes concurrently during mesoderm differentiation. This allows us to track the occupancy of cell identities in N-dimensional (N <= 7) phase space, and to monitor how this occupancy changes with time or change in conditions. We are initially interested in the extent of the allowable space (a reflection of the rigidity of the controlling mechanisms), and how dependent this is on external and internal conditions. In order examine this we are making measurements from large numbers (> 1000) of individual cells at different times and conditions during the differentiation of mouse ES derived mesoderm progenitors to hematopoietic and endothelial lineages. This should provide, not only an idea as to the general nature of the process but also an extensive data set that can provide answers to a range of specific questions and issues. Finally, we hope that this data set, in combination with data directly specifying interactions between components can be combined to construct an explanatory model of the controlling mechanisms.

Poster Board Number: T-3119

DEFINING OSTEOGENESIS TO ELUCIDATE BONE REPAIR MECHANISMS DURING THE NORMAL AND DISEASED STATE

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Osteoporosis is a skeletal degenerative disease which disproportionately affects a large number of postmenopausal women, but can also afflict a smaller percentage of men. This disease is characterized by low bone mineral density (BMD) leading to bone fragility fractures and bone structural deterioration caused by the imbalance between bone resorption by osteoclasts and bone formation by osteoblasts. In postmenopausal osteoporosis disease models, it has been found that bone forming osteoblast cell production is reduced, while bone resorption osteoclast cells remain functioning. Additionally, osteoblasts produced from sources such as bone marrow derived mesenchymal stem cells (BMSCs) is reduced with advancing age. The ability to create a source of osteoblasts would be ideal to restore balance to bone resorption and formation, thus curing the root of this degenerative disease. Our preliminary results demonstrate that we have generated a robust and easy to culture human embryonic progenitor (hEP) cell lines derived from human embryonic stem (hES) cells. To determine the capability of these cells to become osteoblasts, a small scale screen of the hEPs was used to evaluate cell morphology and mineralization for osteogenic properties. Under osteogenic differentiation conditions, a subset of the hEP cells displayed mineralization as detected by Alzarian red staining. The osteogenic potential of the hEP cell lines after 21 days in osteogenic differentiation media was further confirmed by examining gene expression patterns for the osteogenic differentiation markers runx2/cbfa1 (Runx2), and osteocalcin (BGLAP). Interestingly, the phenotype of the BMSCs differed from the osteoprogenitor hEP lines under bone forming conditions. The osteoprogenitor hEPs formed only osteoblasts, however, no chondrogenic or adipogenic differentiation was observed with these cells when cultured under adiogenic, chondrogenic, or osteogenic differentiation media. We have elucidated that different cell signaling pathways are being suppressed and activated in the osteoprogenitor hEPs as compared to the BMSCs. Thus we have identified osteoprogenitor cell lines which may be useful as a surrogate model to study osteoblast differentiation and use as a potential regenerative therapy for osteoporosis.
DERIVATION OF NEURAL STEM CELLS FROM EPIBLAST STEM CELLS
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Pluripotent stem cells can be derived from both pre- and post-implantation embryos. Embryonic stem cells (ES cells), derived from blastocystic are naïve pluripotent and epiblast stem cells (EpiSCs) derived from post-implantation epiblast are primed pluripotent. The phenotypes and gene expression patterns of the two pluripotent stem cells are different each other and EpiSCs thought to be in a more advanced pluripotent (primed pluripotent state) than mouse ES cells (aive pluripotent state). Therefore, we questioned whether EpiSCs are less potential to be differentiated into specialized cell types in vitro. We derived EpiSCs from 5.5-6.5-day post coitum mouse embryos. The EpiSCs could differentiate into all three germ layers in vitro, and expressed pluripotency markers. Interestingly, EpiSCs also were able to efficiently differentiate into neural stem cells (NSCs). The NSCs differentiated from EpiSCs (EpiSC-NSCs) expressed NSC markers (Nestin, Sox2, and Musashi), self-renewed over passage 20, and could differentiate into three neural subtypes, neurons, astrocytes, and oligodendrocytes. Next, we compared global gene expression patterns of EpiSC-NSCs with that of NSCs differentiated from ES cells and brain tissue. Gene expression pattern of brain tissue derived NSCs were closer to ES cell-derived NSCs than EpiSC-NSCs, indicating that the pluripotent stem cell-derived somatic cells could have different characteristics depending on the origin of pluripotent stem cell types.

NEUROTROPHIN SYSTEM: A NEW MOLECULAR PLAYER DURING VASCULAR DIFFERENTIATION OF EMBRYONIC STEM CELLS
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Embryonic stem cells (ESCs) have the ability to self-renew indefinitely and to differentiate in any bodily cell type, including vascular cells. Hence, ESC-based therapies represent a big hope for promoting vascular repair in ischemic disease. Neurotrophins (NTs) are a family of protein ligands (NGF, BDNF, NT4/5 and NT3) which mediate their action by binding tropomyosin receptor kinases (TRKA, B, C). Well known for their actions on the nervous system, NTs were more recently recognized to promote angiogenesis and regulate stem cell biology, including of ESCs. Here, we analyzed the role of NTs during vascular differentiation of ESCs (mouse ES-D3 line) into vascular endothelial cells (ECs) and smooth muscle cells (SMCs). Using a 2D embryoid bodies (EBs) model of differentiation, which enables production of both ECs and SMCs (Jakobsson et al, 2007), we first analyzed the mRNA expression changes in pluripotency and vascular markers, and in NTs and TRKs during differentiation. Pluripotency markers (Nanog, Oct4) halved expression at day 7 of EB-vascular differentiation. Early in EB development, at day 4, mRNA level of SMC markers increased (αSMA: 7 folds, SM22α: 2 folds vs pluripotency) while EC markers mRNA were enhanced from day 7 (CD31: 5 folds, CD144: 4 folds vs pluripotency). Among the NT system, BDNF/TRKB and NT3/TRKC were the most expressed during differentiation. They displayed a differential mRNA expression pattern (qRT-PCR), with an earlier increase of NT3 and its preferential TRKC receptor (8 and 20 folds vs pluripotency, respectively) at day 4, followed by a later increase of BDNF and its TRKB receptor (10 and 4.5 folds vs pluripotency, respectively) near day 7. VEGF (VEGF-A comprise 20ng/ml) is known to induce the expansion of EBs to a peripheral vascular plexus (Jakobsson et al, 2007). We confirmed this data (by immunofluorescence) and discovered that, importantly, recombinant BDNF or NT3 (25ng/ml for both protein) stimulated a comparable organized vascular structure, composed of mural cells surrounding endothelial tubes. Moreover, BDNF increased mRNA level of both EC and SMC markers; while NT-3 enhanced SMC markers, only. The vascular structures were higher and thicker by treating EBs with VEGF (10ng/ml) plus either BDNF or NT3 (12.5ng/ml) thus suggesting synergies between VEGFs and NTs. Inhibition of signalling through either TRKB (small non peptidic compound, ANA-12, 25mM) or TRKC (soluble TrkC receptor domain TrkCd5 neutralizing NT3, 2ug/ml) leded to the formation of a vascular plexus less structured. Interestingly, mRNA levels of both EC and SMC markers were decreased under ANA-12 treatment, although only mRNA of SMC markers was affected by the inhibition of TRKC pathway. These results would suggest a preferential effect of NT3 into the muscular commitment while BDNF would act in both EC and SMC differentiation. In addition to the hereby described role in vascular differentiation, NTs seemed to promote EB expansion. By counting cells at day 10, a crucial time point during EB development, we found significantly higher number of EB cells after VEGF, BDNF or NT3 in comparison with vehicle. This effect was enhanced by the synergic effect of VEGF with either BDNF or NT3. Moreover, ANA-12 and TrkCd5 inhibited cell growth, and so EB expansion. Taken all together, these preliminary data evidence for the first time a role for the NT system in the differentiation process of ESCs into vascular cells.

DERIVATION OF A NOVEL EPIBLAST STEM CELLS FROM SOMATIC EPIBLAST CELLS
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Since ES cell derivation by Evans and Kaufman, several pluripotent stem cell types have been reported, such as embryonic germ (EG) cells, FAB-stem cells, and epiblast stem cells (EpiSCs). Among these, EpiSCs are classified as "primed" pluripotent stem cells to compare with naive pluripotent stem cells, ES cells. Epiblast cells differentiated into three germ layers during in vivo development. Although EpiSCs contribute to three germ layers in in vitro differentiation, they rarely incorporate into ICM of blastocyst and rarely contribute to chimera after blastocyst injection. However, EpiSCs are very heterogeneous population and not well characterized. Here, we established an EpiSC line that can form chimera efficiently. We derived EpiSCs from Oct4-GFP transgenic mouse epiblast, which express GFP by control of Oct4 regulatory region including promoter, distal enhancer (DE), and proximal enhancer (PE). We derived epiblast stem cells from embryonic day 6.5 embryos. All epiblast stem cell lines expressed GFP and self-renewed over 90 passages. Newly derived EpiSCs expressed pluripotency markers Oct4 and Nanog, but did not express germine markers, which were known to be expressed in EpiSCs before; so we called these somatic EpiSCs (sEpiSCs). The sEpiSCs were able to differentiate to all three germ layers and formed teratoma and chimeric embryos without germ cell contribution. sEpiSCs showed completely different gene expression pattern from ES cells and E3 EpiSC line. Therefore, we derived a novel pluripotent cell type that is different from ES cells and EpiSCs. The sEpiSCs could be derived from somatic epiblast.
specific cells, which had been separated from specialized epiblast cells that have a developmental potential into primordial germ cells.

Poster Board Number: T-3123
CONTROLLED OXYGEN MARKEDLY INFLUENCES DIFFERENTIATION OF EMBRYONIC STEM CELLS TO INSULIN PRODUCING CELLS
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Pluripotent stem cells (PSC) hold promise for cell replacement therapy and studying embryonic development. However, efficient differentiation to desired cell types remains a major obstacle. Most PSC research is performed in high, non-physiological O2, but cells during embryonic development are exposed to much lower O2. Here we report a wide-ranging study showing that physiological O2 markedly influences differentiation to insulin-producing cells. We differentiated human embryonic stem cells (hESC) and under O2 markedly influences differentiation to insulin-producing cells. We differentiated human embryonic stem cells (hESC) and under different, well-characterized pO2 environments, controlling cellular oxygen exposure through adhesion culture on highly O2-permeable silicone rubber membranes. We focused our differentiation of hESC to c-peptide+ cells using a modification of the published 5 stage protocol by ViaCyte, Inc (San Diego, CA) (D’Amour 2006 Nature biotech). We found that differentiation under 5% O2 from hESC to definitive endoderm (stage 1), primitive gut tube (stage 2), and posterior foregut (stage 3), followed by 20% O2 to pancreatic endoderm (stage 4) and insulin-producing cells (stage 5) gives rise to a cell population that is 25% positive for both c-peptide and insulin. This result was 5 times greater when compared to differentiation under normoxic conditions (20% O2). The cells cultured under hypoxia passively secreted c-peptide into the medium but were not glucose responsive. All pancreatic endoderm genetic markers were increased for the controlled-hypoxia (5% stage 1-3, 20% stage 4-5) when compared to the normoxia condition (20% stage 1-5). By examining each stage at multiple controlled high and low oxygen levels, we identified O2 conditions that increased the fraction of the intermediate cell type of each cell measured by flow cytometry, or increased expression of genetic markers for those intermediate stages measured by real-time PCR. By combining these oxygen levels appropriately, we achieved a large improvement. Preliminary results on stage 4 cells have indicated potential O2 conditions that result in a 50% c-peptide+ population. Based on these results O2 combined with other directed differentiation protocols is a potentially-straightforward method that could be applied to future cell therapy protocols to generate more of a desired cell type.

Poster Board Number: T-3124
AN ES CELL-DIFFERENTIATION SYSTEM RECAPITULATES A DEVELOPMENTALLY REGULATED NEURON-SPECIFIC PARENT-OF-ORIGIN EXPRESSIVITY
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Genomic imprinting is a phenomenon where mono-allelic gene expression is observed in a parent-of-origin manner. A subset of the imprinted genes acquires a tissue-specific imprinted status during the course of tissue development but mechanisms underlying this epigenetic regulation were largely elusive. We sought to investigate this process of tissue-specific genomic imprinting by utilizing an in vitro differentiation system of embryonic stem (ES) cells. Ube3a gene is expressed from the maternal allele only in neurons. Judging from its expression pattern during development, the involvement of the paternally-expressed non-coding antisense RNA in Ube3a imprinting was anticipated. We established hybrid ES cells between two sub-species of Mus musculus (CS7BL/6 and MSM/Ms) useful to study genomic imprinting mechanism because of the abundant inter-subspecies SNPs, and succeeded in the establishment of an in vitro neuronal differentiation system where neuron-specific imprinting of Ube3a is recapitated. With this system, we revealed that the switch from bi-allelic expression to maternal mono-allelic expression of Ube3a occurs during the late neuronal development, neurite outgrowth period, and that the expression of endogenous antisense transcript of Ube3a locus is up-regulated several hundred-fold at the same period. By analyzing hybrid mouse embryos, we also confirmed Ube3a mono-allelic expressivity from embryonic day 15.5 in the developing nervous system. Concomitantly, marked Ube3a antisense transcriptional up-regulation was also observed, confirming the “epigenetically” faithful differentiation of our hybrid ES cells. Preliminary in situ hybridization studies independently detecting sense and antisense strands showed that the Ube3a-AS transcripts were mostly observed within the cell nuclei of the neural cells. This suggests a cis-acting mechanism of these antisense transcripts. Further studies are under way to elucidate the precise role of these non-coding RNAs. An important implication from this study is that evaluation of the “quality” of the cells derived from in vitro differentiation of ES cells needs scrutinizing epigenetic aspects such as genomic imprinting status found in tissues in vivo, in addition to the evaluation by differentiation gene markers and morphology. These hybrid ES cells and in vitro differentiation system will also allow researchers to investigate broader analyses of neuron-specific genomic imprinting, and our hybrid ES cells can be useful resources for other tissue-specific genomic imprinting and epigenetic analyses.

Poster Board Number: T-3125
MECHANISTIC INSIGHTS INTO DIDO3 FUNCTION IN STEM CELLS
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The death inducer-obliterator (Dido) gene has been identified in genome-wide screens as a potential regulator of embryonic stem cell pluripotency. To explore the role in stem cell biology of Dido3, the largest and most broadly expressed of the three Dido isoforms, we generated embryonic stem cells from a Dido3-specific mouse mutant, using red fluorescence protein (RFP) to replace the only Dido3-specific exon in the Dido locus (Dido3-deltaCT(C-terminal)), that shows implicated epiblast cell differentiation at the onset of gastrulation, causing death at embryonic day 8.5. Functional analysis of mutant ES cells showed indefinite self-renewal capacity, but indicated that Dido3 is necessary for stem cell differentiation in response to withdrawal of leukemia inhibitory factor (LIF). Wild type (wt) Dido3 protein is highly expressed in wt ES cells and is downregulated during differentiation. Mutated Dido3-deltaCT is expressed at comparable levels in mutant ES cells, but is not downregulated during differentiation. Other stemness-related genes showed impaired repression in mutant embryonic bodies (EB), particularly Oct4, a gatekeeper for ES cell differentiation; mutant EB also showed impaired upregulation of genes necessary for differentiation. To study the mechanism of Dido3 function, we stably overexpressed distinct Dido3 protein domains in mutant
Dido3-deltaCT ES cells and monitored expression of stemness and differentiation genes in the EB. Reconstitution with full-length Dido3 restored differentiation capacity in Dido3-deltaCT mutants, with downregulation of Dido3 itself and of other stemness genes, including Oct4, and upregulation of differentiation genes. Overexpression of the CT domain, missing in the Dido3-deltaCT mutant, triggered upregulation of differentiation genes, but not downregulation of stemness genes. In contrast, overexpression of the NT domain (N-terminal common region of the three Dido isoforms) resulted in rapid downregulation of stemness genes and upregulation of differentiation genes. Further experiments revealed Dido3 interaction with the HDAC3-SMRT complex, as well as Wwp2 and RNA polymerase II, suggesting a role in transcriptional repression. Based on these results, we speculate that Dido3 is involved in repression of differentiation genes in stem cells, as well as in correct downregulation of stemness genes at the onset of differentiation.

**Poster Board Number: T-3126**

**NOVEL EPIGENETIC REGULATORY MECHANISM OF OCT4 BY CDK2AP1 IN ESCS**

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Understanding the key regulatory elements in maintenance of stem cell pluripotency is fundamental in using stem cells in clinical applications. Cell cycle regulators are gaining a more prominent role, these include molecules such as p27 that have been shown to play a role in cell cycle kinetics to maintain a pluripotent state and regulate specific genes involved in pluripotency. Similarly, our group has discovered how a novel molecule in cell cycle known as Cyclin Dependent Kinase 2 Associated Protein 1 (CDK2AP1) is involved with key aspects of pluripotency and differentiation. Knockdown of CDK2AP1 in mice leads to an embryonically lethal phenotype, suggesting a critical role in development. Furthermore, mouse embryonic stem cells with CDK2AP1 deletion remain pluripotent with removal of LIF when compared to WT. One possible mechanism is the association of CDK2AP1 with the nucleosome remodeling and deacetylase (NuRD) complex. We have shown that CDK2AP1 interacts with HDAC2 and MBD3, two major players in the NuRD complex. Deletion of CDK2AP1 in mESC shows reduced levels of global DNA methylation and increased levels of global histone acetylation. Recently we have shown CDK2AP1 to regulate Oct4 expression, a master regulator in stem cell renewal and differentiation. In our studies we have shown that CDK2AP1 plays a key role in NuRD-mediated Oct3/4 silencing by epigenetically regulating the Oct4 promoter during differentiation of both mESC and hESC. Detailed analysis of the Oct4 promoter revealed an absence of DNA methylation at the proximal enhancer (PE) region in differentiated Cdk2ap1−/−mESC. In parallel, we have seen an increase in H3K9 acetylation at the same region in Cdk2ap1−/−mESC. We have found CDK2AP1 occupancy at the PE region in mESC as well as hESC embryoid bodies. Furthermore, in mESC we have observed interdependency in CDK2AP1 and MBD3 binding to the OCT4 promoter. In hESC nuclear translocation of CDK2AP1 upon differentiation was distinct from mESC. CDK2AP1 plays a significant role in stem cell differentiation by association with the NuRD complex on specific promoter regions, changing chromatin accessibility and leading to the silencing of the Oct4 promoter during differentiation in mESC. CDK2AP1 itself behaves differently from our IF experiments between human and mouse ESC. We are currently delineating the mechanistic role of CDK2AP1 in hESC, as well as differences and similarities with mESC.

**Poster Board Number: T-3127**

**EPO PREVENT BLOOD-RETINAL BARRIER IN DR THROUGH RECUITMENT OF BMPS**

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Background: Retinal ischemia induced eye diseases such as Diabetic Retinopathy (DR) and Retinopathy of Prematurity (ROP) are the leading cause of blindness in the world. An inadequate blood supply resulting from early vessel loss causes tissue hypoxia, which determines the severity of subsequent pathological vessel growth and the rest usually cause vision loss by abnormal vessel growth. Antiangiogenic compounds (VEGF antibody) often fail to completely inhibit abnormal vascular growth and are associated with underlying complications. Since the potential clinical application, any effective treatment for protection retinal vessel would be beneficial to patients which were suffered by these diseases.

Methods: Firstly, we used intraperitoneal injection STZ to induce early DR and OIR (Oxygen-induced retinopathy) to induce ROP animal model. The mechanism of prevention effect to blood-retinal barrier (BRB) of EPO were studied; Secondly, using lentiviral mediated system to up-regulate or suppress EPO expression level in early DR and ROP animal, we monitored the process of these diseases. Furthermore, using bone marrow transplantation (BMT) and intravitreal injection bone marrow derived progenitor (BMP), we studied the EPO protection mechanism in histological level, which could analyze the vessel cells quantity change of BRB in DR and ROP. Thirdly, after interference of EPO receptor expression by siRNA, we studied the EPO relative signaling pathway in vivo and in vitro. Results: In our study, we found that EPO could prevent the injured BRB in early DR and ROP. EPO also could preserve the DR and ROP retinal apoptosis and electrophysiology function. In retinal flat mount and dissociated retinal FCM experiments, we found that all three cell types containing in the BRB were significantly rescued by EPO gene therapy. In the way of recruit BMP, EPO could improve the BRB morphology and function in both early DR and ROP. In vivo and in vitro studies shown that these EPO specific effects were mediated by its receptors which could activate the downstream signaling pathway. Conclusions: Thus, we will hypothesize that EPO could be serving as a BMP chemoattractant, which can recruit BMP mediated by EPOR and CD131. We found that EPO could recruit BMP migration to the injured retina and maintain vascular hemodynamic stability. The molecular mechanism of these protection effects was further investigated lentiviral mediated siRNA to block the specific receptors. In this study we also found BMP behavior is highly dependent on nitric oxide (NO). In conclusion, it is suggestion that the promising goal of retinopathy treatment is to rebuild and stabilize functional vasculature in hypoxic retinal tissue, rather than to eliminate the abnormal vessels. Furthermore, the advantages of possible cytokine therapy are suitable in wide-use regardless of the underlying genetic defect.
Embryonic stem cells (ES cells) will be valuable resources for clinical therapies because of their unlimited self-renewal ability and potential to generate any differentiated cell type. G protein coupled receptors (GPCRs) play key role in many complex biological processes, including development. However, the role of GPCRs in ES cell pluripotency and differentiation has received little attention. We demonstrated the role of GPCRs on mouse ES cells differentiation including neural or glial differentiation from neural stem cells, and pluripotency. Adrenergic receptor alpha 1a, alpha 2a and alpha 2c were upregulated with the progress of the differentiation from ES cell, whereas adrenergic receptor beta 3 (Adrb3) was dramatically decreased with the progress of the differentiation from ES cell into neural stem cells. The change in the histone modification at the promoter region of Adrb3 was seen in the neural stem cell development from ES cells. Under these conditions, a significant increase in lysine 27 on histone H3 (H3K27) trimethylation at the promoter of Adrb3 was observed in the primary neurosphere derived from ES cells. These results suggest that changes in adrenergic receptors signaling pathway along with epigenetic modification may a play a role in maintaining ES cells.

**Reprogramming**

Poster Board Number: T-3131

**DIRECT GENERATION OF NEURAL PRECURSOR-LIKE CELLS FROM ADULT HUMAN DERMAL FIBROBLASTS.**


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Recent studies have reported direct reprogramming of fibroblasts to mature neurons by the introduction of defined neural genes. This technology has potential use in the areas of neurological disease modelling and drug development. However, the use of induced neurons for large-scale drug screening and cell-based replacement strategies is limited due to their inability to expand once reprogrammed. We propose it would be more desirable to induce expandable neural precursor (iNP) cells directly from fibroblasts. In support of this proposal, we have demonstrated the ability to transform adult human dermal fibroblasts directly into neural precursor-like (iNP) cells. This was achieved by forced expression of the neural stem cell factors Sox2 and Pax6 using either nonviral plasmid transfection or recombinant protein transduction. Further, we have optimized cell culture conditions allowing us to generate iNP colonies in the absence of an animal or human feeder cell layer, an essential requirement for the transfer of reprogrammed cell lines to clinical use. Colony formation was observed within 14 - 30 days with full colony expansion achieved by 60 - 90 days at an efficiency of 0.05%. Daughter colonies were formed after serial passaging. Quantitative PCR demonstrated that iNP colonies express a range of neural stem and precursor genes including Sox2, Pax6, Sox3, Sox1, HoxB9, Nkx6 and Ngn2, comparable to human embryonic stem cell-derived neural precursor cells. Upon differentiation, iNP cells give rise to neurons exhibiting typical neuronal morphologies including multiple arborizing dendrites and expression of the neuronal markers TU-1, NSE and MAP2, with a population of iNP-derived neurons expressing the catecholaminergic neuronal marker TH or the GABAergic neuronal marker GAD. Most importantly, iNP-derived neurons demonstrated the ability to fire action potentials in response to current injection. These results represent a novel virus-free approach for direct reprogramming of human fibroblasts to a neural precursor fate.

**Reprogramming of Human Neuroblastoma Cells Using iPSC Technology**

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Background: Neuroblastoma (NB) is the most common extracranial solid tumor of infancy, which originated from the sympathetic nervous system of the neural crest. Oncogene MYCN amplification is frequently observed and is associated with the aggressiveness of NB. Our recent studies provide novel stratifications of NB which are based on global genomic and RNA expression profiles. These molecular signatures are strongly correlated with patient prognosis, suggesting that each subtype has a specific course of progression. Induced pluripotent stem cell (iPSCs) can be generated from distinct cell types by enforced expression of reprogramming factor (RFP) genes. Recently, induced pluripotent cancer cells (iPCCs) have been established in several cancers and are being used to investigate the stemness of cancer cells. However, generation of iPCCs from NB has never been attempted. Here we aimed to generate and profound study of iPCCs from NB. Methods: We applied the Sendai virus (SeV) vector into SH-SY5Y I-type NB cells for reprogramming, as it is an RNA virus and transgenes do not integrate into the host genome. We carried out 244K human array CGH (aCGH) microarray to examine the gene expression profile of iPCCs. We performed alkaline phosphatase (AP) staining to confirm iPCC generation. To examine the embryonic stem (ES) cell-related genes expressed in iPCCs, immunocytochemistry and quantitative real time RT-PCR were performed. Apoptotic cells were detected using TUNEL assay after treatment with chemotherapeutic agents. Results: SH-SY5Y intermediate (I) type cells show higher expression of RFP genes such as NANOG, OCT4, SOX-2, KLF4 and have stem-like properties. Upon retinoic acid (RA) treatment they undergo differentiation into neuroblastic (N) and substrate adherent (S) type cells. Forty days post transduction of SeV, NB cells form IPS-like colonies and AP staining positive data confirm iPCCs generation. The aCGH data revealed the same genomic aberrations in the iPCCs as their parental cells, indicating that an intact genomic status was maintained during the reprogramming process. Immunocytochemistry data suggested that the iPCCs expressed ES cell surface markers such as SSEA4, TRA-1 60 and TRA-1 81. Quantitative RT-PCR data revealed that endogenous reprogramming factor genes including NANOG, OCT4, SOX2, c-MYC and hTERT were highly
expressed in these colonies. Cis-diaminedichloroplatinum (CDDP) treatment to iPCCs showed a resistant phenotype when compared with their parental cells. Conclusions: We generated iPCCs from human NB which show cancer stem cell (CSCs)-like properties that might create a new opportunity for disease modeling, patient-specific drug screening and personalized cell-based therapies.

Poster Board Number: T-3133

UNDERSTANDING REPROGRAMMING USING THE X CHROMOSOME REACTIVATION

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X chromosome inactivation is an exemplar for epigenetic inheritance. Reactivation of the X inactive chromosome (Xi) can occur both in vivo and in vitro when female somatic cells acquire a pluripotent state, and this provides a model to study the erasure of epigenetic memory in mouse cells. In human, however, the status of the two X chromosomes in female embryonic stem (ES) cells and their reactivation in induced Pluripotent Stem (iPS) cells varies depending upon culturing and reprogramming techniques. The aim of our research is to understand how human somatic cells are reprogrammed towards pluripotency, by examining the X chromosome reactivation in heterokaryons where human fibroblasts or lymphocytes are reprogrammed following fusion with mouse ES cells. This methodology, unlike iPS, allows monitoring early events that occur during successful reprogramming. We have examined known epigenetic marks of the inactive X chromosome during reprogramming and defined the sequence of events that are associated with the X reactivation in the human nuclei, such as chromatin features, XIST localization and biallelic expression of X-linked genes. Delocalization of XIST and loss of H3K27me3 enrichment from the XIST localization and biallelic expression of X-linked genes. Sequence and loss of H3K27me3 enrichment from the XIST localization and biallelic expression of X-linked genes.

Poster Board Number: T-3134

LARGE SCALE ANALYSES OF MOLECULAR SIGNATURES AND BEHAVIORS OF HUMAN PLURIPOTENT STEM CELLS

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There are some variations in differentiation propensity among clones of human embryonic stem (hES) cells and induced pluripotent stem (hiPS) cells. For the promising applications of ES/iPS cells: drug discovery, studies on disease mechanism and cell therapy, we need to select the clones capable of complete differentiation into expected cells with no remaining of undifferentiated cells, preferably without differentiation processes, which are time- and cost-consuming. We established 49 hiPS cells from some types of cells of donors with various age and sex by various methods. And we examined the gene/miRNA expression and methylation status of these and compared with those of 10 hES cells. As a result, we demonstrated that molecular signatures of hES cells and hiPS cells are very similar although some variations exist. To determine whether these variations are related to the differentiation propensity into neural cells or not, we performed neural induction for 3 hESCs and 40 hiPSCs by using modified serum-free floating culture of embryoid body-like aggregates (SFEbQs) methods and examined the percentage of not only the early neural marker, PSA-NCAM positive cells, but also the undifferentiated marker, OCT3/4 positive cells after neural induction. All the tested clones differentiated into the PSA-NCAM positive cells with high efficiency (>80%), but some clones showed more than 10% remaining of OCT3/4 positive cells in at least one experiment. These clones formed teratoma when transplanted into striata of NOD/Scid mice brains after terminal differentiation, so we defined these clones as “bad” clones. Moreover, by analysis for gene/miRNA expressions of “good” and “bad” clones before differentiation, we found some molecular factors by which we can predict clones with without remaining undifferentiated cells after neural differentiation. Combination of these factors will help to delete the “bad” clones which have possibility to form teratoma after transplantation and not suitable for clinical application.

Poster Board Number: T-3135

ACTIVIN AND POLO-LIKE KINASE I SIGNALING REGULATE THE CONVERSION OF MOUSE AND HUMAN CELLS INTO INDUCED NEURONS

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The mammalian nervous system comprises many distinct neuronal subtypes, each with its own phenotype and differential sensitivity to degenerative disease. Although specific neuronal types can be isolated from rodents or engineered from stem cells for translational studies, transcription factor mediated reprogramming might provide a more direct route to their generation. Recent studies have demonstrated that the forced expression of select transcription factors is sufficient to convert mouse and human fibroblasts and stem cells directly into a variety of neuronal subtypes. However, the utility of this approach is currently limited by the low efficiency of conversion. One potential solution is to identify small molecules that increase induced neuron generation. Such chemicals would enable the generation of large numbers of patient-specific neurons for disease studies and provide insight into the mechanisms that regulate neuronal induction by defined factors. To this end, we used a functional reprogramming screen to identify small molecules that increase the rate of transcription factor-mediated conversion of mouse adult fibroblasts into Hb9::GFP + spinal motor neurons. An inhibitor of Activin-like kinases 4/5/7 and a Polo-like kinase I (PLK1) inhibitor each increased induced motor neuron formation by 5-10-fold. In combination, the chemicals increased the rate of induced motor neuron formation by 50-fold. Both small molecules also increased the rate of conversion of human fibroblasts and embryonic stem cells into motor neurons, indicating that these chemicals should enable the generation of human patient-specific motor neurons for disease modeling. After using peptide or small molecule analogues of both chemicals to confirm that Activin and PLK1 signaling are the functional targets of the these molecules during motor neuron induction, we performed pulse treatments at different times during reprogramming to determine when they are most effective. We found that the Activin inhibitor was effective when administered from days 1-5, 6-10, or 11-15. In contrast, the PLK1 in-
hibitor was only active during days 6-10, suggesting it acts on transient intermediates in the cultures. These results suggest that these chemicals act by divergent mechanisms. Because Activin inhibition was effective even after many motor neurons had appeared, we hypothesized that it might enhance motor neuron survival. Indeed, we found that chemical treatment greatly promoted the survival of flow-purified murine and human motor neurons in culture, indicating that Activin inhibition can act by promoting neuronal survival. To determine if Activin inhibition increases conversion into other neuronal types, we transduced fibroblasts with Ascl1, Myt1l, and Brn2, transcription factors that induce the formation of non-motor neurons, and cultured the cells with or without the Activin inhibitor. Chemical treatment increased the number of neurons generated by 10-fold, indicating this approach may be applicable to a variety of neuronal types. We have identified small molecules that increase the rate of direct conversion of mouse and human fibroblasts and stem cells into motor neurons. These results identify the Activin and Polo-like kinase I signaling pathways as major roadblocks to induced neuron formation and indicate that many neurons are lost shortly after conversion. Finally, these chemicals should enable the efficient generation of induced neurons for patient-specific disease modeling.

Poster Board Number: T-3136

EFFICIENT GENERATION OF TRANSGENE-FREE HUMAN AND MOUSE IPS CELLS USING A CELL-PERMEANT TAT-CRE PROTEIN

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Reprogramming of somatic cells using viral transduction of defined transcription factors remains a widely used and efficient method to obtain induced pluripotent stem cells (iPSCs). However, the presence of viral transgenes in iPSCs is undesirable as it raises the possibility of insertional mutagenesis leading to malignant transformation and has also been shown to affect differentiation potential. Various strategies have been employed to address this issue, including non-integrating viruses, RNA transfection, protein transduction and site specific recombinases to excise the transgenes after reprogramming. Here we show efficient generation of transgene-free mouse and human iPSCs through the use of a Cre-excisable polycistronic lentiviral vector expressing the "stem cell cassette" (STEMCCA) comprised of all four transcription factors (OKSM) followed by exposure of the fully reprogrammed iPSC to cell permeable TAT-Cre recombinant protein. Notably highly efficient excision (100% for mouse iPSCs and 60 - 80% for human iPSCs) could be demonstrated following exposure of iPSCs to 4 - 6 μM TAT-Cre for 1 - 2 hours. The high degree of efficiency obtained with protein transduction is in marked contrast to results obtained with electroporation of a plasmid expressing Cre-recombinase (<10%) and also for adenovirus expressing Cre recombinase which has been shown to be effective for mouse iPSCs but not for human iPSCs. Additionally, we present a simple and robust PCR strategy that enables fast identification of deleted clones directly from primary iPSC colonies. Establishment of transgene-free iPSCs required approximately two weeks from the time of addition of the cell-permeant TAT-Cre protein. Factor-free human and mouse iPSCs expressed appropriate morphological and immunocytochemical staining characteristics of pluripotent cells. Factor-free human iPSCs possessed a normal karyotype and were capable of differentiating into derivatives of all three germ layers in vivo. In summary we have established a robust system for highly efficient excision of viral vectors from iPSCs using cell permeant TAT-Cre protein. Efficient delivery of an active recombinant Cre protein to mammalian cells has broad applications not only for somatic cell reprogramming, but also for controlled genetic modification of mammalian genomes.

Poster Board Number: T-3137

ENGINEERING THE REPROGRAMMING OF HUMAN AND MOUSE CELLS TOWARDS THE CARDIAC CELL LINEAGE

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In recent years various groups have successfully demonstrated the transdifferentiation of terminally differentiated cells into alternative cell lineages. In one such case overexpression of MyoD is sufficient to efficiently convert fibroblasts into functional skeletal myocytes. Recent evidence has suggested that this may also be possible for the cardiac cell lineages. The main objective of this work was the identification of a core set of master regulator genes that would activate the efficient transdifferentiation of both human and mouse cells into the cardiac muscle cell lineage. We began by identifying specific transcription factors (TFs) previously shown to actively control cardiogenesis during development (FHL2, GATA4, GATA5, HAND1, HAND2, HEY1, HEY2, HOF, IRX4, MEF2C, Mesp1, MYOCD, NKX2-5, SMARCD3, SRF, TBX5, and TBX20), and subsequently cloning them into an inducible (Tet-On) lentiviral expression vector. To efficiently screen for cardiac reprogramming we utilized lentiviral expression vectors or plasmid DNA molecules allowing the expression of marker proteins under the control of cardiac specific promoters (MYH6gpr) in addition to isolating embryonic fibroblasts from transgenic mice (Myh6.GFP/Myh6.PAC). We subsequently transduced human and mouse primary cells (human dermal fibroblasts, human adipose derived cells, and wild type or transgenic mouse embryonic fibroblasts), as well as established cell lines (NIH3T3 and HELA) with various combinations of the identified transcription factors. We screened for potential transdifferentiation events by cell-specific fluorescent protein (GFP, RFP) expression analysis (FACS and microscopy), RT-PCR analysis, immunofluorescence staining, calcium transient detection (GCaMP3) and electrophysiological cell characterization. One-week post induction of TF transgene expression we readily detected GFP+ or RFP+ cells in samples with particular TF combinations, indicating successful binding and activation of the cardiac specific promoter element. We also measured (qRT-PCR) a significant upregulation of cardiac specific genes including Actc1, Myh6, Myh7, Myl2, Myl7, Nppa, Ryr2, Ttnm2, Casq2, Hcn4, and Pln. Cells also stained positive in a cross-striated pattern when using cardiac specific antibodies (αActinin or Tnntr2). Although no spontaneous contracting was detected, when GCaMP+ cells were co-cultured with neonatal rat ventricular myocytes we readily detected GFP flashing. We conclude that overexpression of a particular set of TFs can successfully initiate the epigenetic reprogramming towards the cardiac cell lineage.
Detailed Program and Abstracts — Thursday, June 14

Poster Board Number: T-3138

DYNAMICS OF LYSINE ACETYLATION DURING THE ONE-CELL STAGE MOUSE EMBRYS AFTER SOMATIC-CELL NUCLEAR TRANSFER

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Although somatic-cell nuclear transfer (SCNT) is a powerful tool for reprogramming differentiated nuclei into totipotency, the developmental rate has been inefficient as other reprogramming technologies of somatic cells like iPSC technology. Treating cloned embryos with trichostatin A (TSA), a HDAC inhibitor (HDACi) has been found to significantly improve their subsequent development. (Kishigami et al. 2006). However, the mechanism for requirement of HDACi in SCNT is not truly understood yet. In pre-implantation embryos, regulation of lysine acetylation through HDAC activity plays a pivotal role in the subsequent embryonic development rates. Further, TSA treatment can induce hyperacetylation of histone and non-histone proteins. To elucidate the mechanism underlying the impact of TSA treatment, we analyzed behaviors of lysine acetylation in fertilized, parthenogenetic and cloned embryos by immunofluorescence. We found that following oocyte activation, lysine-acetylation was increased in both pronuclei and cytoplasm. The presence of TSA significantly enhances lysine-acetylation in the whole cell, suggesting that TSA impact on not only histone but also non-histone protein in cytoplasm. In the pseudo-pronuclei of SCNT embryos, the amount of lysine acetylation was lower than in pronuclei of parthenogenetic embryos, which is consistent with previous reports showing lower histone acetylation in SCNT embryos. Interestingly, lysine-acetylation in the cytoplasm of SCNT embryos was also significantly lower than parthenogenetic embryos, suggesting that somatic-cell injection can induce hypoacetylation. Thus, somatic-cell nuclear transfer impacts on the dynamics of lysine acetylation in embryos, which may contribute to lower developmental potential in SCNT embryos in addition to abnormal gene expression in cloned embryos.

Poster Board Number: T-3139

COMPREHENSIVE ANALYSES OF CHIMERAS AND PROGENY MICE FROM INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem (iPS) cells are generated from somatic cells by introducing small sets of transcription factors. Patients- or disease-specific iPS cells may provide unprecedented resources for drug discovery, studies on disease mechanisms and cell transplantation therapies. Although several assays in vivo and in vitro show that mouse and human iPS cells are similar to embryonic stem (ES) cells in many aspects, their long-term behavior and safeness in vivo remains to be determined. Moreover, up to the present, diverse modified technologies to generate iPS cells have been developed. However it is still unclear which of them are preferable for applications. Chimera formation by injection of cells into the early embryo is one of the most stringent assay to evaluate pluripotency and safeness of mouse iPS cells. In this study, we performed comprehensive analyses of chimeras and progeny mice derived from iPS cells generated with various origins and methods in our laboratory. We herein evaluated 533 chimeric mice and their 484 progenies derived from various IPS cells and 136 several types of control mice. 97% of them were observed until they died. Even among control groups we found significant difference in lifespan. Most chimeras derived from IPS cells generated by the retroviral transduction of Oct3/4, Sox2, and Klf4 with or without L-Myc survived for more than 80 weeks, which are comparable to control mice groups. In contrast, c-Myc integration resulted in a higher incidence of tumors and mortality in both the chimeras and progeny mice. Usage of cMyc with non-integration technology did not show such effect. These data demonstrated that three factor-derived IPS cells, even with proviral integration, are free from either genetic or epigenetic abnormalities which would lead to a shorter longevity in chimeras.

Poster Board Number: T-3140

DIRECT REPROGRAMMING OF MOUSE GALL BLADDER CELLS INTO TRANSPLANTABLE INSULIN-POSITIVE BETA-LIKE CELLS

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Current cell therapies for type 1 diabetes are restricted to transplantation of cadaveric donor islets, but success has been hindered by both lack of donor islets available and by the need for long-term immune suppression in the patient. We hypothesized that reprogrammed gall bladder cells (GBCs) could be used as an autologous cell therapy for type 1 diabetes. Here we show that mouse GBCs can be reprogrammed into transplantable insulin-positive cells using a unique combination of genetic factors and small molecules. First, we show that murine GBCs can be robustly expanded in vitro, allowing the generation of billions of cells from a single gall bladder. These GBCs can be transduced by recombinant adenovirus, although optimal transduction requires complexing of the viral particles with DEAE-Dextran. We determined the combination of Neurog3, Pdx1 and Mafa to be the minimal required transcription factors for robust expression of both Insl1 and Insl2 mRNA in vitro. We also established that addition of retinoic acid and inhibition of notch signaling increased the reprogramming efficiency of GBCs into beta-like cells. Next, using flow cytometry to isolate reprogrammed cells, we confirmed reprogrammed GBCs were differentiating towards the beta cell fate by both RNA and protein analyses of various transcription factors, including Nkx2-2, Nkx6-1, Pdx4, Pax6, Isl1, and Neurod1. Importantly, these reprogrammed cells also began to lose their parent gall bladder phenotype based on decreased expression of many genes, including Muc1, Aap1, Cfr and Krt19. However, although the reprogrammed GBCs were able to produce, process and secrete insulin, they do so in a non-glucose responsive manner. Subsequently, in order to determine the global expression profiles of these cells, we performed RNA-Sequencing analysis to compare reprogrammed GBCs to control GBCs, as well as actual pancreatic beta cells. Interestingly, the analysis confirmed that the reprogrammed GBCs were an intermediate population between gall bladder epithelial and mature pancreatic beta cells. However, reprogrammed GBCs had significant gene expression differences compared to beta cells, and we have identified several key components of beta cell function that are not properly expressed, including genes involved in glucose sensing, insulin vesicle maturation and insulin exocytosis. Finally, these partially reprogrammed...
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**THE EFFECTS OF OCT4 VIRAL VECTOR DELIVERY TO MOUSE BRAIN ON PLURIPOTENCY MARKERS’ EXPRESSION**

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Introduction: There are several reports of somatic cell reprogramming into pluripotent stem cells (iPSCs) with a combination of four transcription factors, Oct4/Sox2/Klf4/c-Myc. Interestingly, NSCs endogenously express Sox2, c-Myc, and Klf4, so they were reprogrammed into iPSCs just with Oct4 although the efficiency was low. Because of the neural stem cell restriction, brain repair is limited. So increasing neural stem cell number or their potencies could be useful in brain repair. Methods: Oct4 lentiviral vector was injected into the lateral ventricle of C57/BL6 mice brain followed by administration of doxycycline (3 µg/ml) for 5 consequent days. Animals were decapitated at day 7 and total RNA was extracted from the tissue collected from the rims of lateral ventricles. After cDNA synthesis, real time PCR performed to analyze the expression of Oct4, Sox2, Nanog, Klf4, c-Myc, alkaline phosphatase, Sox1 and Pax6. Gene expression level was normalized to GAPDH as an internal standard. Moreover, some of the brains were fixed and processed for analyzing expression of Oct4 and Nanog proteins in addition to SSEA1 using immunohistochemistry.

Results: Endogenous expression of Klf4, Nanog, c-Myc, Sox1 and Pax6 mRNAs were increased significantly 7 days after administration of Oct4 lentivector. In addition, increased levels of Nanog and SSEA1 were detected in the brain sections, although Oct4 expression was very low. Conclusion: Exogenous expression of Oct4 vector can induce cells reside in the rim of lateral ventricles to express genes activated in the pluripotency state. This can improve neural stem cell's potencies in SVZ to repair brain degenerations more efficiently.

**THE ROLE OF MICRONRNAS IN REPROGRAMMING OF SOMATIC CELLS INTO MOUSE INDUCED PLURIPOTENT STEM CELLS**

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The reprogramming of adult somatic cells into induced pluripotent stem cells (iPSCs) holds great promise in patient-specific cell therapy and model for drug screening. iPSCs can be generated by somatic cells nuclear transfer (SCNT) or introduction of Oct-4, Sox2, Klf4, c-Myc (OSKM) into somatic cells. However, the clinical application of iPSCs was impeded by the low reprogramming efficiency. In recent years, there is interest on studying the epigenetic modifications during reprogramming. For instance, treatment with compounds affecting epigenetic regulators such as DNA methyltransferase inhibitors and histone deacetylase (HDAC) inhibitors increased the reprogramming efficiency. On the other hand, the ESC-specific microRNAs (miRNAs) such as the miR-290 cluster were found to promote reprogramming. Our laboratory had determined the miRNA profile of mouse oocytes and preimplantation embryos. We found that some mouse ESC-specific miRNAs and some miRNAs known to be involved in mesenchymal epithelial transition (MET) were highly expressed in 1-cell mouse zygotes. We hypothesized that the miRNAs present in the zygotes regulate epigenetic modulators and thereby facilitating nuclear reprogramming. Therefore, a secondary mouse embryonic fibroblasts (MEFs) system carrying doxycycline inducible transcription factors OSKM was used to study the action of some of these miRNAs on reprogramming. Among the selected miRNAs tested, overexpression of miR-101 and miR-135a inhibited doxycyclin-induced reprogramming by almost 5-fold and 2-fold respectively. On the other hand, inhibition of miR-135a expression enhanced the reprogramming efficiency by around 2-fold; while inhibition of miR-101 expression did not affect reprogramming. In silico analysis predicted Enhancer of zeste homolog 2 (Ezh2) and Sirtuin-1 (Sirt-1) as potential targets of miR-101 and miR-135a, respectively. Both Ezh2 and Sirt-1 were highly expressed in mouse embryonic stem cells (mESs), and their expressions decreased time-dependently during in-vitro differentiation of mESC. On the other hand, the expression of Ezh2 and Sirt-1 increased during the reprogramming of MEFs. Interestingly, our data also showed that knockdown of Ezh2 and Sirt-1 expression significant inhibited the reprogramming efficiency, consistent with the inhibitory effects of miR-101 and miR-135a precursors. The identification of the role of miRNAs in reprogramming could help our understanding of the molecular mechanisms of reprogramming.

**PLURIPOTENTIAL REPROGRAMMING AND RE-DIFFERENTIATION RESET GENOMIC IMPRINTING PATTERNS**

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Induced pluripotent stem cells (iPSCs), generated by the overexpression of transcription factors Oct4, Sox2, Klf4 and c-Myc in somatic cells, have the same characteristics as pluripotent embryonic stem cells (ESCs). iPSCs reprogrammed from differentiated cells get through a epigenetic modification during reprogramming and finally have the similar epigenetic state to ESCs. In this study, these epigenetic changes were observed in reprogramming of uni-parenatal parthenogenic somatic cells. Furthermore, we have shown that parthenogenetic pattern of imprinted genes were changed during pluripotential reprogramming. Parthenogenetic neural stem cells (pNSCs) containing only maternal alleles retain the biparental imprinting patterns after reprogramming. However, we have yet to define whether the changed imprinted genes are maintained or reverted to the parthenogenetic state when the reprogrammed cells are differentiated into specialized cell types. To address this question, we compared genome-wide expression profiles of biparental female neural stem cells (iNSCs), parthenogenetic neural stem cells (pNSCs), and NSCs differentiated from parthenogenetic maternal iPSC (miPS-NSCs). Furthermore, this study establishes the correla-
tion between the alteration of genome methylation and activation of imprinting genes in the parthenogenetic cells and reports for the first time that the silenced PWS-related imprinted genes are activated in miPSC-NSCs. Our data demonstrated that pluripotential reprogramming of parthenogenetic somatic cells were able to reset the parthenogenetic imprinting patterns: reprogrammed miPSCs showed erasure of maternal methylation imprints and acquisition of methylation in paternally imprinted genes. Furthermore, the changed imprinting patterns were maintained when the reprogrammed cells are differentiated into specialized cell type.

**Poster Board Number:** T-3145

**FACTORS MEDIATING EPITHELIAL-MESENCHYMAL TRANSITION ENHANCE REPROGRAMMING OF SOMATIC CELLS TO PLURIPOTENCY**

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Somatic cells can be reprogrammed to pluripotency by the expression of four factors: Oct4, Sox2, Klf4, and c-Myc. This process generally requires weeks and is highly inefficient, and has been shown to involve a mesenchymal to epithelial transition (MET). In order to better understand the mechanism of reprogramming, toward the goal of improving its efficiency, we studied the role of factors mediating epithelial to mesenchymal transition (EMT) during reprogramming of mouse and human cells. Knockdown of Twist or Snail in reprogramming of mouse or human fibroblasts decreases efficiency, while overexpression of these factors via ER fusion in both fibroblasts and keratinocytes improves reprogramming efficiency. Utilizing cells from mice expressing a knockin Snail- or Twist-ires-YFP, sorted YFP positive populations reprogram at higher efficiency than negative. We observe nuclear Twist in both fibroblasts and keratinocytes early in reprogramming. Reprogramming efficiency varies across mouse strains, and in strains that reprogram more efficiently, the proportion of cells with nuclear Twist is higher. qPCR on mouse and human samples collected during the course of reprogramming reveals expected increases in pluripotency factors, but surprisingly the expression of Snail and Twist increases early in reprogramming in both mesenchymal and epithelial starting cells, before decreasing to the very low levels seen in iPS cells. Our results indicate that Snail and Twist both enhance the efficiency of reprogramming, and that even epithelial cell populations express mesenchymal factors during reprogramming, implying a pre-MET step. Our results suggest that expression of EMT factors is involved in the creation of a reprogramming-amenable state.

**Poster Board Number:** T-3146

**A ROLE FOR ACTIVATION-INDUCED CYTIDINE DEAMINASE (AID) IN STABILIZING INDUCED PLURIPOTENCY**

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Reprogramming of a somatic cell by ectopic expression of reprogramming factors to yield induced pluripotent stem cells (iPSCs) has the potential to provide personalized regenerative cell therapies and to model diseases “in a dish”. Despite its enormous potential, the generation of iPSCs is a slow asynchronous process with a very low efficiency (<0.1%). One proposed road block to efficiency is incomplete DNA demethylation of pluripotency genes, thereby inhibiting their activation during the process of reprogramming. Activation-induced cytidine deaminase (AID) is primarily known for its role in the generation of antibody diversity in B-lymphocytes. Recently it has been shown that AID can play a role in active DNA demethylation during reprogramming by cellular fusion of mammalian somatic cells. Here we explored the role of AID in direct reprogramming of somatic cells by induction of four factors (Oct4, cMYC, KLF4 and Sox2). Using a polycistronic lentiviral cassette, we expressed the four reprogramming factors in tail tip fibroblasts or embryonic fibroblasts derived from AID-/- mice or littermate controls. We observed that initial stages of reprogramming, measured by the expression of SSEA1 and E-CADHERIN, are functional and even enhanced in AID-/- fibroblasts. The total number of primary colonies obtained by the reprogramming process is significantly enhanced in knockout fibroblasts (p<0.0001) compared to the controls, yet clear differences in the morphology of the colonies are observed. The colonies derived from AID-/- mice display a flatter morphology lacking the sharp edges normally observed in colonies derived from wildtype mice or embryonic stem (ES) cells, and they are relatively heterogeneous with respect to cell size. We also observed that AID expression is induced in wildtype cells transitioning to the iPS state. We analyzed the expression of core pluripotency markers (SSEA1, NANOG and OCT4) at different time points during reprogramming, and observed no difference in the frequency of cells expressing these markers comparing AID-/- and wildtype derivatives three weeks after induction with reprogramming factors. Furthermore, no differences were observed in the expression of pluripotency markers Utft1, Col6a2, Thy1, Utft1, Eras, Klf2, Gbx2, Dpp4 and Tbp, as measured by qRT-PCR. In contrast, 4 weeks after reprogramming was initiated, there is a sharp decline in the frequency of cells expressing pluripotency markers in the AID-/- compared to wildtype derivatives (p <0.001 for SSEA1 and NANOg and p<0.01 for Oct-4). When colonies that appeared morphologically to be pluripotent after three weeks of reprogramming were picked from AID-/- derivatives, approximately 40% differentiated during passaging. The other colonies remained stably pluripotent, as measured by their ability to form embryoid bodies and to differentiate into derivatives representing all three primary germ layers. In contrast, all the wildtype-derived colonies remained pluripotent through passage 50. In conclusion, AID plays a role in regulating the process of induced pluripotency, as shown by lower efficiency of complete reprogramming in AID knockout cells. AID is not essential, and its function may perhaps be compensated by other deaminases. However, in its absence, reprogramming factors generate poorly reprogrammed iPS colonies, many of which are morphologically and genetically unstable.

**Poster Board Number:** T-3147

**CELLULAR MEMORY DISC OF THE REPROGRAMMING CELLS**

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The crucial fact underlying the low efficiency of cellular reprogramming is poorly understood. The cellular reprogramming occurs in nuclear transfer, induced pluripotent stem cell (iPSC), cell fusion, and lineage-switching experiments. Despite these advances, there are three fundamental problems to be addressed: (1) a majority of cells cannot be reprogrammed, (2) the efficiency of the reprogrammed cells is low, and (3) the developed reprogrammed cells
from patient's own cells activate immunogenic responses. These obstacles present major shortcomings for using reprogramming approaches for customized cell therapy. The current hypothesis is that all cells undergo an endogenous and/or exogenous holographic memorization that parts of it dramatically decrease efficiency of the reprogrammed cells, act like a barrier against reprogramming in the majority of cells, and activate immunogenic responses. Accordingly, the focus of this review was mainly to describe the topic of the cellular memory disc. Based on the present theory of memory disc, the cellular memory includes three parts: reprogramming-resistance memory (RRM), switch-promoting memory (SPM), and cultured induced memory (CIM), which effect on the cellular behaviors.

Poster Board Number: T-3148

P27KIP1 REGULATES SOX2 FUNCTION IN STEM CELLS, DEVELOPMENT AND DIFFERENTIATION THROUGH DIRECT TRANSCRIPTIONAL REPRESSION

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The mechanisms involved in the reprogramming of differentiated cells into induced pluripotent stem (iPS) cells by the three transcription factors Oct4, Klf4 and Sox2 remain poorly understood. Recently, we and others have shown that tumor suppressor genes oppose reprogramming, limiting the efficiency of the process. This barrier against reprogramming could potentially represent an antitumor action of these genes and investigating the interplay between tumor suppressors and genes with important functions in the biology of stem cells might provide clues about the molecular mechanisms governing cancer development as well as increasing our understanding of the reprogramming process. While investigating the activity of different tumor suppressor genes during reprogramming, we realized that cells lacking p27Kip1 could be reprogrammed at low but reproducible frequency with only two factors, Oct4 and Klf4. Mouse embryonic fibroblasts derived from p27Kip1-null animals showed an intrinsically high basal level of expression of Sox2. This un-repressed expression of Sox2 correlated with the absence of repressive chromatin marks at the critical Sox2-SRR2 enhancer upstream of Sox2 gene. Moreover, pluripotent stem cells lacking p27Kip1 fail to fully repress Sox2 upon differentiation. At a mechanistic level, we found that upon differentiation, p27Kip1 directly binds to the critical Sox2-SRR2 enhancer together with a p130-E2F4-Sin3a repressive complex and contributes to transcriptional repression of Sox2.

Poster Board Number: T-3149

TRANSDIFFERENTIATION BY BACTERIAL DELIVERY OF MYOD PROTEIN

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Forced exogenous gene expression has been well characterized as an effective method for directing both cellular differentiation and dedifferentiation. However, transgene expression is not amenable for therapeutic application due to the potential for insertional mutagenesis. Protein based techniques provide a safe alternative, but current protein delivery methods are quite limited by labor-intensive purification processes, low protein yield and inefficient intracellular targeting. Such limitations may be overcome by using a naturally occurring bacterial protein injection system. Pseudomonas aeruginosa utilizes a Type III Secretion System (T3SS) to inject bacterial proteins directly into the eukaryotic cell cytoplasm. Our previous studies describe the ability of this system to easily deliver a high quantity of protein to both differentiated and pluripotent cells using a genetically attenuated strain. Using Cre recombinase as a reporter, we have demonstrated high frequency loxP mediated recombination in the chromosome of the recipient cells, suggesting the protein is not only efficiently targeted to the nucleus, but also retains its biological function. MyoD is a key muscle regulatory factor, the over-expression of which is able to induce transdifferentiation of numerous cell types, such as fibroblasts, into functional myocytes. Here we demonstrate transient injection of MyoD protein by P. aeruginosa to be sufficient to induce myogenic conversion of mouse embryonic fibroblasts. In addition to clear morphological changes, muscle specific gene expression has been observed by both immunostaining as well as RT-PCR. These studies serve as a foundation for the bacterial delivery of transcription factors to efficiently modulate concentration-dependent and temporal activation of gene expression to direct cell fate without jeopardizing genomic integrity.

Poster Board Number: T-3150

DIRECT REPROGRAMMING OF PRIMARY LEUKEMIC CELLS INTO IPS CELLS

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The reprogramming of differentiated cells into induced pluripotent stem cells (iPSCs) by introduction of the four defined transcription factors (Oct4, Sox2, Klf4, and Myc) has rapidly become an intensively investigated area in stem cell research due to its enormous potential in regenerative medicine. Noticeably, like malignant transformation, the generation of iPSCs has also been shown to be suppressed by both the p53 and Rb tumor suppressor pathways, indicating that there are some shared mechanisms between tumorogenesis and iPSC reprogramming. However, it has not been definitively demonstrated whether or not primary transformed cells (not established tumor cell lines) can be reprogrammed into iPSCs with a full-term developmental potential in mice. To this end, we established acute myeloid leukemia (AML) by over-expressing the leukemogenic fusion protein, MLL-AF9, in bone marrow-derived hematopoietic cells from doxycycline (Dox)-inducible transgenic mice in which expression of the four reprogramming factors can be turned on by Dox in culture (Brambrink et al., 2008). The sorted leukemic cells were then induced into iPSCs by addition of Dox under mouse embryonic stem cell (ESC) culture conditions. By using genomic PCR, we confirmed that the MLL-AF9 fusion gene was present in the iPS colonies. However, our RT-PCR result indicated the leukemic gene was silent in the iPSCs. The characteristics of these iPSC cell lines were similar to those of normal ESCs, as assessed by the expression of pluriotency genes and the presence of iPSC surface markers. The karyotypes of all tested iPSC lines from the leukemic samples were normal, with 40 chromosomes each. All of these iPSC lines could form teratomas and differentiate into three germ layers. More importantly, the iPSC lines were able to generate chimeric mice with a high integrating rate. In short, our current
study demonstrates that despite the presence of a leukemogenic gene, the primary leukemic cells could be directly converted into iPSC lines in vitro and the MLL-AF9 carrying iPSCs were able to contribute to chimeras in mice. We are currently investigating whether leukemia-derived iPSCs have a full-term developmental potential with germ-line transmission in vivo and what the shared underlying mechanisms are between malignant transformation and iPSC induction.

Poster Board Number: T-3151
REPROGRAMMING FACTOR SELECTION USING THE CORE EXPRESSION MODULE ALGORITHM (CEMA)

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The Core Expression Module Algorithm (CEMA) analyzes the transcriptome of a particular cell type versus many other cell types to identify the unique expression patterns of a cell. The CEMA data generates a list ranking the gene expression of the cell of interest. This information can be used to potentially identify transcription factors for reprogramming any cell line. CEMA and data from 10 different cell types constructs a list of the top unique 200 genes that defines each cell line. Evaluation of gene expression levels parsed the gene list down to 3-10 genes that could be valuable for direct reprogramming. Two different polyclonist retroviruses containing candidate transcription factors for keratinocytes are introduced individually into fibroblast for the reprogramming experiment. After the delivery, the fibroblast displayed positive integration of the vector, expressing the venus YFP reporter protein. Immuno-fluorescent staining confirmed the over-expression of the polycistronic genes. Cells will be cultured keratinocyte media for 2-8 weeks and then assayed for reprogramming. Reprogrammed cell will be collected using Fluorescence-Activated Cell sorting (FACS), and expression profiled by RT-PCR and microarray. To functionally assay reprogramming, the calcium switch assay will be employed. Finally, we will test other types of cells for their ability to be reprogrammed. This test will determine if CEMA facilitates the identification of reprogramming factors.

Poster Board Number: T-3152
GENERATION OF NON-INTEGRATING INDUCED PLURIPOTENT STEM CELLS WITH THE CYTOTUNE™ -IPS SENDAI REPROGRAMMING KIT IN CHEMICALLY DEFINED MEDIUM.

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Human induced pluripotent stem cells (iPSCs) are an important potential source of cells for regenerative medicine due to their inherent ability to differentiate into all cell types of the three germ layers. Generation of iPSCs with a non-integrating reprogramming method and in culture conditions that are completely absent of animal proteins will be ideal for such regenerative and cell therapy applications. Here we demonstrate a highly efficient method to generate non-integrating iPSCs using the CytoTune™ IPS-Sendai Reprogramming Kit. Sendai virus is a RNA virus that replicates only in the cytoplasm and does not integrate into the cellular genome. In addition, we derived non-integrating iPSCs in a chemically-defined medium that does not contain bovine serum albumin. This robust and efficient system of integration-free iPSC generation in defined conditions minimizes the exposure of human cells to animal proteins that may incorporate non-human immunogenic molecules, and will enable human iPSCs for cell therapy-relevant downstream applications.

Poster Board Number: T-3153
MIRNAS ARE REQUIRED FOR INDUCED NEURONAL CONVERSION.

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Our lab has shown that exogenous expression of specific transcription factors can reprogram fibroblast to an induced neuronal cell (IN). Recently, other groups have reported two microRNAs (miRNAs) that enhance IN formation. Because miRNAs are key regulators of biological functions, we wanted to test whether changes in the miRNA population are not only beneficial but also required for the reprogramming process. We have taken advantage of a miRNA deficient mouse embryonic stem cell (mESC line) that lacks DGC8R, a critical component for miRNA processing, to test if miRNAs are required for iN conversion. Wild type (WT) mESCs rapidly convert to Map2+ neurons within 5days upon lentiviral infection with Bm2, Ascl1, and Myt1L (BAM) transcription factors. Meanwhile, DGC8R-/- mESCs do not show any change in their state when infected with the BAM factors and ultimately die during the reprogramming process. This observation is also apparent with other transcription factor combinations, indicating that miRNA expressed developmentally towards iNs requires miRNAs. We have also identified several miRNAs that can enhance the iN conversion in WT mESCs when exogenously expressed along with the BAM factors. We are currently screening miRNAs in the DGC8R-/- mESCs to test whether we can rescue the iN conversion deficiency and ultimately use these miRNAs to increase the iN conversion rate.

Poster Board Number: T-3154
PROANGIgenic FATE OF BONE MARROW DERIVED STEM PROGENITOR CELLS DURING TISSUE REGENERATION IS DIRECTED BY HOXA3 EXPRESSION

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Bone marrow contains a population of multipotent cells with the capability to proliferate, self-renew, and differentiate into different blood cell types as well as non-haematopoietic partners such as endothelial cells. We and others have shown that during wound repair bone marrow-derived (BMD) stem/progenitor cells migrate to injury site and once there support angiogenesis via providing stimulatory signals such as chemokines and growth factors or directly through differentiation into proangiogenic cells. We have previously shown that soon after injury Hoxa3 is upregulated in many cell types such as keratinocytes, endothelial cells, and BMD progenitor cells, leading to enhanced angiogenesis. Here, we show how Hoxa3 influences the angiogenic potential of BMD haematopoietic stem/progenitor cells as well as mesenchymal stem cells (MSCs). Hoxa3...
promotes the differentiation fate of hematopoietic progenitor cells toward pro-angiogenic Gr-1+CD11b+ cells. In acute wounds, these Gr-1+CD11b+ myeloid cells promote angiogenesis; however their function is defective under diabetic conditions. In diabetic animals these cells are unable to migrate normally and have a prolonged retention period in the wound milieu. Their gene expression profile is impaired, they do not have normal proliferative capacity, and are more pro-inflammatory. Hoxa3 transcription factor reprogramming of these cells reverts most of these defects and promotes a more pro-angiogenic phenotype, as their gene expression profile shows. Hoxa3 transcription factor reprogramming also enhances the angiogenic potential of MSCs through increasing their migration towards angiogenic signals such as VEGF and enhancing their tube-like network formation capability during their differentiation into endothelial cells. Altogether this suggests Hoxa3 can reprogramme BMD cells into endothelial progenitor-like cells.

Poster Board Number: T-3155

WHOLE-GENOME IDENTIFICATION OF TARGET TRANSCRIPTS AND MOLECULAR PROCESSES MODULATED BY PLURIPOTENCY-RELATED MICRORNAS: MIR-106A AND MIR-302B

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Increasing evidences have accumulated showing that pluripotency-related microRNAs (miRs) can enhance the efficiency of somatic cells reprogramming into induced pluripotent Stem cells (iPS), when used in association with classic reprogramming factors. Strikingly, transfection of synthetic miRs was shown to directly reprogram somatic cells into iPS cells, opening the possibility of reprogramming without potentially harmful genetic modifications. Nevertheless, the efficiency of synthetic miR-mediated reprogramming is still low, and the molecular mechanisms contributing to the process of reprogramming are only starting to be uncovered. With that in mind, in the present work, we explored the potential roles of the miRs 106a and 302b, centrally involved in miR-mediated reprogramming. For this, transfection of pre-miR, inhibitory anti-miR and corresponding unspecific control molecules were independently transfected into human BJ fibroblasts and into pluripotent NTera2 cells. After 72 hours, whole-genome transcriptomes were obtained by oligonucleotide microarrays. To identify highly confident targets, transcripts downregulated by pre-miR transfection and upregulated by the corresponding anti-miR, in both cell lines, were further compared to the set of predicted targets showing evolutionary conserved miR binding sites (miRorna.org). Molecular changes related to the reprogramming process were identified by comparing the microRNAs-induced transcriptional changes, with those observed upon iPS reprogramming. For this, transcriptomes from two iPS and the fibroblasts of origin were used: a partially reprogrammed cell line generated by our group (lentiviral insertion of OSKM factors). Pathways and biological processes modulated by the miRs were identified using a Functional Annotation Tool (DAVID). Among pathways with a statistically significant enriched number of transcripts, identified as highly confident miR-302b targets, we found: Apoptosis, p53 and WNT signaling (including: MDM4, CDKN1A, CYCS, ATM; IL1R1, LRPP6 and ROCK2). Similarly, for miR-106a, the following pathways were identified: Regulation of Actin Cytoskeleton, Adherens Junction, Focal Adhesion, Axon Guidance and MAPK Signaling (including: FN1, FGFR2, LIMK2, PAK2, ROCK1, ROCK1P1, ITGB1, CTNNAA1, EP300, TGFBR2, ACVR1B, SMAD2, TAOK1, DUSP3, MAP3K2, PTKCA and PPP3CA). Interestingly, analysis based on shared predicted-targets downregulated by miRs 302b and 106a, in both cell lines, and downregulated upon IPS reprogramming, revealed enrichment of central pathway components of TGFbeta (TGFBR2, SMAD2, SMAD3 and ROCK2) an MAPK signaling (MAPK1, DUSP3, MAP3K2, PAK2). Additionally, although not shared predicted-targets, IL1R1, LTB8, ROCK1 and GSK3β were similarly modulated. Finally, several transcripts related to pluripotency and reprogramming (including Nanog, LIN28 and Nodal) were up-regulated by both miRs and confirmed by qRT-PCR. Our results demonstrate that several components of pathways previously implicated in opposing pluripotency, self-renewal and somatic cell reprogramming, are highly likely directly targeted to degradation by miR-106a and miR-302b. Our findings add to the understanding of the molecular mechanisms by which miRs drive reprogramming, and may help in the future development of miR-mediated reprogramming with increased efficiencies. Support: FAPESP, CNPq and FINEP.

Poster Board Number: T-3157

A SYSTEMATIC APPROACH TO IDENTIFY REGULATORS OF COMPLETE REPROGRAMMING

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Temporal gene expression profiling studies of reprogramming mouse embryonic fibroblast cells that were generated using the doxycycline-inducible piggyBac system revealed that reprogramming is a multi-step process that is characterized by Initiation, Maturation and Stabilization phases. Previously, we hypothesized these phases follow each other in a sequential manner and assigned specific markers to each phase of reprogramming. Here we demonstrate that complete Maturation is required for successful transition to Stabilization phase of reprogramming. Using BeadArray and RNA-Seq technologies, we performed clonal transcriptome analysis in a temporal manner during the time course of reprogramming. By comparing gene expression profile of clones that completed Maturation and transited to Stabilization phase to clones that did not, we discovered a gene expression signature associated with successful reprogramming. To determine whether gain of the identified signature is required for transition to Stabilization phase and completion of reprogramming, we established conditions for a systematic RNAi screen to evaluate the function of the candidate genes. In this assay, we took advantage of piggyBac secondary reprogramming model and assessed the effect of specific gene knockdown on complete cellular reprogramming. I will present the integrative approach we employed to highlight the significance of some key signaling pathways required for completion of somatic cell reprogramming. Furthermore, the approach and the result of the RNAi screen as well as follow up studies on potential regulators of complete reprogramming will be presented.
Detailed Program and Abstracts — Thursday, June 14

Poster Board Number: T-3158

RECRUITMENT OF THE TRANSCRIPTION MACHINERY FACILITATES REPROGRAMMING

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In order to generate induced pluripotent stem cells (iPSCs), Oct4, Sox2, Klf4 and C-Myc need to compete with the endogenous transcription factors for recruiting the transcriptional machinery. Oct4 is considered to be a key player in the pluripotency network and it is essential for the iPSCs generation. We hypothesize that facilitating the access of Oct4 to the transcriptional machinery could increase the reprogramming efficiency. As it has been reported that the adenoviral protein E1A is able to link Oct4 to the basal transcription initiation complex, we decided to include E1A in the reprogramming cocktail. Our data show that E1A increases the iPSC colony formation in neural stem cells and in mouse embryonic fibroblasts. In summary, our results suggest that the recruitment of the transcriptional machinery is a limiting step during the reprogramming process.

Poster Board Number: T-3159

C/EBPA INDUCES B CELL TO MACROPHAGE TRANSDIFFERENTIATION BY ESTABLISHING A STABLE MYELOID TRANSCRIPTION FACTOR NETWORK

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C/EBPa induces B cell to macrophage transdifferentiation by establishing a stable myeloid transcription factor network. C/EBPalpha is a transcription factor capable of inducing the transdifferentiation of committed B cell precursors into macrophages at 100% efficiency. Using an inducible pre-B cell line carrying C/EBPaER we have explored the mechanism by which the myeloid phenotype is established. This line becomes stably committed to a macrophage fate after 24 hours of C/EBPa activation followed by inducer washout. Gene expression profiling over time, combined with gain and loss of function experiments, showed that C/EBPb and PU.1 are required to establish the macrophage state and that endogenous C/EBPa is required to maintain this phenotype. ChIPseq experiments showed that C/EBPa and C/EBPb bind to promoter-distant sites of upregulated genes within 3 hours after induction, and that the transcription factors remain bound to these sites at all later time points. About a third of the sites are already bookmarked by PU.1 in pre-B cells, another third shows a recruitment of PU.1 and the last third remains free of PU.1 binding. The upregulation kinetics of genes in the vicinity of these putative enhancers is significantly influenced by their transcription factor occupancy, as is also reflected by the presence or absence of histone marks that correlate with gene activation (H3K27Ac). Interestingly, the genes closest to the enhancers bookmarked with PU.1 are poised as they are already expressed in pre-B cells at basal levels and then become further activated during transdifferentiation. In contrast, genes corresponding to the two categories of unmarked enhancers are silent in pre-B cells and after C/EBPa binding become de-repressed. In conclusion, C/EBPa induces a stable switch from pre-B cells into macrophages by activating its partners C/EBPb and PU.1. Together the three factors form a stable transcription factor network that first establishes and then maintains the macrophage state.

Poster Board Number: T-3160

MOLECULAR MECHANISMS OF ASTROGLIA-TO-NEURON CONVERSION BY FORCED EXPRESSION OF NEUROG2

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Direct conversion of differentiated cells into neurons is emerging as a promising alternative for cell-based therapies of neurodegenerative diseases. Compared to induced pluripotency, direct conversion is relatively efficient, faster, does not give rise to pluripotent intermediates, and allows for the generation of different types of neurons, depending on the combinations of factors employed. We previously focused our attention on the reprogramming potential of glial cells because they are lineage-related to neurons through common radial glia ancestors and are distributed throughout the mammalian brain, thus representing an endogenous source of potential reprogrammable cells. Indeed, we could recently show that retrovirus-mediated expression of a single proneural gene - Neurog2 or Mash1 [Ascl1] - can directly and efficiently convert astroglia of postnatal mouse cerebral cortex into fully functional neurons in vitro. In order to understand the molecular mechanisms underlying the transgene-mediated conversion of astroglia into neurons, we generated an inducible system in which the Neurog2 cDNA is fused to a modified estrogen receptor-binding domain (ERT2). Therefore, the resultant protein is expressed but it remains inactive unless Ngn2ERT2-expressing cells are treated with tamoxifen, upon which inhibition is released. This allows for a temporally defined onset of the proneural gene's transcriptional activity. Indeed, Neurog2ERT2-transduced astrocytes generated neurons upon tamoxifen treatment, while no neurons were observed in control-treated Ngn2ERT2-transduced cells, thus providing an experimental system for further molecular analyses. To gain insight into the early events of Neurog2-mediated reprogramming, we collected Neurog2ERT2-transduced astrocytes at various time points after tamoxifen treatment - 4, 24 and 48 hours - and performed a microarray-based expression analysis. Interestingly, more than 100 genes were significantly upregulated already 4 hours after tamoxifen application: amongst them, some neurogenic transcription factors are maintained at later stages after induction, while others are only transiently upregulated. Interestingly, none of these genes has a neurogenic effect on astroglia on its own, but they enhance Neurog2-mediated conversion efficiency, and rescue the neurogenic conversion in a model of failed reprogramming. Experiments are ongoing to assess whether these factors are essential for Neurog2-mediated reprogramming, and whether, when expressed altogether, they are sufficient to induce direct neuronal reprogramming in absence of Neurog2.

Poster Board Number: T-3161

DIRECT CONVERSION OF FIBROBLASTS INTO NEURAL STEM CELLS BY DEFINED FACTORS

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Recent advances in stem cell biology have revealed that cell type-specific transcription factors could reset the somatic memory of differentiated cells via direct reprogramming into cells of specific
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identities, such as neurons, cardiomyocytes, blood progenitor cells, hepatocytes, and epiblast stem cells, i.e., without the cells having to pass through an induced pluripotent stem cell state. However, the direct reprogramming of differentiated cells into self-renewing somatic stem cell types has not yet been described. Here we show that a combination of transcription factors can induce mouse fibroblasts to acquire a neural stem cell (NSC) identity. The induced neural stem cells (iNSCs) showed morphology, gene expression, epigenetic features, differentiation potential, self-renewing capacity, as well as in vitro and in vivo functionality similar to those wild-type NSCs. Therefore, our data suggest that specific defined factors can induce the direct reprogramming of differentiated somatic cells into specific somatic stem cell identities.

Poster Board Number: T-3162

NUCLEAR WAVE1 IS NECESSARY FOR TRANSCRIPTIONAL REPROGRAMMING BY XENOPUS EGGS AND OOCYTES

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Nuclear reprogramming of differentiated cells holds great promise for regenerative medicine. Reprogramming of differentiated cells can be very efficiently achieved by nuclear transfer to eggs and oocytes. Eggs/oocytes utilize natural reprogramming activity that is used for sperm nuclei to ensure normal embryonic development. However, such egg/oocyte factors and mechanisms involved in reprogramming are not well understood. We have previously shown that nuclear actin which naturally polymerizes in the germinal vesicle (GV), the Xenopus oocyte nucleus, plays a key role in reprogramming. Actin polymerization is regulated by actin-binding proteins. We therefore screened actin-binding proteins in the GV as candidate reprogramming factors. We find that WAVE1, which is predominantly expressed in the brain in an adult body, is present in the oocyte nucleus and required for efficient transcriptional reprogramming from mouse nuclei transplanted into the GV. WAVE1 transcript variants are expressed in oocytes and one variant containing the WHD-Basic domain is sufficient to bind to active RNA polymerase II. Interestingly, interaction between nuclear WAVE1 and RNA polymerase II is observed in embryos at the time of zygotic genome activation, at which major transcriptional reprogramming is induced in development, and moreover WAVE1 is important for embryonic development. These results unravel an unexpected role of an actin-binding protein, WAVE1, in reprogramming. This is also a first clear demonstration that a reprogramming factor stored in the oocyte nucleus is used for reprogramming both somatic nuclei and fertilized embryos.

Poster Board Number: T-3163

STRATEGIES FOR IMPROVING DOPAMINE NEURONS DERIVATION WITH DEFINED FACTORS

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Parkinson’s Disease (PD) is a neurodegenerative disease characterized by a loss of movement control due to the degeneration of midbrain dopaminergic (DA) neurons in the substantia nigra pars compacta, the brain region that controls voluntary movements. PD predominantly affects elderly people and with an increasing aging world population, it is critical to improve current treatments for this disease. Previous studies utilizing DA neurons derived from ES, IPS and MEF cells have shown various degrees of improving Parkinsonian behavior after transplantation into rodent models. Using Ascl1 (Mash1), Nurrol1 and Lmx1a, we report a ~8.6% efficiency of obtaining TH+ cells from MEFs cells. These cells, however, are not positive for another marker PITX3 which is considered a unique defining marker of true midbrain DA cells. A screen of more DA transcription factor yields indicated candidates that express both TH and PITX3, but the number of double positive cells were few. Current work is focusing on improving the efficiency of obtaining TH+/PITX3+ cells and the performance of these cells in vivo.

Poster Board Number: T-3164

THE ROLE OF INDUCED PLURIPOTENT STEM CELLS IN CONSERVATION OF ENDANGERED SPECIES

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Abstract: Induced pluripotent stem cells (iPSCs) are generated from somatic cells by direct molecular reprogramming and are capable of unlimited expansion and differentiation into a wide variety of cell types. Mouse iPSCs have been shown to be capable of generating all fetal cell types. These results suggested to us that iPSCs could be valuable tools in the conservation of endangered species. We recently reported on the successful application of reprogramming technology toward the generation of iPSCs from primary fibroblast cultures banked in the Frozen Zoo at the San Diego Zoo’s Institute for Conservation Research. We made iPSCs from two critically endangered species, the drill, Mandrillus leucophaeus, and the northern white rhinoceros, Ceratotherium simum cottoni (Ben-Nun et al., 2011). The Frozen Zoo is an extensive collection of cryopreserved primary fibroblast cultures, collected from over 8600 individual vertebrates from approximately 800 species. During these reprogramming efforts, we determined that Moloney murine leukemia virus-based retroviral vectors could effectively deliver the reprogramming factors into the northern white rhinoceros fibroblasts when pseudotyped with vesicular stomatitis virus G envelope protein (VSV-G), but not with the amphotrophic envelope protein. The VSV-G pseudotyped retroviral vectors also efficiently transduced the drill fibroblasts. We were able to reprogram both the rhinoceros and drill fibroblasts using the human OCT4/POU5F1, SOX2, KLF4, and MYC cDNA sequences, suggesting that retroviral vectors carrying human reprogramming factor sequences and pseudotyped with VSV-G may be widely applicable for generating iPSCs from a variety of species. We are currently using the same methods to reprogram fibroblasts from the Javan Banteng and Somali Wild Ass. The Javan Banteng, Bos javanicus, is a species of wild cattle native to Asia. Due to hunting and habitat destruction, the population of wild Javan bantengs has dwindled and they are currently listed as an endangered species1 (Timmins, 2008)1. The Somali wild ass, Equus asinus somaticus, is critically endangered with fewer than 200 individuals living in the wild (IUCN Red List of Threatened Species (Moehlman, 2008)). In 2003, it was demonstrated in a collaboration between the San Diego Zoo’s Institute for Conservation Research and Advanced Cell Technology that somatic cell nuclear transfer of the nucleus from a Javan Banteng fibroblast into an oocyte from a domestic cow could be used to generate a viable animal, using a domestic cow as a surrogate. These results suggest that we may be able to use germ cells, embryos, or surrogates from domestic animals to explore the developmental capabilities of iPSCs from endangered animals.
**Tissue Engineering**

**Poster Board Number: T-3165**

**EFFECT OF PURIFICATION ALGINATE SPONGES WITH CHONDROCYTES FOR THE ARTICULAR CARTILAGE TISSUE ENGINEERING**

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Abstract: Adult articular cartilage tissue has poor capability of self-repair. Therefore, a variety of tissue engineering approaches are motivated by the clinical need for articular repair. Non-Purify alginate and purify alginate were fabricated by seed cartilage cell on the sponge. After 1, 3, 7, 10 and 14 day of cell seeding, cell proliferation activity was measured via MIT assay. Morphological observation, histology, biological assay for collagen type I, II and aggrecan, and PCR were performed at each time point 1, 2 and 3 weeks. In result, the cell viability was better in purify alginate sponge than non-purify alginate sponge. Scaning electron microscope (SEM) results showed that more attach cell in the purify alginate sponge with lapse of time. DMMB results showed that the highest content of glycosaminoglycan (GAGs) and collagen at purify alginate sponge. Compression results indicated that purify alginate sponge was the highest intensity. This study suggested that purify alginate sponge may serve as a potential cell delivery vehicle and a structural basis for tissue engineered articular cartilage. This research was supported by WCU (R31-20029). Keywords: Purification alginate sponge, alginate, chondrocyte WCU BIN Fusion Tech. Secretariat Dept. of BIN Fusion Tech., WCU, Eng. 8th Bldg. rm. No.408, Chonbuk National University, 664-14, Dukjin, Jeonju, 561-756 Korea Tel: +82 63 270 4434 begin_of_the_skype_highlighting +82 63 270 4434 end_of_the_skype_highlighting Fax: +82 63 270 4254 E-mail: bkwcu@jbnu.ac.kr

**Poster Board Number: T-3166**

**VARIATION RATIO OF DBP/ALGINATE MICROCAPSULES WITH CHONDROCYTES FOR THE ARTICULAR CARTILAGE TISSUE ENGINEERING**

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Abstract: Adult articular cartilage tissue has poor capability of self-repair. Therefore, a variety of tissue engineering approaches are motivated by the clinical need for articular repair. 0, 0.1, 0.3, 0.5 and 1% DBP/Alginate microcapsules were fabricated by seed cartilage cell into microcapsules. After 1, 5, 7, 10 and 14 days of cell seeding, cell proliferation activity was measured via MTT assay. Morphological observation, histology, biological assay for collagen type I, II and aggrecan, and PCR were performed at each time point 1, 2 and 3 weeks. In result, the cell viability was higher 1% DBP/alginate microcapsules than the other DBP/alginate microcapsules. DMMB results showed that the highest content of glycosaminoglycan (GAGs) and collagen at 1% DBP/alginate microcapsules. Compression results showed that 1% DBP/alginate microcapsules was the highest intensity. This study suggests that 1% DBP/alginate microcapsules may serve as a potential cell delivery vehicle and a structural basis for tissue engineered articular cartilage. This research was supported by WCU (R31-20029). Keywords: Gellan gum hydrogel of GG reacted with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to obtain a cross-linked GG. From the FT-IR analyze, the mechanism of EDC cross-linking in the GG was the condensation reaction between the carboxyl and hydroxyl group. Because of polysaccharide hydrogels normally possess poor mechanical strength, the results obtained in this study indicated that GG was suitable for potential applications in tissue regeneration. In vitro biocompatibility tests, hydrogel ex- hibited nontoxic effects. When implanted in to rat subcutaneous tissue, implanta- tion showed that hydrogel caused slight inflammation in the first few days after operation. In the tissue engineering, cross-linked GG demonstrated good physical properties and biocompatibility, and has great potential for future use. This research was supported by WCU (R31-20029) and MBC (0405-BOO1-0204-0006). Keywords: Gellan gum, Hydrogel, Biocompatibility WCU BIN Fusion Tech. Secretariat Dept. of BIN Fusion Tech., WCU, Eng. 8th Bldg. rm. No.408, Chonbuk National University, 664-14, Dukjin, Jeonju, 561-756 Korea Tel: +82 63 270 4434 begin_of_the_skype_highlighting +82 63 270 4434 end_of_the_skype_highlighting Fax: +82 63 270 4254 E-mail: bkwcu@jbnu.ac.kr

**Poster Board Number: T-3167**

**STRUCTURE AND PROPERTIES OF INJECTABLE GELLAN GUM HYDROGEL**

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Abstract: Gellan gum (GG) is an exopolysaccharide produced by Sphingomonas elodea. In this study we prepared three dimension-al hydrogel of GG reacted with 1-ethyl-3-(3-di-methylaminopropyl) carbodiimide (EDC) to obtain a cross-linked GG. From the FT-IR analysis, the mechanism of EDC cross-linking in the GG was the condensation reaction between the carboxyl and hydroxyl group. Because of polysaccharide hydrogels normally possess poor mechanical strength, the results obtained in this study indicated that GG was suitable for potential applications in tissue regeneration. In vitro biocompatibility tests, hydrogel exhibited nontoxic effects. When implanted in to rat subcutaneous tissue, implantation showed that hydrogel caused slight inflammation in the first few days after operation. In the tissue engineering, cross-linked GG demonstrated good physical properties and biocompatibility, and has great potential for future use. This research was supported by WCU (R31-20029) and MBC (0405-BOO1-0204-0006). Keywords: Gellan gum, Hydrogel, Biocompatibility WCU BIN Fusion Tech. Secretariat Dept. of BIN Fusion Tech., WCU, Eng. 8th Bldg. rm. No.408, Chonbuk National University, 664-14, Dukjin, Jeonju, 561-756 Korea Tel: +82 63 270 4434 begin_of_the_skype_highlighting +82 63 270 4434 end_of_the_skype_highlighting Fax: +82 63 270 4254 E-mail: bkwcu@jbnu.ac.kr

**Poster Board Number: T-3168**

**EFFECT OF GELLAN GUM HYDROGEL FOR CARTILAGE REGENERATION**

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Abstract: Injectable hydrogels have been studied for potential applications for articular cartilage regeneration. In this study, our aim is to research effect of gellan gum hydrogel for cartilage regeneration on the cell viability and extracellular matrix (ECM) secretion. The preparation of gellan gum hydrogel was designed by varying the contents with 1, 2 and 3 wt%. Hydrogels using 1, 2 and 3 wt% of gellan gum were fabricated with cross-linking by EDC (1-ethyl-3-(3-dimethyl aminopropyl)carboadiimide). Cell viability (MTT), glycosaminoglycan (GAG) assay, histological examination were evaluated to analyze the effect of the gellan gum hydrogels on the cell viability and ECM secretion. The gellan gum hydrogels cross-linked by EDC became more increase the rate of cell growth than non cross-linked gellan gum hydrogels. Also the phenotype of the cells maintained better in gellan gum hydrogels having 3 wt% gellan gum contents than the others. These results demonstrated that the gellan gum would significantly affect the cell metabolism. This research was supported by WCU (R31-20029) and MBC (0405-BOO10204-0006). Keywords: Gellan gum, Cartilage, disc, EDC(1-ethyl-3-(3-dimethyl aminopropyl) carbodi mide)
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**Poster Board Number: T-3169**

**THE INFLAMMATORY RESPONSES TO PLGA/DBP/SIS SCAFFOLDS IN VITRO AND IN VIVO**

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Poly (lactide-co-glycolic acid) (PLGA) has been widely applied to tissue engineering as a good biocompatible materials. However, there degradation products can decrease the pH in the surrounding tissue, which can result in inflammatory reaction. We hypothesized that the PLGA implant might have an inflammatory responses that could be reduced by impregnating demineralized bone particles (DBPs), porcine small intestinal submucosa (SIS) and silk into the PLGA. We manufactured three different materials of PLGA scaffolds, with each materials containing 20 wt% of DBPs, SIS and silk of PLGA. For biocompatibility test, NIH/3T3 mouse fibroblasts were cultured in the PLGA/DBP/SIS/Silk scaffold for 3 days. The PLGA/DBP/SIS/Silk scaffolds had no adverse effect on NIH/3T3 cell attachment and did not affect cell viability. The inflammatory potential of PLGA was evaluated using messenger ribonucleic acid expression of tumor necrosis factor alpha (TNF-α) and reactive oxygen species (ROS) on a mouse leukemic monocyte macrophage cell line (RAW264.7). This study showed that DBP/SIS/Silk scaffolds reduction of inflammatory reaction of PLGA. This research was supported by WCU (R31-20029) and MBC (0405-B001-0204-0006).

**Poster Board Number: T-3170**

**THE EFFECTS OF HESPERIDIN ON THE INFLAMMATORY RESPONSE; IN VITRO AND IN VIVO**

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Hesperidin, a flavonoid present in fruits and vegetables, possesses anti-inflammatory and chemopreventive effects. In this work, hesperidin-incorporated Poly (lactide-co-glycolic acid) (PLGA) films were manufactured to evaluate the effects of hesperidin on the inflammatory responses. In vitro inflammatory responses to murine macrophage RAW264.7 cells were cultured on the PLGA/hesperidin film for 1, 3 and 5 days. Hesperidin films minimally stimulated the cells to generate less production of tumor necrosis factor alpha (TNF-α) and reactive oxygen species (ROS) than PLGA films. The **in vivo** responses to PLGA/hesperidin films implanted in the axilla skin of rat. Fibrous wall thickness and macrophage were evaluated by hematoxylin and eosin (H&E) and ED-1 immunohistochemical staining, respectively. Based on the low level of inflammatory responses, hesperidin has great potential for biomedical applications. This research was supported by WCU (R31-20029) and MBC (0405-B001-0204-0006).

**Poster Board Number: T-3171**

**PROLIFERATION AND DIFFERENTIATION OF SCHWANN CELL IN SIS SPONGE OF VARIOUS CONTENTS**

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The porcine small intestinal submucosa (SIS) consists of some growth factors which can stimulate cell activity without immune rejection responses. Thus, SIS had been widely used as a biomaterial. In this study, we evaluated the effect of content SIS sponge on proliferation and differentiation of schwann cells (SC). SCs were harvested from the femurs and tibias of female Fischer rat. SIS sponge were prepared by freeze-drying method from 1, 2 and 3 wt% SIS solution. The SCs were seeded in SIS sponge with 2×10^5 cell/sponge concentration. The effect of SIS sponge on the proliferation and differentiation of SCs were assessed in culture using the MTT assay, SEM, swelling test. RT-PCR was conducted to confirm mRNA expression of S100, NSE, p75 and NF for nerve marker. In addition, immunochemical evaluation for cellular proliferation and differentiation by H&E and anti-NF staining. Theses studies revealed that SIS sponge has a positive effects on the SCs growth and the phenotype maintain through the study. This research was supported by WCU (R31-20029) and SCRC (SC4110).

**Poster Board Number: T-3172**

**SPINAL CORD REGRODUCTION USING BONE MARROW STROMAL CELL WITH PLGA/DBP NERVE CHANNEL CONTAINED ALGINATE**

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Poly (L-lactide-co-glycolide) (PLGA) is a biodegradable synthetic polymer with acceptable mechanical strength and well-controlled degradation rate. Also, it can be easily fabricated into many shape. Demineralized bone particle (DBP) that affects to cell proliferation and differentiation has been used as biomaterial. Bone marrow stromal cells (BMSCs) exhibit multiple traits of a stem cell population. We proposed to use PLGA nerve channel impregnated with DBP and Alginate by tissue-engineering principles for the repair of spinal cord injury. The spinal cord was completely transected horizontally at two levels (T7 and T8) and PLGA/DBP nerve channel seeded the cells were implanted in the lesion. For histological and immunochemical evaluation, the implants were removed after 2, 4 and 8 weeks and H&E and anti-NF staining. Motor functional outcome measurements using the BBB scoring, sensory test and motor functional recovery test were performed every week for 8 weeks post injury. This study suggested that BMSCs and Alginate contained to PLGA/DBP nerve channel may have an important role for spinal cord regeneration of tissue engineering area. This research was supported by WCU (R31-20029) and SCRC (SC4110).

**Poster Board Number: T-3173**

**TISSUE ENGINEERING TECHNOLOGY INDISPENSABLE FOR CELL-BASED REGENERATIVE MEDICINE**

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A new therapeutic trial based on the natural healing potential of body itself to induce tissues regeneration and repairing, has been recently expected. The natural healing is based on the potential of cells for their proliferation and differentiation. Now several stem cells with high potentials have been available through the recent development of stem cell biology. However, only when the cells are transplanted without considering any their local environment, we cannot always expect the cell-based tissue regeneration. This is because in the body, generally cells survive and biologically function by interacting with their local environment. The environ-
Liver fibrosis is one of the intractable diseases. Now a clinically possible therapy for liver fibrosis is liver transplantation. However, the lack of donor liver is the large problem. Another therapeutic choice is cell transplantation. Mesenchymal stem cells (MSC) can be readily isolated from patients and possess the potential of good proliferation. It is reported that MSC have an inherent ability to naturally recruit to an injury tissue. In addition, the dual release of two signaling molecules can be achieved by the hydrogel. When both stromal derived factor (SDF)-1 and bone morphogenic protein (BMP)-2 were released in the subcutaneous tissue, ectopic bone formation was observed to a significantly great extent compared with that of either release. The SDF-1 release enhanced the recruitment of cells from the bone marrow, while the osteogenic differentiation of cells recruited was promoted by the BMP-2 release. The DDS technology can manipulate the body distribution and fate of cells, which enhances the natural healing potential for cell-induced tissue regeneration. The biomaterials technology of regenerative medicine is also applicable to the basic researches of stem cells biology. The further development of stem cell biology will accumulate the scientific knowledge of regenerative medicine, resulting in enhancing the therapeutic efficacy of cell-based tissue regeneration. In this paper, several applications of DDS and cell scaffold technologies to the tissue regeneration therapy as well as the basic research of stem cell biology are introduced to emphasize significance of tissue engineering technologies in regenerative medicine.

**Poster Board Number: T-3174**

**LIVER ANTI-FIBROSIS THERAPY WITH MESENCHYMAL STEM CELLS GENETICALLY ENGINEERED BY SPERMINE-INTRODUCED PULLULAN**

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Liver fibrosis is one of the intractable diseases. Now a clinically possible therapy for liver fibrosis is liver transplantation. However, the lack of donor liver is the large problem. Another therapeutic choice is cell transplantation. Mesenchymal stem cells (MSC) can be readily isolated from patients and possess the potential of good proliferation. It is reported that MSC have an inherent ability to naturally recruit to an injury tissue. In addition, MSC have some characteristics suitable for cell therapy. They can differentiate into different lineages of cells, such as osteocytes, chondrocytes, adipocytes, myocytes, epithelial cells, and neurons, while their differentiation into cardiocytes and hepatocytes, is experimentally demonstrated. Another characteristic is to secret bioactive factors, such as tumor necrosis factor, interleukine-6, and hepatocyte growth factor (HGF), which positively affect the biological functions of the surrounding cells. Among them, HGF shows anti-apoptotic, migration, proliferation, and anti-fibrotic functions. However, considering their transplantation for cell therapy, the low viability of cells and the consequent poor functions are of therapeutic problem. As one trial to break through the problems, the cells genetically engineered to enhance their functions were promosing. From the clinical viewpoint, the genetically engineering of cells through their transfection with non-viral carriers is preferable. This study is undertaken to design a novel gene carrier of cationic polysaccharide for the receptor-mediated gene transfection. MSC are reported to express an asialo-protein polysaccharide receptor. We prepared a spermme-introduced pullulan of non-viral vector and the therapeutic effect of MSC genetically engineered by spermme-introduced pullulan was investigated. Spermine was chemically introduced to the hydroxyl groups of pullulan by N,N'-carbonyldimidazole activation method. MSC were genetically engineered by transfection with the complex of the spermme-introduced pullulan and a plasmid DNA of HGF. HGF secretion from MSC transfected was evaluated. The biological activity of HGF secreted was assayed in terms of hepaocytes proliferation. The anti-fibrotic effect of genetically engineered MSC was evaluated for a rat model of liver fibrosis. When MSC were transfected by the complex of HGF plasmid DNA and the spermme-introduced pullulan, the MSC secreted HGF with biological activity for hepatocytes over 1 week. After intravenous injection into rats with liver fibrosis, the HGF-secreting MSC accumulated in the liver and decreased the fibros area to a significantly great extent compared with the original, non-transfected MSC. It is concluded that the transplantation of genetically-engineered MSC is a promising therapy for liver fibrosis.

**Poster Board Number: T-3175**

**ELESTROSPRAYING TECHNIQUE EFFECTS ON HUMAN MESENCHYMAL STEM CELLS**

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Electrospinning (ES) is a method widely used in bioengineering to produce nanofibrous scaffolds that mimic the extracellular matrix. These scaffolds act as supports for growing stem cells (SCs) and this combination offers a promising tool for the recovery of organs and tissues. Normally, the scaffolds are produced by ES and subsequently the cells are seeded onto their surface for a tridimensional culture. Alternatively, by combining ES and electrospaying (EP) methods, SCs can be incorporated into the tridimensional scaffolds while the nanofibers are being produced. EP is a technique that can be used for processing SCs in suspension, which is subjected to an electric field. The suspension fluid is then passed through a thin needle and is fragmented, generating droplets containing cells. This ensures that the cells are evenly distributed throughout the scaffold structure to form a 3D system. In this study, the effects of EP method on mesenchymal stem cells (MSCs) from deciduous teeth were evaluated in terms of viability, proliferation, plasticity and genotoxicity. The MSCs, in the sixth passage, were suspended in culture media at 3x106 cells/mL and electrospayed into a petri dish containing HDLMME media. The parameters: 15 kV, 4 cm and
A strategy to optimize tissue regeneration is the use of nanofiber matrices containing growth factors (GFs). This is to ensure correct distribution of stem cells, enhancing their proliferative and differentiation capacity at the injury site, thus preventing the cells from migrating to other locations. The bioavailability of these factors can be obtained by incorporation GFs into nanofibers by electrospinning. The aim of this work was to produce aligned nanofiber matrices with incorporated epidermal growth factor (EGF) and to evaluate the influence of these scaffolds when mesenchymal stem cells (MSCs) are cultivated and differentiated into neural precursors into the scaffolds. The polymer solution consisting of poly(lactic-co-glycolic acid) (PLGA) was produced at a concentration of 15% (w/w) using 1,1,1,3,3,3-Hexafluoro-2-propanol with 0.2% of Span 80, forming the oil phase. An aqueous solution of PBS containing 0.1% of albumin and 1mg/mL of EGF was mixed with the oil phase to form the emulsion. Aligned scaffolds with emulsion, without EGF (PLGA, group 1) and with EGF (PLGA/EGF, group 2) were produced using a cylinder rotating at 2,500 rpm. The control group was the cells cultured on wells. The matrices were evaluated for morphology and fiber diameter by scanning electron microscopy (SEM). The MSCs were extracted from human deciduous teeth pulp, called SHEDs (Stem cells from Human Exfoliated Deciduous teeth) and used in the experiments in the 5th passage. The dental pulp was removed and the cells were treated with collagenase at 37°C for 60 minutes, centrifuged, washed and prepared for cell culture. For neuronal differentiation, 5x10⁵/cm² and 10x10⁵/cm² cells were seeded in culture plates of 24 wells (control group) and on scaffolds, respectively, and then treated with DMEM supplemented with 30µM of retinoic acid. After 14 days, the differentiation was evaluated by phase contrast microscopy and immunofluorescence using primary antibodies, nestin and GFAP. PLGA and PLGA/EGF scaffolds showed aligned fibers, homogeneous morphology and absence of beads, with an average diameter of 548±29nm and 329±87nm, respectively. By confocal microscopy, it is possible to observe that SHEDs adhere on scaffolds following the same orientation of aligned fibers in group 1 and 2. Concerning neuronal differentiation, samples cultivated on the control group showed cells with neural phenotype, labeling positively for nestin and GFAP markers. The differentiation on group 1 and 2 is still in progress. The small and more variable diameter of the PLGA/EGF (329±87nm) is interesting because it mimics the collagen fibers of the extracellular matrix, where the diameter varies from 50 to 300nm. Through observation of the SEM photographs, it is possible to observe that the scaffolds have a high alignment and porosity. It allows for a three-dimensional arrangement of the cells in the scaffold and permits cellular growth and differentiation. SHEDs are capable of differentiating into neural precursors on the plates and adhere on aligned PLGA/EGF. Despite the neuronal differentiation in scaffolds not being complete, it is speculated that SHEDs will also be able to differentiate under the matrices and increase the expression of nestin and GFAP, due to the stimulus provided by the scaffolds, especially in group 2. Therefore, SHEDs cultivated on aligned PLGA/EGF can be a possible option for tissue engineering to treat neurological diseases.
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NGF LOADED POLYMERIC NANOFIBER STANDARIZATION IN ASSOCIATION WITH HUMAN MESENCHYMAL STEM CELLS FOR NERVE TISSUE ENGINEERING

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The use of polymeric matrices or scaffolds composed of nanofibers produced by electrospinning (ES) can serve as templates in which the cells can adhere, proliferate and differentiate, contributing to the development of a new tissue. To optimize the process of tissue regeneration, the cell growth in the scaffolds can be stimulated through the controlled release of bioactive molecules, such as neurotrophic factors. The nerve growth factor (NGF), for example, can be highlighted when associated to nerve tissue engineering models. The main objective of this work is to develop poly (lactic-co-glycolic acid) (PLGA) nanofiber matrices loaded with NGF and evaluate the behavior of mesenchymal stem cells (MSCs) on these three-dimensional devices. The MSCs were extracted from human deciduous teeth pulp (nDTP), called SHEDs (Stem cells from Human Exfoliated Deciduous teeth). To permit NGF incorporation, the scaffolds were produced by emulsion, as follows: organic phase consisting of PLGA 15% (w/w), sorbitan monooleate (Span-80) 0.2%, 1,1,1,3,3,3-hexafluoro-2-propanol and 0.1 % aqueous phase containing PBS, NGF and Bovine Serum Albumin. The following scaffolds were developed, characterized and evaluated: (1) NGF loaded aligned scaffolds, (2) NGF loaded random scaffolds, (3) aligned scaffolds without NGF and (4) random scaffolds without NGF. Cultured cells on plate wells were used as the control group. The physicochemical analysis fiber morphology and diameter by scanning electron microscopy (SEM), NGF incorporation assessment by differential scanning calorimetry (DSC) and NGF loading efficiency by ELISA (enzyme-linked immunosorbent assay) were carried out. Cell adhesion on the scaffolds was assessed by DAPI fluorescence (3 and 6 hours of incubation) and cell morphology through confocal microscopy. In all groups, nanofibers with homogeneous morphology without beads were observed. The average diameter was 559±198 nm in group 1, 673±32 nm in group 2, 548±29 nm in group 3 and 754±300 nm in group 4. DSC analysis indicated a greater trend toward increased viability rate compared to the control group. This indicates a satisfactory interaction between the cells and the scaffolds. Cells settled on the scaffolds were observed by confocal microscopy, showing extension of actin filaments. The low efficiency of factor incorporation corroborates with previous results from the literature. NGF release profile from scaffolds, citotoxicity and viability studies will be performed, along with neural differentiation analysis by gene expression of the following markers: nestin, β-III tubulin, Neuron Specific Enolase (NSE), Glial Fibrillary Acidic Protein (GFAP) and Olig 2. These preliminary results suggest appropriate cell adhesion on scaffolds, indicating a good environment for cell growth, which will possibly be enriched by NGF released from the scaffolds. As is well known, growth factors are potent molecules, which even at low concentrations can improve cell behavior and stimulate differentiation. Therefore, using nanotechnology for the incorporation of NGF in the scaffolds, the association of these scaffolds and MSCs is a promising strategy for nerve tissue engineering.
EVALUATION OF BONE REGENERATION IN RAT CALVARIA USING POLYMERIC NANOFIBER MATRICES SEEDED WITH STEM CELLS FROM HUMAN DENTAL PULP OF DECIDUOUS TEETH

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Bone loss is a challenge for reconstructive surgery, where the use of different sources of bone grafts with physical, chemical and biological limitations is common. Currently, bioengineering combines knowledge from different areas seeking new forms of tissue production, including for use in reconstructive surgery. The aim of this study was to associate nanotechnology with stem cell therapy, to study bone formation in bone defects created in rat calvaria. Scaffolds of acid poly (lactic-co-glycolic acid) (PLGA) were produced by electrospinning technique and characterized for their morphology. Five samples of human stem cells from deciduous teeth (SCDT), in electrospinning technique and characterized for their morphology. Five samples of human stem cells from deciduous teeth (SCDT), in a process of rhizolysis were grown until the 5th passage. They were seeded onto culture plates (control) and scaffolds (test) for adhesion and cell viability assays. To evaluate their ability to promote bone formation, 15 Wistar rats were used, in which critical defects were produced with a 8.0 mm diameter in the calvaria. The animals were divided into three groups (n=5): I- only scaffolds; II- scaffolds/SCDT; III- scaffolds/SCDT, maintained for 13 days in osteogenic medium. Sixty days postoperatively, the calvarias were removed for histometric analysis of the amount of new bone. This study was approved by the Ethics Committee of Federal University of Rio Grande do Sul (n° 19273). Through the images obtained by scanning electron microscopy, it was observed that the fibres formed (mean diameter of 315.9 ± 70.2 nm) were smooth and randomly distributed throughout the scaffold structure, which presented a large number of interconnected pores and a thickness of 37 ± 5.3 μm. Using cell adhesion assay, the same observations were found in the control and test groups, with 44.8 ± 23.1 and 51.6 ± 20 cells per field respectively and with no statistical difference between the groups. Cell viability increased progressively in the beginning of the experiment up to 14 days, followed by a decrease on day 21. Cell viability remained similar between the test and control groups in the different experimental periods with no statistical difference. Histological examination showed that group III, using osteogenic medium, showed 17 ± 4.31% of new bone formation at the site of the original defect, with statistical difference compared to the other groups (9.39 ± 2.55% - group I and 10.7 ± 3.22% - group II). In the light of these results, it is concluded that the PLGA scaffolds produce favorable results in relation to the interaction with the SCDT. The use of PLGA scaffolds and SCDT in association with osteogenic medium, prior to implantation has been shown to have the ability to promote bone formation and, therefore, it is appropriate for use in bioengineering.

THREE DIMENSIONAL PROLIFERATION AND MIGRATION OF HUMAN MESENCHYMAL STEM CELLS

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Thus far, human mesenchymal stem cells (hMSCs) have been shown to sense, respond and adapt to various sources of physical, chemical and biological stimuli. Though how these factors converge in three dimensions (3D) to govern cell proliferation and reinforce tissues’ homeostasis remains unknown. In this study, we investigated the multidimensionality of hMSCs proliferation in a clinically-relevant size scaffold (70 µl). To achieve strict control of hMSCs cultures of three human donors, spatial (i.e. surface area and hMSCs position) and culture (i.e. cell number/cm²) parameters were chemically and physically manipulated by surface modification and scaffold fabrication techniques in two dimensions (2D) and 3D. We began by exploring hMSCs proliferation in time, which led to the finding that hMSCs displayed significantly lower specific growth rates in 3D than in 2D. Ostensibly, hMSCs’ specific growth rate in 3D is strongly influenced by molecular gradients (e.g. glucose) present in scaffolds which are time- and space-dependent. p21 and p27 cell cycle regulators and cell death assays indicated that despite steep gradients in scaffolds, hMSCs did not die or stop dividing. Furthermore, we assessed nutrient availability through glucose kinetics and unraveled higher glucose consumption rates in 3D than in 2D, corroborating that hMSCs’ phenotype in time is dimension-dependent. Additional glucose/cell in 3D suggested a shift in the carbon balance towards the production of extracellular matrix components. Therefore, total collagen and glycosaminoglycans (GAGs) were measured to verify the direction of carbon in glucose molecules. However, neither collagen/cell nor GAGs/cell were higher in 3D. Upon microscopic inspection, we observed that hMSCs consistently migrated into millimeter-size circular patterns in the scaffold’s pores which are structurally sustained by GAGs. Thus, another possibility was that extra glucose/cell in 3D is necessary for organized hMSCs migration, which constitutes an energy demanding process. Interestingly, the circular shape of hMSCs organization in the scaffold’s pores correlated with the shape of concentration gradients of glucose, suggesting a chemotaxis-guided hMSCs migration towards regions of high concentration of molecules. These data implied that nutrient availability has a morphogenetic effect, which prompts hMSCs proliferation and migration. To understand how the molecular gradients in the 3D environment influence hMSCs, we are performing a genome-wide microarray analysis of cultures to connect molecular and physical mechanisms of hMSCs proliferation and migration. These are relevant to understand the homeostasis of tissues and to implement stem-cell-driven tissue and organ regeneration.
Objective: Pancreatic islets are highly vascularized, which is important in their ability to quickly secrete insulin in response to changes in blood glucose. Islet transplantation is an alternative to whole pancreas transplantation in curative therapy of type I diabetics. The outcome of engraftment of islet, however, remains disappointing. Rapid and adequate islet revascularization is crucial for the survival and function of transplanted islets. Here, we describe the induction of functional vascular networks into an islet-like tissue through the vascular-mesenchymal interactions. Methods: Pancreatic beta-cell lines (MIN6) were three-dimensionally (3D) cultivated with endothelial cells and mesenchymal cells. Timelapse confocal microscopy analyses were performed to visualize the dynamic cellular organizing process with the use of fluorescence labeled cells. In vitro pre-vascularized constructs were transplanted under the transparency window of immunodeficient mice. The process of functional vascular network formation was intravital monitored for over 1 month. Generated constructs were harvested and histologically examined. Results: We successfully integrate the premature vascular network into be-ta cell clusters following the 3D co-cultivation in vitro. After transplantation in immunodeficient mice, generated clusters were permeated with host blood vessels in vivo. The patency of human vascular structures was proved by rhodamine dextran infusion. These functional vascular structures were remained for over 2 weeks. Vascular quantification showed the dense vascular networks compared with control samples. Histological analyses revealed the reconstitution of basement membrane proteins, which contributes maintenance of beta-cell functions. Discussion: One ultimate goal of regenerative medicine is to reconstitute 3D vascular structures with fully functioning mature cells. Vascularization is essential to maintain cell viability during tissue growth, induce structural organization and promote vascularization after implantation. We successfully introduced the vascular networks into the islet-like tissue. Our preliminary results should facilitate future efforts towards the establishment of highly efficient islet transplantation techniques including long-term survival and function of islet grafts.

Poster Board Number: T-3182

ALIGNED VASCULAR GRAFTS USING INDUCED HUMAN PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS MODULATE CELL MORPHOLOGY AND VIABILITY

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Cell-based approaches to restore or regenerate a healthy vascular endothelium by enhancing angiogenesis are promising for the treatment of ischemic vascular disease. We have previously shown that human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) can enhance blood flow and neovessel formation in animal models of peripheral arterial disease. However, in order to replace large conduit vessels, bioequivalent vascular grafts that sustain viability and mimic the longitudinal alignment of native endothelial cells (ECS) are desired. Therefore, the purpose of this study was to examine the effect of aligned nanofibrillar biomaterials on iPSC-EC behavior and survival. We developed a novel method to produce highly organized nanofibrillar collagen scaffolds and characterized their effect on the morphology and survival of iPSC-ECs. Shearing of purified monomeric collagen I solution in the liquid crystal state enables the collagen molecules to self-assemble into parallel-oriented nanofibrils with controllable fibril diameters. Uniform areas of aligned collagen fibrils were fabricated, as confirmed by atomic force microscopy and scanning electron microscopy. Human adult dermal fibroblast-derived iPSCs were differentiated into endothelial lineage in the presence of differentiation media containing BMP4 and VEGF, and then purified based on the expression of a mature phenotypic EC marker, VE-cadherin. The iPSC-ECs were expanded and characterized for EC phenotype by the expression of known EC phenotypic markers, uptake of acetylated-LDL, and the formation of tube-like structures in matrigel. When cultured on 30-nm diameter nanofibrils, the iPSC-ECs became elongated and reorganized their F-actin cytoskeletal assembly along the direction of the fibrils, mimicking the longitudinal orientation of healthy ECs in straight segments of vessels.

Poster Board Number: T-3183

INCREASE OF VASCULARIZATION USING ALLODERM ASSOCIATED WITH DIFFERENT SOURCES OF MESENCHYMAL STEM CELLS FOR PALATAL WOUND IMPLANTATION

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BACKGROUND: Tissue shortage complicates the surgery of cleft lip and palate anomalies. The healing of defects on the palate impairs growth of the dento-alveolar complex due to scar tissue formation. Implantation of substitutes into the wound area might overcome this adverse effect. It is known that palatal wounds treated with a dermal substrate (allograft) heal with fewer indications of scar tissue formation and evoke only a mild inflammatory reaction. The aim of this study was to compare the tissue response in concern to revascularization properties using AlloDerm associated with mesenchymal stem cells obtained from different sources: dental pulp, levator palatal muscle and adipose tissue to assist in faster tissue integration. This can be helpful to find a good source of stem cell to be associated with AlloDerm for future clinical application in patients with cleft palate to increase the vascularization process. Methods: Adipose-derived stem cells (ASC) were obtained from liposuction patients without cleft lip and palate. Dental pulp stem cells (DPSC) and levator palatal muscle-derived stem cell (EPMDSC) were obtained from cleft lip and palate patients. The cells were isolated using previously described methodology and they were characterized as mesenchymal stem cells (positive for CD90, CD166, CD29, and negative for CD45 and CD31). Cells were seeded (10^5 cells) onto 1cmX0.5cm AlloDerm membranes 24 hours before the surgery. Four membranes: one associated with DPSC, one associated with EPMDSC, one associated with ASC, and one control (AlloDerm alone) were implanted subcutaneously on the dorsal side of athymic mice. The mice were euthanized nine days after the transplantation and H&E, immunohistochemistry for hematopoietic precursors (CD34), and immunofluorescence for human nuclei were performed to assess revascularization and the presence of the human cells in the new tissue. Results: Revascularization using AlloDerm was confirmed. Additionally, the association of mesen-
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chymal cells to this scaffold can increase the revascularization. Two sources of stem cell, ASC and EPMDS cell, are the most potent cells to be associated with AlloDerm and increase the vascularization. Conclusions: Our findings show that mesenchymal stem cells seeded on allograft increase the vascularization, and that the best sources of stem cells are adipose tissue and levator palate muscle derived stem cell. Since levator palate muscle derived stem cells can be obtained from palatal surgery this can be the best mesenchymal stem cell to be associated with allograft and increase the vascularization potential to be used in cleft lip and palate patients because levator palate muscle is a non invasive source of stem cell.

Poster Board Number: T-3184

ESTABLISHMENT OF IMMORTALIZED HUMAN ERYTHROID CELL LINES ABLE TO PRODUCE ENUCLEATED RED BLOOD CELLS

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Red blood cell (RBC) transfection is one of the most standard transplantations. However, the shortage of RBCs for transfusion has been a serious problem. In vitro production of RBCs offers a potential means to solve this point and provides the additional advantage of freedom from infection or contamination by microorganisms. Hence, such in vitro production may become a standard procedure for generation of RBCs for clinical use in the future. We previously reported on the establishment of immortalized mouse erythroid progenitor cell lines able to produce enucleated RBCs. Here, we introduce a promising protocol for establishing immortalized human erythroid progenitor cell lines from iP cells or umbilical cord blood stem/progenitor cells. These immortalized cell lines express erythroid-specific markers and many of the markers are upregulated after induction of differentiation in vitro. Most importantly, all these immortalized cell lines can produce enucleated RBCs after induction of differentiation in vitro. To our knowledge, this is the first report to demonstrate the feasibility of using immortalized human erythroid cell lines as an ex vivo source for production of enucleated RBCs.

Poster Board Number: T-3185

DEVELOPMENT OF HIGH EFFICIENT GENE DELIVERY SYSTEM USING EPISOMAL VECTOR AND MAGNETIC NANOPARTICLES FOR PRODUCTION OF iPSCS

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The development of eukaryotic expression vectors has provided a direct and convenient way of introducing novel genetic information into cultured cells. Heterologous expression of recombinant proteins has subsequently found widespread use for overproduction of therapeutically important proteins and for studies of gene regulation. Episomal vector is the nonviral autonomous replicon that was constructed for mammalian cells, expected to be applied to the magnetic nanoparticles can enhance the transfection efficiency. This result suggests the magnetic force applied to the magnetic nanoparticles can enhance the transfection efficiency.

Poster Board Number: T-3186

FUNCTIONAL BIOARTIFICIAL CARDIAC TISSUE GENERATED FROM MURINE AND HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES - IMPROVED TISSUE FORMATION BY ASCORBIC ACID AND APPLICATION OF GROWING STRETCH

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Introduction: Acute infarction of the myocardium results in the formation of a non-contractile fibrous scar, eventually leading to end-stage heart failure. The aim of our study was to develop Bioartificial Cardiac Tissue (BCT) from pluripotent stem cells for future reconstructive therapies. For a functional contractile tissue, highly purified stem cell-derived cardiomyocytes are needed. We report the efficient generation and antibiotic-based selection of cardiomyocytes from transgenic lines of murine induced pluripotent stem cells (miPSC), human embryonic stem cells (hESC) and human induced pluripotent cells (hiPSC). All cell types were successfully implemented for cardiac tissue engineering based on contractile BCTs. We investigated the role of fibroblasts, ascorbic acid and mechanical stimuli on tissue formation, maturation and functionality, which was demonstrated by direct force measurement, gene expression analysis and electrophysiology. Methods and results: In a proof-of-concept-study murine iPSC-derived pure (>99% troponin T+) 3D cardiomyocyte aggregates (Cardiac bodies) were generated from transgenic cells stably expressing a Zeocin resistance gene under control of the cardiac MYH6 promoter. Addressing the limitation of cardiomyocyte loss after single cell dissociation, intact cardiac bodies were applied for BCT generation. Notably, direct fusion of non-dissociated cardiac bodies resulted in a structurally and functionally homogenous syncytium. Continuous in situ characterization of BCTs for 21 days identified three critical factors for fusion of cardiac cells and formation of a functional BCT: i) addition of fibroblasts and ii) ascorbic acid supplementation supported ECM remodeling and CB fusion, while iii) increasing static stretch supported sarcomere alignment and cardiomyocyte coupling. All factors together considerably enhanced contractile forces of murine BCTs. We have generated human BCTs based on antibiotic-based selected human ESC- and human iPSC-derived cardiomyocytes together with morphologically inactivated human foreskin fibroblasts in a 3-dimensional collagen matrix. Again, tissue formation and force development was strongly promoted by the fibroblast-mediated remodeling of the matrix. The resulting tissue contained aligned cardiomyocytes with cross-striated morphology, and presence of Ki67+ cardio-
myocytes demonstrated proliferative capacity up to 21 days after tissue formation. Human BCTs showed Frank-Starling curves of an increase in their active forces in response to stretch. The maximal active forces developed were up to 8.5 mN/mm² for hESC-BCTs and 3.2 mN/mm² for hiPSC-BCTs on d21. Field potential recordings showed propagation of the electrical stimulus in a functional syncytium and a chronotropic response to the β-adrenergic agent isoproterenol. Conclusion: Our data demonstrate the successful generation of functional murine and human engineered cardiac tissue starting from purified populations of stem cell-derived cardiomyocytes. These constructs show cell alignment and maturation and are able to contract synchronously, generating 70-times higher forces than previously reported for engineered human cardiac tissue. This makes them suitable for cardiac disease modeling, and broadens the path towards the development of cardiac patches to be used for future regenerative and replacement therapies for the heart. This work was funded by the Cluster of Excellence REBIRTH (DFG EXC62/1).

Poster Board Number: T-3187

SMART THERMO-RESPONSIVE POLYMERS AS A TOOL TO ASSESS UNDIFFERENTIATED AND MATURE CELL RESPONSE TO SUBSTRATE STIFFNESS AND NANOPATTERNING.

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The sensitivity of mature and undifferentiated cells to substrate mechanophysical properties has been investigated by a number of independent research groups so far. The results demonstrated a clear dependency of cell behaviour and phenotype on substrate composition, but also on matrix mechanical features and nanostructure. The ability of living cells to activate specific signaling pathways in response to mechanical stimuli has been recently shown to be highly cell-specific, this evidence being likely to reflect differences in their physiology in vivo. Indeed, human mesenchymal stem cells (hMSC) can be committed to differentiate to a specific lineage when the stiffness of a given tissue is matched by the substrate, while tissue- resident progenitors were shown to be more sensitive to pore geometry and surface patterning in the micro-scale. Contractile cells are likely to be more responsive to substrate characteristics: in embryonic cardiomyocytes, substrate stiffness was shown to affect contractility, with tissue-like Young modulus appearing more suitable for beating activity; consistently, neonatal cardiac cell maturation was demonstrated to be impaired on stiff substrates, while the formation of functional contractile units in skeletal myotubes was found to be optimal on substrates having a tissue-like stiffness. Given the ability of cells to sense the substrate they grow on and their acknowledged capacity to convert such “feelings” in a biological behavior, the possibility that scaffolds developed for a specific in vivo application could interfere with host cell behavior after implantation cannot be neglected. Synthetic materials can be tailored to mimic the tissue three-dimensional organization in the micro- and nanoscale, while their mechanical properties are usually far from those of the host tissue. In the present investigation, inert poly-e-caprolactone (PCL) planar layers were manufactured to obtain thermo-responsive films displaying tuneable surface features (with Young modulus ranging from kPa to MPa values and controlled nanopattern) without changing matrix chemistry. The substrates were challenged with human mesenchymal stem cells, murine skeletal myoblasts, human normal dermal fibroblasts and neonatal murine cardiomyocytes to study the possible effects of substrate stiffness and nanostructure on such cell adhesion, survival and phenotype without changing biological cues. Dynamic experiments demonstrated a cell-specific response to the changes in surface stiffness and nano-pattern, with hMSC displaying the highest responsivity to matrix features.

Poster Board Number: T-3188

PHYSICAL FACTORS IN DIRECTING MURINE EMBRYONIC STEM CELL DIFFERENTIATION INTO OSTEOGENIC AND CHONDROGENIC LINEAGES

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In recent years, significant advances have been made to elucidate the signal transduction pathways involved in murine embryonic stem cell (mESCs) differentiation. It has also become clear that ESCs behave differently if placed in 3-dimensional scaffolds as opposed to static culture conditions. Aside from biochemical factors, it is now apparent that extra-cellular matrices (ECM) have the potential to direct stem cell differentiation, and this effect is not only based on protein composition, but in response to factors such as ECM stiffness, intrinsic forces, and extrinsic mechanical stimuli exerted on the ESC/ECM construct. The integrin family of proteins are ideally situated to play a major role in these ESC/ECM interactions, since they are transmembrane proteins that directly bind to the ECM and initiate signalling pathways that can effect gene regulation. To date, though the regulation of mESCs to mechanical stimuli has been the focus of several studies, the function of mechanosensitive proteins remains largely undetermined. Therefore, the effect of compressive loading on mESC differentiation was investigated using chondrogenesis and osteogenesis as model systems. Furthermore, the role of Arg-Gly-Asp (RGD) dependent integrin family members in this process was investigated. The ESC/ECM constructs were prepared by resuspending 106 mESC within 3D collagen I gels. To identify the role of RGD-dependent integrins, RGD peptide (cyclo Arg-Gly-Asp-d-Phe-Cys) was added to mESCs cultured under static conditions. To examine the effect of cyclic compressive loading, a FX-4000™ Compression Plus System was modified and the ESC/ECM constructs were subjected to a loading regime of 1 Hz and 5% strain for 40 hours with or without the RGD blocking peptide. The morphology, proliferation rate, viability and gene expression were examined. RGD had a significant effect on cellular morphology in static culture. Cells showed a tendency to detach and to form cell aggregates, most apparent at a concentration of 0.5 mM RGD. Treatment with RGD did not significantly influence cell viability and proliferation rate in comparison to non-treated cells. At an early stage of differentiation, cyclic compression down-regulated the pluripotent genes Sox2, Rex1, and Nanog, but not Oct4. In addition, when the integrin-blocking RGD peptide was introduced at the same time points, a similar gene expression response was observed that was independent of the loading regime. Interestingly it was found that compressive loading induced chondrogenesis but not osteogenesis by day 15 of differentiation. Furthermore, the addition of RGD suppressed OCN and Col2 gene expression but up-regulated Sox9. When integrin gene expression was examined, it was observed that mechanical stimuli upregulated the collagen-dependent (α1, β1) and RGD-dependent (αv, β3) integrin subunits on day 1 of differentiation. By day 15, however, the αv subunit was up-regulated and the α1, β1, β3 subunits were down-regulated, with the addition of RGD inhibiting this effect at all stages of differentiation. Overall, these ESC/ECM constructs and RGD supplementation provide a
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model system to study the response of mESCs to physical factors and have contributed to a better understanding of integrin signaling in mESC mechanotransduction pathways.

**Poster Board Number: T-3189**

**IS FIBRIN GLUE ESSENTIAL FOR ATTACHMENT OF HYPERDRY AMNIOTIC MEMBRANE PATHES OVER THE BONY SURFACE OF MASTOID CAVITIES IN CANAL WALL DOWN TYPANOPLASTY?**

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Fibrin glue is usually used as a reliable bioadhesive of the grafting materials in ear surgery. Because of adhering well to wound, the human amniotic membrane (AM) has been widely used clinically for membranous allograft in the field of dermatology without fibrin glue. In this study, the feasibility of the AM fixation without fibrin glue on the bony surface of mastoid cavity was evaluated in canal wall down (CWD) tympanoplasty. In 11 ears of 11 patients, the AM was attached onto the bony surface of the mastoid cavity using fibrin glue (FG+) group. In 7 ears of 7 patients, the AM was simply attached onto the bony surface of the mastoid cavity without fibrin glue (FG-) group. The time for graft epithelization were compared in both groups. In both groups, complete epithelization of the mastoid cavity took place in all patients. The mean time of epithelization in the FG(+) group was 32.4 days, while in the FG(-) group it was 31.9 days. Complete epithelization of the AM graft was not significantly different between two groups. It is suggested that fibrin glue is an inessential adhesive for attachment of the AM in the CWD tympanoplasty.

**Poster Board Number: T-3190**

**IMPLANTATION OF BONE MARROW-DERIVED CELLS INTO IRRADIATED RAT URINARY BLADDERS RECONSTRUCT FUNCTIONAL SMOOTH MUSCLE LAYERS AND NERVE FIBERS**

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Aims: We investigated to determine if bone marrow-derived cells implanted into radiation-injured rat urinary bladders could reconstruct tissue structures and recover bladder functions. Methods: Recipient female Sprague-Dawley (SD) rats were anesthetized and covered with an iron shield except for a 1-cm diameter circle bordering at the pubic bone to allow radiation of the pelvic region containing the urinary bladder. The region was radiated with 2 Gy once a week for 5 weeks, and then kept for 2 weeks. Bone marrow cells were harvested from femurs of donor male GFP-transfected SD rats and cultured for 7 days. Two weeks after the last radiation exposure, the adherent, proliferating bone marrow-derived cells were implanted into the irradiated urinary bladders (n=10). For controls, cell-free solutions were similarly injected (n=10). Four weeks after cell implantations or control injections, cystometric, histological, and immunohistochemical investigations were performed. Results: Just prior to implantation, irradiated urinary bladders showed that smooth muscle layers and nerve fibers were disorganized. The proportions of smooth muscle layer (8±1%) and nerve fiber (3±1%) areas were significantly decreased compared to un-irradiated urinary bladders (20±1%, 12±3%, respectively). In the cystometric investigations, the voiding interval of irradiated rats was irregularly prolonged, 7.92±1.09 min, and the residual volume, 0.13±0.03 ml, was significantly higher compared to the sham-irradiated rats (5.50±0.43 min and 0.05±0.01 ml). After 4 weeks, smooth muscle layers and nerve fibers in the cell-free control urinary bladders remained similar to the pre-implanted irradiated urinary bladders; however, the cell-implanted urinary bladders contained reconstructed smooth muscle layers and nerve fibers in amounts similar to the sham-irradiated urinary bladders. The proportions of the smooth muscle layer (19±1%) and nerve fiber (9±1%) areas in the cell-implanted urinary bladders were significantly higher than that in the cell-free injected controls (11±0.4%, 1±0.3%, respectively). Some GFP-positive implanted cells differentiated into smooth muscle and nerve cells and formed the reconstructed tissues. The voiding interval of cell-implanted rats, 5.46±0.33 min, was regular and similar to the un-irradiated rats, and less than the cell-free injected controls, 7.39±0.54. The residual volume, 0.04±0.01 ml, was decreased compared to the cell-free injected controls, 0.15±0.05 ml, and was similar to the un-irradiated rats. Conclusions: The bone marrow-derived cells implanted into radiation-injured urinary bladders reconstructed tissue structures. Some of the implanted cells differentiated into smooth muscle or nerve cells and formed the reconstructed tissues. The cell-implantation re-acquired regular voiding intervals and low residual volume.

**Poster Board Number: T-3191**

**HEPATIC CELLS: AN ALTERNATIVE SOURCE TO REPOPULATE THE BIOARTIFICIAL LIVER**

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Introduction: Severe hepatic failure is the result of long-term liver injury. Liver transplantation is the only efficient treatment, but is currently limited by organ shortage. The demand for new livers continues overcoming the availability. In this context, the creation of a bioartificial liver might solve this clinical problem. Aims: This work aims to produce a 3D whole-liver scaffold with intact components of extracellular matrix (ECM) and vascular system and also evaluating the repopulation of this ECM using hepatic cells. Methods: Wistar rats were used for liver excision surgery. Twenty minutes before this procedure, heparin was administered. Livers were perfused through the portal vein using an infusion pump at 4 mL/min with cold saline for 1 h. Then, livers were washed with PBS for 30 minutes. Subsequently, livers were perfused with Triton X-100 1% for 30 min, and then with SDS 1% for 36h. After complete decellularization, livers were washed with distilled H2O for 15 min, Triton X-100 for 30 min to remove residual SDS and then were preserved at 4°C for 7 days. To analyze the ECM integrity post decellularization, DAPI, H&E and sirius red stainings and electronic scanning microscopy and immunofluorescence to detect collagen type I, III, albumin, laminin, osteopontin and fibronectin, were performed. Toluidine blue was used to examine the vasculature. For recellularization, cells were obtained from normal liver. These cells were cultured over slices of the decellularized liver in a 24-well plates and immunofluorescence assays to detect albumin, HNF and CK-18 were performed. Results: Toluidine blue showed that the vascular system was totally preserved. Macroscopy, microscopy and histological staining showed that the decellularization process preserves the structure and components of the ECM. After 30 days,
cells were detected in the decellularized tissue. Conclusions: Our decellularization method was efficient in removing resident cells and preserving the liver's ECM and vascular system. Moreover, liver cells were able to adhere on liver 3D scaffold generating new perspectives for building a bioartificial liver.

Poster Board Number: T-3192

COMPLETE DENTAL PULP REGENERATION BY TRANSPLANTATION OF PULP STEM CELLS WITH G-CSF

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Dental pulp is critical for homeostasis of teeth, regeneration of pulp following deep caries and/or pulp inflammation is the conservation of dental function. The long term goal of endodontic treatment following root canal treatment in pulpitis and resultant improvement of dental function. The long term goal of endodontic treatment following deep caries and/or pulp inflammation is the conservation and restoration of teeth including dental pulp. A promising approach is stem cell therapy to regenerate the dentin-pulp complex for the conservation and total restoration of structure and function of teeth. We succeeded in complete pulp regeneration after transplantation of dental pulp CD105+ or CD31- SP stem cells with high angiogenic and neurogenic potential into pulpectomized teeth with stromal cell-derived factor 1 (SDF1) in dogs. CD31- SP cells are isolated after labeling with DNA binding dye Hoechst 33342 and flowcytometry. CD105+ cells are isolated by magnetic antibody beads method and have to be cost effective. The unfractinated total pulp cells by a single colony method are not optimal for pulp regeneration since the regenerated tissue is less in volume compared to fractionated stem/progenitor cells. Thus, we have developed novel isolation methods ensuring safety and efficiency from small amounts of pulp tissue to permit clinical trials. In addition to stem/progenitor cells, migration/homing factors should be produced in GMP facilities for clinical investigation. In the present study we have examined granulocyte colony-stimulating factor (G-CSF), a widely used hematopoietic stem cell mobilization cytokine, as a cell migration factor instead of SDF-1. The isolated dental pulp stem cells by our novel method highly expressed stem cell markers, angiogenic/neurogenic factors compared with unfractinated total pulp cells. In addition they exhibited potent chemotactic and mitogenic activities and multilineage differentiation potential including vasculogenic and neurogenic lineages. Autogenous transplantation of the pulp stem cells with G-CSF were performed after pulpectomy in dogs. The root canal was filled with regenerated pulp tissue including vasculature and nerves by day 14. The odontoblast-like cells attached to the dentinal wall in the root canal, and produced dentin-like tissue extending their processes into dentin tubules by 60 days. The transplanted pulp stem cells had high migratory and proliferative effects due to G-CSF. Angiogenic/neurotrophic factors were expressed by the G-CSF-treated cells and they localized in the vicinity of newly formed capillaries, suggesting possible trophic effects. Transplantation of pulp stem cells with G-CSF yielded significantly more regenerated tissue compared with transplantation of stem cells alone or G-CSF alone. When unfractinated total pulp cells were transplanted, less tissue was observed compared with pulp stem cells, followed by evidence of matrix formation and mineralization on day 60. It is noteworthy that the regenerated tissue demonstrated expression of authentic dental pulp cell markers. In conclusion, dental pulp stem cell therapy with G-CSF is of utility for pulp regeneration following pulpectomy, demonstrating safety and efficacy to permit clinical applications in the near future.

Poster Board Number: T-3193

REGENERATION OF HOLLOW ORGANS A PLATFORM APPROACH

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Recent successes in regenerative medicine and tissue engineering of hollow organs have leveraged regenerative constructs composed of: (1) a biodegradable, custom fabricated scaffold based on synthetic, biopolymers approved by regulatory agencies (2) a population of smooth muscle-like cells. As acellular constructs do not mediate regenerative outcomes, cell sourcing is fundamental for hollow organ engineering. Mesenchymal stem cells (MSC) are under active investigation for applications in regenerative medicine, but may not be ideal for industrial-level organ engineering. We demonstrate that smooth muscle-like cells (SMC) for hollow organ regeneration are isolatable from cell sources alternate to the primary organ. Transcriptional, proteomic and functional methodologies identify SMC as a cellular sub-population with markedly distinct phenotypic and functional properties relative to MSC. We have demonstrated that implantation of regenerative constructs within human or animal subjects catalyzes the de novo regeneration of a neo-tissue/organ. We provide further evidence from multiple organ systems that this methodology represents a broadly applicable organ regeneration platform for tissue engineering multiple, disparate laminarily organized hollow organs. Specifically: 1) Esophagus. Using rodent models of esophageal injury, we demonstrated that patch regenerative constructs based on this platform technology mediate complete regeneration of esophageal wall by 10 weeks post-implant in rodents. These results are foundational for on-going attempts to regenerate tubular esophageal constructs in pre-clinical large animal models and eventually for clinical trials in human patients requiring esophageal replacement secondary to esophageal cancer. 2) Small intestine. Leveraging rodent models of small intestine injury, we show that patch and tubular regenerative constructs mediate regeneration of small intestine tissue with laminarily organized neo-mucosa and smooth muscle layers as early as 8 weeks post-implant in rodents. Follow-up studies with pre-clinical small animal models will lay the framework for clinical trials in human subjects presenting with short bowel syndrome. 3) Urinary conduit. In a pre-clinical GLP study, we demonstrated that implantation of tubular regenerative constructs with SMC sourced from autologous adipose or peripheral blood into a porcine cystectomy model triggers de novo regeneration of a hollow neo-organ composed of urinary-like neo-tissue histologically identical to native bladder. The neo-urinary conduit (NUC) represents the first of a class of entirely novel neo-organs that have hitherto not existed in nature. Remarkably, this engineered neo-organ functions as expected in vivo to efflux urine. Tengion is currently conducting Phase I clinical trials of NUC constructs in human patients requiring urinary bypass secondary to radical cystectomy as a consequence of bladder cancer. Unlike other tissue engineering approaches, this organ regeneration platform does not require use of cadaveric organs or stem cell populations potentially relictual to industrial scale process development. Taken together, we believe that the ability to create hollow organs de novo from biodegradable, synthetic scaffolds seeded by autologous SMC sourced alternate to the primary organ will greatly facilitate translation of hollow organ tissue engineering technologies into clinical practice.
DIFFERENTIAL ANGIOGENESIS, NEUROGENESIS AND PULP REGENERATION FROM PORCINE CD31- SIDE POPULATION CELLS ISOLATED FROM DENTAL PULP, BONE MARROW AND ADIPOSE TISSUE

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Background: Pulp CD31- side population (SP) cells have high angiogenic, neurogenic and pulp regeneration potential. However, decrease in the abundance and volume of pulp tissue with age makes the pulp stem cell therapy and its clinical utility challenging. In this study, we investigated the comparative regenerative potential of porcine pulp, bone marrow and adipose tissue-derived CD31- SP cells to evaluate an alternative cell source for pulp regeneration.

Methodology/Principal Findings: The primary porcine pulp cells, adipose cells and bone marrow cells were separated from the same individuals, and were labeled with Hoechst 33342 and further anti-porcine CD31. The frequency of CD31- SP cells in pulp, bone marrow and adipose tissue was 0.9%, 0.3% and 0.1%, respectively. Flow cytometric analyses of cell surface antigen markers demonstrated the "stemness" of bone marrow and adipose CD31- SP cells as pulp CD31- SP cells. Bone marrow and adipose CD31- SP cells, however, had less expression of angiogenic/neurotrophic factors and less migration activities compared with pulp CD31- SP cells. The angiogenic and neurogenic potential was similar in the three cell populations. In mouse hindlimb ischemic models, transplantation of bone marrow and adipose CD31- SP cells resulted in significantly less increase in the blood flow and density of capillary formation compared with pulp CD31- SP cells. In rat brain ischemic models, recovery of motor function and decrease in infarct size were less in bone marrow and adipose CD31- SP cells compared with pulp CD31- SP cells. In tooth root models of subcutaneous transplantations, bone marrow and adipose CD31- SP cells induced vasculogenesis, neurogenesis and pulp regeneration with biomarker expression of syndecan and thyrotropin-releasing hormone degrading enzyme (THR-DE), although yielded less regenerated tissue compared with pulp CD31- SP cell transplantation on day 28. The GOT-stained porcine transplanted tissues were not co-localized with RECA1 positive capillaries in all the three transplantsations. The transplanted cells were not stained with Ki67 on day 28, indicating little proliferation. Some of the cells in the regenerated tissues were not stained with GOT. Highly expression of angiogenic/neurotrophic factors, such as vascular endothelial growth factor-A (VEGF-A), matrix metalloproteinase (MMP)-3, granulocyte-monocyte colony-stimulating factor (GM-CSF), brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in the transplanted cells were found by in situ hybridization on day 28. Conditioned media from bone marrow and adipose CD31- SP cells showed similar angiogenic, neurogenic and chemotactic activities on NIH3T3 cells in vitro as that from pulp CD31- SP cells. Conclusion/Significance: These results suggested potent trophic effects of bone marrow and adipose CD31- SP cells as pulp CD31- SP cells to enhance homing of stem/progenitor cells in the tooth roots, contributing to pulp regeneration. Thus, bone marrow and adipose CD31- SP cells may be alternative cell sources of clinical utility for pulp regeneration, although its regenerative potential is inferior to pulp CD31- SP cells.

REGENERATING KIDNEY STRUCTURE AND FUNCTIONALITY BIORESPONSE OF RODENT MODELS OF RENAL DISEASE TO IMPLANTATION OF NEOKIDNEY AUGMENT PROTOTYPES COMPOSED OF SELECTED RENAL CELLS AND BIOMATERIALS

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Chronic kidney disease (CKD) is continued loss of renal function over time. Current renal therapies include dialysis and kidney transplant. An unmet need exists for new treatments to restore renal function thus delaying dialysis/transplant. Efforts to regenerate diseased kidneys have focused on application of mesenchymal stem cells (MSC). However, we have identified populations of tubular epithelial cell-enriched primary renal cells (Selected Renal Cells, SRC) that positively impact aspects of disease phenotype in rodent CKD models, potentially by interfering with onset of tubulointerstitial fibrosis and mobilizing host renal stem cell populations. Addition of biomaterials may facilitate cellular engraftment while creating space for vascularization and cellular infiltration during regeneration. Here, we report on development of Neo-Kidney Augment (NKA) product prototypes, comprised of SRC/biomaterials combination constructs which facilitate regeneration of kidney tissue. Methods: Identification of biomaterials appropriate for renal tissue engineering was accomplished by iterative screening and evaluation of bio-response to implantation of SRC/biomaterial constructs within rodent models as follows: (1) Healthy adult rodents (n=200) were used to identify biomaterials associated with minimal inflammatory and fibrotic outcomes, cellular infiltration and neo-kidney tissue induction (2) Removal of single kidney from rodents (hemi-nephrectomy) facilitates detection of systemically acting toxicological effects. Hemi-nephrectomized rodents (n=20) were injected with SRC/biomaterials NKA prototypes within renal parenchyma of the remnant kidney. Physiological indices derived from whole blood, serum and urine chemistries were evaluated at 2 and 4 week time points post-implantation. Animals were sacrificed at 4 weeks post-injection and remnant kidney examined histologically for evidence of inflammatory or fibrotic bio-response (3) Removal of single kidney and 5/6 of remnant kidney induces disease physiologies within rodents mimicking aspects of chronic kidney disease (CKD) in humans, permitting evaluation of bio-response to implantation of SRC/biomaterials NKA prototypes within a clinically relevant small animal model. 5/6 nephrectomized rodents (n=20) were implanted and evaluated as described above for hemi-nephrectomized rodents. Results: Of the biomaterials candidates examined, implantation of gelatin-based hydrogels within healthy rodent renal parenchyma was associated with minimal fibrotic and inflammatory outcomes and concomitant neovascularization, cell and tissue infiltration and biomaterial degradation within 4 weeks post-implantation; by 8 weeks, neo-kidney tissue was observed, suggesting regenerative response induction in vivo. Implantation of SRC/biomaterials NKA prototypes within remnant renal parenchyma of hemi- or 5/6 nephrectomized rodent kidneys presented minimal evidence of inflammatory, necrotic or fibrotic bio-response and did not significantly affect key renal physiological indices.
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Therefore, NKA prototypes based on SRC/gelatin-based hydrogels are well tolerated by remnant kidney in rodent hemi- or 5/6-nephrectomy models. Conclusions: Together with previous studies, these data establish SRC/gelatin-based hydrogel NKA prototypes for tissue engineering of CKD kidneys. Evaluation in canine CKD models is in progress prior to start of GLP animal studies.

Poster Board Number: T-3196

HEPATOGENIC DIFFERENTIATION OF MENSTRUAL BLOOD DERIVED STEM CELLS ON A THREE DIMENSIONAL NANOFIBROUS SCAFFOLD

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Introduction: In few recent years, such problems with well-known stem cell sources as low availability, painful access or limited proliferative ability have impelled scientists to take advantages of menstrual blood stem cells (MenSCs) in tissue engineering and regenerative medicine. However, only little information is available about hepatogenic differentiation potential of MenSCs. In the present study, we examined the differentiating potential of MenSCs into hepatocytes on a three dimensional nanofibrous scaffold fabricated from Poly (ε-caprolactone) (PCL). Methods: MenSCs were isolated from menstrual blood samples by discontinuous density gradient centrifugation and plastic adherence. After characterization of seeded MenSCs on matrigel coated PCL nanofibers, differentiation ability of cultured cells into hepatocyte using a novel three-stage protocol was evaluated by biochemical and molecular experiments. Results: Flow cytometric analysis illustrated that MenSCs were strongly positive for mesenchymal and negative for hematopoietic stem cell markers. MenSCs also exhibited marked expression of OCT-4. Based on the result of scanning electron microscopy, MTT assay and Hematoxilin & Eosin staining, cells adhered, distributed and expanded on the scaffold. The cultured MenSCs on scaffold in presence of hepatic conditioned media significantly expressed mature hepatocyte mRNA such as albumin, tyrosine amino transferase and cytochrome P7A1 on day 30 of differentiation judged by RT-PCR. Moreover, unlike undifferentiated MenSCs, great synthesis and production of albumin protein by differentiated MenSCs was shown by immunofluorescent staining and enzyme-linked immunosorbent assay using monoclonal antibody against albumin protein. Conclusion: The fabricated scaffold supports MenSCs differentiation into hepatocyte-like cells. So, a new stem cell-scaffold construct has been suggested for treatment of liver diseases using hepatic tissue engineering

Poster Board Number: T-3197

EMERGENCE OF PLURIPOTENT STEM CELLS CONTROLLED BY KLF5

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In our previous study, we generated Klf5 knock-out mice and showed that Klf5 is indispensable for blastocyst development and the derivation of ESCs from the inner cell mass (ICM)(Ema et al., 2008). Recent studies indicate that Kruppel-like factors (Klf5) are essential for both maintenance of ES cell self-renewal and reprogram-
**IPS Cells**

**Poster Board Number: T-3201**

**DEVELOPMENT OF EFFICIENT INDUCTION METHODS FROM HUMAN IPS/CSCS INTO INTERMEDIATE MESODERM BY USING LOW MOLECULAR WEIGHT COMPOUNDS**

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The increased prevalence of chronic kidney disease has caused a rise in the number of dialysis patients, and is associated with elevated morbidity and mortality. Development of kidney regeneration therapy is required because most patients with chronic kidney disease never recover renal function. Kidney is derived from one of the early embryonic germ layers, intermediate mesoderm (IM), and directing pluripotent stem cells, such as induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs), into IM lineage is a crucial step for kidney regeneration. In this study, to identify small molecules that can efficiently induce the differentiation of human iPSCs/ESCs into IM lineage cells, we screened a chemical library of about 1,800 low-molecular weight compounds using human iPSC lines that contain an allele of an IM-specific marker gene OSR1 into which a green fluorescence protein (GFP) gene was knocked-in by homologous recombination. We identified two chemical compounds with the inducing ability and established a differentiation protocol using the combinational treatment of these compounds that directs the differentiation of human iPSCs/ESCs into IM lineage cells. The combinational treatment with these compounds induces nearly 90% of OSR1+ cells from human iPSCs for only 6 days, in the same way as that achieved by our combinational treatment of growth factors for 11 days. Moreover, the generated OSR1+ cells produced the substantial expression of other IM markers, and had the ability to differentiate into the cells expressing the specific markers for renal cells constituting the adult kidney in vitro and in vivo. Furthermore, we elucidated a part of the mechanisms involved in the differentiation of human iPSCs/ESCs into IM lineage cells. In conclusion, we have identified two low-molecular weight compounds that can induce a robust differentiation of human iPSCs/ESCs into IM cells with the similar developmental potential to their in vivo counterpart. Our differentiation methods using chemicals alone can produce faster, more consistent and lower-cost generation of IM cells than growth factor-based protocols, which may contribute to understanding the mechanisms of kidney development and supplying the cell sources for the regenerative medicine strategies for chronic kidney disease.

**Poster Board Number: T-3202**

**EFFICIENT GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS IN A FEEDER-FREE AND XENO-FREE CONDITION**

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Due to an extraordinary speed of development in the iPSC research, we know that iPSCs share many key properties with ESCs including pluripotency, self-renewal, morphology, colony formation, and gene expression profiles. These similar traits of iPSCs to ESCs are thought to lead the cells to clinical applications in the foreseeable future. One important safety-related issue for clinical application of iPSCs is the generation and expansion of the cells in the absence of animal-derived products in the media. It was reported that HESCs incorporated significant amount of nonhuman sialic acid, Neu5Gc, in the cell surface proteins which may result in failure in transplantation due to immune rejection. Furthermore, iPSCs cultured in animal-derived products can be a source for nonhuman pathogen transmission to human. In that sense, conventional mouse embryonic fibroblast (MEF)-based culture methods are not suitable for clinical application. In this study, we were able to generate hiPSCs efficiently in a feeder-free & xeno-free culture condition. Our study provides valuable information which would expedite clinical application of human pluripotent stem cells to treat many incurable diseases. This research was supported by grant SC170 from the Stem Cell Research Center of the 21st Century Frontier Research Program, and Stem Cell Research Program (2010-0020350) funded by the Ministry of Education, Science and Technology of Republic of Korea.

**Poster Board Number: T-3203**

**‘THREE-IN-ONE’ SENDAI VIRUS VECTOR IS HIGHLY PROMISING FOR EFFICIENT GENERATION OF TRANSGENE-FREE HUMAN IPS CELLS.**

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[Abstract] Induced pluripotent stem cell (iPSC) technology is a promising technology for drug discovery and regenerative medicine. It is important to select an efficient and safer gene transfer vector for the generation of high-quality iPSCs. Previously, we reported efficient generation of transgene-free, vector-free iPSC cells using Sendai virus (SeV) vector, a non-integrating cytoplasmic RNA vector, carrying the OCT3/4, SOX2, KLF4 and c-Myc gene, independently. To further increase the efficiency, we created SeV vectors that carried three genes encoding reprogramming factors per vector and tested the gene order to examine the efficiency of iPSC induction. Methods and Results: The order of the genes for the generation of iPSCs: KLF4-OCT3/4-SOX2 (SeV-KOS) and OCT3/4-SOX2-KLF4 (SeV-OSK) in combination with c-Myc (SeV-c-Myc) were examined, because the expression level is affected by the gene order. We found that the efficiency was up to 4% by using SeV-KOS when we used BJ human neonatal fibroblast cells as a source of parental cells. It was ten times higher than that by SeV-OSK or previous vector constructs using four genes cocktail. However, the efficiency was much improved when SeV-OSK vector was used together with additional SeV vector carrying KLF4 (SeV-OSKK). These results suggested that a certain high level of KLF4 expression was necessary for the efficient iPSC generation. Next, we created a temperature-sensitive SeV vector carrying KOS (SeV-KOS/TS12). We tested the efficiency of iPSC generation together with two kinds of SeV-c-Myc vectors (Sev-c-Myc/TS12 and SeV-c-Myc/TS15, respectively). TS15 is more temperature-sensitive than TS12. When we used a combination of SeV-KOS/TS12 and SeV-c-Myc/TS12, iPSCs were generated efficiently (up to 3%) at 37°C and vectors were removed by culturing at 39°C for a week. When SeV-KOS/TS12 and SeV-c-Myc/TS15 were used, iPSCs were generated efficiently at 36°C (up to 2%) and iPSCs became vector-free by culturing at 37°C within almost 5 passages. We confirmed that iPSCs induced by using SeV-KOS and OSKK showed normal karyotype and had similar properties of ES cells such as pluripotency and ES marker expression. Conclusion: These results suggest that SeV vectors carrying three genes encoding reprogramming factors in one vector backbone is a promising vector to efficiently generate transgene-
and vector-free iPSCs. Acknowledgements: This work was in part supported by JST, PRESTO.

Poster Board Number: T-3204

REGULATORY CIRCUITS COMMON TO HEMATOPOIETIC AND EMBRYONIC STEM CELLS DIRECT BULK NON-INTEGRATED REPROGRAMMING OF LINEAGE-COMMITTED HUMAN MYELOID PROGENITORS

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Factor-driven reprogramming of human somatic cells using non-viral, non-integrating methods is slow and inefficient. We previously reported the derivation of non-integrated, transgene-free cord blood-derived induced pluripotent stem cell (CB-iPSC) lines that were generated at efficiencies between 1 - 4% of input cells using a novel bone marrow stromal cell (BMSC) co-culture system and an EBNA-based episomal system. In designing this BMSC-CD34+ co-culture reprogramming system, we capitalized on the principle that the innate epigenetic plasticity of hematopoietic progenitors can be positively influenced by stem cell niche signals. We now report the optimized non-integrated reprogramming of lineage-commit ted CB myeloid progenitors with a single plasmid episome expressing only the four Yamanaka factors (SOX2, OCT4, KLF4, MYC), and with unprecedented bulk efficiencies up to 50%. Contrary to prior studies that suggested stem-progenitors are more amenable to reprogramming, lineage-committed CD33+CD38+CD45+CD34-myeloid cells, and not primitive CD34+ hematopoietic stem-progenitors (HSPC) were the main targets of this highly permissive myeloid cell reprogramming. When myeloid progenitors were FACs-purified from HSPC populations, CD34+CD38hiCD33+ myeloid progenitor cells (16.7±3.7%) revealed significantly more amenable to pluripotency induction compared to CD34+CD38low HSPC populations (0.3±0.3%). The true reprogramming efficiency of myeloid progenitors was assayed by enumerating alkaline phosphatase+ TRA-1-81+ hiPSC that emerged from plasmid-nucleofected CB cells, and by enriching only for hematopoietic cells that had been successfully transfected via GFP co-nucleofection. These studies revealed the reprogramming efficiency of lineage-committed GFP+CD34+CD45+ myeloid progenitor that was 51±10.5%. Further mechanistic studies revealed that the conversion of myeloid populations into NANOG+TRA-1-81+ hiPSC was directed en masse by a synergy between the Yamanaka factors (SOX2, OCT4, KLF4, MYC) and extrinsic stromal micro-environmental signals. Highly efficient pluripotency induction correlated not to endogenous expression of core pluripotency factors (e.g. OCT4, SOX2, NANOG) in myeloid cells, but instead to de novo expression of transcriptional circuits that commonly regulate epigenetic plasticity and self-renewal in both hematopoietic progenitors and embryonic stem cells (ESC). Using a modular bioinformatics approach, we observed that CB myeloid progenitors expressed activated ESC-like levels of the OCT4 interactome and MYC-regulated circuits, and inactivated ESC-like Polycomb group (PCG)-regulated networks that were poised de novo in partially-reprogrammed states. These findings solve the problem that hiPSC emerge only from rare factor-expressing somatic cells, and open the cellular biology of large synchronized populations of reprogrammed cells to full kinetic analysis. These data also provide the first evidence that induced pluripotency may require activation of a molecular framework that commonly regulates self-renewal and differentiation in both hematopoietic progenitors and ESC, and that is already expressed at high, partially-reprogrammed levels in myeloid progenitors.

Poster Board Number: T-3205

GENETICALLY IDENTICAL HUMAN EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS REVEAL SIGNIFICANT DIFFERENCES IN DIFFERENTIATION CAPABILITIES TO MESODERMAL LINEAGES

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It has been extensively evidenced that both hESC and hiPSC have the capability of differentiating into many different cell types of the three germ layers. However, it has recently emerged upon their further scrutiny, that hESC and hiPSC exhibit significant differences in their comparative differentiation capabilities and epigenetic properties. It is hypothesized these differences may be due to the fact that each cell line reported thus far has been derived from genetically individual sources. In an attempt to identify the underlying cause of the differences observed between hESC and hiPSC, we have terminally differentiated hESC to definitive fibroblasts then reprogrammed these cells to hiPSC, creating genetically identical, or isogenic, hESC and hiPSC. Upon characterization of the generated hiPSC lines, we examined the mRNA expression and DNA methylation patterns of all cells in their undifferentiated state. We then differentiated these cells, in parallel with the parental hESC from which they were derived, to cells of the mesodermal lineage, specifically cardiac and hematopoietic cell types. Cardiomyocytes and hematopoietic cells were then compared with respect to their mRNA and protein expression patterns. Our results showed there were significant differences in differentiation capacities and expression patterns, even though in an undifferentiated state, the DNA methylation and pluripotent gene expression levels were found to be highly similar. This suggests that other factors, such as somatic cell source and/or the process of reprogramming itself, creates the observed differences between hiPSC and hESC. The creation of hiPSC derived from isogenic sources is a novel way to pinpoint differences in hiPSC vs. hESC not related to genetic background.

Poster Board Number: T-3206

IMMORTALIZED HUMAN FORESKIN FIBROBLAST FEEDER CELLS WITH ENDOGENOUS SECRETION OF BASIC FGF SHOWS UP-REGULATION OF TGF BETA AND IGFII : KEY FACTORS OF HUMAN EMBRYONIC STEM CELLS/INDUCED PLURIPOTENT STEM CELL PLURIPOTENCY AND SELF RENEWAL

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Recently, it has been shown that several members of TGF-β family and IGF-II are necessary for the maintenance of pluripotency and self renewal in Human Embryonic Stem Cells (hESCs). Both the components have been proposed to cooperate with FGF pathway to maintain pluripotency and self renewal by establishing a regulatory stem cell niche. With the enhanced understanding of hESC culture conditions it has been found that the supplementation with b-FGF is still required to sustain pluripotency. Immortalized Human Foreskin Fibroblast (I-HFF) feeder cell line (established & kind gift
from Prof. Anis Feki, Laboratory of Stem Cell Research, Geneva University Hospital, Switzerland) stably transduced with Bmi, hTERT, GFP and secreted form of b-FGF gene was used. The I-HFF cell lines have been successfully validated and proven to support the growth and maintenance of hESC and Induced Pluripotent Stem Cells (iPSC). The present study was designed to evaluate the minimum requirement of exogenous b-FGF to be added to meet the standard regulation of key factors involved in the regulation of pluripotency compared to naïve Human Foreskin Fibroblast cells used as feeders. I-HFF cells and HFF cells after mitomycin inactivation were cultured for additional 5 days using varying concentration of exogenous b-FGF at 10 ng/ml, 5 ng/ml, 2 ng/ml and 0 ng/ml for I-HFF and with 10ng/ml standard concentration for HFF. The cultures were harvested at Day 02 (1st harvest) and Day 05 (2nd harvest), mRNA was isolated and reverse transcribed, initially the presence of FGFR receptor 4 was checked with RT-PCR on both the feeders. The semi quantitative PCR was done to observe any difference in the expression of both TGF-β and IGF-II genes at all concentration of exogenous b-FGF and without b-FGF in I-HFF cells. Furthermore, using SYBR green chemistry the fold expression of both the genes was quantified using GAPDH as a reference gene for all the parameters. The fold expression was negligible when I-HFF without b-FGF was compared with I-HFF at all other b-FGF concentration used for both the genes, corresponding with the semi quantitative PCR results. Next, I-HFF was compared to the HFF without exogenous b-FGF, a 2.38 and 2.85 fold increase was observed for TGF-β and IGF-II genes respectively. Likewise, I-HFF at all b-FGF conc. and without b-FGF regime was compared with HFF at 1ng/ml b-FGF conc. in both the harvests. Interestingly, we also found a sequential expression in the fold expression of both the genes between 1st and 2nd harvest, indicative of a reproducible trend. These finding indicate the likely hood of using the conditioned media (CM) of I-HFF feeder cells in the maintenance of feeder free culture of hESC, without the variability of batch to batch feeder cell variation. This preliminary study indicates the fact that I-HFF can be a better alternative to the naïve HFF and for hESC culture maintenance without additional requirement of exogenous b-FGF.

Acknowledgement: We thank Prof. Anis Feki for providing us immortalized Human Foreskin Fibroblast Feeder cell line.

Poster Board Number: T-3207

MODELING CONGENITAL CENTRAL HYPOVENTILATION SYNDROME USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Congenital Central Hypoventilation Syndrome (CCHS) is a rare disorder of respiratory control that shows significant neural crest (NC)-pathology. CCHS is highly correlated with mutations in the PHOX2B gene. PHOX2B is a paired-like homeodomain transcription factor. CCHS-associated PHOX2B mutations show preservation of the DNA homeodomain structure, and therefore may continue to bind DNA; however, how this interaction affects gene regulation remains unknown. The ultimate goal of this project is to generate an in vitro model of CCHS using induced pluripotent stem (iPS) cells derived from a CCHS autopsy subject that suffered from a severe case of intestinal aganglionosis. To this end, we validated full cellular reprogramming of fibroblasts by molecular marker expression. Furthermore, we differentiated the PHOX2B mutated iPS cells into all three human germ layers.

Poster Board Number: T-3208

THE PLURIPOTENCY MARKER, ZFP42 (REX1) IS HIGHLY UPREGULATED UNDER HYPOXIC CULTURE CONDITIONS IN THE MULTILINEAGE-DIFFERENTIATING STRESS-ENDURING (MUSE) SUBPOPULATION OF PRIMARY HUMAN FIBROBLASTS.

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Hypoxia conditions are known to support both pluripotency in embryonic stem (ES) cells as well as to enhance the efficiency of reprogramming of somatic cells to induced pluripotent stem (iPS) cells. Biopsy derived fibroblasts are an easy accessible source for obtaining patient specific cells for reprogramming. However, studies have shown that the efficiency of reprogramming of such cells is very low, usually in the range of 0.01 - 0.05%. Interestingly, recent data have shown that only a small subpopulation of human dermal fibroblasts are prone to be reprogrammed into iPS cells, whereas the major part of the cells are not completely reprogrammed and thus are maintained in a partially pluripotent state. Investigations of human primary fibroblasts, mesenchymal stem cells as well as native bone marrow cells, all seem to possess a few percent of this subpopulation, which are characterized by heterogeneous expression of the Stage Specific Embryonic Antigen 3 (SSEA-3), an embryonic/pluripotency marker. These cells, called MUSE (multilineage-differentiating stress-enduring) cells, can efficiently be isolated by FACS sorting as cells positive for both SSEA-3 and the mesenchymal cell marker CD105. Since MUSE cells only constitute a small subpopulation of a primary fibroblast cultures, we have initiated an investigation of culture conditions promoting the proliferation and expansion of these cells without loss of the SSEA-3+/CD105+ phenotype. Using FACS sorting we have investigated whether cultivation of primary human fibroblasts under hypoxic conditions affect the CD105+/SSEA3+ phenotype. Also, we tested the effect of the addition of bFGF on the CD105+/SSEA3+ phenotype. To obtain a better understanding of this subpopulation, we have used qPCR analysis to study how different growth conditions affect the expression of specific genes and miRNA’s known to be important for reprogramming and maintenance of pluripotency. The data shows that both hypoxic conditions and addition of bFGF increase the number of CD105+/SSEA-3+ cells in culture in an additive manner. Furthermore, several genes known to be important for pluripotency and self-renewal have induced expression. Interestingly, we find that the Zinc finger protein, ZFP42 (REX1), known to be involved in self-renewal in ES cells, is highly upregulated, more than 20-fold (qPCR), in hypoxic conditions as well as by addition of bFGF.
**Detailed Program and Abstracts — Thursday, June 14**

**Poster Board Number: T-3209**

**INDUCED PLURIPOTENT CANCER CELLS FROM INDIVIDUAL PATIENTS AS A METHOD FOR STUDYING ONCOGENESIS OF HUMAN MALIGNANT MELANOMA.**

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Melanoma is thought to arise through a series of genetic and epigenetic events. Primary it involves numerous irreversible changes within the human genome, such as chromosomal number alterations, gene amplifications and mutations. Recently, however, it has become widely recognized that epigenetic events may occur at much higher frequency compared to gene mutations and thus may have a greater impact on the process of melanoma tumorigenesis. Furthermore, several studies showed the reversion of the metastatic phenotype of aggressive cancer cells and revealed the convergence of embryonic and tumorigenic signaling pathways, which may help to identify new targets for therapeutic intervention. Generation of cancer-specific iPSC cells, also called induced pluripotent cancer (iPC) cells, provides a valuable experimental platform to the model of oncogenesis. In our work we have established and characterized primary melanoma cell lines from human biopsies. Established cell lines were analyzed for the endogenous expression of the pluripotency markers and then reprogrammed into ES-like state using individual ectopic expression of reprogramming factors for each primary cell line. It was demonstrated that established iPC cell lines express pluripotent stem cell markers, and, despite of transformation status, primary melanoma cell lines can be converted to the ESC epigenetic state. Established iPC cell lines could serve as a model for oncogenic transformation study and hold great potential for drug screening.

**Poster Board Number: T-3210**

**DISTURBANCE OF A DEVELOPMENTAL SWITCH IN SCHIZOPHRENIA: A HUMAN NEURONAL CELL THROUGH SPECIFIC PHOSPHORYLATION OF DISC1**

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Schizophrenia (SZ) is a severe brain disorder that results in mental dysfunctions and significant social disability in affected individuals. Deficits associated with neurodevelopment have been suggested but the molecular mechanisms remain elusive. A major obstacle to address this question directly has been in the difficulty of accessing relevant tissues/cells for study. Thus, we have systematically collected tissue/cells and established a panel of human cell lines, including olfactory immature neurons and induced pluripotent stem cells (iPSCs) from patients with schizophrenia as well as normal controls. We have generated iPSCs from skin fibroblasts from sporadic SZ patients and controls by the retroviral transduction of four factors: OCT4, SOX2, KLF4 and c-MYC. We then differentiated iPSCs to neurons via embryio body and neurosphere formation using previous method with slightly modification. Our recent study indicated that phosphorylation at a specific site (serine-710 (S710) in mouse) on DISC1 acts as a molecular switch from maintaining proliferation of mitotic progenitor cells to differentiation and migration of postmitotic neurons (Ishizuka et al, Nature, 2011). We hypothesized that deficits in the phosphorylation of human DISC1 at serine-713 (S713) that corresponds to mouse DISC1-S710, may participate in the pathology of schizophrenia. By using newly generated a phospho-specific antibody against human DISC1-S713, we observed a significant decrease in this phosphorylation in schizophrenia-derived olfactory immature neuronal cells compared with those from normal controls. To validate this deficit associating with the molecular switch, we examined iPS-derived neuronal precursor cells and neurons and compared them between schizophrenia and controls. Our strategy will provide important clues for understanding neurodevelopmental processes associated with schizophrenia.

**Poster Board Number: T-3211**

**MODELING A HUMAN GENETIC DISORDER OF THE AUTONOMIC NERVOUS SYSTEM IN IPS CELLS**

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Congenital Central Hypoventilation Syndrome (CCHS) is a rare disorder of respiratory control that shows significant neural crest (NC)-pathology, affecting cranial, cardiac, and trunk NC cell lineages. CCHS is highly correlated with mutations in the PHOX2B, a paired-homeodomain transcription factor. CCHS-associated PHOX2B mutations show preservation of the DNA homeodomain structure, and therefore may continue to bind DNA; however, how this interaction affects gene regulation and ultimately cellular function remains unknown. Interestingly, PHOX2B mutations also cause a NC tumor (Neuroblastoma), and it is unclear how this tumor would be related to CCHS. We have successfully generated induced pluripotent stem (iPS) cells derived from a CCHS autopsy subject and can differentiate these cells into NC cells. CCHS-derived NC cells show a hyper-proliferative and abnormal migratory phenotype. Currently, we do not know which NC cell subpopulation contributes to the hyper-proliferative and/or migratory phenotype, since unique gene signatures of NC subpopulations remain unknown. To identify the unique gene signatures of NC subpopulations we are performing RNA-seq from genetically marked NC cells in mouse embryos, as well as in human iPS-derived NC cells using several different NC differentiation methods. With unique gene signatures, we can then determine the contribution of each NC cell subpopulation in CCHS and hopefully improve regenerative therapies and tissue repair with implications for multiple NC diseases.
EFFICIENT AND STABLE EXPRESSION OF NON-VIRAL VECTORS IN HUMAN IPS CELLS: TOWARDS A GENE THERAPY APPROACH FOR HEMOPHILIA.

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Hemophilia A is a congenital bleeding disorder caused by a mutation in the coagulation factor VIII (FVIII). Successful secretion of FVIII protein from engineered cells within a patient’s body could circumvent the current replacement therapies that require frequent infusions of FVIII concentrates. For such cell therapy, induced pluripotent stem (iPS) cells derived from the patient or an HLA-matched donor are an attractive cell source because of enormous cell growth potential, pluripotency, and low immunogenicity. However, the large size of the FVIII gene prohibits efficient delivery by conventional viral vectors, and strong silencing activity in pluripotent stem cells hinders long-term, stable expression of transgenes. To overcome these issues, we took advantage of the piggyBac transposon vector system. During the evaluation of piggyBac vectors in iPS cells, we noted a loss of reporter expression after long-term (2~3 month) of culture, presumably due to transgene silencing. To stabilize transgene expression, we incorporated a human insulator element, called D4Z4, into our piggyBac vector. Unexpectedly, full-length D4Z4 inhibited the transposition of the vector in target cells. However, 5’ or 3’ fragments of D4Z4 permitted transposition as efficiently as the native vector. After more than 5 months of culture, we show that either the 5’ or 3’ fragment of D4Z4 is sufficient to maintain stable expression in human iPS cells. In addition, similar anti-silencing activity was also observed in de novo DNA methyltransferase-null ES cells, suggesting that the activity of the insulator is DNA methylation independent. Next, we cloned a 4.4 kb B domain-deleted FVIII cDNA, as well as 7.0 kb full-length FVIII cDNA into our piggyBac vectors. Even though the transposition rate tends to be lower with longer transgenes, we confirmed expression of the transgene and blood coagulation activity in the culture media of transgenic iPS cells. Lastly, we injected our piggyBac vectors into hemophilia A mice via tail vain by hydrodynamic injection, and successfully detected stable expression of human FVIII protein in plasma for several months without developing the anti-FVIII antibodies. We expect our engineered vectors will facilitate long-term expression of therapeutic transgenes in human pluripotent stem cells and their derivatives.

Screening Human Induced Pluripotent Stem Cell Derived Neurons for Histone Deacetylase Inhibition

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The vast majority of high throughput in vitro model systems in neuroscience drug discovery rely on target over expression in non-neuronal cell lines or lower throughput rodent embryonic primary neuronal cultures as human neurons have been difficult to ethically obtain in a reliable and consistent process. The stem cell field has made great strides filling this gap with the discovery of induced pluripotent stem (iPS) cells and protocols for differentiating brain region specific neurons. However, just like with primary cells there are questions concerning variabiliy during differentiation and donor differences to consider especially when looking at drug targets with potential splice variants or subunit heterogeneity. For the application of a differentiated cell model to drug discovery it is critical to have a robust and reliable process of differentiation that will allow the comparison of results over the several years of a discovery project. In this study we characterized Neurons differentiated from human iPS cells in order to understand the application of these cells as a physiologically relevant model system for neuroscience drug discovery. We have developed a high content imaging assay to study the role of epigenetics on synaptic plasticity by measuring changes in the acetylation of histone 4 lysine 12 as well as simultaneously monitoring effects on neurite outgrowth and branching.

Modeling Human Myotubular Myopathy Via Induced Pluripotent Stem Cells Derived from Autopsy Skeletal Muscle Tissue

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INTRODUCTION: Congenital muscular dystrophies (CMDs) are a group of genetic disorders that affect children at birth and clinically manifest as a "floppy" infant lacking muscular tone. In this study, we focus on X-linked myotubular myopathy (XLMTM), a severe form of CMDs. Although the deletion or point mutation of the MTM1 gene has been implicated in the etiology of this disorder, to date its pathogenesis is poorly understood. Recent progress in our understanding of human induced pluripotent stem cells (iPSCs) opened new avenues for modeling human diseases. This dynamic recapitulation of a disease process will help gain insight into disease pathogenesis and might potentially aid in finding specific therapeutic interventions such as gene-based therapy. In this study we generate iPSCs from somatic cells of a patient with XLMTM, which, to our knowledge, has not been demonstrated thus far. In addition, we show differentiation of iPSCs into myocytes and their functional integration. METHODS: Skeletal muscle tissue was collected during the autopsy of a patient with XLMTM and an age-matched healthy individual. The collected muscle tissue was dissected and cultured for three weeks with the goal to isolate fibroblasts. These fibroblasts were transformed with a polycistronic lentiviral vector construct expressing human OCT4, SOX2, KLF4 and cMyc/mCherry. Formed colonies were characterized by immunofluorescent stains, RT-PCR, karyotyping, and microarray studies. Generated iPSCs are injected into SCID mice to demonstrate teratoma formation. Cells were also grown in culture for differentiation into muscle cell lineages and injected in vivo to test their potential for differentiation and integration. RESULTS: After two weeks of transduction, a large number of colonies had formed. The colonies show positive immunostaining of Oct4, Tra1-60, and Nanog, as well as expression of human pluripotency genes hSox2, hNanog, hOCT4, and hDNMT3B as revealed by RT-PCR. These results support the pluripotent nature of the cells. In addition, teratomas composed of all three germ layers formed when iPSCs were injected into SCID mice. The iPSCs also demonstrate ability to differentiate into cells expressing muscle markers in vitro. CONCLUSIONS: Our study has demonstrated the successful generation of iPSCs from autopsy tissue of a patient with XLMTM - a new tissue source for generating human iPSCs. These patient-specific iPSCs possess the ability to differentiate in vitro and in vivo, providing an effective model for myopathy studies.
Detailed Program and Abstracts — Thursday, June 14

Poster Board Number: T-3215

MESENCHYMAL PROGENITORS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Introduction: Mesenchymal stem cells (MSCs) have a high potential for therapeutic efficacy in treating diverse musculoskeletal injuries and cardiovascular diseases, and ameliorating the severity of graft-versus-host and autoimmune diseases. However, most of these clinical applications require substantial quantities of cells, but the number of MSCs that can be obtained from a single donor is limited. Moreover, cultured MSCs gradually lose their potency, and eventually cease proliferation following extensive cultivation. Reports on the derivation of mesenchymal progenitors from human embryonic stem cells (ESCs), and more recently also from human induced pluripotent stem cells (iPSCs), are thus most intriguing, since the infinite proliferative capacity of ESCs opens the possibility to generate large amounts of highly uniform batches of MSCs. Materials & Methods: In the present study we derived mesenchymal progenitor cells (iMPs) using three different protocols: (1) embryoid body formation, (2) indirect co-culture with MSCs, or (3) forced differentiation of colonies. Results: Starting from an existing human iPSC cell line and a newly established cell line reprogrammed from human MSCs, respectively, we derived iMPs exhibiting typical MSC/ fibroblastic morphology. These cells could be expanded more than 20 passages (over 35 population doublings) before reaching senescence and were able to differentiate in vitro along the osteogenic (Alizarin Red staining, alkaline phosphatase activity), chondrogenic (GAG assay), and adipogenic (Oil Red-O staining) lineages. Discussion: Until now, there are currently no standard protocols for the derivation of mesenchymal progenitors from pluripotent cells and verification of their mesenchymal nature. Our system of using iPSC cells reprogrammed from human MSCs allows us to compare key characteristics of iMPs with the original MSCs from the same donor and draw more specific conclusions on their similarities and differences than would be possible with iMPs and MSCs from different sources and donors.

Poster Board Number: T-3216

A TRIAL OF IN VITRO RECONSTITUTION OF HUMAN SKIN USING TRANSGENE-FREE INDUCED PLURIPOTENT STEM CELLS

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Some defined factors which are important for self-renewal of embryonic stem cells (ESCs) have been shown to reprogram both mouse and human somatic cells into ESC-like pluripotent cells, named induced pluripotent stem cells (iPSCs). Induction of reprogramming by these defined factors had been mostly carried out by co-infection with retroviral vectors. The main problems of this retrovirus-based method were oncogenicity and mutagenesis. Among several DNA transposons, piggyBac transposon does not leave ‘footprint’ mutations upon excision. This means removal of the transposon from the host genome without changing any nucleotide sequences. Using this system, we generated hiPSCs from human dermal fibroblasts. After the removal of piggyBac transposon, these hiPSCs can be transgene integration-free and mutation-free, which are invaluable for therapeutic applications. Next, we tried to differentiate these hiPSCs into keratinocytes and obtained a homogeneous population of cells that showed phenotypic characteristics of basal keratinocytes (K5/K14 positive). Finally, we performed three-dimensional culture of these induced-keratinocytes and could observe a pluristratified epidermis. Our results indicated the great promise of hiPSCs in the dermatological regenerative medicine.

Poster Board Number: T-3217

INVESTIGATION OF THE X CHROMOSOME USING INDUCED PLURIPOTENT STEM CELLS FROM HUMAN X ANEUPLID MODELS

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The goal of this project is to develop a model system to identify genes located on the human X chromosome, which require two copies in normal early development. Turner Syndrome females have complete or partial loss of their second sex chromosome leading to a 45, X karyotype. Three percent of pregnancies start out with a 45,X karyotype, however 99% of these pregnancies are spontaneously terminated. Turner Syndrome accounts for 1 in 2,000 live births, making it one of the most common human chromosomal abnormalities and the only viable monosomy. Turner females have a wide variety of clinical characteristics, including short stature, premature ovarian failure and subsequent infertility. Triple X syndrome (47,XXX) is on the opposite end of the spectrum, with presence of an additional X chromosome. Triple X syndrome occurs in 1 in 1000 life births. Triple X females may be taller on average and have learning disorders, but these women have normal sexual development. Because of the developmental consequences of monosomy X, we hypothesize that induced pluripotent stem cells (iPSCs) derived from Turner Syndrome females will accurately replicate the haploinsufficiency and X chromosome aberrations observed in the original patient, making them, along with Control and Triple X iPSCs, an ideal model with which to study the role of the X chromosome in early development and pluripotency. To address this hypothesis, one Turner syndrome and two Control fibroblast lines were reprogrammed using two sequential retroviral transductions of the four Yamanaka factors (OCT4, SOX2, KLF4, CMYC). To optimize iPSC line generation, a lentiviral vector carrying all four Yamanaka factors in one single excisable vector was used to reprogram the previous lines, and three additional Turner Fibroblast lines (Fetal, Neonate and Child). We observed increased reprogramming efficiency amongst all lines with the use of Valproic Acid (VPA), a histone deacetylase inhibitor. However, Turner Syndrome Child 1 was only reprogrammable with the addition of VPA. Turner iPSC lines exhibit a 45, X karyotype, while the control iPSC lines maintain a normal 46,XX karyotype. All lines express the key pluripotency markers. All lines developed teratomas with tissues characteristic of all three germ layers when injected into SCID mice. In vitro differentiation resulted in beating cardiomyocytes and differentiated cells were positive for cells of the three germ layers. Thus, initial characterization of all iPSC lines did not indicate differences in pluripotency between Turner Syndrome and Control iPSC lines. To further investigate possible differences amongst the X chromosome aneuploid iPSCs and Control iPSCs we used fluorescence activated cell sorting.
and the microfluidic technology of the Fluidigm system to analyze gene-expression at the single cell level. Using this method we can accurately analyze the expression of 96 genes for each individual pluripotent cell. The iPSC lines were sorted for two pluripotency cell surface markers (SSEA4 and TRA-1-60), then analyzed for the following sets of genes: Pluripotency, X-inactivation, Pseudautosomal, Escape from X-inactivation, Germ Cell, and the three germ layers. To gain a whole transcriptome view of expression in the X chromosome aneuploid and Control iPSC lines, we performed RNA-Seq. Alterations in gene expression between the X chromosome aneuploid iPSC lines may indicate causes of the early lethality in Turner Syndrome females and causes of symptoms in Triple X females.

Poster Board Number: T-3218
DONOR AGE AFFECTS TO GENERATE IPS CELLS FROM HUMAN SKIN FIBROBLASTS
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Human induced pluripotent stem cells (hiPSCs) represent a unique opportunity for the field of regenerative medicine because they offer the prospect of generating unlimited quantities of cells for autologous transplantation, with a potential application for use in treatments for a broad range of disorders. Although somatic cells can be reprogrammed into iPSCs by the expression of certain defined embryonic factors, several factors, including age and health status, have been found to show large variations in the efficiency of hiPSC generation. Reprogramming results in the early activation of the pluripotency marker alkaline phosphatase (ALP). This study examined the relationship between the donor age of human skin fibroblasts and the emergence of ALP-positive colonies by iPSC generation when using an episomal approach. Three skin fibroblast lines from normal Japanese donors of varying ages were used; including the TIG-35 (Fetus), TIG-120 (from a 6-year-old donor) and TIG-108 (from a 40-year-old donor) fibroblast lines. Immunofluorescent staining showed all fibroblast lines to be positive for CD13 and CD90. The cells were cultured at an initial seeding density of 1x10^4 cells/cm^2, and the doubling time (DT) at 1-3 days was estimated based on their respective growth curves, which showed the TIG-35 fibroblasts to grow faster (22.0 ± 0.2 hours) than the TIG-120 fibroblasts (34.3 ± 0.9 hours) and TIG-108 fibroblasts (38.8 ± 1.7 hours). The saturation density (cells/cm^2) declined with age (TIG-35: 9.68 ± 0.14 x 10^4, TIG-120: 5.36 ± 0.29 x 10^4, TIG-108: 3.96 ± 0.39 x 10^4). The colony formation capacity of TIG-3S fibroblasts was higher than that of TIG-120 fibroblasts and TIG-108 fibroblasts. Episomal vectors with pCXLE-HOCT3/4-shp53-F, pCXLE-hSK and pCXLE-HUL (Okita et al., Nat. Methods, 2011) were electroporated into three skin fibroblast lines and then the lines were cultured with fibroblast growth factor to maintain the colony formation and the emergence of ALP-positive colonies. The transfected cells were reseeded onto SNL feeder cells after 6 days, and thereafter were maintained in an medium to induce hiPSCs. The transfected cells were reseeded onto blast lines and then the lines were cultured with fibroblast growth factor to maintain the colony formation and the emergence of ALP-positive colonies. The colony formation capacity of TIG-3S fibroblasts was higher than that of TIG-120 fibroblasts and TIG-108 fibroblasts (34.3 ± 0.9 hours) and TIG-108 fibroblasts (38.8 ± 1.7 hours).

The invention of induced pluripotent stem cell (iPSC) technology has enabled the derivation of patient-specific iPSC lines from a broad range of patient samples. This technology offers the potential for personalized medicine and has the potential to revolutionize the field of regenerative medicine. However, the efficiency of iPSC generation is affected by several factors, including donor age. This study aimed to investigate the relationship between donor age and iPSC generation efficiency using an episomal approach.

Poster Board Number: T-3219
CONSTRUCTION OF SPECIFIC NONVIRAL VECTOR FOR EX VIVO GENE TRANSFER AND CORRECTION OF HUMAN B-GLOBIN GENE IN INDUCED PLURIPOTENT STEM CELLS DERIVED FROM A B-THALASSEMIC PATIENT
Dormiani, Kianoush1, Mir Mohammad Sadeghi, Hamidic2, Ghaedi, Kamran3, Karbalaei, Khadeejeh4, Forouzanfar, Mahboobeh4, Lachini, Lianna5, Nasr- Esfahani, Mohammad Hossein4, Baharvand, Hossein4
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Corriged human β-globin gene named pHBB. This vector comprises two main parts: a bacterial backbone for efficient amplification in E. coli cells and a eukaryotic antibiotic resistance ORF, puromycin. Second part contains one expression cassette including β-globin gene enhancers, promoter and complete β-globin gene sequence along with its 3’ and 5’ UTRs. Moreover this vector encompasses a specific phiC31 integrase site (attB) which its co-transfection with another vector encoding phiC31integrase enable it to integrate into specific sites named pseudo attP sites in the human genome. On the other hand, two loxP sites flanking of bacterial backbone and puromycin resistant ORF were designed in this vector that facilitate deletion of these sequences by TAT-NLS-Cre recombinase after integration into the genome of target cells. This character reduces possible host immune response to the vector and potential genotoxicity. The structure of the vector was confirmed through several steps of digestion experiments and sequence analysis. Functional analysis of the plasmid vector was successfully achieved by stable transfection of the vector into genome of a hematopoietic cell line, KG62, which is β-globin promoter specific line and able to express β-globin gene. Then keratinocyte derived iPSCs (KiPSCs) are created by using episomal vectors as safe harbors for long time acceptable gene expression. Finally the efficiency of recombinant β-globin production by the vector was determined in hematopoietic cells derived from transected KiPSCs. Thus pHBB can be assumed as an ideal vector for ex vivo gene transferring into β-thalassemic patient specific iPSCs to compensate β-globin expression defect by producing 15% or more normal hemoglobin concentrations after hematopoietic differentiation.

Detailed Program and Abstracts — Thursday, June 14
Poster Board Number: T-3219
CONSTRUCTION OF SPECIFIC NONVIRAL VECTOR FOR EX VIVO GENE TRANSFERRING AND CORRECTION OF HUMAN B-GLOBIN GENE IN INDUCED PLURIPOTENT STEM CELLS DERIVED FROM A B-THALASSEMIC PATIENT
Dormiani, Kianoush1, Mir Mohammad Sadeghi, Hamidic2, Ghaedi, Kamran3, Karbalaei, Khadeejeh4, Forouzanfar, Mahboobeh4, Lachini, Lianna5, Nasr- Esfahani, Mohammad Hossein4, Baharvand, Hossein4
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The invention of induced pluripotent stem cell (iPSC) technology has enabled the derivation of patient-specific stem cells and holds promise for cell-based therapies. Recent studies have revealed the potential of iPSC cell generation combined with gene therapy for treatment of different disease in human. In this study, we designed and constructed a nonviral tissue-specific plasmid vector as a gene transfer vehicle to express wild type β-globin gene named pHBB. This vector comprises two main parts: a bacterial backbone for efficient amplification in E. coli cells and a eukaryotic antibiotic resistance ORF, puromycin. Second part contains one expression cassette including β-globin gene enhancers, promoter and complete β-globin gene sequence along with its 3’ and 5’ UTRs. Moreover this vector encompasses a specific phiC31 integrase site (attB) which its co-transfection with another vector encoding phiC31integrase enable it to integrate into specific sites named pseudo attP sites in the human genome. On the other hand, two loxP sites flanking of bacterial backbone and puromycin resistant ORF were designed in this vector that facilitate deletion of these sequences by TAT-NLS-Cre recombinase after integration into the genome of target cells. This character reduces possible host immune response to the vector and potential genotoxicity. The structure of the vector was confirmed through several steps of digestion experiments and sequence analysis. Functional analysis of the plasmid vector was successfully achieved by stable transfection of the vector into genome of a hematopoietic cell line, KG62, which is β-globin promoter specific line and able to express β-globin gene. Then keratinocyte derived iPSCs (KiPSCs) are created by using episomal vectors as safe iPSCs with intact genome. The vector used for site-specific recombination (SSR) in pseudo attP sites of iPSC cell genome by electroporation. Some of these pseudo attP sites can act as safe harbors for long time acceptable gene expression level. Finally the efficiency of recombinant β-globin production by the vector was determined in hematopoietic cells derived from transected KiPSCs. Thus pHBB can be assumed as an ideal vector for ex vivo gene transferring into β-thalassemic patient specific iPSCs to compensate β-globin expression defect by producing 15% or more normal hemoglobin concentrations after hematopoietic differentiation.
No treatment currently exists to restore lost neurological function after stroke or spinal cord injury. A growing number of studies have highlighted the potential of stem cell and more differentiated neural cell transplantation as intriguing therapeutic approaches for neural repair after stroke. In this study, we used human induced pluripotent stem cell (iPS-NPs) for transplantation in a rat middle cerebral artery occlusion (MCAO) model of stroke or a balloon-induced spinal cord compression (SCI) model. Neural precursors were derived from iPS through the micro-aggregate stage using 300 ng/ml noggin and 20 uM SB. Prior to in vivo experiments, iPS-NPs were differentiated in vitro and the results analyzed. Subsequently, the percentage of cells expressing markers of neural precursors, including oct3/4, sox2, SSEA-4, SSEA-1, TRA-1-60, CD24, CD133, CD56, betaIII-tubulin, NF70, nestin, CD271 and CD29, was assessed by FACS in undifferentiated and predifferentiated (FGF and EGF omitted from the culture medium (CM) for 7 days) NPs. In vitro we were able to differentiate iPS-NPs into GABA-ergic neurons and, with less efficiency, into motor neurons. Female Sprague-Dawley rats were subjected to focal cerebral ischemia by reversible right MCAO for 90 min, while male Wistar rats were used for SCI. A suspension of iPS-NPs (300 000 cells in 3ul of CM) was transplanted into the lesions 7 days after MCAO or SCI (n=14, n=12); the control groups (n=8) were injected with saline. Metabolic profiles in the striatal tissue of both hemispheres were assessed by magnetic resonance spectroscopy (MRS) in the MCAO model. Four months after MCAO, MRS revealed that the concentrations of brain metabolites (glutamate, glutamine, N-acetyl-aspartate, creatine, taurine, choline and inositol) in grafted animals returned nearly to the values found in nonlesioned animals. Functional recovery in rats with an MCAO lesion was regularly assessed after transplantation by the apomorphine-induced rotation test, the tape-removal test or the rotating pole test. Rats with SCI were tested using the BBB (motor) and plantar (sensory) tests. The grafted animals in the stroke model displayed a decreased number of clockwise rotations in the apomorphine rotation-induced test and performed better in the tape removal test. Animals with SCI that received cell transplants significantly improved their BBB score when compared to control animals. iPS-NPs robustly survived in both models of injury, maintained their neural phenotype, migrated toward the lesioned area and survived there for 2-4 months after transplantation. In addition, some of the cells differentiated into more mature and tissue-specific neurons (NSE-, MAP2- and DARPP32-positive cells). No tumor formation was observed throughout the study. In conclusion, these results suggest that iPS-NPs undergo further differentiation after transplantation, integrate into the neural tissue, partially improve functional outcome and can serve as a tool for cell transplantation therapy.
blasts were differentiated to IPS-NSCs by embryoid body formation or SMAD inhibitors treatment, and propagated as neurospheres. NSCs derived from human fetal neural tissues (fetal-NSCs) were used as an additional normal control. The in vitro chemosensitivity to 11 reagents (procarbarzine, 6-MP, CDDP, VP-16, gefitinib, vincristine, Ara-C, ACNU, temozolomide, 5-FU, and MTX) was examined using two assays. The number of viable cells was estimated by measuring the total ATP content in the cells, and the number of non-viable cells was determined by the release of lactate dehydrogenase (LDH) from cells with damaged membranes. All three GSC lines, the IPS-NSCs, and the fetal-NSCs showed similar chemosensitivities to procarbarzine and gefitinib. However, the GSCs, IPS-NSCs, and fetal-NSCs responded differently to the other reagents. These findings suggest that GSCs and normal NSCs show different chemosensitivities to several regents. Examining these differences will increase our knowledge about the cellular properties of GSCs and provide useful information for the development of new drugs that target GSCs.

**Poster Board Number: T-3223**

**SYSTEMATIC SHRNA-MEDIATED SILENCING OF GENES IMPLICATED IN AUTISM SPECTRUM DISORDERS AND SCHIZOPHRENIA IN IPSC-DERIVED HUMAN NEURONS**

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Autism spectrum disorders (ASD) and schizophrenia are highly heritable disorders of human cognition and behavior. Advances in sequencing technology have allowed for better refinement of genetic etiology of complex disorders, and numerous genes and microdeletion/microduplication regions have been associated with both disorders, with some overlap. However, it remains challenging to establish a causal role for genetic variants associated with disease risk. One aim of this study is to investigate important implicated genes in these related disorders by identifying aberrant phenotypes in a genetic knock-down system. This is particularly relevant for elucidating the relative contributions of genes encompassed within microdeletion/microduplication syndromes such as 16p11.2 or 22q11.2, where the presumed causative gene(s) has not been found. To complement mutation analysis studies, we developed and characterized a human neural progenitor cell line derived from induced pluripotent stem cells (iPSC-NPCs) taken from fibroblasts from a psychiatrically screened healthy male. iPSC-NPCs were karyotypically normal, differentiated into all central nervous system cell types assessed, and were capable of synapse formation upon long-term differentiation. We selected over 100 genes associated with ASD and/or schizophrenia and targeted each gene with > four short-hairpin (sh) RNAs/gene, delivered by lentivirus along with a series of control shRNAs not known to target any human gene. In total, over 400 stable cell lines were generated and all cell lines were validated by qPCR in comparison to control shRNAs. A battery of phenotypic assays at both the progenitor and mature neuron stage were developed that assess a range of phenotypes including cell growth, cell extension, and intracellular calcium release. Changes in these cellular phenotypes occur upon knockdown of several of the GWAS-nominated candidate genes suggest that these genes may play a functional role in microdeletion/microduplication syndromes. These results can help the research community prioritize and direct further investigation of the function of genes associated with ASD and schizophrenia.

**Poster Board Number: T-3224**

**EXOGENOUS SIGNALS OPPOSITELY AFFECT ADIPOCYTE DIFFERENTIATION AND NEURAL DIFFERENTIATION OF HUMAN ES/IPS CELLS**

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Efficient differentiation of human pluripotent stem cells (hPSCs) into specific cell lineage is required for disease modeling and for regenerative medicine. We have previously reported adipocyte and neural differentiation from human ES cells (hESCs) and iPS cells (hiPSCs). In this study, we found the efficient differentiation of hiPSCs into adipocytes by regulating the concentration of knockout serum replacement (KSR) during embryoid body formation, which is commonly used as serum replacement supplement for maintaining hPSCs. Although KSR has been known to contain growth factors which may affect the differentiation of PSCs, its effects on the differentiation of hPSCs into adipocytes and neural lineage are still unknown. Here, we investigated how the concentration of KSR affects adipocyte and neural differentiation of hPSCs (5%-30% in the medium). Considering the differentiation propensity of hPSCs, we used 4 cell lines (2 lines of hESCs and 2 lines of hiPSCs). We evaluated the efficiency of adipocyte differentiation by Oil red O staining and the expression of adipocyte marker genes, PPARy2 and CEBPα, and examined neural differentiation potential by the expression of neural marker genes, PAX6 and NCAM. We observed high concentrations of KSR induced adipogenesis but inhibited neural differentiation in a concentration-dependent manner, except for one hESCs line, KHE3. KHE32 differentiated into adipocytes efficiently even in low concentrations of KSR, but did not differentiate into neural lineage effectively. Taken together, these results indicate that KSR, basically, negatively regulate neural differentiation but positively regulate adipocyte differentiation. Because KSR contains high concentrations of insulin (about 6.7μg/ml at 10%KSR in medium), it may have triggered a series of transcription factors regulating adipogenesis and then may have promoted the adipocyte differentiation.

**Poster Board Number: T-3225**

**TROPHOBLAST DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS TREATED WITH BMP4**

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Models appropriate for studying human trophoblast development are limited, especially ones to study transition between the undifferentiated cell and early trophoblast stages. Treating human embryonic stem cells (ESC) with the growth factor, BMP4 (termed hESC/BMP4), is a powerful means for generating trophoblast and provides a series of temporal and spatial snapshots of the changes that accompany initial differentiation from precursor pluripotent cells to the subsequent formation of sublineages as they arise from cytotrophoblast precursors. Attempts to improve differentiation to trophoblast can be achieved by blocking the signaling systems essential for maintaining pluripotency such as activin A and FGF signaling simultaneously. This involves exposure to A83-01 (an inhibitor of activin A signaling superior in both specificity and potency to SB431542) and PD173074, a fibroblast growth factor receptor inhibitor. The hESC/BMP4 system improves our understanding of the mechanisms that underpin the initial emergence
of trophoblast from its stem cell precursors as well as when and how the various trophoblast sub-lineages differentiate. If the hESC/BMP4 system can be applied to induced pluripotent stem cells (iPSC), it opens ways to study connections between genetic/epigenetic background and pregnancy disorders associated with trophoblast phenotype that have been difficult to address until now. In other words, it might allow a recapitulation of features of a prior placental phenotype after a baby is born. In our work, iPSC are routinely established by either retroviral or lentiviral transduction of reprogramming factors into somatic cells. However, as continued expression of the transgenes may interfere with downstream differentiation processes, alternative methods have been used to generate integration-free iPSC. Here we have developed a dozen lines of human iPSC from mesenchyme outgrowths of umbilical cords collected at birth either by integrating virus carrying tetracycline-inducible lentiviral vectors or by an episomal plasmid-based approach initially developed in the Yamanaka’s laboratory to establish integration-free iPSC. Four iPSC lines were established from umbilical cord mesenchyme infected with virus carrying the OCT4/SOX2/KLF4/cMYC/NANOG/LIN28 reprogramming genes at ~0.1% efficiency. Eight other primary cultures were transfected with episomal plasmids, which reprogrammed cells with about one-tenth the efficiency of retroviruses. Three of the former and one of the latter cell lines were tested for differentiation towards trophoblast in response to addition of BMP4 and the activin A/FGF inhibitors. The cell lines produced CG, P4, and PGF, indicators of advanced trophoblast sub-lineages, as efficiently as H1 and H9 hESC, with no evidence for generation of mesendoderm derivatives. Our data demonstrate that iPSC derived from umbilical cords can be converted efficiently and unidirectionally to trophoblast. This approach will allow us to examine features of sub-lineage differentiation, including invasiveness, hormone production, oxygen sensitivity, and response to various stressors implicated in placental diseases such as preeclampsia.

Poster Board Number: T-3226

APPLICATION OF DRUG-CONTROLLABLE GENETIC SWITCH FOR GENERATION OF HUMAN IPS CELLS.

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Human induced pluripotent stem cells (iPSCs) are generated by reprogramming of somatic cells through enforced expression of embryonic transcription factors. However, clinical applications require that expression of introduced transgenes must be permanently switched off in the iPS cells and obtained differentiated progenies. Here, we took advantage of epigenetic switch that relies on doxycycline(dox)-controllable binding of tTRKRAB transrepressor to tetO element. In the presence of dox tTRKRAB is sequestered from tetO allowing for transgenes expression. In contrary, dox removal allows for tTRKRAB binding to tetO that results in tight transcriptional repression of proximal promoter through heterochromatin formation. In order to apply this system for reprogramming, the tetO element was inserted into pSTEMCCA lentiviral vector carrying OCT4, SOX2, KLF4 and cMYC under control of EF-1alpha promoter. Co-transduction of human skin fibroblasts with obtained pSTEMCCA-tet and pLV-HK carrying tTRKRAB cDNA followed by culture in presence of doxycycline allowed for expression of reprogramming factors and thus efficient generation of human iPSC clones. Obtained clones were picked and further cultured in the absence of dox. Tight repression of introduced transgenes in all human iPS clones was analysed by RT-qPCR and confirmed full functionality of our system. Pluripotent phenotype of iPSC cells was revealed by analysis of endogenous embryonic genes expression using RT-PCR and cell surface protein markers by immunofluorescence. tTRKRAB-mediated epigenetic repression persisted through prolonged culture of obtained iPSC cell lines. Importantly, expression of introduced transgenes remained undetectable after differentiation into embryonic bodies. Our results confirm that our epigenetic switch effectively prohibits re-expression of embryonic transgenes in human iPSC cells and their differentiated progenies paving the way for their applications in various fields of regenerative medicine, disease modelling and drug discovery.

Poster Board Number: T-3227

PERICELLULAR MATRIX OF DECIDUA-DERIVED MESENCHYMAL CELLS IS A VERSATILE HUMAN SUBSTRATE FOR BOTH FEEDER-CELL-FREE GENERATION AND LONG-TERM MAINTENANCE OF HUMAN-INDUCED PLURIPOTENT STEM CELLS

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Human-induced pluripotent stem cells (hiPSCs) have been generated from various somatic cells by inducing defined sets of transcription factors, and the properties of these hiPSCs are similar to those of human embryonic stem cells (hESCs). Generally, the use of hiPSCs should make great contributions to the field of regenerative medicine and to our understanding of the molecular pathogenesis underlying many intractable diseases. The clinical promise of cell-based therapies using hiPSCs has driven an intense search for ideal cell sources, reprogramming methods, and cell culture systems. However, the details remain to be investigated. hiPSCs/hESCs are generated and maintained on living feeder cells, such as mouse embryonic fibroblasts or SNL cells, or on feeder-free culture substrates with Matrigel, fibronectin, vitronectin, or human recombinant laminin-511. Clinical applications require quality-controlled xenobiotic-free culture systems to minimize health risks from animal-derived pathogens and immunogens. Therefore, the use of human-derived primary living cells, such as human fibroblasts or amnion-derived cells, is a hopeful approach, although there are difficulties to overcome. Previously, we reported that the pericellular matrix of decidual-derived mesenchymal cells (PCM-DM) is an ideal human-derived material for maintaining hiPSCs/hESCs. The activity of PCM-DM in maintenance of hiPSCs/hESCs is similar to that of Matrigel and the preparation of a PCM-DM is easy and reproducible, as decidua-derived mesenchymal cells (DMCs) can be prepared and expanded in large quantities. Moreover, we reported success generating hiPSCs from DMCs on PCM-DM via the retroviral transduction of four factors: OCT4, SOX2, KLF4, and c-MYC. The established hiPSCs had stable cellular properties, such as alkaline phosphatase (ALP) activity, hESC-specific genes and surface markers, and differentiation ability in vitro and in vivo for long culture periods, when early and later passages were compared. This study
conducted a global gene expression analysis of hiPSCs cultured on PCM-DM at early and later passages in order to estimate their cellular properties in detail. We found that the expression levels of GDF3, FGF4, UTF1, and XIST varied with culture time, although the expression of these genes did not affect pluripotency. Moreover, cluster analysis showed that the differences in gene expression patterns between clones were less than those between early and later passages for each clone, and established hiPSCs tended to resemble control hiPSCs and hESCs cultured on feeder cells in terms of gene expression patterns. We also confirmed copy number variation (CNV) at early and later passages. There was no correlation between CNV and gene expression patterns, although some CNV involved genes that are often reported in hiPSCs/ hESCs cultured for long periods. These findings suggest that PCM-DM is a practical, human-derived substrate that can be used not only for the stable maintenance of hiPSCs, but also for their generation.

**Poster Board Number: T-3228**

**VALIDATION OF IN VITRO METHODS FOR DETECTION OF RESIDUAL UNDIFFERENTIATED CELLS IN RETINAL PIGMENT EPITHELIAL CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS**

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Human pluripotent stem cells (hPSCs) have the ability to differentiate into a variety of cells and to self-renew in vitro. Because of these two characteristics, it has been expected that they would provide new regenerative medicine/cell therapy. Recently, it has been getting big attention that clinical trials have been conducted with retinal pigment epithelial (RPE) cells derived from human embryonic stem cells (hESCs) to treat patients with dry age-related macular degeneration and Stargardt’s macular dystrophy in the US. For the clinical use of products derived from hPSCs, it is essential to improve both the efficacy and safety of the final product. In order to develop safe hPSC-based treatments, the hurdle of tumorigenicity arising from undifferentiated cells must be overcome. To address the issue of tumorigenicity, some recent publications have advocated the development of protocols for the derivation of hPSC and have outlined methods for the elimination of residual hPSC. However, to date, there are no capable methods for detection of a small number of residual undifferentiated cells. To locate residual undifferentiated cells, in vivo teratoma formation assays have been performed with immunodeficient animals, but more than several hundred cells are necessary for human pluripotent stem cells to form a tumor in immunocompromised mice. Therefore, highly sensitive tumorigenicity assays and their standardization are necessary for detecting a small population of residual undifferentiated cells in products derived from hPSCs. To solve this issue, we evaluated three methods for derivation of residual undifferentiated human induced pluripotent stem cells (hiPSCs) in hiPSCs-derived RPE stem cells: soft agar colony formation assay, flow cytometry and qRT-PCR. These assays were used on cell mixtures that contained defined numbers of undifferentiated hiPSCs in primary RPE cells. Although the soft agar colony formation assay unsuitable for detecting residual undifferentiated hiPSCs, the flow cytometry assay using anti-TRA-1-60 antibody detected 0.1% undifferentiated hiPSCs that were spiked in primary RPE cells. Moreover, qRT-PCR with a specific probe and primers targeting Lin28 transcripts can detect at least 0.01% contamination of residual undifferentiated hiPSCs in primary RPE cells. Based on these results, we examined whether qRT-PCR for Lin28 was applicable in the detection of residual undifferentiated cells in differentiated RPE cells from hiPSCs. As a result, RT-PCR using probes and primers targeting Lin28 transcripts can detect levels as low as 0.002% residual undifferentiated cells in hiPSC-derived RPE cells. Namely, that a single hiPSC in 50,000 RPE cells is detectable. We expect our findings to contribute to the process of validation and quality control of hiPSCs-based cell therapy products and to promote the application of regenerative medicine in the treatment of a wide variety of diseases in the near future.

**Poster Board Number: T-3229**

**GENERATION OF INTEGRATION-FREE HUMAN DENTAL-PULP-DERIVED IPS CELLS FROM HLA-HOMOZYGOUS DONORS USING SENDAI VIRUS RNA VECTORS.**

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[Abstract] Efficient and integration-free methods to generate human iPSCs are desirable for the future application of regenerative medicine with decreasing the risk of tumorigenesis. Here we show that Sendai virus (SeV) vectors, non-integrating and cytoplasmic-replicating RNA virus vectors, efficiently generated integration-free human dental-pulp-derived iPSCs (DP-iPSCs). We have established multiple DP-IPSC lines from two putative human leukocyte antigen (HLA)-homozygous donor cells (DP-74 and DP-94) that match ~20% of the Japanese population at major HLA loci. We used SeV vectors carrying four reprogramming factors independently, OCT3/4, SOX2, KLF4 and c-MYC, or two sets of three-in-one vector in different order of OCT3/4, SOX2 and KLF4 genes (KOS or OSK), with or without MYC. The highest induction efficiency was achieved when using three-in-one vector (KOS) with c-MYC. However, the phenotypes of obtained IPS colonies were similar: all the colonies tested were transgene-free and showed human ES markers and pluripotency. We conclude that SeV vectors may provide human iPSCs suitable for autologous and allogamous stem-cell therapy in the future. [Grant acknowledgement] This work was in part supported by JST, PRESTO.

**Poster Board Number: T-3230**

**NOVEL REAL-TIME PCR-BASED DNA DAMAGE QUANTIFICATION METHOD REVEALS HUMAN PLURIPOTENT STEM CELLS TO BE SELECTIVELY PROTECTED AGAINST ROS-INDUCED DNA DAMAGE**

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In this study, we present a method for real-time PCR-based quantify of DNA damage in human stem cells (long-run DNA damage quantification, LOR-DQ). The LOR-DQ method enables highly accurate, time-saving, non-radioactive, high throughput DNA damage determination. Human induced pluripotent stem cells (hiPSCs) represent a promising tool for regenerative medicine, circumventing embryonic stem (ES) cell research-associated ethical issues and ES cell-mediated xenograft complications. Tissues derived from patient-specific iPSC cells open up new avenues for the development of therapies for various diseases and provide a valuable tool for use in embryo-toxicity assays. Pluripotent stem cells have been described to be highly vulnerable to DNA damaging...
agents, such as ionizing radiation (IR) or alkylating chemicals. Applying the LORD-Q method, we demonstrate that human iPSCs are selectively less vulnerable to DNA damaging agents or radiation compared to their parental somatic cells. At sublethal dosages, hiPSC cells exhibit a higher repair capacity of genomic and mitochondrial DNA damage than human fibroblasts. Furthermore, iPSC cells are resistant to high dosages of UV radiation and reactive oxygen species. In contrast, treatment with low dosage double-strand break-inducing agents lead to substantial DNA damage initiated apoptosis. Taken together, we demonstrate that our newly developed method is a sensitive tool to monitor DNA damage repair kinetics. We found that hiPSC cells are protected against high dosages of ROS and UV radiation, but are vulnerable to DNA double-strand break-inducing agents. In addition, LORD-Q allows to discriminate between DNA damage and other cytotoxic effects caused by harmful agents or radiation. Future applications of this method may reveal the mechanisms protecting or sensitizing stem cell DNA, respectively.

**Poster Board Number:** T-3231

**THE ORIGIN OF SOMATIC MUTATIONS IN HUMAN INDUCED PLURIPOTENT STEM CELLS**

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Defined transcription factors can induce epigenetic reprogramming of adult mammalian cells into induced pluripotent stem cell lines (iPSCs). Recent studies have revealed that iPSC lines acquire fixed point mutations enriched in cancer-related genes immediately after the process of reprogramming. However, the source of these reprogramming-associated mutations remains unclear. It is unknown if many reprogramming-associated mutations occur during the reprogramming process or are inherited from rare mutations in the progenitor cell population. Here we characterize in detail the origin of reprogramming-associated mutations using novel sequencing techniques. We demonstrate that the iPSC and embryonic stem cell (ESC) genomes are relatively stable and uniform at the point mutation level during short-term culture, and that subcloning does not inherently introduce mutations. We also demonstrate a novel analysis technique that allows enhanced high-throughput detection of low-level progenitor cell mutations that have become fixed in iPSC lines. With these results, we are able to separate an elevated mutation rate during reprogramming from inheritance of rare progenitor mutations. Our findings will lead to a greater understanding of the origin of reprogramming-associated point mutations, and will aid in the development of safe iPSCs for clinical use.

**Poster Board Number:** T-3232

**SITE SPECIFIC GENOME EDITING OF HUMAN INDUCED PLURIPOTENT STEM CELLS BY PNA**

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Targeted genome editing of human induced pluripotent stem cells (iPSCs) is a useful tool in utilizing the potential of iPSC technology. Nuclease based genome editing technologies, such as Zinc-finger Nucleases (ZFNs) or TAL Effector Nucleases (TALENs), are demonstrated to be effective and efficient in human iPSCs. However, the routine use of these technologies. Here, we utilize an artificial DNA to define a digestion site to engineer a specific locus of the genome in human iPSCs. In this system, peptide nucleic acid (PNA) is used to invade into the target sequence of double strand DNA for exposing single-strand DNA (ssDNA) regions in both sides. Exposed ssDNA regions are digested by a ssDNA specific nuclease or chemical ssDNA cutter Cel(V)/EDTA complex to induce double strand break (DSB). We confirmed the activity and specificity of this system by digesting a plasmid DNA in vitro. Next, we optimized the transfection condition of Cy3-labeled PNA molecules in human iPSCs by electroporation. PNAs were efficiently introduced into nuclei assessed by confocal microscopy. No severe cytotoxicity of Cel(V)/EDTA complex was observed under working concentrations. Currently, we have been investigating the digestion activity and specificity of the system using the extracted genomic DNA. We expect this system might offer an alternative tool for editing human genome of stem cells, such as introducing a point mutation or facilitating homologous recombination.

**Poster Board Number:** T-3233

**IN VITRO NEPHROGENESIS OF HUMAN INDUCED PLURIPOTENT STEM CELLS**

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In vitro differentiation of human induced pluripotent stem cells (hiPSC) towards the renal lineage might require careful direction by providing cues in the culture medium for the activation of transcription factors related to the development of the nephros. The selection of these factors is provided by the current knowledge of organogenesis of the kidney. Attempts to induce mouse pluripotent stem cells to mature kidney cell types by applying growth factors like BMP, activin A or retinoic acid have been partially successful in deriving cells of the renal lineage, but their specific developmental identity with respect to nephrogenesis could not be deduced. Also, in vitro organ cultures from embryonic rudiments have demonstrated the necessity of multiple signals from the environment, usually accomplished only by usage of conditioned media from ureteric bud or metanephric mesenchyme cultures or by direct co-cultures. Thus, the exact combination and timely order of many morphogens and extracellular matrix components to direct renal differentiation in vitro has to be determined. The aim of our study is to screen growth factors and matrix signals to develop a renal differentiation protocol. Our methodology utilizes high content screening (HCS) to examine the effect of different concentrations, combinations and sequential addition of molecular environments on iPSC differentiation. As a tool for screening, we generated a hiPSC line that is stably transfected with a transposon containing an eGFP gene driven by the brachyury promoter as an indicator for the mesodermal differentiation and a histone (H2B)-mCherry marker that is utilized in image analysis for cell identification. Using this approach, we successfully identified conditions for the derivation of mesodermal cells that are positive for markers typical for intermediate mesoderm. Screens for further differentiation of these intermediate mesoderm cells along the renal lineage to generate cells that can be used for in vitro applications like drug screening or cell based therapies will be presented.
MOdelling neurogenesis impairment in Down syndrome disease using induced pluripotent stem cells derived from human monozygotic twins discordant for trisomy 21

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Down syndrome (DS), caused by a trisomy of chromosome 21, is the most common chromosomal disorder, having an incidence of 1 in 800 live births. It results in varying degrees of physical and mental retardation. At present, even though a few genes and anomalies are known, the pathogenic mechanisms underlying the abnormalities remain obscure. The defects described in DS are thought to be closely associated with a group of genes mapped to a specific region of human chromosome 21q22 called Down syndrome critical region (DSCR). Here, we report the generation and the characterization of induced pluripotent stem cells (iPSCs) from fibroblasts obtained from human monozygotic twins discordant for trisomy 21: Twin-N-iPSCs for the normal and Twin-DS-iPSCs for the DS-affected iPSCs. To document their development potential in vitro, iPSC cells were also characterized to confirm their potential to differentiate into neuronal progenitor cells (NPCs, day 21 of neuronal differentiation) and mature neurons (day 40 of neuronal differentiation). The derived iPSCs were evaluated to confirm the disease-specific genotype of their parental somatic cells. As revealed by karyotype and high-resolution array-based comparative genomic hybridization (aCGH) analysis, contrary to Twin-N-iPSCs, Twin-DS-iPSCs showed the characteristic trisomy 21. Neuronal differentiation of Twin-DS-iPSCs in vivo revealed an abnormal teratoma formation in NOD-SCID mice. Moreover, in vitro, Twin-DS-iPSC-derived neurospheres showed a reduced number of NPCs by comparison with the normal ones. This effect was associated with a decrease of cell proliferation and an increase of cell death. When NPCs were further induced to mature into neurons, we found structural changes in the architecture and density of neurons, glial cells and oligodendrocytes populations together with alterations in the expression of genes involved in lineage specification in neurogenesis and brain development. Furthermore, we provided new insights into the molecular mechanisms underlying these defects. In conclusion, these findings establish iPSCs generated from human monozygotic twins discordant for trisomy 21 as a unique model to recapitulate DS phenotype in vitro, study the detailed mechanisms involved in the pathogenesis of DS and design new therapies.
ALS-RELATED GENE EXPRESSION CHANGES IN DEFINED POPULATIONS OF HUMAN IPS-DERIVED MOTOR NEURONS

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ALS is a uniformly fatal disease of adults characterized by degeneration and death of motor neurons in the spinal cord. The motor neurons most severely affected are those that innervate the distal limb muscles, which derive during embryogenesis from the lateral motor column (LMC) at brachial and lumbar levels. There is much data to suggest that motor neuron degeneration in ALS results in part from a toxic cellular environment. Nevertheless, there is clear evidence in mouse models for determinants of disease onset that are motor neuron-intrinsic. We are using expression data from human motor neurons in vitro and in vivo to identify gene expression differences between ALS and controls, focusing on the vulnerable limb-innervating populations. Cultures of motor neurons from human ESC/iPS lines typically contain a majority of other cell types, a serious confound for gene expression studies. The only protocols for human motor neuron purification to date involve stable Hb9-GFP reporters, which are not feasible to generate for large collections of iPS lines. We therefore developed a method for motor neuron enrichment that is applicable to any hiPSC line. Following infection with a lentiviral vector expressing Hb9-RFP (a gift from C. Marchetto and F. Gage), mixed cultures were allowed to mature and then FACS sorted. The resulting populations contained ~70% of neurons positive for the marker ISL1. Enriched motor neurons from 4 ALS lines with SOD1 mutations and 2 controls were profiled on Affymetrix microarrays. We used a combination of microarray and computational methods to identify differentially expressed genes and resulted in amino acid changes. As expected, mtDNA expression studies. The only protocols for human motor neuron purification to date involve stable Hb9-GFP reporters, which are not feasible to generate for large collections of iPS lines. We therefore developed a method for motor neuron enrichment that is applicable to any hiPSC line. Following infection with a lentiviral vector expressing Hb9-RFP (a gift from C. Marchetto and F. Gage), mixed cultures were allowed to mature and then FACS sorted. The resulting populations contained ~70% of neurons positive for the marker ISL1. Enriched motor neurons from 4 ALS lines with SOD1 mutations and 2 controls were profiled on Affymetrix microarrays. These motor neurons were immature and had not been exposed to ALS-related stressors so, as expected, many genes showed little change. However, a subset of genes showed strong differences in expression levels between ALS and controls. Many of these genes are related to ALS-relevant properties such as hyperexcitability, excitotoxicity and specificity for limb innervation. This suggests that motor neuron-intrinsic differences related to ALS appear early during maturation and therefore may be accessible to study in culture. In order to study the influence of these and other pathways on ALS-related phenotypes in culture, it will likely be necessary to focus on the most vulnerable, limb-innervating, motor neurons. Using the Hox cofactor FoxP1 as a marker for LMC motor neurons we have developed a protocol for enrichment of such cells using early neuralization and modulation of hedgehog signaling. This combination of small molecules generates cultures containing up to 50% of human motor neurons in only 3 weeks. Of these, >70% were limb-innervating motor neurons of the LMCm division, which innervates flexor muscles of the distal limb. These will be a valuable tool for many studies of human motor neuron phenotypes in ALS. This work was funded by Project A.L.S., P2ALS, NYSAl, Helmsley Foundation and NINDS (NRSA fellowship to DHO).
ASSESSMENT OF AN IMAGING PROTOCOL FOR THE REAL TIME SELECTION OF HUMAN INDUCED PLURIPOTENT STEM CELL COLONIES USING LIVE CELL MICROSCOPY AND IMAGE RECOGNITION SOFTWARE

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The ability to reprogram somatic cells to an embryonic stem cell-like state has had landmark impact on basic biological research, drug screening, and drug discovery. However, induced pluripotent stem (iPS) cell colony selection remains technically challenging, while characterization of pluripotency is costly and time consuming. Tools that can reliably select fully reprogrammed iPS colonies in real time would be extremely useful to standardize selection, and drive down costs through the selection and expansion of fewer colonies. In addition, it is desirable that such a tool could work using only brightfield imaging as not all reprogramming technologies make use of reporter genes and thus can generate fluorescence images. Previously, we have created a custom image recognition analytic using CL-Quant (Nikon Corporation, Yokohama) for the classification of human iPS colonies in fibroblasts undergoing reprogramming using Klf4, Oct3/4, Sox2 and c-Myc, and achieved a classification accuracy of 99.83% (just 7 errors from 4077 colonies) using only the phase contrast images without fluorescence information. In this study, we have acquired 9 time-lapse, 20 x 20 composite image sequences of the complete 10cm dish at 2x and 4x magnifications. These image sequences show the reprogramming of Spinal Muscular Atrophy (SMA) fibroblast lines imaged at 6 hour intervals for four weeks using a cell culture observation system, BioStation CT (Nikon Corporation, Yokohama). To assess CL-Quant classification performance, iPS-like colonies are selected manually at four weeks post transduction on the basis of their morphological appearance and viral-GFP silencing. We assess 1) the best time point for CL-Quant selection using single time point metrics, 2) the improvement (if any) in selection accuracy from using temporal metrics characterizing the colonies over time from their earliest formation events, and 3) the most useful temporal metrics for colony selection and illustrate their time courses using data and images. Our hypothesis is that incorporating temporal metrics can improve the performance over single point metrics alone in selection of fully reprogrammed iPS colonies. In conclusion, we present an optimal imaging protocol for the real time selection of iPS colonies.

DNA METHYLATION ANALYSIS OF MOUSE INDUCED PLURIPOTENT STEM CELLS USING BISULPHATE TREATED WHOLE-GENOME SEQUENCING.

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iPS cells have several advantages for stem cell research and regenerative medicine including a potentially limitless supply of patient-matched cells for cell-based therapies without the requirement for human embryonic donor tissue. iPS cells may be used for therapeutics, in vitro modelling of human complex diseases, and drug development. Somatic cell reprogramming involves epigenetic remodelling of chromatin architecture including methylation, conferring iPS cells with characteristics similar to embryonic stem (ES) cells. However, it remains unknown whether the re-establishment of an ES-cell-like DNA methylation pattern occurs throughout the genome, and how the methylation pattern changes during the reprogramming process. To address these questions, we utilized a secondary inducible reprogramming system developed in mouse using piggyBac-mediated transposition of the four reprogramming factors[1]. Here we report whole-genome DNA methylation profiles at single-base resolution for mouse ES cells, secondary mouse embryonic fibroblast (MEF), secondary factor independent iPS cells induced from these MEFs, and cells undergoing reprogramming at Day 8, using whole genome bisulphite sequencing. Hiseq 2000 was used for the whole genome sequencing, and Bismark was used as an alignment tool. We defined differentially methylated regions (DMRs) and partially methylated domains(PMDs) in Cpg, CHG, and CHH context. iPS cells show significant reprogramming variability, including somatic memory and aberrant reprogramming of DNA methylation. 1. Wolten K, Michael IP, Mohseni Pet al. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature. 2009; 458:766-770.
Scalable culture systems for propagation/differentiation of induced pluripotent stem cells (iPSCs) are strongly expected for their uses in regenerative medicine or cell-based drug screening. Generally, stirred suspension bioreactors are widely used in mass production, but their direct use for iPSCs culture and differentiation usually results in unignorable cellular damage by shear stress or extensive and inhomogeneous cell aggregation that causes uncontrollable differentiation. To overcome these problems, some researchers reported the hydrogel-based encapsulation. However, these reports are still in a preliminary stage and few reports refer to the biological effect of microcapsule to iPSCs differentiation. In previous ISSCR meeting, we showed three types of micro (Ø= 600 µm) encapsulation system, calcium alginate gel with or without alginate-PLL layers, and with or without EDTA treatment (Naked group, Coated group, and Hollow group respectively[1]), and that iPSCs leaked from the NAKED beads. In this report, we evaluated leakage and undifferentiation of iPSCs in hydro-gel based microcapsule. We encapsulated mouse iPSCs (IPS-MEF-Ng-20D-17; Riken BRC Cell Bank) that have GFP under the control of Nanog promoter. After 10 days culture, DNA amount measurement and RT-PCR were performed. As a control, same evaluation was performed in direct suspension culture (suspended group). In the NAKED group, cell leakage was observed and we obtained ununiform aggregates (250-900 µm) outside and small uniform aggregate (100-300 µm) inside. After 10 days, cells expanded 3-fold but 30% of cells were leaked from the NAKED beads. The large (Ø = 1 mm) NAKED beads could only just regulate cell leakage to some extent (10-15% leakage). On the other hand, that leakage was remarkably suppressed by PLL coating (2-3% leakage). It showed that NAKED beads are not suitable for mass culture and investigating biological effect of microcapsule because of the cellular leakage from the capsules, which few reports refer to. We obtained 6 times of cells from COATED beads and 9 times of cells from Hollow beads and SUSPENDED. In COATED beads, cells made different size of lens-shape aggregates (300-600 µm). This is because cells were surrounded by hydrogel and couldn’t get together. On the other hand, uniform spherical aggregates (400-550 µm) were observed in Hollow beads, because cells could move and get together in the Hollow beads. On the other hand, these experiments showed issue of low expansion for mass culture, which is common to microencapsulation. In order to apply microencapsulation for mass culture, it is necessary to modify material or encapsulation process, which promote cell growth. In SUSPENSION system, cells expanded more quickly than encapsulated systems. However, various sizes of aggregates (150-350 µm) and some large cell clumps (550-1000 µm), which lost nanog-GFP fluorescence, were observed. RT-PCR experiment and nanog-GFP fluorescence observation showed that cells kept undifferentiated for 10 days in each type of capsules, but decrease of undifferentiation marker gene expression was observed in SUSPENDED group. Throughout these experiments, we concluded that microencapsulation is better to keep undifferentiation than suspension system but expansion is not enough for mass culture. Now we are investigating early differentiation in microcapsule with a PLL layer.

Cyclin D1 is one of important molecule involved in alteration of cell cycle regulators in normal and cancer cells. Expression of Cyclin D1 can be attributed to many factors including increased transcription, translation, and protein stability. Cyclin D1 plays roles as well as a key sensor and integrator of extracellular signals of cells in S to G1 phase that mediate its function through binding the CDKs for modulating local chromatin structure of the genes that are functions in regulation of cell proliferation and differentiation. Although many studies reported that Cyclin D1 is frequently overexpressed in cancers, but its expression and biological functions in stem cells are not clear. Cell reprogramming can convert somatic cells to ESC-like cells that was named "induced pluripotent stem cells (iPSCs)", by using direct transduction of a cocktail composed of only four pluripotent transcription factors: Oct4, Sox2, KIf4 and c-Myc. We speculate that Cyclin D1 may play novel function in cell reprogramming processes and in regulation of cell proliferation and differentiation in stem cells. In our study, we performed Cyclin D1 gene expression in mouse induced pluripotent stem cells (miPSCs), mouse embryonic stem cells (mESCs) and mouse bone marrow-derived mesenchymal stromal cells (mBMSCs) with comparing mouse embryonic fibroblasts (MEF) using quantitative reverse transcription polymerase chain reaction (qRT-PCR). To compare MEF for Cyclin D1 mRNA level, 54% of expression in BMSCs and 28% of expression in mESCs were accounted respectively. A significant decreased Cyclin D1 mRNA expression was detected in iPSCs. Our results indicated an alteration of Cyclin D1 gene expression in different pluripotency of stem cells. We hypothesize that Cyclin D1 gene expression would be inhibited by novel mechanisms in processes of cell pluripotency and reprogramming.

Somatic nuclei are reprogrammed by fusion with embryonic stem cells (ESCs) and nuclear transfer into an enucleated oocyte, suggesting that ESCs and oocytes contain a factor capable of reprogramming somatic cells. In recent works, reprogramming of somatic cells to a pluripotent state was achieved by defined factors which are essential to the ESC identity. However, the defined factors-mediated reprogramming was shown to be lower efficiency and slower kinetics than that of the cell fusion- and the nuclear transfer-mediated reprogramming. In addition, comprehensive analyses revealed that induced pluripotent stem cells (iPSCs) generated by four transcription factors (4F; Oct3/4, Sox2, KIf4 and c-Myc)-based method are very similar but not identical to ESCs. These results prompt us to hypothesize that any additional factors are required for efficient and proper iPSC generation. Here we demonstrate that

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Poster Board Number: T-3242

PROLIFERATION, MORPHOLOGY AND UNDIFFERENTIATION OF MURINE IPS CELL IN ALGINATE BASE MICROCAPSULE

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Poster Board Number: T-3243

CYCLIN D1 GENE EXPRESSION IN MOUSE BMMSCS, ESCS AND IPSCS

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Cyclin D1 is one of important molecule involved in alteration of cell cycle regulators in normal and cancer cells. Expression of Cyclin D1 can be attributed to many factors including increased transcription, translation, and protein stability. Cyclin D1 plays roles as well as a key sensor and integrator of extracellular signals of cells in S to G1 phase that mediate its function through binding the CDKs for modulating local chromatin structure of the genes that are functions in regulation of cell proliferation and differentiation. Although many studies reported that Cyclin D1 is frequently overexpressed in cancers, but its expression and biological functions in stem cells are not clear. Cell reprogramming can convert somatic cells to ESC-like cells that was named “induced pluripotent stem cells (iPSCs)”, by using direct transduction of a cocktail composed of only four pluripotent transcription factors: Oct4, Sox2, KIf4 and c-Myc. We speculate that Cyclin D1 may play novel function in cell reprogramming processes and in regulation of cell proliferation and differentiation in stem cells. In our study, we performed Cyclin D1 gene expression in mouse induced pluripotent stem cells (miPSCs), mouse embryonic stem cells (mESCs) and mouse bone marrow-derived mesenchymal stromal cells (mBMSCs) with comparing mouse embryonic fibroblasts (MEF) using quantitative reverse transcription polymerase chain reaction (qRT-PCR). To compare MEF for Cyclin D1 mRNA level, 54% of expression in BMSCs and 28% of expression in mESCs were accounted respectively. A significant decreased Cyclin D1 mRNA expression was detected in iPSCs. Our results indicated an alteration of Cyclin D1 gene expression in different pluripotency of stem cells. We hypothesize that Cyclin D1 gene expression would be inhibited by novel mechanisms in processes of cell pluripotency and reprogramming.

Poster Board Number: T-3244

PROMOTION OF MOUSE IPSC GENERATION BY HIGHLY EXPRESSED GENES IN BOTH ESCS AND OOCYTES

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Somatic nuclei are reprogrammed by fusion with embryonic stem cells (ESCs) and nuclear transfer into an enucleated oocyte, suggesting that ESCs and oocytes contain a factor capable of reprogramming somatic cells. In recent works, reprogramming of somatic cells to a pluripotent state was achieved by defined factors which are essential to the ESC identity. However, the defined factors-mediated reprogramming was shown to be lower efficiency and slower kinetics than that of the cell fusion- and the nuclear transfer-mediated reprogramming. In addition, comprehensive analyses revealed that induced pluripotent stem cells (iPSCs) generated by four transcription factors (4F; Oct3/4, Sox2, KIf4, and c-Myc)-based method are very similar but not identical to ESCs. These results prompt us to hypothesize that any additional factors are required for efficient and proper iPSC generation. Here we demonstrate that
mouse iPSC generation is promoted by highly expressed genes in both ESCs and oocytes. Using the BioGPS database, we identified genes highly expressed in mouse ESCs and oocytes, and cloned 39 candidate genes into the pMXs retrovirus vector. Mouse iPSCs were generated from mouse embryonic fibroblasts (MEFs) containing the Nanog-GFP-IREs-Puro’ reporter by retroviral transduction of 4F and an additional gene. We counted the numbers of GFP-positive colonies after 12 days transduction, and found that 6 genes could promote miPSC generation over six fold compared with the control. We next examined whether the gene X, which promotes miPSC generation most efficiently and reproducibly among these six genes, was associated with 4F. Co-immunoprecipitation assay showed that the gene X was associated with Sox2, Klf4, and c-Myc but not with Oct3/4, suggesting that the gene X promotes miPSC generation through Sox2, Klf4, and c-Myc. These findings propose genes that highly expressed in both ESCs and oocytes have iPSC generation-promoting potency and provide new insights into the mechanisms underlying somatic cell reprogramming.

Poster Board Number: T-3245

A COMPARISON BETWEEN IPSCS AND ESCS REVEALS REPROGRAMMING-ASSOCIATED POINT MUTATIONS

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In order to address the issue of whether IPS cells generation is mutagenic; we compared the point mutation profiles between iPSCs and ESCs with genome-wide sequencing. To exclude factors that could influence point mutation analysis, we established ESC clones and genome integration-free iPSC clones under identical culture condition besides the use of feeder for ESCs generation. Two iPSC clones, 2A-4F-118 and 2A-4F-136, and two ESC lines, B6ES2-2 and B6ES2-9, were used for current study, and their doubling times were indistinguishable and germline transmission was verified for all cell lines. Genome sequences including intergenic regions were determined with pair-end method using HiSeq2000. Consequently, over 90% of entire genome were covered and 55% of it were analyzed at least 10-fold redundancy. Although it must be needed to obtain the corresponding parent and donor cells for identifying single nucleotide variations (SNVs) in iPSCs or ESCs, in human obtaining of them for ESCs is almost impossible. In the present analysis, we employed an inbred strain, C57BL/6J, of which whole genome sequences have been determined, and collected corresponding parent and donor cells in advance. As a result of the genome sequencing of samples and their corresponding parent cells, we could compare 31.9-46.4% of entire genome with high redundancy. 269 and 307 for 2A-4F-118 and 2A-4F-136, and 31 and 13 for B6ES2-2 and B6ES2-9 were thereby identified. All SNVs showed a heterozygous pattern in iPSCs and ESCs but showed a homozygous pattern in the corresponding donor embryo and cells. Next, we conducted Sanger sequencing on 104 SNVs candidates randomly chosen from the pool of candidates for testing our informatics screening and then all of them were verified; all candidates were observed in ESCs or iPSCs in a heterozygous fashion, but not detected in their parent cells. We therefore concluded that SNV candidates predicted by informatics were true SNVs. Our data demonstrate 8.7-23.6 fold higher frequency in iPSCs than ESCs, and suggest the presence of more than 1,000 point mutations in iPSCs genomes. Furthermore, surprisingly, we found unique point mutation profiles in iPSC genomes. Point mutation profiles of iPSCs exhibit transition preference. Although in ESCs 30.8-35.5% of point mutations were transversion, in iPSCs those increased by 52.8-57.3%. While transition was also revealed to be increased, transversion rate was increased drastically. In addition, transversion preference was observed only during genome reprogramming, was not observed before and after the reprogramming process. Our observation through the comparison between iPSCs and ESCs clearly demonstrated that iPSCs generation is mutagenic, and the presence of reprogramming-associated point mutations that occur in a transversion-predominant manner. This shed light on the mechanism of iPSCs generation. While this raise concerns for the potential for the clinical use of iPSCs, a mechanistic understanding could resolve the issue.

Poster Board Number: T-3246

INvolvement of Adrenergic Receptors on Proliferation and Differentiation of Mouse Induced Pluripotent Stem Cells

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[Introduction] Previous studies reported that adrenaline stimulates the proliferation of mouse embryonic stem (ES) cells through adrenergic receptors (AR). Mouse induced pluripotent stem (iPSC) cells display properties of self-renewal and pluripotency similar to ES cells, but the effects of AR on the proliferation of iPSCs remains unknown. On the other hand, it was reported that cyclic AMP (cAMP) activation in mesenchymal stem cells may induce neural differentiation. As it is known that activation of β-AR increases cAMP formation, it is hypothesized that stimulation with β-AR on mouse iPS cell may be involved in neural differentiation. In the present study, we examined whether AR stimulation regulates the proliferation or neural differentiation of mouse iPSCs. [Materials and Methods] Mouse iPSC cells were cultured under feeder-free conditions in the presence of leukemia inhibitory factor (LIF). Twenty-four hours after re-plating, the cells were treated by l-adrenaline, l-phenylephrine (l-AR agonist), or prazosin (l-AR antagonist) for 8–24 h. Proliferation or DNA synthesis of mouse iPS cells was examined by MTT assay or by BrdU incorporation assay. Mouse iPSC cell differentiation was initiated by embryoid body (EB) formation under LIF-free condition. The EBs were stimulated with all trans retinoic acid (ATRA; 1 μM) and/or l-isoproterenol (β-AR agonist; 0.1–3 μM) for 4 days and then transferred to fibronectin-coated dishes. The differentiation potential from mouse iPSC cells into neural progenitor cells was evaluated by Nestin expression using immunofluorescence staining or western blot analysis. [Results] The stimulation with l-adrenaline or l-phenylephrine (300 nM) significantly enhanced the DNA synthesis and proliferation of the mouse iPSC cells. Pretreatment with prazosin (1 μM) significantly reduced the DNA synthesis and proliferation enhanced by l-adrenaline or l-phenylephrine. Although treatment with l-isoproterenol (3 μM) alone did not affect Nestin expression in the differentiated cells, the treatment significantly enhanced ATRA-induced Nestin expression. The effect of l-isoproterenol was significantly inhibited by pretreatment with atenolol (a specific β-AR antagonist; 1 μM). [Conclusion] These results suggest that β-1-AR stimulation may enhance the proliferation of mouse iPSC cells and β-1-AR stimulation may enhance the neural differentiation of the cells.
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**Poster Board Number: T-3247**

**A COMPARISON BETWEEN IMMUNOGENICITY OF IPSCS AND OF ESCS**

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Recently, an important study that showed an immunogenicity of induced pluripotent stem cells (iPSCs) was reported. Using an inbred mouse strain, C57BL/6, induced T-cell-dependent immune response to teratoma was demonstrated in syngenic recipients. Here we established many lines of embryonic stem cells (ESCs). In addition to integration-free iPSCs from C57BL/6 for obtaining a conclusion on this issue. Fully reprogrammed state was verified by germline transmission test for the most lines. First, we performed teratoma formation test. Slightly efficient formation was observed in iPSCs, but basically little difference in incidence was observed between iPSCs and ESCs. Furthermore, we could not detect meaningful T-cell-infiltration not only in the teratomas derived from iPSCs but also from ESCs. In addition, we examined the expression of Homad1 and Zg16, but their expressions in the teratomas derived from iPSCs were lower than those in ESCs. Thus, unexpectedly, even using a large number of ESCs and iPSCs, we could not detect any differences between these two types of pluripotent stem cells. Second, we assessed the immunogenicity of the differentiated cells derived from iPSCs and ESCs, dermal tissue and bone marrow. Donor tissues were prepared from 2N chimera and/or 4N chimera mice; to completely exclude the recipient derived cells from donor tissues, we used 100% chimera only that were generated by aggregation with GFP-mice embryos. The tissues that were confirmed to be GFP-negative, were transplanted. Consequently, even in the cases focusing on differentiated cells, we also observed little difference not only in incidence but also in T-cell response; almost all transplantations were succeeded and very few T-cells were observed within the transplanted tissues. Additionally, transplantation of bone marrow cells into recipient mice without X-ray irradiation, was also succeeded and hematopoietic reconstitution was achieved two months later. Thus, in the present study we could not observe immunogenicity of iPSCs and ESCs-derived tissues. There is little difference in immunogenicity between iPSCs and ESCs.

**Poster Board Number: T-3248**

**NEURONAL DIFFERENTIATION OF MOUSE INDUCED PLURIPOTENT STEM CELLS USING CONDITION MEDIUM OF DORSAL ROOT GANGLION AND ROCK INHIBITOR**

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Mouse induced pluripotent stem (iPS) cells were directly generated from mouse fibroblasts by introducing four genes, Oct3/4, Sox2, c-Myc, and Klf4. The iPS cells exhibit the morphology and growth properties of embryonic stem (ES) cells and ES cell marker genes, and may have a pluripotent ability to differentiate into various cell lineages in vitro including neurons. The in vitro differentiation of iPS cells provides new perspectives for studying the cellular and molecular mechanisms of early development and the generation of donor cells for transplantation therapies. Because we have already reported the differentiation of mouse ES cells into neurons using conditioned medium of dorsal root ganglia (DRG-CM), we investigated the differentiation of iPS cells into neurons using DRG-CM and ROCK inhibitor. Recently, it was reported that ROCK inhibitor (Y-27632), a specific inhibitor for Rho-dependent protein kinase (ROCK), permits the survival of dissociated human ES cells by efficiently blocking the dissociation-induced cell death (apoptosis). Mouse iPS cells (APS0001; RIKEN Cell Bank, Saitama, Japan) were grown on a mitotically inactivated mouse embryonic fibroblast feeder layer in the presence of leukemia inhibitory factor (LIF). The iPS cells appeared to be maintained in an undifferentiated state until passage 17-19. To form iPS cell colonies, iPS cells were transferred to nonadhesive plastic dishes. The number of iPS cell colonies (approximately 200 μm in diameter) increased until 6 of cultivation. To achieve the directed differentiation of iPS cells into neurons, iPS cell colonies were cultivated in DMEM/F-12K medium containing DRG-CM. The iPS cell colonies effectively differentiated into neurons when they were cultivated for 6-12 days with the culture medium containing 5-10% DRG-CM. Gene expression analysis using RT-PCR appeared that nestin (a marker for neural stem cell) gradually increased and TH (a marker for dopaminergic neuron) increased at day 9. On the other hand, we investigated the growth of dissociated iPS cells by the addition of Y-27632. When iPS cells were plated 500 cells/well with a growth medium containing 10 μM of Y-27632, the iPS cells proliferated very well. Then, we investigated the differentiation of dissociated iPS cells into neurons by the addition of Y-27632. Dissociated iPS cells were plated 5000 cells/well in a gelatin-coated 96-well culture plate with a differentiation medium containing 10 μM of Y-27632. After 12 days of cultivation, many iPS cells differentiated into neurons compared to control. In addition, the iPS cells efficiently differentiated into neurons by the addition of both 10 μM of Y-27632 and 10 ng/ml of nerve growth factor (NGF). These results show that iPS cells could be effectively induced to differentiate into neurons using DRG-CM in a similar manner as ES cells and the addition of Y-27632 to the culture medium is useful to the growth and differentiation of iPS cells.

**Poster Board Number: T-3249**

**MOUSE IPS CELL TRANSPLANTATION WITH WHISKER STIMULATION INCREASES ENDOGENOUS MIGRATION IN A NEONATAL STROKE MODEL**

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Stroke is a leading cause of death and disability world wide. More specifically, neonatal stroke affects 26 of 100,000 live births each year, yet there are limited treatments available to repair stroke-damaged tissue. Induced pluripotent stem (iPS) cell transplantation is currently being explored as a possible therapeutic. In previous work, transplantation of embryonic stem (ES) cells has been shown to increase the recruitment of progenitors to the infarct. Similarly, in our previous work using the whisker barrel cortex stroke model, we demonstrated that whisker stimulation also increases progenitor migration to the infarct area suggesting that increasing thalamocortical afferent signals to the damaged barrel cortex may serve as a guiding signal to migrating progenitors for regeneration. Therefore, we hypothesize that iPS cells and whisker stimulation together promote migration and neurogenesis in a neonatal rat stroke model. Whisker barrel cortex mini-stroke was induced in P7 neonatal rat pups through a temporary occlusion of the common carotid artery and permanent occlusion of the middle cerebral artery. Seven days after stroke, 400,000 iPS cell-derived neural
progenitor cells were transplanted into the stroke penumbra. The rats received daily BrdU injections to label proliferating cells and whisker stimulations 3 times a day for 5 minutes each. Rats were sacrificed 21 days after stroke and examined for BrdU co-labeled with NeuN (neuronal marker) or Collagen IV (vessel marker) in the infarct penumbra. Pluripotent iPSCs were stained for trophic factor expression in vitro and terminally differentiated IPS cell-derived neurons were patch-clamped to record sodium and potassium currents. Rats transplanted with IPS cell-derived progenitors exhibited greater numbers of BrdU-, NeuN/BrdU-, and collagen IV/ BrdU-labeled cells in the penumbra compared to rats with stroke only. Rats that received both whisker stimulation and transplantation had the greatest number of BrdU-labeled, NeuN/BrdU and collagen IV/BrdU cells in the ischemic penumbra. Immunocytochemistry of iPSCs in vitro showed expression of several trophic factors involved in neurogenesis, cell survival, and cell migration including SDF-1, FGF, EPO, and GDNF. Mature IPS cell-derived neurons were patch-clamped and exhibited neuronal functionality with inward sodium and outward potassium currents in vitro. IPS cell-derived progenitor transplantation and whisker stimulation may promote neurogenesis and angiogenesis after an ischemic barrel cortex infarct in neonatal rats. The increased NeuN/BrdU and collagen IV/ BrdU cells suggests increased neurovascular remodeling with whisker stimulation and IPS cell-derived neural progenitor transplantation. Transplantation of IPS cell-derived neural progenitors may contribute additional trophic support that increases endogenous migration to the infarct, endogenous neurogenesis, and transplant cell survival. The combination of whisker stimulation and transplantation therapy shows great potential as a synergistic therapeutic strategy for neonatal ischemic stroke.

Poster Board Number: T-3250

A HIERARCHY OF REPROGRAMMING POTENCY IN MOUSE LIVER

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Ectopic expression of certain transcription factors can reprogram somatic cells to a pluripotent state. It has been shown that hematopoietic stem cells can be reprogrammed with a higher efficiency than differentiated blood cells. Similar findings have not been demonstrated in other primary organ systems. Moreover, molecular characteristics in the cellular hierarchy of tissues that influence reprogramming capacities need to be delineated. Here, we analyzed the influence of the differentiation stage of freshly isolated, murine liver cells on the reprogramming efficiency. Liver progenitor cell (LPC)-enriched cell fractions from fetal and adult liver showed a significantly increased reprogramming efficiency after transduction of 3 or 4 reprogramming factors. Transfection efficiency corrected reprogramming rates of fetal LPCs were 275 fold higher compared to un-sorted fetal liver cells when 3 reprogramming factors were transfected. LPC-derived IPS cells showed all hallmark features of pluripotency. The increased reprogramming efficiency of LPCs compared to differentiated liver cells occurred independent of proliferation rates but associated with the endogenous expression of reprogramming factors (Klf4 and c-Myc) and two members of the BAF-complex (Baf155 and Brg1) mediating epigenetic changes during reprogramming. Knockdown of BAF-complex members abolished the increased reprogramming efficiency of LPCs vs. non-LPCs. The current study provides the first functional evidence that LPCs carry intrinsic, cell proliferation-independent characteristics allowing highly efficient reprogramming compared to differentiated liver cells.
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Poster Board Number: T-3252

ESTABLISHMENT OF IPS CELLS FROM AMYOTROPHIC LATERAL SCLEROSIS MODEL MICE AND MOTOR NEURONAL DIFFERENTIATION

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Subject: To unravel pathogenesis of amyotrophic lateral sclerosis (ALS), methods using induced pluripotent stem cells (iPS cells) are promising. We establish iPS cells from ALS model mice, mutant superoxide dismutase1 (SOD1) transgenic mice, induce neural differentiation and unravel pathogenesis of ALS. Methods: We introduced known four factors (Oct3/4, Sox2, Klf4, c-Myc) into mouse embryonic fibroblasts obtained from crossbreeding of SOD1G93A mice with Nanog-GFP-IRE-PS-Puror mice using retroviral vectors and cultured them on SNL feeder cells. We picked ES cell-like colonies, cultured them and confirmed establishment of iPS cells using ES cell markers. Then we induced direct differentiation using retinoic acid and Smoothened agonist and confirmed motor neuronal identity by immunocytochemistry. Results: We established iPS cells from mutant SOD1 mice and control mice and confirmed motor neuronal differentiation. Conclusions: These results indicate that iPS cells from ALS model mice possess properties of ES cells with mutant SOD1 and may contribute to establishment of ALS model(s) in vitro.

Poster Board Number: T-3253

COMPARATIVE ANALYSIS OF DOPAMINERGIC NEURON DIFFERENTIATION EFFICACY FROM MOUSE EMBRYONIC STEM CELLS AND PROTEIN- BASED IPS CELLS

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During the progression of Parkinson’s disease, stem cells can act as therapeutic agents to recover, or regenerate injured nervous system. In this study, two types of stem cells; mouse embryonic stem cells (mESCs) and protein-derived iPS cells (P-iPSCs), generated by non-viral methods, were differentiated into midbrain dopaminergic (mDA) neurons and compared its efficiency. In the undifferentiated stage, P-iPSCs expressed pluripotency markers without any difference to that of mESCs elucidating the fact that protein-based reprogramming was successful and stable as authentic ES cells. All two types of cells reached terminal matured mDA neurons while P-iPSCs showed more mDA neuron positive markers in protein levels as well as in mRNA levels when compared with mES cells. To investigate the mechanism of significantly advanced induction of mDA neurons in P-iPSCs, we analyzed histone modifications by genome-wide ChIP sequencing analysis and their corresponding microarray results. We found Wnt signaling was up-regulated while SFRP1, known as its counter-actor was suppressed more in P-iPSCs than mESCs. Moreover, dramatic change of expression level of epigenetic regulator, Sirt1 may be associated with differentiation efficiency. In 6-OHDA-induced Parkinson’s rat model, both types of transplanted neural precursor cells showed rescued motor activity and migration in damaged striatum. Our results demonstrate that P-iPSCs can be an ultimate source for patient-specific cell therapy and efficient to replace damaged neurons in vivo as well.

Poster Board Number: T-3254

DISRUPTED STEM CELL HOMEOSTASIS IN PREMAUTERLY AGING MICE WITH MTDNA MUTAGENESIS

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A proof-reading deficient mitochondrial DNA polymerase gamma in the transgenic mtDNA mutator mouse results in accumulation of point-mutations in the mtDNA, defects in the respiratory chain complex subunits and a mild respiratory chain deficiency in aged mice. The mtDNA mutator mouse develops several premature aging symptoms, such as anemia, reduced subcutaneous fat, kyphosis, alopecia, osteoporosis, sarcopenia and reduced life span. The mild respiratory chain phenotype seen in post-mitotic tissues does not explain the aging related symptoms seen in proliferating tissues. We have shown a disrupted somatic stem and progenitor cell homeostasis in mutator neural stem (NSC) and hematopoietic progenitor (HPC) cell compartments. This stem cell dysfunction is present already during embryonic life, long before any symptoms arise, or before any evidence of respiratory chain dysfunction. Further, this stem cell dysfunction can be ameliorated by treatment with antioxidant (N-acetyl-cysteine; NAC), suggesting that an imbalance in the redox regulation or a subtle increase in ROS (reactive oxygen species) production underlies the stem cell phenotype. Based on our results on somatic stem cells, we suggest that the mutator mouse have two separate dysfunctions. 1) The mild respiratory chain dysfunction that manifests in post-mitotic tissues of aged mice, and 2) an early imbalance in the cellular ROS/redox regulation that causes somatic stem cell dysfunction. To provide further evidence for a “stemness” defect in mutators we set out to generate induced pluripotent stem (iPS) cells from mutators. While we were successful in reprogramming mutator fibroblasts, the efficiency of reprogramming was significantly reduced when compared to wild-type cells. Further, mutator fibroblasts treated with NAC showed a significant increase in colony formation when compared to untreated cells. The established mutator iPS cell lines had ES-like characteristics, but showed severe growth defect as well as reduced ability to self-renew. While the growth defect was not affected by the antioxidant treatment, the treated mutator iPS lines showed self-renewal ability comparable to wild-type iPS lines. These data indicate that both somatic and pluripotent stem cells, are especially sensitive to mitochondrial dysfunction and that the mechanism involves ROS/redox homeostasis.

Poster Board Number: T-3255

CO-TRANSPLANTATION OF MOUSE IPS DERIVED CARDIOMYOCYTES AND MESENCHYMAL STEM CELLS FOR THE REGENERATION OF INFARCTED MYOCARDIUM

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Introduction: Cardiomyocytes generated from induced pluripotent stem cells (iPS-CM) hold great promise as a sustainable and autologous source of cells with potential for the regeneration of infarcted myocardium. The goal of this study was to analyze the efficacy of a cell transplantation strategy using iPS-CM in combination with
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mesenchymal stem cells (MSC). Methods: A murine iPS cell line was genetically engineered to express antibiotic resistance and EGFP under the control of cardiac α-myosin-heavy-chain promoter and, additionally, firefly luciferase driven by a constitutive ubiquitin C promoter. iPS-CM were generated by puromycin selection in a mass culture differentiation system and their purity and quality was assessed by flow cytometry, immunocytochemistry and qPCR. Murine MSC were isolated from bone marrow and cell identity was confirmed by in vitro differentiation and flow cytometry. A myocardial infarction was induced in syngeneic mice by cryoinjury followed by intra-myocardial transplantation of 5x10⁶ iPS-CM, MSC or a combination of both. The retention of cells over the course of four weeks was assessed by in vivo bioluminescent imaging of iPS-CM and detection of paramagnetically labeled MSC using a clinical 3T magnetic resonance imaging (MRI) scanner. MRI was also used for the assessment of cardiac pump function (left ventricular ejection fraction, LV-EF) at weekly intervals. Immunohistochemical analyses were performed to locate the transplanted cells on a microscopic level. Results: Puromycin-selected iPS-CM were more than 95% pure, devoid of contaminating pluripotent stem cells and did not cause teratoma formation in immunodeficient animals. iPS-CM expressed α-actinin and cardiac troponin T and exhibited the sarcomeric organization typical of immature cardiomyocytes. MSC expressed CD44 (64.1±4.1%) and Sca-1 (98.1±0.9%) and showed in vitro adipogenic, chondrogenic and osteogenic differentiation potential. After transplantation into cryo-infarcted hearts LV-EF increased significantly for iPS-CM transplanted and iPS-CM/MSC co-transplanted animals as compared to MSC transplanted and sham operated animals (LV-EF, four weeks after transplantation - sham: 44.2±2.6%; iPS-CM: 51.8±3.3%; MSC: 47.6±1.9%; iPS-CM+MSC: 55.7±2.3%; sham vs. iPS-CM, sham vs. iPS-CM+MSC, MSC vs. iPS-CM+MSC, all p<0.001; n=8). Paramagnetically labeled MSC could be detected within the site of transplantation in the ventricular wall for four weeks. The bioluminescent signal from iPS-CM decreased within the first week below the level of detection. However, immunohistochemical analyses of hearts four weeks after transplantation revealed the presence of patches of EGFP-, α-actinin- and connexin 43-positive iPS-CM, indicating structural maturation and functional integration. Conclusion: Intramyocardial co-transplantation of iPS-CM and MSC into infarcted hearts resulted in improved recovery of heart function, as compared to transplantation of single cell populations, demonstrating the potential of combined transplantations of iPS cell-derived cardiac cells and adult stem cells for regenerative therapies. However, large fractions of transplanted iPS-CM were lost within the first week after injection, either due to their poor survival or mechanical extrusion from the site of injection. Therefore, additional optimization is required for achieving improved engraftment of transplanted cells and better therapeutic efficacy.

Poster Board Number: T-3256

EXPERIMENTAL TRANSPLANTATION OF HUMAN IPS CELLS DERIVED NEURAL CELLS IN A MOUSE MODEL OF HEMIPLEGIA.

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[Introduction] Stroke is the leading cause of adult disability due to the limited repair capacity of neural cells. Neural cell transplantation is a promising strategy to overcome this limitation. We have found that monkey embryonic stem (ES) cell derived neural cell transplantation were effective to restore the motor functions of stroke model mice. In this study, we tried to generate neurons from human inducible pluripotent stem (hiPSC) cells and to transplant the cells to hemiplegic model mice. [Method] 1. Culture of hiPSCs: A hiPS cell line was obtained from RIKEN Cell Bank (cell name: 253G1, cell number: HPS0002) and was maintained according to RIKEN cell preparation manual. 2. Cell differentiation: We developed embryonic bodies (EBs) from undifferentiated hiPSCs by 4-day floating culture. EBs were transferred to fibronectin-coated dishes and cultured for 24 hours. We added retinoic acid, sonic hedgehog and noggin in the dishes twice and cultured for 72 hours. RT-PCR analysis and immunocytochemistry were performed to evaluate their differentiation. 3. Stroke mouse model and cell transplantation: Cryogenic injury was made in motor cortex by the direct compression of a chilled metal probe to a frontal bone at a point where the bone was dug with a drill in advance. One week later, the Burr hole mark was made near the probe compression point and hiPSC cell derived neural cells were injected into peri-ventricular area under the injured motor cortex layer. We used the rotarod test and the beam walking test to evaluate the motor functions before and after transplantation. Three weeks later from the transplantation, we conducted pathological analysis and immunohistochemistry. For statistical analysis, MANOVA was used and p<0.05 was considered as significant. [Results] 1. Cell differentiation: The cells derived from hiPSCs expressed neural markers in RT-PCR and confocal immunofluorescence analysis as well. The former showed that the cells expressed genes of beta III tubulin, nestin, neurofilament and Islet1. The latter disclosed that the cells were 30-50% neural cell adhesion molecule (NCAM) positive, 90% nestin positive and 90% beta III tubulin positive. 2. Transplantation: All mice showed severe hemiplegia after cryogenic injury. The hiPSC cell derived neural cells survived and migrated to the injured motor cortex from the transplanted area. Motor functions were significantly improved in the beam walking test throughout the observation period. [Conclusions] We successfully generated hiPSC cell derived neural cells which retained their function after transplantation in vivo. We found that the clinical application of hiPSC cell derived neural cells looked promising to restore the motor functions of patients with hemiplegia.

Poster Board Number: T-3257

4D-NUCLEOFECTORTM SYSTEM: CELLULAR REPROGRAMMING WITH AN EYE TOWARDS THE CLINIC

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Pluripotent stem cells hold great promise for cell replacement therapies. While human embryonic stem cells (hESCs) are limited to allogeneic therapies, human induced pluripotent stem cells (hiPSCs) can be applied to both allogeneic and autologous therapies; the latter having the advantage of utilizing a patient’s own cells. Recent advances in the field have shown that various adult cell types can be reprogrammed to a pluripotent state by introduction of defined transcription factors. This process of cellular reprogramming, however, has been robust only when the transcription factors are delivered by viruses thereby diminishing applicability of hiPSCs to cell therapy. Lonza’s Pluripotent Stem Cell Innovation Center is focused on developing tools and technologies that apply to both research and clinical applications. For the latter, the generation of hiPSCs under cGMP conditions is a necessity. As an important first step towards cGMP-grade hiPSCs, the example shown below combines Lonza’s 4D-Nucleofector™ system with a “zero-footprint” reprogramming technology. Here we demonstrate efficient generation of hiPSCs from blood cells using the 4D Nucleofector™.
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System. Lonza hiPSCs share characteristics with human embryonic stem cells (hESCs), including the hESC-associated markers POU5F1, NANOG, TRA-1-81 and TRA-1-60. In addition, Lonza hiPSCs show alkaline phosphatase activity, a normal karyotype, and the ability to efficiently differentiate into cells of all three germ layers. Notably, Lonza hiPSCs show no trace of exogenous DNA integration, confirming that cells were reprogrammed with a “zero footprint” technology. These results contribute to Lonza’s goal of generating iPSCs under cGMP conditions for cell therapy applications.

Poster Board Number: T-3258
SUPPRESSED NEUTROPHIL DEVELOPMENT IN HEMATOPOIESIS OF INDUCED PLURIPOTENT STEM CELLS DERIVED FROM A SEVERE CONGENITAL NEUTROPENIA PATIENT WITH ELA2 MUTATION

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Severe congenital neutropenia (SCN) is a rare disorder characterized by severe neutropenia present at birth, an arrest of neutrophil differentiation at the promyelocyte or myelocyte stage, and a propensity to develop acute myeloid leukemia and myelodysplasia. Mutations of the ELA2 gene encoding neutrophil elastase have been identified in the majority cases of SCN, but the mechanisms which disrupt neutrophil development in SCN with ELA2 mutation have been unclear. To understand the mechanisms, we established three human induced pluripotent stem (iPS) cell clones (SPN0101, SPN0102 and SPN0103) from bone marrow stromal cells of a patient having heterozygous mutation in ELA2 gene at exon 5, 707 region by transfection with retrovirus vector which expressed human OCT3/4, SOX2, KLF4, and c-MYC (SCN-iPS cells). The silencing of exogenous genes and the capability to differentiate into three germ layers by teratoma formation were confirmed in the three SCN-iPS clones. We also identified the ELA2 gene mutation in SCN-iPS cells same to that in the patient. We then examined the hematopoietic differentiation of SCN-iPS and control iPS cells which were generated from healthy donors by the same method to SCN-iPS cells, using coculture system with a murine stromal cell line (AGM-3 cells). The cocultured cells were harvested at day 12, and CD34+ cells were separated. Hematopoietic colony assay was performed using these CD34+ cells. Although number and size of erythroid and mixed-lineage colonies derived from SCN-iPS cells were almost similar to control, those of myeloid colonies derived from SCN-iPS cells were significantly less and smaller than control. In particular, we could detect few number of G colonies from SCN-iPS cells. Since SCN patients need granulocyte colony-stimulating factor (G-CSF) treatment to increase peripheral neutrophils, we conducted the hematopoietic colony assay with G-CSF alone to examine the sensitivity of granulopoiesis derived from SCN-iPS cells and control iPS cells to G-CSF. Meloid colony formation reached a plateau at 1 to 10 ng/mL of G-CSF in control iPS cells, while the number and size of myeloid colonies gradually increased at up to 1000 ng/mL in SCN-iPS cells, but did not attain the control level. In suspension culture with myeloid differentiation-oriented cytokines including 10 ng/mL of G-CSF, CD34+ cells from control iPS cell increased 23.3-fold for 2 weeks, and mature neutrophils predominantly occupied in the cultured cells. By contrast, CD34+ cells from SCN-iPS cells gradually decreased, and few neutrophils, but mainly monocyctic cells were contained in the culture. We finally carried out microarray analysis using CD34+ cells stimulated by myeloid differentiation-oriented cytokines for 2 days to identify the genes which led to impaired granulopoiesis in SCN-iPS cells. As a result, LEF-1, C/EBP alpha, Cyclin D1 and BCL2 were downregulated in the cultured cells from SCN-iPS cells compared with those from control iPS cells. These results demonstrated that the development of neutrophils was selectively impaired in the hematopoiesis derived from SCN-iPS cells, and that the stimulation of higher concentration of G-CSF compensated the impaired development of neutrophils to some extent, indicating SCN-iPS cells can provide a useful tool to understand pathogenesis of SCN with ELA2 mutation.

Poster Board Number: T-3259
PATIENT DERIVED IPSCL TO STUDY ALPORT’S DISEASE

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Alport’s Syndrome is an inherited disease caused by mutations in COL4A3, COL4A4 and COL4A5 genes which code for type IV collagen α3, α4 and α5 chain genes, respectively. Approximately 85% of Alport’s cases are X-linked dominant, caused by mutations in the COL4A5 gene. The disease is associated with glomerulonephritis, endstage kidney disease, and hearing loss due to loss of basement membrane function. There is no known cure and treatment options are symptomatic only. With the aim of generating an in vitro model of the disease we generated provirus free iPSC from fibroblasts obtained from a skin biopsy of a patient with Alport’s Syndrome. iPSC were generated using a CRE excisable six factor polyclonistic lentiviral reprogramming cassette that we developed and colonies were picked between day 14 and 18 of reprogramming. The provirus was excised using CRE-RNA and excision was confirmed by absence of a PCR product for the lentiviral backbone and the SOX2-KLF4 junction in the reprogramming plasmid. Both iPSC and provirus iPSC were fully characterized for stem cell pluripotency including endogenous gene expression, immunofluorescent imaging of colonies, EB formation, teratoma formation of all three germ layers and routine karyotype analysis. The genotype of the patient was unknown, so after extensive sequencing of the 6,455 Kbp COL4A5 gene we discovered a novel frame shift mutation, 2598delG. This mutation leads to truncation of the COL4A5 protein from 1690 amino acids to 816 amino acids. The Alport’s iPSC at early passage in particular have a distinct phenotype, the iPSC initially attach to matrigel and then ball up with a tendency to form EBs. Passage with Accutase and ROCK inhibitor is able to prevent this from occurring. Furthermore, EBs are poorly defined. Alport’s Syndrome is associated with an increase in matrix metalloproteinase production, particularly MMP9. Using zymography we assessed the activity of gelatinases, including MMP9 a 92 kDa type IV collagenases or gelatinase B, and found a significant increase in MMP9 activity in Alport’s patient derived iPSC when compared to wild type iPSC (P<0.05). As wild-type bone marrow derived cells have been shown to significantly improve renal function in a COL4A3/- mouse model
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of Alport’s Syndrome we differentiated the iPSC into mesenchymal stem cells. These cells were fully characterized using a panel of markers by Flow Cytometry and for their capability to differentiate to adipocytes, osteoblasts and chondrocytes. These disease-linked iPSC-derived mesenchymal stem cells enable further study of the mechanisms pertaining to Alport’s disease and to assess their potential for therapeutic approaches for COL4A5 deficiency. In conclusion we have generated the first iPSC from a patient with Alport’s Syndrome. Correction of this novel disease mutation via homologous recombination advances the development of cell based therapies and create a valuable congenic positive control for in vitro models of this disease.

Poster Board Number: T-3260

MODELING OF ALPERS SYNDROME CAUSED BY POLG MUTATION BY INDUCED PLURIPOTENT STEM CELLS

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The fibroblasts from one patient with Alpers syndrome caused by POLG (mitochondrial DNA polymerase gamma subunit) mutation were isolated, and the induced pluripotent stem cells were derived from the patient specific fibroblasts (AHS-iPS). Then, with a 21-day, 5-staged in vitro development protocol, the AHS-iPS was induced towards hepatocyte-like cells displaying various hepatic functions. As controls, hES and normal iPS were also induced towards hepatocyte-like cells. Based on these works, the defective phenotypes of mitochondria are investigated in the hepatocyte-like cells. Further, by treating the hepatocyte-like cells with valproic acid, the underlying mechanism of AHS would explain the onset of lethal acute liver failure in AHS patients.

Poster Board Number: T-3261

MODELING VASCULITIS SYNDROME USING MICROSCOPIC POLYANGITIS MPA SPECIFIC IPSCS

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Microscopic polyangiitis (MPA) is an autoimmune disease characterized by pauci-immune, necrotizing, small-vessel vasculitis without clinical or pathological evidence of necrotizing granulomatous inflammation. The disorder presents with rapidly progressive glomerulonephritis as the most frequent manifestation, while pulmonary hemorrhage is among the most life-threatening complications. Although previous reports describe that the activation of neutrophils and vascular endothelial cells is involved in the development of vascular inflammation in animal models, the mechanisms of vasculitis remain largely unknown in human. Here we report the derivation of induced pluripotent stem cells (iPSCs) from skin fibroblasts of three patients with MPA by retroviral transduction of four transcription factors: OCT3/4, SOX2, KLF4 and c-MYC, or three factors: OCT3/4, SOX2 and KLF4. MPA-iPSCs are similar to human embryonic stem cells (ESCs) in their morphology, cell behavior and the expression of surface antigens and marker genes for the undifferentiated state. In addition, the disease-specific iPSCs show multipotent differentiation ability into three embryonic germ layers both in vitro and in vivo. Notably, we have confirmed that MPA-iPSCs can be induced to differentiate into vascular endothelia and neutrophils in vitro, cell types associated with the pathogenesis of MPA. Furthermore, the iPSC-derived endothelia and neutrophils showed some aspects of immunological functions involving the production of cytokines/chemokines. These results suggest that MPA-iPSCs can be used to create a novel in vitro disease model for vasculitis syndrome, supplying resources to study disease mechanisms, screen new drug compounds and develop novel therapeutic strategies.

Poster Board Number: T-3262

GENERATION OF IPS CELLS FROM PATIENT FIBROBLASTS CARRYING MITOCHONDRIAL RESPIRATORY CHAIN COMPLEX DEFECT

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Mitochondrion is a cellular power plant producing energy necessary for cellular metabolism. Impaired mitochondrial respiratory activity causes numerous disorders from metabolic defects to severe and fatal symptom. Although various mutations on mitochondrial respiratory chain complex have been reported, metabolic mechanisms of m.3398T>C mutation on MTND1 gene have been poorly understood. In this study, fibroblasts of a mitochondrial respiratory chain complex disease patient carrying m.3398T>C mutation were reprogrammed to the pluripotent state by ectopic expression of four transcription factors (OCT4, SOX2, c-MYC, and KLF4). These disease-specific IPS cells with normal karyotypes expressed pluripotency markers at transcription and protein levels. Also, it was confirmed by DNA methylation analysis that promoter regions of endogenous pluripotency marker genes were epigenetically reprogrammed in the IPS cells. Like human ES cells, the disease-specific IPS cells had immature mitochondrial cristae. These IPS cells can be used as a cellular model for studying metabolic mechanisms of the mitochondria-related disease in human.

Poster Board Number: T-3263

HUMAN INDUCED PLURIPOTENT STEM CELLS: MODELING PARKINSON’S DISEASE

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Parkinson’s disease (PD), the second-most common neurodegenerative disease, is characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta in the midbrain. Patients present with a loss of TH+ neurons, as well as aggregation of the protein a-synuclein in the surviving dopamine neurons. Because the majority of PD cases are sporadic, it is often defined as an idiopathic syndrome. However, about five percent of PD cases are considered familial and are characterized by the presence of known mutations in genes related to the development of PD. Studies into finding underlying factors for the development of PD are hindered by the lack of a relevant human model. The derivation of iPSCs from PD patients provides us with a starting point for developing a human in vitro model of the disease. The study of this model may provide clues into the molecular mechanisms that underlie the disease. We obtained fibroblasts from patients with a multi-generational family history of PD, as well as from patients with sporadic PD. Fibroblasts from a non-PD patient were also obtained, for use
as a control. These patient fibroblasts were transformed using a lentivirus carrying Yamanaka’s transcription factors. We were able to successfully derive and characterize the resultant induced pluripotent stem cells (iPSCs). Under neural differentiation conditions, the iPSC lines were able to reach the neural precursor stage, expressing relevant early neural precursor markers, as well as beginning to form neural rosettes. The availability of these iPSC lines will give us the opportunity to evaluate, side by side, if there are any differences in neutralization capacity, ability to form dopaminergic neurons, or susceptibility to exogenous culture chemicals. These results may provide insights on the mechanisms underlying the development of PD.

Poster Board Number: T-3264
INDUCED PLURIPOTENT STEM CELLS DERIVED FROM IDIOPATHIC PARKINSON’S DISEASE PATIENTS DIFFERENTIATE INTO MIDBRAIN DOPAMINERGIC NEURONS
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Induced pluripotent stem cells (iPSCs) are promising source for cell replacement therapies. However, several problems remain to be solved before they can be used in clinical settings. Such problems include the use of animal-derived materials such as feeder cells, immune rejection by the hosts, and re-activation of virally transduced genes. As for immune rejection, autologous transplantation of iPSCs derived from somatic cells of patients can be a solution. Recent studies showed that iPSCs derived from patients with sporadic Parkinson’s disease (PD) could differentiate into dopaminergic (DA) neurons. Upon transplantation into PD model rats, they successfully reduced amphetamine- and apomorphine-induced rotational movements, indicating their contribution to functional recovery. In this study, we show that iPSCs derived from patients with idiopathic PD can differentiate into dopaminergic neurons using our feeder-free floating culture method. First we generated iPSCs from the dermal fibroblasts of these patients by reprogramming with episomal vectors. Polymerase chain reaction (PCR) confirmed that resulting iPSCs had no genomic integration of those vectors. On the day 0 of neural induction, pluripotent iPSCs were seeded onto 96-well plates in GMEM supplemented with 8% KSR. With the addition of Nodal and BMP inhibitors from day 0 to day 12, most of the cells in the floating culture were positive for nestin, an early neural marker, at day 12. From day 12 onwards, the medium was replaced with Neurobasal medium supplemented with B-27, and DA neurons were induced by the addition of Purmorphamine, fibroblast growth factor 8 (FGF8), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), dibutyryl cyclic AMP, and Ascorbic acid. Immunohistochemistry and quantitative PCR indicated midbrain DA neuronal identity of the generated neurons. In conclusion, we differentiated iPSCs derived from PD patients into midbrain DA neurons with our feeder-free differentiation method. iPSCs generated with episomal vector method and our floating culture method with minimal animal-derived materials would be more suitable for clinical settings than reported methods.

Poster Board Number: T-3265
PATIENT-SPECIFIC STEM CELL-DERIVED MODELS FOR ALZHEIMER’S DISEASE - A DANISH CONSORTIUM
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The groundbreaking finding that somatic cells can be reprogrammed into iP5 cells has opened up for novel ways of identifying patient specific medicine. The technology is a step towards development of personalized medicine and is expected to increase the low proportion (<10%) of drug candidates, entering clinical studies for central nervous system (CNS) disorders. A focused Danish Consortium has recently initiated its development of in vitro models for well-characterized Alzheimer disease (AD) patients based on iP5 technology. The Consortium involves leading pharmaceutical competences within neurodegeneration as well as leading clinical and scientific competences within neural development, pluripotent stem cells and disease modeling. The overall goal for the Consortium is to establish a pipeline all the way from the AD patient to the in vitro-derived mature neuron and its use for targeted drug screening compatible with a pharmaceutical screening approach. The Consortium will be focusing on AD patients with identified mutations like e.g. PSEN1/2 and MAPT. Proper selection of optimal subpopulations of patient fibroblasts for reprogramming, based on epigenetic and marker characteristics, will be an important first step in the process. Controlled expansion (optimal O2 tensions and growth factor addition) of subpopulations of either patient derived fibroblasts- or blood (CD34+) cells for integration-free reprogramming (miRNA/episomal based) has already been established (see Holst et al. poster). Transfection of normal human dermal fibroblasts (NHDF; Lonza) was compared between different liposome-based methods including Lipofectamine 2000 (invitrogen), X-tremeGene (Roche), and Magnetofection (OZ Biosciences), and a Neon electroporation device (Invitrogen). Results were evaluated by flow cytometry using constitutively expressed RFP or GFP. The Neon electroporation method yielded superior transfection efficiencies of >60% with lower overall cell death (50%) when using a single pulse (1600 V for 20 ms). This method is currently being tested with episomal vectors (Yu et al., 2009), with and without P53 siRNA (Okita et al., 2011), Minicircles (Jia et al., 2010), or a Minicircle containing the miR-302/607 cluster (Anokye-Danso et al., 2011). The ratio of iP5-like cells is analyzed by flow cytometry every week over a 4-week period using the pluripotency markers SSEA-3/4 and Tra-1-60. The number of iP5-like colonies will eventually be visualized by live staining with Tra-1-81 and picked. Another major task of the Consortium is to establish an efficient and reproducible differentiation process to cholinergic neurons in a xenofree and defined environment. The first differentiation trials have demonstrated that human iPSCs within cultured in a xenofree environment can be differentiated towards the neuroepithelial lineage. In our trials (combining SB431542 and Noggin) a strong upregulation of the early neural marker, Pax6, has been observed at both mRNA (qPCR, >100 fold) and protein level. Exchange of Noggin with a specific analogue is being explored as the final xenofree process for the establishment of neuroepithelial cell populations intended for further cholinergic directed differentiation of AD derived iPSCs. We believe that the involvement of strong scientific, clinical and pharmaceutical partners in the Consortium will pave the way for a
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process, generating AD specific in vitro models as well as discoveries of new AD disease mechanisms.

Poster Board Number: T-3266

INVESTIGATION OF THE PATHOGENESIS OF PARKINSON’S DISEASE BY PD PATIENTS DERIVED-INDUCED PLURIPOTENT STEM CELLS

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Parkinson’s disease (PD) is one of the common neurodegenerative diseases. It is preferentially happened to elderly people, and leads to the movement disorder. The hallmarks of PD are progressive loss of midbrain dopaminergic neurons and the appearance of Lewy bodies in the affected neurons. At present, the etiology of PD is not well understood. Some familial forms of PD are caused by mutations on the PD-related genes, Parkin, PINK1, LRRK2, and SNCA. However, most of the cases of PD are idiopathic and may reflect the interactions of environmental and genetic factors. The hurdle of studying PD pathogenesis is the inaccessibility of brain tissues from PD patients. Recent advances in cellular reprogramming now allow an alternative approach for investigating PD. PD patients’ skin fibroblasts can be converted to the pluripotent state, and further differentiated to the specific disease cell type, dopaminergic neurons. Such PD affected neurons in culture represent a promising departure point for exploring the pathogenic factors of PD. Here we have generated disease specific human induced pluripotent stem cells (hiPSCs) from 3 individual idiopathic PD patients’ fibroblasts and one healthy person fibroblast. We applied a single lentiviral vector (hSTEMCCA-loxP) which encodes OCT4, KLF4, SOX2, and c-MYC, and subsequently discarded the transgenic plasmid using cre-loxP recombination. The genomic constitutions of PD-hiPSCs were found identical to their parental fibroblasts by DNA fingerprinting. The characteristics of PD-hiPSCs resemble human embryonic stem cells (hESCs) with the expression of pluripotency marker genes and the capability of differentiating into 3 germ layers. Moreover, some of the PD-hiPSCs passed the teratoma tests, 3 different lineage tissues could be observed in vivo. Neural precursors were derived from PD-hiPSCs, and then applied for transcriptome analysis. Our results revealed some differentially expressed genes involved in calcium homeostasis and neural development. In the future work these genes will be examined in PD derived neural precursors and differentiated dopaminergic neurons. Meanwhile, PD affected dopaminergic neurons will be analyzed in morphology and function compared to normal dopaminergic neurons in order to discover novel molecular players involved in Parkinson’s disease.

Poster Board Number: T-3267

DEVELOPMENT OF A NEW METHOD: THE CLONING FOR THE HEREDITARY UNKNOWN DISEASE GENE USING PATIENT-DERIVED IPS CELLS.

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Linkage analysis is a well-established and powerful method for mapping disease genes of Mendelian inheritance. Although linkage analysis is a powerful tool, it depends on the number of pedigree information. So analysis with large number of pedigrees points out few candidate genes. While one or two families analysis produces many candidate genes. Recently, it is possible to scan of the personal whole genome using the next generation sequencer. Although this new technology finds some DNA base changes rapidly and efficiently, these results cannot tell us whether the DNA base change is the disease mutation or very rare variant in small numbers of family analysis. To address this problem, we generate the patient-derived IPS cell and try to identify disease gene from one family only.

Poster Board Number: T-3268

UTILIZING PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS TO MODEL ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that affects motor neurons in the brain and spinal cord. Generation of treatments for ALS and the understanding of mechanisms that cause the disease have been hampered by an inability to culture motor neurons isolated from patients and by the complex genetic nature of most cases. Patient-specific induced pluripotent stem (iPS) cells, generated by epigenetic reprogramming of somatic cells, provide an invaluable tool for in vitro disease modeling, and could be used to address these problems. We have previously shown that iPS cells are similarly variable in their genomic qualities to ES cells as well as in their ability to differentiate into physiologically active motor neurons. Here we addressed whether iPS cells can be used to probe ALS in a cell culture system. We specifically focused on familial ALS cases that carry a mutation in the gene SOD1, which is responsible for around 25% of all genetic ALS. We show that motor neurons derived from these lines exhibit a variety of phenotypic differences to ones generated from healthy individuals. In particular, ALS-motor neurons decrease in numbers over long-term culture, with the largest motor neurons being selectively lost. Although ALS-derived motor neurons do not form large SOD1 aggregates, a typical pathological phenotype in post mortem patients, they exhibit signs of ER stress and mitochondrial impairment as well as altered electrophysiological properties. These results, for the first time provide an insight to the functional defects that physiological levels of mutant SOD1 may lead to in patient motor neurons and broadly demonstrate that iPS cell technology can be used to probe an adult-onset neurological disease such as ALS.

Poster Board Number: T-3269

DERIVATION OF AUTISM SPECTRUM DISORDER-SPECIFIC INDUCED PLURIPOTENT STEM CELLS FROM PERIPHERAL BLOOD MONONUCLEAR CELLS

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Autism spectrum disorders (ASDs) comprise an array of complex neurodevelopmental disorders. One of the major constraints in ASD research is the lack applicable human disease models. Patient-specific induced pluripotent stem cells (iPSCs) hold great potential for deciphering the molecular pathophysiology of human genetic disease by giving rise to genetically relevant disease-affected cell
types that can be studied in vitro. iPSCs are most commonly derived from fibroblasts, which are obtained through skin biopsies. Among certain populations of individuals, including children with ASDs, the necessity for skin punch biopsies to extract fibroblasts make them an unsuitable source of cells for reprogramming. To circumvent this limitation, we have derived iPSC lines from peripheral blood mononuclear cells (PBMCs) isolated from the whole blood of autistic children. Furthermore, the autism iPSCs could differentiate into GABAergic and dopaminergic neurons, which are neural subtypes frequently affected in ASDs.

Poster Board Number: T-3270

PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS FROM CARDIAC PROGENITORS RECAPITULATE THE MODELS FOR CARDIAC CHAMBER DISORDERS

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Background- In contrast to human, zebrafish can efficiently regenerate the lost cardiac muscles shortly after injury. Although the studies have indicated the genetic fingerprint essential for cardiac regeneration in human. Single ventricle (SV) physiology is a rare but fatal congenital heart defect in human. Here, we propose that the creation of disease-specific induced pluripotent stem (iPS) cells from patients with congenital heart malfunction allows investigation on disease phenotype and may provide an opportunity to uncover the genetic evidence for myocyte injury in human. Methods- Disease-specific iPS cells were generated from 15 patients include 10 patients with SV physiology and 5 bi-ventricle (BV) control hearts. Myocardial tissue specimens were minced to isolate cardiac progenitor cells (CPCs) and the cells were infected with a combination of retroviruses encoding the human transcription factors Oct3/4, Sox2, Klf4, and c-myc. Alkaline phosphatase staining and immunofluorescent analysis were performed to verify the authenticity of generated iPS clones. RNA was isolated from the cells for quantitative PCR and whole-genome expression analysis. Bisulfite sequencing was assessed by using the primers amplify the Oct3/4 and Nanog promoter regions. Results- In culture, CPCs from SV hearts showed an enhanced proliferative potential compared with those from BV patients. Although iPS colonies from most individuals can be readily identified by embryonic stem (ES) cell-like morphology, ES cell-like colonies formed from SV-derived CPCs can be expanded more efficiently than those from BV patients (70% vs. 20% of subjects analyzed). Bisulfite sequencing analysis showed that Oct3/4 and Nanog promoter regions were demethylated in both types of iPS cells generated. Likewise, iPS cells from CPCs could formed teratoma when injected the cells into immunocompromised mice. Whole genome analysis revealed that Nkx2-5, Tbx5, and Me3.2c, those are core transcriptional networks involved in mammalian heart development, were comparable between the CPCs isolated from SV and BV patients. We also found that inductive signals specific for second heart field development, including Isl1, Hand1, Wnt3a, BMP type receptor, and Bap1 were significantly downregulated in BV-derived CPCs; however, Notch1 and Foxh1 expressions increased remarkably compared with BV-derived CPCs. Surprisingly, GATA4 which is responsible for zebrafish heart regeneration after injury was decreased in SV-derived CPCs. Both types of CPCs demonstrated similar calcium oscillation before lineage induction. Upon cardiac differentiation, transcriptional factors include alpha-MHC, Nkx2-5, Tbx5, Hand2, and Isl1 were significantly upregulated in BV-derived iPS cells, whereas these genes remained unchanged but GATA4 expression was enhanced in SV-derived iPS cells. Conclusions- Our results suggest that factor-based reprogramming of patient-derived cardiac progenitors can efficiently generate disease-specific iPS cells. CPCs and iPS cells from single ventricle hearts have distinct characteristics during cardiac differentiation. Verification of genes prerequisite for zebrafish heart regeneration by using iPS cells may enable disease investigation and also facilitate the development of new cardiac regeneration therapy in human.

Poster Board Number: T-3271

DISEASE CHARACTERIZATION USING LONG QT SYNDROME SPECIFIC INDUCED PLURIPOTENT STEM CELLS

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[Background] Although previous reports have indicated that long QT syndrome-specific iPS cells (LQTS-iPSCs)-derived cardiomyocytes recapitulated disease phenotypes, those patients were previously diagnosed with mutated channel profiles. In reality, most patients have no such specific information. To address whether iPSCs could be used for personalized disease characterization, we generated iPSCs from a sporadic LQTS patient with unknown disease cause. [Methods and Results] (1) We generated iPSCs from control (n = 2) and a patient with LQTS, and differentiated into cardiomyocytes through embryoid body (EB) formation. (2) Electrophysiological analysis of the LQTS-iPSCs-derived EBs using multi electrode array system revealed that the duration of the field potential (FPD) was markedly prolonged compared with the control (388.9 ± 44.3 msec vs 202.3 ± 16.3 msec, P<0.01). (3) We tested several drugs affecting QT prolongation to clarify the electrophysiological properties. The IKr blocker E4031 significantly prolonged FPD (% change 1.21 ± 0.02, P<0.01) and induced frequent severe arrhythmias including torsades de pointes, only in LQTS (n = 10/16). These data strongly suggested a functional impairment in the patient’s IKs channel; genotype analysis for KCNQ1 revealed a novel heterozygous mutation, 1893delC. (4) Patch clamp analysis and immunostaining confirmed a dominant-negative role for 1893delC in IKs channel through a trafficking deficiency. (Conclusions) LQTS-iPSCs-derived cardiomyocytes recapitulated the disease phenotypes, and they can be utilized for identification of the disease cause and genotype. This study demonstrated that iPSCs could be useful to characterize the disease, drug responses, diagnosis and genotyping in patients with sporadic LQTS, which in turn may facilitate medical therapies in the clinical settings.
POSTER BOARD NUMBER: T-3272

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM PRIMARY HEMATOLOGICAL MALIGNANCIES.
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Induced pluripotent stem cells (iPSCs) can be generated by the expression of defined transcription factors not only from normal tissue, but also from malignant cells. Bone marrow cells from a chronic myelogenous leukemia (CML) patient were reprogrammed by introducing the transcription factors Oct3/4, Sox2, KLF4, and c-myc. Two CML derived iPSCs (CML-iPSCs) were generated. Although CML-iPSCs expressed the bcr-abl, they were resistant to imatinib. Comprehensive analysis of DNA methylation revealed the very similar methylation pattern between normal and CML-iPSCs. Then we differentiated them into hematopoietic progenitors. They showed the hematopoietic marker CD45 and immature marker CD34, and recovered the sensitivity to imatinib, which recapitulated the feature of initial CML disease. The phosphorylation state of ERK1/2, AKT, and STAT5 were evaluated after imatinib treatment in CML-iPSCs. The phosphorylation of ERK1/2 and AKT were unchanged after treatment, whereas the phosphorylation of STAT5 was decreased in CML-iPSCs although it was not activated in normal iPSCs. The phosphorylation of CrKL, which is the direct target of the bcr-abl, was decreased in both CML-iPSCs and CML-iPSCs derived hematopoietic cells although it was not activated in the normal iPSCs. These results showed that the signaling for iPSCs maintenance compensates for the inhibition of bcr-abl in CML-iPSCs and that bcr-abl dependence was lost in CML-iPSCs. Thus, this system can act as a new platform for the elucidation of the disease mechanism and drug screening.

Poster Board Number: T-3273

SPONTANEOUS NEURONAL DIFFERENTIATION IN TUBEROUS SCLEROSIS COMPLEX DERIVED PLURIPOTENT STEM CELLS
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Tuberous Sclerosis Complex (TSC) is a multiorgan disease caused by mutation or deletion of either the TSC1 (encodes hamartin) or TSC2 (encodes tuberin) genes. Hamartin and tuberin bind to one another and deficiency of either protein is sufficient to cause TSC. Although multiple downstream signaling pathways appear to be controlled by the TSC1/2 genes, inhibition of the mTOR kinase appears to be central. Inhibition of the mTOR complex 1 (mTORC1) in fact has become an established and potent therapy for at least a subset of the clinical manifestations seen in TSC. The pathological hallmark of TSC is hamartomas (collections of disorganized non-malignant cells) that appear to underlie the severe disease manifestations that are seen in most patients. Neurological features are particularly severe with many patients suffering from epilepsy, mental retardation and autism. Pathological examination of brain hamartomas (“tubers”) reveal large neurons and glial cells that are highly dysmorphic. The prevailing model of TSC pathogenesis posits a germline mutation in TSC1/2 genes followed by a “second hit” mutation/deletion in the other TSC1 or TSC2 allele. Such loss of heterozygosity is then required for the formation of hamartomas in brain (“tubers”), kidney, heart, lung and skin. While experimental support for this model has been verified with non-brain hamartomas, loss of heterozygosity in cortical tubers has been extremely difficult or not possible to demonstrate. To study the role of the TSC1/TSC2 genes during human development and to begin to unravel complex genotype/phenotype relationships and the role of mTORC1 inhibition, we have generated induced pluripotent stem cells (iPSC) from multiple patients with TSC using both viral and plasmid-based reprogramming methods. Biochemical and genetic analyses indicate increased mTORC1 signaling in TSC patient derived iPSC. TSC patient derived iPSC exhibited rapid growth and spontaneously generated immature neurons even under stem cell maintenance conditions. Compared to control iPSC, spontaneously generated neurons were much more frequently seen and had elongated, multipolar neurites. Treatment with the mTORC1 inhibitor rapamycin reversed these phenotypes. In addition, TSC patient derived iPSC had altered media requirements for growth factors in a cell-autonomous manner. Finally, loss of heterozygosity was not found in TSC patient derived iPSC lines. These last findings are highly significant as it suggests that abnormal differentiation of human neural progenitor cells and neurons may be due to haplo-inssufficiency of the TSC1 or TSC2 genes. If validated, these findings would possibly change therapeutic approaches to the treatment of TSC and related disorders.

Poster Board Number: T-3274

IN VITRO MODELING OF NEUTROPHIL DEVELOPMENT AND FUNCTION USING IPSCS: CLINICAL VALUES IN UNDERSTANDING THE PATHOPHYSIOLOGY AND APPLICATIONS IN THE TREATMENT OF CHRONIC GRANULOMATOUS DISEASE.
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Introduction: Neutrophil (NEU) differentiation is a dynamic process that can be recapitated in vitro using induced pluripotent stem cells (iPSCs). This process is susceptible to manipulation by cytokines, allowing for the study of these cells at specific stages in their development. Most importantly from a clinical perspective, by generating patient autologous iPSCs, it is possible to recapitulate a specific disease phenotype. Chronic granulomatous disease (CGD) is a congenital NEU disorder characterized by the impaired generation of reactive oxygen species (ROS). The transplantation of gene modified CD34+ cells offers the best hope of a permanent cure but carries the inherent risk of genotoxicity. In this study, we aim to highlight the clinical value of iPSCs as a disease model in elucidating the underlying pathophysiology and in assessing the effective recovery of cell functions following gene modification.

Methods: Peripheral blood (PB) CD34+ cells were isolated from two CGD patients with gp91phox or p47phox deficiency and from a healthy donor. Cells were reprogrammed using a Sendai virus vector expressing Oct4/Sox2/KLF4/c-Myc. Self-inactivating lentiviral or alpharetroviral vectors were used to insert either gp91 or p47 cDNA into iPSCs. Neutrophil differentiation was induced using VEGF and
G-CSF, WG, MPO and ALP staining was done to assess cell morphology. The immunophenotypic profiles of differentiating cells were assessed by staining for macrophage and neutrophil specific antigens. ROS production was analyzed by using the DHR flow cytometry assay following NEU stimulation with PMA. Neutrophil extracellular traps (NETs) were visualized by staining with SYTO 13 and anti-MPO antibody. Neutrophils isolated from healthy donors served as the control. Results: Mature PB-NEUs and control iPSC-NEUs displayed the classic multi-lobed appearance of the nucleus. ROS were generated at comparable levels and both populations were able to form NETs. However, CGD iPSC-derived NEUs (gp91phox and p47) appeared to show impaired development. This was suggested by the fact that at day 7 of the differentiation culture, only a proportion of the population stained positive for ALP, which is a protein found in secretory vesicles. At the same time point, nearly all control iPSC-NEUs stained positive for ALP. CGD iPSC-derived NEUs were ROS negative at all time points and displayed impaired formation of NETs. The loss of these functions however was recovered in NEUs differentiated from gene modified CGD iPSCs: Conclusion: This is the first report to show that in vitro differentiated NEUs have the capacity to form NETs. Only mature NEUs with a complete repertoire of cellular components and normal ROS generating capacity possess this property. Along with other matching characteristics to PB NEUs, these results may be taken as validation of the accuracy with which this model may be utilized to mimic NEU physiology and development in vivo. Indeed, in this instance it has been utilized to uncover a previously unreported impairment in CGD NEU development. Further study is required to determine the implications of this. In addition, we have shown that vector mediated gene transfer can recover the characteristic loss of function associated with CGD NEUs. It is possible that this disease model may also be used to study insertion site profiling for example. This will allow therapeutic vectors to be evaluated for clinical safety thus minimizing the potential risk of genotoxicity and possible harm to patients.

Poster Board Number: T-3275

HIGH THROUGHPUT SCREENING OF NEUTRAL LIPID STORAGE DISEASE, MYOPATHY SUBTYPE IPS CELLS IDENTIFIES MODULATORS OF INTRACELLULAR LIPID METABOLISM THAT REDUCE DISEASE PHENOTYPE IN VIVO.

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In this study, fibroblast cultures from patients (subject with autism), and non-affected controls have been established; subsequently this study, fibroblast cultures from patients might be circumvented proving the effectiveness of treatment. The invasive nature of collecting primary neuronal tissue from patients might be circumvented through the use of iPSCs and their subsequent neuronal differentiation. With the successful reprogramming of human fibroblasts into ES cell-like state (aka induced pluripotent stem cells, iPSC) by Yamanaka et al in 2007, this methodology has subsequently been successfully employed to derive cultured neural cells from patients with ALS, Parkinson disease, and other disorders. These break-throughs make it possible for us to generate a cell culture model of autism spectrum disorder by application of iPSC reprogramming of human fibroblasts and subsequent neural differentiation. In this study, fibroblast cultures from patients (subject with autism), and non-affected controls have been established; subsequently these cells are reprogrammed into an ES cell-like state (aka induced pluripotent stem cells, iPSC). The reprogrammed cell colonies are cloned, propagated, and induced to differentiate in vitro into neuronal cultures. Based on our underlying assumption that synaptic transmission is aberrant in autism, these patient-specific neuronal cultures will be utilized for neuronal network analysis by using the photoconductive-stimulation system described in Gutierrez et al. Briefly, spontaneous or pulse-stimulated activity of networks is measured by optical techniques, and the structural basis of these patterns will analyzed by fractal dimension analysis. By use of these approaches we have the capacity to characterize the arrangement and complexity of their axonal architecture. This approach has been
employed to demonstrate differences in hippocampal cultures of a rat model carrying the neurologin mutation R471C-NL3 which has been identified in a subgroup of patients with autism spectrum disorders. This study represents the pilot attempt to evaluate membrane excitation and signal transduction in neural cells derived from patients with autism.

**Poster Board Number: T-3277**

**NEURAL DIFFERENTIATION OF MULTIPLE SCLEROSIS IPS CELL LINES**

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The recent introduction of technologies capable of reprogramming human somatic cells into induced pluripotent stem (iPS) cells offers a unique opportunity to study many aspects of neurodegenerative diseases in vitro that could ultimately lead to novel drug development and testing. Here, we report for the first time that human dermal fibroblasts from a patient with relapsing-remitting Multiple Sclerosis (MS) were reprogrammed to pluripotency by retroviral transduction using defined factors (OCT4, SOX2, KLF4, and c-MYC). The MSiPS cell lines resembled human embryonic stem (hES) cell-like colonies in morphology and gene expression and exhibited silencing of the retroviral transgenes after four passages. MSiPS cells formed embryoid bodies that expressed markers of all three germ layers by forming embryoid bodies in vitro and teratomas in vivo. The T21 AF-iPS still maintained the abnormal and unique chromosomal karyotypes, three pairs of chromosome 21. We found the expressed protein level of APP in neuron progenitor cells (NPCs) derived from T21 AF-iPS cells to be significantly increased compared with NPCs from normal AF-iPS cells. We also demonstrate that the expression of miR-155 and miR-802 in T21 AF-iPS-NPCs were highly expressed in presence low expression of MeCP2. Our findings demonstrate that T21 AF-iPS cells serve as a good source to further elucidate neurogenesis of DS and onset of Alzheimer’s disease.

**Poster Board Number: T-3278**

**MODELING TRISOMY-21 SYNDROME WITH IPS CELLS FROM AMNIOTIC FLUID CELLS**

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Trisomy 21 (T21) Syndrome is a common chromosomal abnormality caused by an extra copy of chromosome 21 that contributes to the cognitive impairments associated with Down Syndrome (DS). The human gene for amyloid precursor protein (APP) is located on chromosome 21. APP is a ubiquitously expressed transmembrane protein whose cleavage product, the β-amyloid (Aβ) protein, is deposited as amyloid plaques in the aged brain, and in the neurodegenerative conditions of Alzheimer disease’s (AD) and DS. Recent study demonstrated that in DS patient’s, neurons overexpress miR-155 and miR-802 lead to low levels of the target protein, methyl-CpG-binding protein (MeCP2), which is important for the maturation of neuron cells. Generation of diseased induced pluripotent stem (iPS) cells has unique values in developing human genetic models in vitro. This technology holds the promise of increased understanding of complex disease. Here we have generated human iPS cell lines from second trimester amniotic fluid (AF) cells with T21 by lentiviral delivery of Yamanaka factors. We report that T21 AF-iPS cells were characterized for expression of pluripotent markers such as alkaline phosphatase activity, Oct4, Sox2, Nanog, TRA-1-60, TRA-1-81, SSEA-3, SSEA-4 and the abilities of differentiation into three germ layers by forming embryoid bodies in vitro and teratomas in vivo. The T21 AF-iPS still maintained the abnormal and unique chromosomal karyotypes, three pairs of chromosome 21. We found the expressed protein level of APP in neuron progenitor cells (NPCs) derived from T21 AF-iPS cells to be significantly increased compare with NPCs from normal AF-iPS cells. We also demonstrate that the expression of miR-155 and miR-802 in T21 AF-iPS-NPCs were highly expressed in presence low expression of MeCP2. Our findings demonstrate that T21 AF-iPS cells serve as a good source to further elucidate neurogenesis of DS and onset of Alzheimer’s disease.
Autosomal recessive polycystic kidney disease (ARPKD) is one of the most common pediatric renal cystic diseases, with an estimated incidence of 1 in 20,000 live births. The disorder is characterized by non-obstructive fusiform dilatations of the renal collecting ducts resulting in enlarged spongiform kidneys and ductal plate malformation of the liver leading to congenital hepatic fibrosis. Most patients with ARPKD present by the age of 1 year with oligohydramnios caused by decreased fetal urine output and related hypoplastic lungs, but others present later in life when the clinical symptomatology is dominated by either renal failure or hepatic dysfunction, or both. The pathogenesis of congenital hepatic fibrosis as well as renal cyst formation remains largely unknown, and no therapeutic strategies have been established. Here we have generated induced pluripotent stem cells (iPSCs) from skin fibroblast samples of a 10-year-old female patient with ARPKD by transducing five transcription factors, OCT4, SOX2, KLF4, L-MYC and LIN28 with non-integrating episomal vectors. ARPKD-iPSCs are similar to human embryonic stem cells (ESCs) in their morphology, cell behavior, and the expression of surface antigens and marker genes for the undifferentiated state. In addition, the disease-specific iPSCs show multipotent differentiation ability into three embryonic germ layers both in vitro and in vivo. Notably, we have confirmed that ARPKD-iPSCs can be induced to differentiate into bile duct epithelia and renal collecting duct cell lines in vitro, cell types affected in ARPKD. Using these differentiation systems and microarray analyses, we are identifying the molecules involved in the onset or progression of liver fibrosis and cystic kidneys by comparing the expression profiles of the hepatic and renal cells differentiated from ARPKD-iPSCs with those from normal Japanese iPSCs. These results suggest that disease modeling using patient-specific iPSCs can be used for studying the molecular mechanisms in the pathogenesis of ARPKD.

**Poster Board Number: T-3280**

**MODELING FOR AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE USING PATIENT-SPECIFIC IPSCS.**

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**Poster Board Number: T-3281**

**REAL TIME SCORING OF HUMAN INDUCED PLURIPOTENT STEM CELL DIFFERENTIATION POTENTIAL USING LIVE CELL MICROSCOPY AND IMAGE RECOGNITION SOFTWARE.**

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The ability to reprogram somatic cells to an embryonic stem cell-like state has had landmark impact on basic biological research, drug screening, and drug discovery. Ultimately, achieving the promise of induced pluripotent stem (iPS) cell research requires the controlled differentiation of cells to specific cell types. However, it is well known that there can be large differences in target cell type yields for iPS lines even from the same patient. Previously, we have created an image recognition tool that can be used to classify iPS-like colonies in microscopy image sequences with a high degree of accuracy, and in a related poster here we more thoroughly assess an imaging protocol for the automated selection of fully reprogrammed iPS colonies using live cell microscopy and image recognition. Another question we are interested in is whether these technologies can be used to score iPS colonies, both fully and partially reprogrammed (piPS, viral-GFP positive colonies with iPS like morphology), for having a differentiation bias towards specific cell types in real time. In this study, we have acquired 8 time-lapse, 20 x 20 composite image sequences of the complete 10cm dish at 2x and 4x magnifications showing the reprogramming of colonies from fibroblast lines of Spinal Muscular Atrophy patients imaged at 6 hour intervals for four weeks using a cell culture observation system, BioStation CT (Nikon). 40 iPS-like and piPS colonies were then picked expanded and differentiated towards cardiomyocyte lineages in embryoid bodies and their differentiation propensities assessed using QPCR. For each colony, a microscopy image sequence showing colony formation is quantified using an image recognition tool in CL-Quant (Nikon). Our hypothesis is that live cell imaging metrics can be used to score colonies in terms of their differentiation bias. Here we present the results from these 40 cell lines together with the image-based measurements that correlate with cardiomyocyte outcomes.

**Poster Board Number: T-3282**

**XENO-FREE CULTURE SYSTEMS FOR PLURIPOTENT STEM CELLS.**

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In recent years, there has been an increasing demand for qualified media and reagents that are free of animal derived ingredients for culturing pluripotent stem cells (PSCs). An important consideration for PSCs to be cultured in defined feeder-free and xeno-free conditions is that animal origin products may increase the risk of non-human pathogen transmission and immune rejection, limiting their use in downstream applications. To overcome these issues, we developed and tested a set of xeno-free reagents for PSC culture. The CTS™ culture system enables a complete and comprehensive solution for successful culturing of hESCs and iPSCs in KnockOut™ SR XenoFree CTS™ (KSR XF) (culture medium) and KnockOut™ SR Growth Factor Cocktail (supplement) with CELLStart™ CTSTM (xeno-free matrix) that meet cell therapy compliant requirements and regulations. Cells grown in these conditions express normal pluripotent markers, maintain normal karyotype, and retain their ability to differentiate into cells of the three germ layers. Additionally, we also successfully derived iPSCs under xeno-free conditions using a non-integrating CytoTune™ -iPS Sendai Reprogramming Kit, and expanded the iPSCs under xeno-free conditions. This xeno free system will help researchers to transition towards translational and clinical research for PSC. CTS™ regulatory-compliant reagents such as KSR XF, GFC supplement and CELLStart™, will facilitate standardization and optimization of cell culture processing, minimize exposure of PSC lines to non-human animal origin material and render safety in cell therapy applications.
DIFFERENCE IN GLOBAL GENE EXPRESSION PROFILE AND STABILITY BETWEEN ES AND IPS CELL-DERIVED NEURAL STEM CELLS

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Induced pluripotent stem cell (iPS) cells, which were reprogrammed from somatic cells by overexpressing reprogramming factors, undergo unlimited self-renewal and have differentiation potential into various types of cells like embryonic stem cells (ESCs). Direct differentiation into a specialized cell type from iPS cells holds considerable promise for regenerative medicine as well as basic research. Neural stem cells (NSCs) are self-renewing multipotent populations and have the capacity for neural differentiation into multiple cell lineages such as neurons, astrocytes, and oligodendrocytes. Many researchers have reported that NSCs have therapeutic effects in neurological disease following transplantation. Here, we induced differentiation of iPS cells into NSCs in vitro and in vivo, which were compared with ESC-derived and brain-derived NSCs. NSCs differentiated from ESCs and iPS cells were morphologically indistinguishable from brain-derived NSCs and rapidly propagated in the presence of EGF and bFGF, and stained positive for NSCs markers Nestin and Sox2. Moreover, these iPS cell-derived NSCs showed the tripotent capacity of differentiation into neurons, astrocytes, and oligodendrocytes. However, global gene expression pattern of iPS cell-derived NSCs (both in vivo- and in vitro-derived) was distinct from the brain-derived NSCs. Moreover, iPS cell-derived NSCs were spontaneously aggregated during the long-term passaging and formed ES cell-like colonies, which finally expressed Oct4-GFP. The spontaneously reverted GFP-positive cells (iPS-NSC/iPS) expressed similar levels of pluripotency markers (Oct4, Nanog) to ESCs and iPS cells, and were able to form germline chimeras. This reprogramming may be due to spontaneous re-activation of transgenes inserted in iPS cell genome when iPS cells were differentiated into NSCs. Taken together, iPS derived NSCs were morphologically and characteristically similar to brain-derived NSCs, but differ in global gene expression pattern and tend to revert into pluripotent state spontaneously.

ULTRASTRUCTURE OF PORCINE INDUCED PLURIPOTENT STEM CELL-LIKE COLONIES AND DERIVED EMBRYOID BODIES

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The generation of induced pluripotent stem cells (iPSCs) from terminally differentiated cells provides exciting possibilities for designing patient-specific in vitro disease models and producing immune-compatible tissues for transplantation. iPSCs have been produced from both mouse and human, however, the derivation of bona fide iPSCs from pigs is still pending. Porcine iPSCs were extremely useful, since autologous transplantation in porcine biomedical models could serve as an intermediate step between mouse and man. The present study aimed to characterize the ultrastructure of porcine iPSC-like colonies reprogrammed from porcine neural progenitor cells (NPCs) by lentiviral transduction with a polycistronic construct encoding the transcription factors OCT4, SOX2, KLF4, and C-MYC. Two cell lines with embryonic stem cell (ESC)-like morphology, capable of prolonged clonal propagation, were analyzed at passage 24 as well as after embryoid body (EB) formation at this passage. Transmission electron microscopy was applied to look for stem cell characteristics and signs of differentiation. The iPSC-like colonies consisted of naïve cells with large ovoid nuclei containing abundant euchromatin and, typically, one to two distinct nucleoli. The cytoplasm contained lipid droplets, free ribosomes, and small rounded mitochondria with few cristae as the most prominent structures. Additionally, many cells had well-developed rough endoplasmic reticulum. Microvilli were commonly protruding between cells and at the borders of colonies, and the basal body and axoneme of a cilium were also found. Apoptosis was occasionally seen at the periphery of colonies, and phagocytosed apoptotic bodies were present in the cytoplasm of some cells. On one occasion, columnar epithelial differentiation had occurred at the colony surface, where adjacent cells were linked with tight junctions and desmosomes. Overall, these observations are indicative of an undifferentiated ESC-like morphology of the porcine iPSC-like colonies, although apoptosis and signs of spontaneous differentiation were also noted. EBs produced from the iPSC-like colonies presented cells with a radically altered ultrastructure. The nuclei were extensively lobulated and contained electron-dense blocks of heterochromatin. The cytoplasm was rich in organelles; especially the smooth endoplasmatic reticulum and Golgi compartments were abundant, and intermediate filaments and microtubules composed a meshwork throughout the cells. Tight junctions and desmosomes were commonly found in the EB periphery. Furthermore, mitoses were evident. In two cases, neural rosettes consisting of radially arranged columnar cells, linked with tight junctions and desmosomes and enclosing a central cavity into which abundant cilia protruded, were observed. No other morphologically distinct cell types were detectable in the EBs, underscoring the inherent capability of the NPC-derived iPSCs for neural differentiation. It is tempting to speculate that this phenomenon may be due to an epigenetic memory of the NPCs potentially retained through incomplete iPSC reprogramming. In conclusion, porcine NPC-derived iPSC-like cells have an undifferentiated ultrastructure compatible with the potential state of pluripotency, whereas EBs derived from them revealed an inherent capability of neural differentiation.
pluripotent stem cells (iPSCs), using transfection by electroporation of a single gene encoding a defined factor of homeodomain transcription factor OCT4. These iPSCs are derived from preimplant ICM, but they do not become naïve but become primed ones from unknown reasons. When you make PSCs from animals (monkeys, pigs, rabbits, etc) other than mice by the conventional method, you will obtain primed ones which are PSCs from animals (monkeys, pigs, rabbits, etc) other than mice by modified conditions. Emergent colonies were round-form, which were similar to those of mouse PSCs. The cells typically expressed pluripotency markers and developed teratomas in immunodeficient mice, and thus they were iPSCs. The naïve state of porcine iPSCs was demonstrated by LIF-dependency, negative MHC class I, and two active X chromosomes (XaXa) when female, and characteristic gene expression profiles. In addition, the porcine iPSCs grow as rapidly as mouse PSCs. They can be easily passaged without a ROCK inhibitor. Thus, the porcine iPSCs are easy to handle, just like naïve mouse PSCs. Most importantly, when injected into the morulae, the porcine iPSCs contributed to the in vitro embryonic development to the blastocysts (11/24, 45.8%). They also contributed to the in utero fetal development (11/71, 15.5% at day 23; 1/13, 7.7% at day 63). Currently, we are trying to generate offspring chimeras.
**Poster Board Number: T-3288**

**GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM TRANSGENIC PIG PRODUCED FOR XENOTRANSPLANTATION**

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The generation of embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC) has great potential for the application of stem cell technology. However, many barriers are in the usage of human ESC or iPSC as a source of cell therapy, especially a major obstacle is no means to test the efficacy and safety of the therapy. The generation and application of porcine iPSCs (piPSCs) as a large animal model may be one of strategies to overcome these barriers in human regenerative medicine. Here, to our knowledge we report for the first time the generation of piPSC from genetically modified pig, alpha1,3-Galactosyltransferase knock-out (−/−) (GaIT K0) and CD46 (membrane cofactor protein) knock-in (CD46 KI). Fibroblasts were isolated from the ear skin of a 10-day-old NIH miniature pig (GaIT KO/CD46 KI). After 1 or 2 passage, fibroblasts were transduced with cocktail of 6 human factors (POUSF1, NANOG, SOX2, C-MYC, KLF4, and LIN28) and cultured on a mitotically inactive mouse embryonic fibroblast (MEF) monolayer. Reprogrammed somatic cells expressed the classical pluripotency markers (POUSF1, NANOG, SOX2, and SSEA1). Similar to mouse ESCs, piPSCs were negative for SSEA3, Tra-1-60, and Tra-1-81. Further these cells could form embryoid body (EB) and differentiate into 3 germ layers in vitro. Our piPSCs may provide useful source as a large animal model for studying approaches that can reduce an immune-rejection of cell or organ transplantation.

**Poster Board Number: T-3289**

**IPS CELL REPROGRAMMING FROM FIBROBLASTS IS INDEPENDENT OF PIWI PROTEINS**

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Piwi protein functions in the regulation of stem cell function, germ line specification, transposon silencing, and the maintenance of genomic integrity across animal phylogeny. They bind to Piwi-interacting RNAs (piRNAs) and act as regulators of epigenetic programming. In addition, work in Drosophila shows that Piwi promote developmental robustness via the suppression of phenotypic variation. Given the pivotal role of Piwi/piRNAs in epigenetic modulation, we investigated the potential role(s) of Piwi proteins in induced pluripotent stem (IPS) cell reprogramming. The mouse genome encodes three homologs; Miwi, Mili, and Miwi2. We show that all three mouse Piwi genes are expressed in embryonic stem cells at significantly higher levels than in fibroblasts, with Mili being the highest. However, mouse embryonic fibroblasts derived from triple knockout (lacking Miwi/Mili/Miwi2) embryos were able to form as many as iPSc cell colonies as wildtype control cells. Furthermore, these cells expressed pluripotency markers and were capable of differentiating into the three germ layers in teratoma assays. Our results indicate that Piwi proteins are dispensable for direct reprogramming of mouse fibroblasts.
Poster Board Number: T-3291

OVEREXPRESSION OF PGC-1 BY ADENOVIRAL VECTOR PROMOTED ADIPOGENIC DIFFERENTIATION IN INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells (iPSCs) are novel stem cell populations induced from mouse and human adult somatic cells through reprogramming by transduction of defined transcription factors. Peroxisome proliferator-activated receptor gamma coactivator-1a (PGC-1α) plays an important role during brown fat development. However, the potential roles of PGC-1α in regulating mitochondrial biogenesis and the differentiation of iPSCs are still unclear. Here, we investigated the effects of adenovirus-mediated PGC-1α overexpression in iPSCs. PGC-1α overexpression resulted in increased mitochondrial mass, reactive oxygen species production, and oxygen consumption. Microarray-based bioinformatics showed that the gene expression pattern of PGC-1α-overexpressing iPSCs resembled the expression pattern observed in adipocytes. Furthermore, PGC-1α overexpression enhanced adipogenic differentiation and the expression of several brown fat markers, including uncoupling protein-1, cytochrome C, and nuclear respiratory factor-1, whereas it inhibited the expression of the white fat marker uncoupling protein-2. Furthermore, PGC-1α overexpression significantly suppressed osteogenic differentiation. These data demonstrate that PGC-1α directs the differentiation of iPSCs into adipocyte-like cells with features of brown fat cells. In addition, these results suggest that PGC-1α enhances adipogenesis but prevents osteogenesis.

Poster Board Number: T-3292

GENERATION OF INTEGRATION-FREE INDUCED PLURIPOTENT STEM CELLS FROM ADULT COMMON MARMOSET FIBROBLAST

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Transgenic non-human primate models for Human disease are available only in the common marmoset (Callithrix jacchus). In this species, the embryonic stem cells (ESC) and the induced pluripotent stem cells (iPSC) have been already established. They are useful for preclinical non-human models on regenerative medicine including xeno-auto graft. Although we have been generated marmoset iPSC from fetal liver cells, other tissue have not been generated iPSC. As the control for preclinical study of iPSC therapy, it is important that generation of integration-free iPSC from adult marmoset tissues that can be collected by low invasive method would contribute in preclinical non-human primate model study on regenerative medicine.

Poster Board Number: T-3293

GENERATION OF IPSC FROM ADULT PROLIFERATING CNS PROGENITOR CELLS.

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Purpose. We have recently showed that RPE (retinal pigment epithelium) cells can be isolated from adult cadaver donor eyes and grown to form a functional epithelial monolayer. Furthermore, we have recently shown that the human RPE is a plastic cell with inducible stem-like properties. In this study, the adult RPE, which is only the proliferative CNS cell that can be readily obtained by minor surgery, is used as a unique tissue source for iPSC production. Because the RPE is the disease-affected tissue in Age-Related Macular Degeneration (AMD), the leading cause of blindness in people over 50, there is good reason to think that RPE will produce a superior iPSC-based model of AMD. Methods. We routinely isolate adult RPE from cadaver donor eyes of different ages and sex. iPSC were derived from human adult RPE using a Sendai virus-based reprogramming strategy and characterized according to standard protocols, including immunostaining, qPCR and in vitro differentiation into the three germ layers. Results. Adult human RPE were successfully used as donor tissue to derive iPSC expressing pluripotency markers, including Tra-1-60, Nanog and SSEA4, and able to give rise to the three germ layers in vitro. No residual donor RPE cells were detected in the iPSC cultures. Conclusions. We were able to obtain iPSC from elderly donor using a mix of 4 factors, demonstrating that we can successfully reprogram RPE from donors with age ranging from 56 to 91 year old. To our knowledge, these are the first adult human CNS-derived iPSC, and the collection is particularly valuable as it includes elderly donors. In contrast to other sources of CNS stem cells in the hippocampal dentate gyrus and the subventricular zone, the RPE can be obtained relatively easily from live patients with minimal surgery, or from donor cadaver eyes. RPE-derived iPSC could be used to reveal the molecular pathways involved with aging and that underlie AMD. The essentially unlimited iPSC supply makes large-scale studies and drugs screens possible.
Introduction: Recently, we have reported the effectiveness of transplantation of mouse as well as human iPSC-derived neural stem cells (hiPSC-NSs) for spinal cord injury (SCI) in rodents. From the viewpoint of a clinical trial, it is critical to determine the effectiveness of human iPSC-NSs in non-human SCI model. The purpose of this study is to determine the effectiveness and safety of transplantation of human iPSC-NSs into the injured spinal cord of common marmosets. Methods: We used a pre-evaluated safe human iPSC clone (hiPSCs), and neural differentiation of hiPSCs were induced through embryoid body using original protocol. A moderate contusive SCI was induced at the C5 level in adult common marmosets. 9 days after the injury, hiPSC-NSs that have been differentiated into NeuN positive neurons, GFAP positive astrocytes and Olig1 positive oligodendrocyte progenitor cells. It was noteworthy there was no tumor formation at least for 12 weeks after transplantation. Luxol fast blue and eriochrome cyanine stainings showed a significant decrease in demyelinated areas at the lesion epicenter in the transplanted group compared to the vehicle control group. Furthermore, quantitative analyses revealed that there were significant differences in CaMK2-alpha and RT97 positive areas at the epicenter between the transplanted and vehicle control groups. Quantitative RT-PCR revealed the expression of the vascular endothelial growth factor (VEGF), which is the source of the angiogenic signals, were significantly higher in the vehicle control group. Quantitative RT-PCR revealed the expression of the VEGF, which is the source of the angiogenic signals, were significantly higher in the vehicle control group.

Conclusion: Grafted human iPSC-NSs safely survived and differentiated into neurons, astrocytes, and oligodendrocytes without any tumorigenicity, thereby promoting functional recovery after SCI. Our results suggest that pre-evaluated safe hiPSC-NS therapy has major potential for the treatment of SCI in the clinical setting.
teratomas. Dox-induction in in vitro cultured somatic cells, including mouse embryonic fibroblasts (MEFs), generates bona fide iPSCs, however, the kinetics, efficiency, and quality of generation correlates with the design of the transgene encoding the reprogramming factors. Differences in early transgene and protein expression levels manifest as disparate effects on cell proliferation, morphology, and colony formation, presumably through modulation of the mesenchymal-epithelial transition (MET). Late-stage expression levels impact endogenous pluripotency gene expression and iPSC quality. We are now employing these composite systems in MEFs to reveal direct effects on transcriptional regulatory networks mediated by the reprogramming factors. Our data implies discrete reprogramming factor requirements at each stage of the process, and prospective refinement of current reprogramming standards.

Poster Board Number: T-3297

EFFICIENT METHOD TO GENERATE VECTOR-FREE INDUCED PLURIPOTENT STEM CELLS FROM CD34+ AND PBMCs USING THE CYTOTOUNE™ -IPS SENDAI REPROGRAMMING KIT

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The most common source of human cells for the generation of induced pluripotent stem cells (iPSCs) have been fibroblasts. However, fibroblasts are obtained from skin biopsies which require invasive surgical procedures and limit the ability to generate scalable iPSCs from existing tissue sources. While peripheral blood mononuclear cells (PBMCs) and CD34+ can be readily obtained from blood, existing methods encounter technical challenges to convert peripheral blood cells to iPSCs consistently and with high efficiency. In addition, the conventional methods use integrating virus or plasmid based method to reprogram, which could potentially result in multiple insertions and risk of tumorigenicity. Here we demonstrate a highly efficient method to reprogram PBMCs or CD34+ to iPSCs using the CytoTune™ -IPS Sendai Reprogramming Kit which utilizes non-integrating Sendai virus vectors. The iPSCs were characterized and showed absence of trans-genes and viral vector by real-time TagMan™ Assays. The IPS clones express proper pluripotent markers and differentiate into three lineages. These studies demonstrate an easy to use method to generate iPSCs from peripheral blood and facilitate the transition to high production of iPSCs.

Poster Board Number: T-3298

IDENTIFICATION OF TRANSCRIPTION FACTORS INVOLVED IN THE REPROGRAMMING PROCESS

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One of the most important roles of transcription factors is to regulate cell fate decision. In many cases, however, how transcription factors work to decide the cell fate is veiled in mystery. The reprogramming process from mouse embryonic fibroblasts (MEFs) to induced pluripotent stem cells (iPSCs) also remains incompletely understood. Our goal is to identify reprogramming specific changes of gene expression and their master regulators. Our microarray analysis identified 532 genes whose expression levels showed more than two fold upregulation in the reprogramming process, and these genes were classified into three groups according to their expression patterns. Then we attempted to predict transcription factors responsible for the gene expression changes in each group. Our promoter analyses and the subsequent screening processes identified two candidates, Foxd1 and Foxo1. Foxd1 was previously shown to be required for the development of retina and the morphogenesis of kidney. In the reprogramming process, downregulation of Foxd1 by RNA interference decreased the number of iPSC cell colonies, and reduced expression levels of the predicted downstream target genes of Foxd1. Another candidate Foxo1 was previously shown to be involved in several intracellular signaling pathways, and required for the maintenance of ES cell pluripotency. Downregulation of Foxo1 also resulted in the decreased reprogramming efficiency. Studies on how Foxd1 and Foxo1 work are in progress.

Poster Board Number: T-3299

ESTABLISHMENT AND FUNCTIONAL ANALYSIS OF IPS CELLS WITH DRUG-INDUCIBLE SYT-SSX FUSION GENE

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Purpose: Synovial sarcoma (SS) is a malignant soft tissue tumor harboring a tumor-specific fusion gene, SYT-SSX, which consists of SYT (at 18q11) and either SSX1 or SSX2 (both at Xp11). Based on the high sensitivity and specificity of this fusion gene, the fusion product is deeply related to tumorigenesis of SS. SYT-SSX chimeric proteins have been reported to regulate the transcription of specific target genes, although the roles are not clearly known. Forced expression of SYT-SSX gene induced cell death in normal cells except Rat1, which was successfully transformed into tumorigenic cells. These results suggest that only cells on particular lineages can be permissive for SYT-SSX fusion protein. To identify such cell-lineage, we established human induced pluripotent stem cells (iPSCs) containing drug-inducible SYT-SSX fusion gene, which can differentiate into every type of human cells. Materials and Methods: Human iPSCs established either by retroviral or plasmid vectors were used as parental cells. PiggyBag transposon system with Tet-ON construct was used to improve the integration efficiency. After cloning SYT-SSX fusion gene into Tet-ON construct, PiggyBag vector was co-transfected with a vector expressing transposase gene. Appropriate clones were selected by the response after doxycyclin treatment. Results: We succeeded to establish iPSCs with inducible SYT-SSX that showed minimum leaky expression and efficient Dox-concentration-dependent induction of SYT-SSX fusion gene. The morphology of such iPSC cells was indistinguishable from that of parental cells, whereas induction of SYT-SSX fusion gene dramatically changed their morphology within 48 hours after induction. Expression of stem cell marker genes was also significantly inhibited by SYT-SSX fusion gene. Conclusion: Human iPSCs with drug-inducible SYT-SSX fusion gene were successfully established. This system will be a useful tool to analyze the role of SYT-SSX fusion protein in tumorigenesis and also the cell-of-origin of SS.
Pluripotent stem cells such as embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) are commonly identified and characterized based on biomarker expression. While commonly used surface marker expression provides a method for screening and identifying emerging iPSC clones for further expansion, detailed characterization of the established iPSC clones is necessary before its use for downstream applications. Several platforms are available for gene expression analysis varying in content and complexity. To determine the optimal method and minimal set of genes required for definitive characterization of pluripotency, iPSCs were generated using the integration-free CytoTune™-iPS Sendai Reprogramming kit. Established clones were expanded on feeder-based and feeder-free culture conditions and confirmed to have normal karyotype, express pluripotent surface markers and possess Tri-lineage differentiation potential. These clones were included in detailed transcriptome analysis using medium density TaqMan® OpenArray® qPCR platform and Illumina whole genome array. Some samples were further characterized by Next Generation sequencing RNA Seq using Ion PGM™ Sequencer. Analysis of the data indicates that iPSCs generated using the CytoTune™-iPS Sendai Reprogramming kit are similar to a control iPSC line generated using non-integrational episomal vectors and H9 ESC, but distinct differences were observed between feeder-dependent and feeder-free iPSC lines. Such differences in gene expression were not observed in H9 ESC grown on feeder dependent vs. feeder free conditions. Functional analysis of differential expressed genes showed that genes related to DNA replication and cell cycle was more richly represented in feeder-dependent conditions than feeder-free lines. To determine if the difference was due partial reprogramming of the feeder-free systems, later-passage cells were analyzed. Comparison of massively parallel methods to identify novel markers and utilization of large scale arrays to confirm such markers paves way to distill the content into focused set of genes that can be validated on medium density qPCR OpenArray® fixed content panels for high throughput put analysis of multiple samples.

**Poster Board Number: T-3301**

**INDUCED PLURIPOTENT STEM CELL AND ES CORE FACILITY OF THE ERASMUS STEM CELL INSTITUTE FOR REGENERATIVE MEDICINE**

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The discovery of induced pluripotent stem cells (iPSC) in 2006 had an enormous impact on study of human disease and provides starting material in disease models for regenerative medicine and for drugs screening. Several methodologies have been published to generate iPSC lines, but the process is labor intensive and requires defined standard operating procedures to provide a consistent and reproducible outcome of the reprogramming process. The iPSC & ES core facility of Stem Cell institute at Erasmus medical center was established in 2010 with the mission to generate iPSCs from mouse & patient material to accelerate research in the stem cell field supporting the research groups at the Erasmus medical center and academic communities. We are currently using a Polyclonergic lentiviral reprogramming construct including a fluorescent marker (dTomato) 1 for reprogramming human fibroblasts and four retroviral reprogramming factors 2 to reprogram mouse fibroblasts. We have generated normal and disease specific iPSC lines using fibroblast cells obtained from skin biopsies of schizophrenia, fragile X patients, & patients with X-chromosome abnormalities. In addition, several mouse embryonic fibroblast cells representing different mouse models were reprogrammed. Our quality control involves characterization of our iPSC lines by morphology, cell surface markers, expression of pluripotent markers such as Nanog, Klf4, Sox2, Oct3/4, c-Myc, Fgf4, Rex1; their capacity to differentiate and karyotype & mycoplasma analysis. In mouse X chromosome reactivation happens in female cells concomitantly with the reprogramming process providing a convenient readout for pluripotency. In human this relationship is less clear. To establish the activity of both X chromosomes in our female iPSC cell lines, we will use a combination of RNA-FISH analysis detecting XIST RNA, which associates with the inactive X, and immunohistochemistry detecting the histone modifications, H3K4me3 & H3K27acetyl, specific for the inactive X chromosome. As a core facility, we aim to deliver state of the art iPSCs with the best available reprogramming protocols. We also facilitate the iPSC research of customers with on site training of tissue culture practice, by providing tissue culture protocols, and the facilitation of the iPSC related research. In near future, we aim to extend our service including new donor cell types such as blood and stromal cells. References: 1. Lentiviral Vector Design and Imaging Approaches to Visualize the Early Stages of Cellular Reprogramming. Molecular Therapy vol. 19 no. 4 apr. 2011 2. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663-676.

**Poster Board Number: T-3302**

**ACTIVATION OF INNATE IMMUNITY IS REQUIRED FOR EFFICIENT NUCLEAR REPROGRAMMING**

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The generation of induced pluripotent stem cells (iPSCs) by Yamanaka and colleagues, first reported in 2006, was a seminal development in regenerative medicine. However, much remains to be understood about the underlying mechanisms of reprogramming of somatic cells to iPSCs. Consequently there is concern regarding potential clinical applications in the absence of mechanistic insights. Although retroviral overexpression of the reprogramming transcription factors (Oct4, Sox2, Klf4 and c-Myc) generates iPSCs, the integration of foreign DNA into the host genome could silence indispensable genes or induce genomic dysregulation. One approach to overcoming these limitations is to express the transcription factors as cell-permeant proteins, yet to date this has proved difficult. Moreover, human somatic cells have not been reprogrammed using purified cell permeant peptides (CPPs). In seeking to develop this alternative approach, we discovered a striking and consistent difference in the pattern of gene expression induced by viral versus protein-based delivery of the reprogramming factors. The difference in the pattern of gene expression suggested to us...
that a signaling pathway required for efficient nuclear reprogramming was activated by the retroviral, but not CPP approach. We suspected, and then confirmed, that the viral vector was more than a mere vehicle for genes encoding the reprogramming factors. In both gain- and loss-of-function studies, we find that activation of toll-like receptor 3 (TLR3) plays a role in the efficiency of nuclear reprogramming. Stimulation of TLR3 causes rapid changes in the expression of epigenetic modifiers, with chromatin remodeling and changes in gene expression, which favor induction of pluripotency. These studies highlight the gaps that remain in our understanding of nuclear reprogramming, and for the first time suggest a collaborative contribution of inflammatory pathways in the induction of pluripotency.

Poster Board Number: T-3303

PATTERNED NEURAL AND CARDIAC DIFFERENTIATION OF ONE EB OF NANOG-IPS USING A MICROFLUIDIC DEVICE

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Embryonic stem (ES) or induced pluripotent stem (iPS) cells are widely applied in fundamental research because of their ability of self-renewal and differentiation into many derivatives. Regulation over two kinds of differentiation simultaneously in one EB (embryoid body) is necessary and important in fundamental and biomedical fields at present. So far, there are still no these kinds of reports because of some practical difficulties in mimicking in vivo surroundings. It is very difficult to imitate over two kinds of chemical gradients in micro-scale in vitro by only conventional methods of using dishes or plates, which play important roles in directing in vivo differentiation spatiotemporally. Some microfluidic devices had been fabricated for culturing and collecting stem cells, even for controlling simple differentiation. However, great physical shear or strain generated by flow would affect cellular proliferation or differentiation when chemical gradient was maintained continuously in space. Therefore, it is expected to regulate over two kinds of differentiation spatiotemporally by new methods (new devices). For the purpose, a microfluidic device was designed, which is composed of two flow channels, and an EB seeding channel located between the flow channels. An EB can be seeded and cultured at the channel crossing, where three PDMS pillars stand for preventing the EB from flowing away. The flow rate can be too small to affect the EB metabolism. At the same time, chemical gradients can be kept at the crossing point. The hypothesis was testified by theoretical simulation using COMSOL software. The theoretical simulation showed that the chemical gradient was kept at the crossing even when the flowing velocity was 0.1μL/min corresponding to the shear force of 0.07mPa, which is small enough to avoid its influence on the cells. After seeding an EB of mouse nanog-GFP iPS at the crossing, the flow channels were connected to syringe pumps, EB differentiation was induced by perfusing a medium containing FBS (20%) for cardiac differentiation and/or a medium containing N2 supplement for neural differentiation through the two flow channels respectively. After 4 days of culture, it was shown that dynamic culture in a microfluidic device promoted not only proliferation but also differentiation. It was also found that patterned differentiation was induced in one EB when the two kinds of media were perfused simultaneously. Immuno-staining experiments showed that a half of the EB derivatives was cardiac-troponin stained, interestingly, the other half was b3-tubulin stained. The experimental results of real time PCR indicated that gene expression of cardiac troponin and b3-tubulin was simultaneously observed in one EB. These results suggested that the chemical gradients for inducing cardiac and neural differentiation were kept at the crossing continuously and simultaneously, which effectively directed the two kinds of differentiation. The experimental methods of using the microfluidic device shows the advantages in maintaining a low level of shear, and keeping two chemical gradients simultaneously, which suggested that it is possible to control the patterned differentiation in one EB for future biomedical research.

Poster Board Number: T-3304

KDM2B ENHANCES IPS CELL GENERATION BY FACILITATING GENE ACTIVATION IN EARLY STAGE OF REPROGRAMMING

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Reprogramming from somatic cells to induced pluripotent stem (iPS) cells by transcription factors is by nature an epigenetic process of cell fate change. Previous studies have shown that the low reprogramming efficiency can be enhanced by introduction of additional factors. To investigate the mechanism of reprogramming, we aimed to identify epigenetic modulators promoting iPS cell generation. Here we report that Kdm2b, a histone H3 lysine 36 dimethyl (H3K36me2)-specific demethylase, promotes iPS cell generation. The capacity of Kdm2b to promote iPS cell generation depends on its demethylase activity and DNA binding property, but is independent of its role in regulating senescence and cell proliferation. We show that Kdm2b functions early in reprogramming and enhances the activation of early responsive genes by localizing onto and demethylating its target genes. Restricting the upregulation of early activated genes abrogates the capacity of Kdm2b in promoting reprogramming. Our studies not only identify Kdm2b an epigenetic factor important for iPS cell generation, but also uncover the mechanism of how Kdm2b contributes to the reprogramming process.

Poster Board Number: T-3305

NOVEL STRATEGY OF EPIGENETIC REPROGRAMMING WITH THE MYOD TRANSACTIVATION DOMAIN FOR IPS CELL GENERATION

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Induced pluripotent stem cells (iPSCs) can be created by reprogramming differentiated cells through introduction of defined genes, most commonly Oct4, Sox2, Klf4, and c-Myc (OSKM). A major difficulty of producing induced pluripotent stem cells (iPSCs) has been the low efficiency of reprogramming differentiated cells into pluripotent cells. Here we show that 30% of mouse embryonic fibroblasts (MEFs) were reprogrammed into iPSCs when they were transfected with a fusion gene composed of Oct4 and the transactivation domain of MyoD (called M3O), along with Sox2, Klf4 and c-Myc (SKM). Transduction of M3O and SKM into fibroblasts effectively remodeled patterns of DNA methylation, chromatin accessibility, histone modifications, and protein binding at pluripotency genes, raising the efficiency of reprogramming differentiated cells into pluripotent cells. In addition, M3O facilitated chromatin remodeling of pluripotency genes in the majority of transfected MEFs, including the cells that did not become iPSCs. These results identified that one of the most critical barriers to iPSC creation is poor chromatin accessibility and
The generation of induced pluripotent stem cells (iPSCs) that can differentiate into all cell types of the body like embryonic stem cells (ESCs) has brought us expectations for the human iPSCs to be used as a cell source for human regenerative medicine without any ethical problems. However, the inefficient generation of iPSCs (0.01â^¼0.1%) limited the utility of iPSCs. Previous studies have shown that Phosphoinositide 3-kinase (PI3K) pathway plays important roles in self-renewal and proliferation of embryonic stem cells (ESCs). The tumor suppressor, phosphatase and tensin homolog deleted on chromosome 10 (Pten), negatively regulates PI3K pathway and its inhibition leads to the activation of PI3K pathway. Recent reports have shown that suppression of Pten improves self-renewal, cell survival and proliferation of mouse and human ESCs. However the contributions of PI3K pathway or its negative regulator, Pten, to the process of reprogramming remain unknown. Here, we report that the activation of PI3K pathway by the inhibition of Pten, improves the reprogramming efficiency for iPSC generation. In this study, we retrovirally expressed four reprogramming factors Oct3/4, Klf4, Sox2 and c-Myc (OKSM) in Pten deficient mouse embryonic fibroblasts (MEFs). Continuous activation of PI3K pathway by the loss of Pten and expression of PI3K pathway enhances the generation of iPSCs by the co-expression of Pten inhibitor, bpV(HOpic) dramatically improves the efficiency of iPSC generation. Furthermore, we report that the activation of PI3K pathway by the inhibition of Pten, improves the reprogramming efficiency for iPSC generation. In this study, we retrovirally expressed four reprogramming factors Oct3/4, Klf4, Sox2 and c-Myc (OKSM) in Pten deficient mouse embryonic fibroblasts (MEFs). Continuous activation of PI3K pathway by the loss of Pten and expression of PI3K pathway enhances the generation of iPSCs by the co-expression of Pten inhibitor, bpV(HOpic) dramatically improves the efficiency of iPSC generation. Moreover, these iPSCs expressed immature cell markers such as SSEA1 and Nanog, and showed a normal karyotype. The AP activity of AP+ colonies from MEFs expressing CS-Pten or myr-AKT compared to controls. These results indicate that the activation of PI3K pathway enhances the generation of iPSCs by the co-expression of OKSM. Continuous activation of PI3K pathway by the loss of Pten may lead to transformation of cells. Thus to reduce the risk of cell transformation, we transiently activated PI3K pathway by the use of Pten inhibitor, bpV(HOpic), during the reprogramming process. The efficiency of iPSC generation (bpV-iPSCs) by the retroviral transduction of OKSM. The efficiency of iPSC generation was examined by AP activity. The number of AP+ colonies in the presence of bpV(HOpic) was twice higher than controls (approximately 9%). These bpV-iPSCs expressed immature cell markers such as SSEA1 and Nanog, and showed a normal karyotype. Moreover, these bpV-iPSCs have the ability to differentiate into three-germ layers in vitro and in vivo. Furthermore, bpV-iPSCs contributed to germline-competent chimeric mice. These results indicate that bpV-iPSCs shared the similar pluripotency like ESCs. Notably we have also found that inhibition of Pten efficiently promoted the human iPSC generation. Overall activation of PI3K pathway by the use of Pten inhibitor, bpV(HOpic) dramatically improves the efficiency of iPSC generation from mouse and human somatic cells, and this technology may directly contribute to establish human regenerative medicine using iPSCs.
Detailed Program and Abstracts — Thursday, June 14

**Pre-clinical and Clinical Applications of Mesenchymal Cells**

**Poster Board Number:** T-3311

**RECONSTRUCTION OF HUMAN ELASTIC CARTILAGE BY PROGENITOR CELLS IN THE EAR PERICHONDRIUM**

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**Introduction:** Future cell-based therapies such as tissue engineering will benefit from a source of autologous cartilage progenitor cells. In human auricular elastic cartilage, the cartilage progenitor cells have not yet been identified. It is expected that the cells will have new possibilities for better cartilage reconstruction and be a useful cell resource in place of mature chondrocytes. The human auricular elastic cartilage consists of the chondrium, and the perichondrium that has been known for regenerating cartilage clinically. Therefore, it is presumed that the cartilage progenitor cells of auricular cartilage might exist in the perichondrium. The purpose of this study is to identify cartilage progenitor cells of human auricular elastic cartilage and utilize for cartilage reconstruction. Methods: The perichondrium, the interlayer, and the chondrium were harvested from pieces of retained auricular cartilage of microtia patients. Cells isolated from their tissues were defined as the perichondrocytes, the interlayer cells and the chondrocytes. To analyze the proliferative ability, clonogenicity assay and long-term cell growth assay were done. In order to characterize surface marker profiles, we performed flow cytometry. The hematopoietic stem cell markers, and the MSC markers were analyzed. The multipotency was tested in vitro chondrogenic, adipogenic and osteogenic differentiation assays. To study cartilage regeneration capacity in vivo, perichondrocytes and chondrocytes induced cartilage differentiation in vitro were implanted in NOD/SCID mice and after 1, 3 months estimated histologically. Results: The perichondrocytes isolated from the perichondrium showed the highest proliferative ability of cells isolated from the others in both clonogenicity and long-term cell growth assay. Among the mesenchymal stem cell markers, CD44 and CD90 expressions showed significant differences. Higher expressions of CD44 and CD90 were observed in perichondrocytes compared with those of chondrocytes. The perichondrocytes expressed not only proteoglycan and collagen II but also lipid vacuoles and calcium by each induction mediums. Perichondrocytes and chondrocytes implanted in NOD/SCID mice reconstructed cartilage and their matrix was consisted of proteoglycan and collagen II histologically similarly. The perichondrocytes-derived cartilage has had perichondrium and the cell density not changed after 3 months, but the chondrocytes-derived cartilage not perichondrium and the density decreased. Conclusion: Our study suggests that the perichondrocytes might be cartilage progenitor cells, because they had not only higher proliferative capacity than the chondrocytes but also multipotency to differentiate into osteocytes and adipocytes. Surface marker profiles indicated that these progenitor cells were potentially enriched by combinations of CD44 and CD90. Moreover, we demonstrated that cultured human perichondrocytes could reconstruct cartilage in vivo and the perichondrocytes-derived cartilage might maintain the cartilaginous phenotype in the long term in comparison with chondrocytes. The utilization of progeni-

**Poster Board Number:** T-3309

**REPROGRAMMING COCHLEAR CELLS INTO INDUCED PLURIPOTENT STEM CELLS WITH OCT3/4, SOX2 AND KLF4**

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The loss of regenerative ability of hair cells in the mammalian contributes to the permanent sensorineural hearing loss. In embryonic or neonatal mammalian inner ear, cochlear stem/progenitor cell populations (otospheres) have been identified, while cochlear stem cells appear to diminish in number after birth and become quiescent in adult. Yamanaka’s group has established the method for reprogramming of somatic cells into induced pluripotent stem (iPS) cells by introduction of transcription factors, the established iPS cells has provided great potential for tissue specific regeneration. The aims of present study were to examine whether transcription factors can induce reprogramming of otosphere cells, which may result in generation of iPS cells from cochlear cells. We introduced transcription factors, Oct3/4, Sox2 and KLF4 into otospheres derived from postnatal day-1 mouse cochlear epithelia using retrovirus, and analysed alterations in cell characteristics. After transduction, otospheres generated colonies that displayed embryonic stem cell (ESC)-like morphology and expressed pluripotent markers of iPS cells. iPS cells derived from otospheres (OiPS cells) are able to differentiate into cells from three germ layers in vitro and contributed to the formation of teratoma in vivo. These findings demonstrate that transduction of transcription factors is capable of inducing reprogramming of otospheres, suggesting that the method for generation of iPS cells might be utilized for full or partial reprogramming of cochlear cells.

**Poster Board Number:** T-3310

**GENERATION OF GERMLINE TRANSMISSION COMPETENT IPSCS IN 7 DAYS**

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Somatic cells can be reprogrammed into induced pluripotent stem cells (iPSCs) by defined factors. However ,the efficiency is considerably low and the process is time-consuming. We first exploited a chemical defined medium, named iCD1, in which the MEFs can be reprogrammed with high efficiency and fast kinetics by Oct4/Sox2/KLF4. Utilizing blastocysts injection ,here we report that the iPSC generated at D7 post-infection in iCD1 has the ability to contribute to chimera mice and germline transmission. Thus ,we established a technical scheme to determine fully reprogramming ,and first proved that somatic cells can be fully reprogrammed in a 7 day procedure with only three reprogramming factors.
tors from human auricular perichondrium will not only improve our understanding of basic cartilage biology, but will lead to novel therapeutic strategies, including long-term tissue restoration, for patients with craniofacial defects.

Poster Board Number: T-3312

TRANSPLANTATION OF HUMAN ADULT STEM CELLS PROTECTS AGAINST SOCIAL DEFICIT EXHIBITED BY SUB-CHRONICALLY PHENCYCLIDINE TREATED MICE

Barzilay, Ran, Ganz, Javier, Segal, Hadar, Ben-Zur, Tali, Bren, Ziv, Hinden, Noa, Tarasenko, Igor, Taler, Michal, Lev, Nirit, Sadan, Ofer, Gil-Ad, Irit, Weizman, Abraham, Offen, Daniel

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Background: Stem cell based regenerative therapy is a promising therapeutic approach, bringing hope for patients affected with incurable diseases. Mesenchymal stem cells (MSCs) represent an attractive cell source for regenerative medicine strategies for the treatment of brain diseases, exerting neuroprotection and inducing neurogenesis. Schizophrenia (SCZ) is a devastating brain disease with poor prognosis, especially due to the debilitating negative symptoms. Beside a neurodevelopmental element, the pathophysiology underlying SCZ involves neurodegeneration, oxidative stress and impaired neurogenesis. In the current study, we sought to explore the prospect of intracerebral MSCs transplantation for treating the phencyclidine (PCP) SCZ mouse model. PCP was administered subcutaneously to C57bl mice (10mg/kg daily for 2 weeks). Adult human MSCs transplantation into the hippocampus was conducted on the day of first PCP administration. Social preference test was conducted 10 days following the last PCP administration. Results: Transplantation resulted in a significant reduction in the impairment of social phenotype induced by the PCP insult, as observed by the social preference test. Immunohistochemical analysis revealed that the human cells survived in the mice brain throughout the course of the experiment (25 days). Western blot analysis of the hippocampus showed a decrease in cleaved caspase3 and an increased Bcl-2 protein levels in the brains transplanted with MSCs. Moreover, proteins involved in glutamate metabolism and oxidative stress which were affected by the PCP insult were positively regulated by the MSCs transplantation. Interestingly, co-culture of MSCs with mouse astrocytes in vitro showed that MSCs exert effect that counteracts changes in astrocytes gene expression following exposure to PCP. Conclusions: Delivery of MSCs to distinct brain regions, known to be involved in the pathophysiology of SCZ, is beneficial in attenuating the behavioral deficits exerted by the PCP insult in mice. We hereby suggest a novel therapeutic approach for the treatment of SCZ negative symptoms.

Poster Board Number: T-3314

DIFFERENTIATED MESENCHYMAL STEM CELL TRANSPLANTATION IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATIENTS A PHASE II/III CLINICAL TRIAL

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Background: Amyotrophic Lateral Sclerosis (ALS) also known as Lou Gehrig’s disease, is a fatal, rapidly progressive, neurodegenerative disease caused by motor neuron degeneration. Despite extensive efforts no treatment is currently available that slows, stops, or reverses disease progression. Bone marrow derived Mesenchymal stromal stem cells (MSC) represent an attractive cell source for the treatment of neurological diseases, exerting neuroprotection and possibly inducing neurogenesis. A previous pilot study from our group at Hadassah has shown the safety of the intravenous and intrathecal administration of unmodified MSC in ALS patients and provided some indications of clinical benefit in terms of stabilization of the disease. We have developed a protocol for MSC differentiation into cells that secrete neurotrophic factors (NTF), based on the Brainstorm’s NurOwn™ technology. These MSC-NTF cells, demonstrated neuroprotective effects in various animal models of neurodegenerative diseases, including ALS. Aims: To evaluate the safety and tolerability of treatment with autologous MSC-NTF cells in ALS patients utilizing the intramuscular (IM) way of administration of the cells at the early stage of the disease and the intrathecal (IT) transplantation in patients with more advanced/progressive disease. Method/Design: This Phase I/II clinical study will include in total 24 ALS patients, assigned to 2 treatment groups based on the severity of their disease: 12 patients with early ALS (ALSFRS score of >30), and 12 patients with advanced ALS (ALSFRS score of 15-30). Patients from both groups are followed for three months prior to transplantation. Autologous bone-marrow derived MSC-NTF cells are transplanted, by IM or IT injections to patients with early or advanced ALS, respectively. After transplantation patients are followed up clinically (detailed neurological examination and ALSFRS evaluation) on a monthly basis for a post-treatment follow-up period of 6 months. Respiratory function tests, MRI of the muscles and EMG will be used as additional surrogate markers of the disease activity. Results: Four ALS participants were recruited in the second half of 2011. MSC were isolated, expanded ex-vivo, purified and induced to differentiate into cells secreting neurotrophic factors (MSC-NTF cells). After a one month follow-up of the first four patients treated with MSC-NTF cells, no treatment-related adverse events were observed, indicating a short term safety of the treatment. Conclusion: Preliminary results of our ALS trial with autologous MSC-NTF cells after ex-vivo differentiation indicate that the used treatment protocols appear to be safe. Further analysis of the data during the following months of the trial, is needed to confirm the safety and efficacy of the proposed therapy. Trial registration: ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT01051882). The study is sponsored by Brainstorm Cell Therapeutics Ltd.

Poster Board Number: T-3315

DOPAMINERGIC NEURONS DERIVED FROM HUMAN DENTAL PULP STEM CELLS

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Aim / Background: Parkinson’s disease, one of the serious neurodegenerative diseases, is caused by the loss of dopaminergic neuron. In order to overcome Parkinson’s disease, stem cell therapy was suggested as an alternative treatment. Human dental pulp stem cells (hDPSCs) are one of the adult multipotent stem cells, which can differentiate into three germ layers including neurons. As hDPSCs can obtain noninvasive way by deciduous tooth, they could be a suitable autologous stem cell source. We identified that hDPSCs could differentiate into dopaminergic neurons under appropriate in vitro conditions. Methods: hDPSCs were cultured with dopaminergic neuron differentiation media and the dopaminergic neuronal characters were identified at each stage. With morphological changes, stem cell properties and potential to differentiation into dopaminergic neuron of hDPSCs were verified with immunocytochemistry (ICC), flow cytometry, Real time PCR and

ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT01051882). The study is sponsored by Brainstorm Cell Therapeutics Ltd.
ELISA. Results: Differentiation of hDPSCs into dopaminergic neuron was verified according to the 5 stages. hDPSCs were proliferated at stage 1 and neurospheres were formed at stage 2. Subsequently at stage 3 and 4, NESTIN-positive cells were selected and expanded. Lastly at stage 5, cells were induced into dopaminergic neuron. On the protein level, the expression of stem cell marker (SSEA4) was observed at early stages and gradually decreased. The expression of early neural stem cell marker (NESTIN) and early neuronal marker (TUJ1) were maintained through the stages. The late neuron markers (GFAP and MBP) were highly expressed from stage 3. Most importantly, the dopaminergic neuron marker (Tyrosine Hydroxylase, TH) expression was found at stage 5. Flow cytometry results showed that expression of neuron-related markers was increased during the stages, while expression of SSEA4 was decreased. On the gene expression level, the expressions of SSEA4 (stem cell marker) and VIMENTIN (neural stem cell marker) were constant, while, the expressions of OCT4, NANOG (stem cell markers), NESTIN, PAX6 (neural stem cell markers) were observed gradually increased by time course, and the expressions of early (TUJ1, MAP2) and late neuronal markers (GFAP, MBP, O4) were significantly decreased at stage 5. TH was highly expressed from stage 4 and matured at stage 5. The quantification of TH was carried with ELISA and the value was 0.289±0.063ng/ml at stage 5.

Conclusions: We identified that hDPSCs induce efficient neural differentiation, and the induced neurons contain TH-positive ones producing a significant amount of TH. Therefore, hDPSCs can be an alternative cell source of autologous cell therapy for Parkinson’s disease.

**Poster Board Number: T-3317**

MICRONRNAS SECRETED ISCHEMIC HEART INHIBIT SURVIVAL OF TRANSPLANTED RAT MESENCHYMAL STEM CELLS

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Stem cell therapy for repair of myocardial injury has essential limitations due to the poor survival of stem cells after cell transplantation. After transplantation of mesenchymal stem cells (MSCs), cell survival is inhibited by various factors secreted from ischemic surroundings after myocardial infarction. Recently microvesicles (MVs) have been known to mediate cellular communication. The goals of this study are to identify effects of miRNA secreted from ischemic heart for MSC survival. We demonstrated that MVs were secreted by ischemic hearts using transmission electron microscopy and MVs containing various miRNAs were isolated from hypoxic cardiomyocyte by real-time PCR analysis. To determine the roles of miRNAs in MVs isolated from hypoxic cardiomyocytes for MSCs survival, we investigated the survival of hypoxic MSCs after transfection of miRNA mimic in vitro. In various miRNAs-treated MSCs, the survival rate was 2-folds lower and cells were arrested in G1/S checkpoint. In addition to, the ratio of Bcl-2/Bax decreased 2-fold but caspase-3 activation was increased. Annexin V/PI assay was consistent results with the cell survival. These results indicated that secreted MVs from ischemic heart inhibited survival of implanted MSCs and provide evidence that the elimination of miRNAs secreted from ischemic hearts might be a novel strategy for increasing successful stem cell therapy via improving the survival of engrafted MSCs.

**Poster Board Number: T-3318**

RAT MESENCHYMAL STEM CELLS PROTECT HYPOXIC CARDIOMYOCYTES VIA SECRETION OF MICRONRNA-125B MICROVESICLES

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Microvesicles (MVs), an extracellular vesicle, are secreted from all cells with microRNAs (miRNAs), which act mediators for cell-to-cell...
Detailed Program and Abstracts — Thursday, June 14

Poster Board Number: T-3319

HUMAN STEM CELL THERAPY FOR THE TREATMENT OF RADIATION-INDUCED NORMAL TISSUE DAMAGE

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In Europe, per year, 1.5 million patients undergo external radiotherapy. The late adverse effect of radiotherapy concern 5 to 10% of them. Eradication of these manifestations is crucial. We are strongly implicated in the field of regeneration of healthy tissue after radiotherapy or radiological accident. In 2004, in collaboration with Saint-Antoine Hospital (Paris, France), our first success in cell therapy was the correction of deficient haematopoiesis in two patients. The intravenous injection of Mesenchymal Stem Cells (MSC) has restored bone marrow micro-environment necessary to sustain haematopoiesis after total body irradiation. From 2006 until 2011, we have obtained foremost success in cell therapy of radiation induced burns. In collaboration with the Percy hospital (Clamart, France) we have evidenced for the first time, the efficiency of MSC therapy in the context of acute cutaneous and muscle damage following irradiation in five patients. In 2007, we have also demonstrated that MSC treatment is a promise approach for the medical management of gastrointestinal disorder after irradiation. Three patients were successfully treated for consequences of over exposure for pelvic radiotherapy in 2007 and one patient in 2011. Evaluation of stem cell therapy combining different source of adult stem cells is under investigation. Key message of your presentation in 30-40 words: Clinical trials for treatment of radiation - induced normal tissue damage after radiotherapy or radiological accident using Mesenchymal Stem Cells (MSC) to restored haematopoiesis, acute cutaneous skin and muscle damages or consequences of pelvic radiotherapy. Key words: stem cell therapy, radiotherapy, healthy tissue, gastrointestinal disorder.

Poster Board Number: T-3320

LET-7B MODULATES SURVIVAL OF HYPOXIC HUMAN MESENCHYMAL STEM CELLS VIA THE REGULATION OF PARP

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Although mesenchymal stem cells (MSCs) represent a suitable source for therapy of heart failure, the poor survival of MSCs limited the efficacy of cell therapy. Cleaved Poly (ADP-ribose) polymerase(cPARP)-1 plays an important role in cellular injury such as ischemia-reperfusion of the heart. PARP-1, an endogenous enzyme, repairs the broken DNA strand in nucleus due to oxidative stress, chromatinremodeling or cell death. But the cleavage of PARP by caspase-3 inhibits PARP’s DNA-repairing abilities. MicroRNAs(miRNAs) are short non-coding RNA and key regulator in various biological functions including cell differentiation, remodeling, cell proliferation, and cell death by negatively regulating gene expression. In this study, we found that caspase-3 was a possible target of let-7b, inhibiting the cleavage of PARP. Overexpression of let-7b increased cell survival by the regulation of PARP activity in MSCs. In MSCs transfected with let-7b mimic in hypoxic condition, the apoptosis-related factors such as p-AKT, p-MEK, p-ERK, Bcl-2, Bax, cytochrome-c, cPARP and Annexin V/PI were well regulated. These results indicate that let-7b regulates expression of PARP for repairing DNA strand and for increasing cell survival in hypoxic MSCs by inhibiting caspase-3 activity.

Poster Board Number: T-3321

HUMAN ADULT ADIPOSE-DERIVED STROMAL VASCULAR FRACTION AND HIGH DENSITY PLATELET CONCENTRATES IN BIOCELLULAR REGENERATIVE MEDICINE

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Biocellular regenerative medicine values the heterogeneous, undifferentiated cell population with fat tissues. High numbers of undifferentiated cells, ease and safety of access, and the native 3D adipose matrix, favors fat as a stem/stromal cells source. In translating from lab to bedside, an appreciation of potential uses of the cellu lar, structural, chemical and paracrine interactions within elements of the stromal vascular fraction (SVF) has gained attention. Understanding the potentials of platelet growth factors and signal proteins have led to combining the cell source, native adipose biomatrix (scaffolding), and signaling elements for wound healing and regeneration in vivo. Paper studies the components of AD-SVF from a cellular, structural, chemical, and mechanical viewpoint. As a huge microvascular bed, fat provides a vast pool of undifferentiated cells with complex signaling mechanisms. Comparative studies remain challenging, as standardization of terms and protocols remains elusive. Paper brings an understanding of the between various microenvironments. The makeup of the AD-SVF is key to
the potential uses in tissue maintenance and repair. Importance of regulatory feedback and the niche to cellular proliferation and differentiation is presented. The ability to transfer the intact niche (microenvironmental) components within liposaprical samples, offers real potential in the clinical setting. Advantages of non-ma-nipulated tissues relative repair potentials and the ability to deliver these elements within a "same surgical procedure" are outlined. Research activities have sought to isolate, concentrate and expand the component cellular groups. We believe provision of the entire "smorgasbord" of undifferentiated, multipotent cells and their native scaffolds may be of greater value than any one component. Letting the niche dictate its needs may be more effective than isolation of some parts. Highly concentrated platelet-rich plasma addition offers a diverse bioactive stimulus, providing an array of growth factors, signal proteins, cytokine/chemokines and lipokines. This is believed to enhance healing and regenerative capabilities acting primarily in a paracrine function. A true biocellular mixture can be safely and effectively delivered with the living biocasclotfolding of the adipose tissue complex. It appears that available perivascular and ECM elements (AD-SVF), the heterogeneous, and tissue specific conditions offer a major importance for total organism function, homeostasis, and regeneration. Tissue repair, homeostasis, and regenerative capabilities represent an inseparable interaction resulting in integral functions of the microenvironment it supports. Understanding the multiple factors involved in human homeosta-sis and self-repair is rapidly providing information which will alter many existing paradigms in the practice of medicine and surgery in the coming years. Permitting the niche to dictate the cellular fates and use of many variable inflammatory elements is providing a safe and effective clinical option in many musculoskeletal and reconstructive applications.

Poster Board Number: T-3322
SUCCESSFUL ISOLATION AND EXPANSION OF HUMAN SYNOVIIUM-DERIVED MESENCHYMAL STEM CELLS (MSCS) GREW OUT FROM TISSUE EXPLANT BY USING CHEMICALLY DEFINED SERUM FREE MEDIA STK1 AND STK2
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Synovium-derived mesenchymal stem cells (MSCs) provide a superior cell source for cartilage regeneration because of their capacity for self-renewal and chondrogenic potential. For effective cell-based therapy, it is important consistently to obtain sufficient numbers of transplantable cells with minimal safety concerns. Traditionally, MSCs were isolated from synovium tissue by using collagenase and/or other proteases and expanded in media containing fetal bovine serum (FBS) or autologous serum. However, enzymatic digestion is time consuming, especially when applied to large volumes of tissues, and it decreases cell viability due to proteolytic activity. In addition, the sensitivity of tissues to collagenase shows large inter-individual and sample-to-sample variations. On the other hand, there are potential risks associated with the use of FBS in clinical settings. Thus, the increasing number of clinical protocols for cell-based therapy underscores the need for non-enzyme treatment and serum-free media. In the present study, we developed new methods for isolation of human synovium-derived MSCs from tissues, and isolated MSCs were expanded in a chemically defined serum-free medium STK1 and STK2 (DS Pharma Biomedical Co., Ltd., Japan). STK1 was used in primary cultures; meanwhile STK2 was used after the 1st passage. We found that synovium tissue fragments could adhere onto plastic culture surfaces within a very short time and that fibroblast-like cells actively migrated from these explants. Morphologic analysis and surface markers expression of these cells (positive for CD44, CD73, CD90, CD105, CD166, negative for CD34, CD45, HLA-ABC) indicated their mesenchymal origin. After in vitro expansions, these cells were successfully induced into adipogenic, osteogenic, and chondrogenic lineages. The growth rate and colony-forming efficiency of explants-derived cells were higher than those of cells obtained by collagenase treatment. Furthermore, after primary culture, explant-derived cells yielded larger numbers of cells than those obtained by the conventional digestion method. At the 5th passage and 4-6weeks after seeding, total cell number increased >1,000,000,000-fold in serum-free cultures of explant-derived cells with STK1 and STK2. In conclusion, we obtained multipotent MSCs from the synovium tissue by combination of primary explant cultures and chemically defined serum-free media STK1 and STK2. This method is simple, time saving, reliable and gives a high yield of cells compared to the conventional methods, and can be used in stem cell therapy.

Poster Board Number: T-3323
EFFECTS AND SAFETY OF HUMAN ADIPOSE TISSUE DERIVED STEM CELLS ON OSTEOARTHRITIC CARTILAGE EXPLANTS IN VITRO.
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Introduction. Osteoarthritis (OA) is a chronic joint disease characterized by progressive destruction of articular cartilage and degeneration of the extracellular matrix. Matrix enzymes, such as metalloproteinases (MMPs) play a crucial role in the modulations of degradation and cell-matrix interactions. MMPs are controlled through activation of proenzymes and the inhibition of active enzymes by tissue inhibitors of metalloproteinases (TIMPs). Many studies imply the therapeutic potential of mesenchymal stem cells (MSCs) for cartilage repair. Adipose tissue derived stem cells (ADSCs) are close to MSCs from bone marrow in their characteristics, moreover, ADSCs are much easier available. Therefore, the development of ADSC therapy in OA, using their paracrine and chondroprotective effect would be advantageous. The present study is a part of the international project designed for elaboration of clinical procedure for the therapeutic application of ADSCs in OA. Materials and methods. To evaluate effects of human ADSCs for OA cartilage we performed in vitro ADSC co-cultures with articular cartilage explants (CE) for 3 and 7 days. To further reproduce the OA conditions co-cultures were also stimulated with IL-1β. ADSCs were isolated under GMP conditions, and all cultures were performed with incomplete chondrogenic medium supplemented with human platelet growth factor-enriched plasma. Secretion of TIMPs, MMPs, and other factors was analyzed in coculture supernatants by ELISA. CE were examined by histochemical and immunohistochemical methods. Expression of genes associated with chondrogenesis was analyzed in CE by RT-qPCR. Results. Factors, including fibronectin, hyaluronic acid, TIMP-1, TIMP-2 and TIMP-3 as well as MMP-2, which are known for their beneficial effect on OA cartilage, were produced by CE and particularly by ADSCs on day 3 and
to the higher level on day 7. On the contrary to the other MMPs, the suppressive role of MMP-2 in arthritis has been reported. No production of VCAM-1, MMP-1, MMP-3, MMP-9 and MMP-13 which are harmful in arthritis was determined in supernatants of ADSCs. Co-cultures resulted in changes of analyzed gene expression profile in CE, with the most pronounced up-regulation of COL1A1 gene, as compared to CE alone. Addition of IL-18 to co-cultures differentially modulated production of MMPs and TIMPs, as well as gene expression pattern, however, effects of ADSC on CE essentially remains similar. Histological and immunohistochemical examination have shown a tendency to extracellular matrix improvement in the osteoarthritic CE cocultured with ADSC, suggesting their chondroprotective effects of those cells. Conclusions. Secretory profile and results of histological analysis suggest safety and possible beneficial effects of ADSC in prevention of cartilage from degrada- tion during OA. The up-regulation of COL1A1 gene in the presence of ADSCs implies stimulated initial stage of OA cartilage healing. Presence of IL-1β may modify interaction between ADSC and CE in a co-culture system.

Poster Board Number: T-3324
MESENCHYMAL STEM CELLS: APPLICATION FOR ENHANCING THE ENGRAFTMENT OF HUMAN HEMATOPOIETIC STEM CELLS
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To overcome the limitations of allogeneic hematopoietic stem cell transplantation (HSCT), such as graft rejection and graft versus host disease (GVHD), we conducted a study to identify a strategy for enhancing HSC engraftment during HSCT. Cotransplantation experiments with mesenchymal stem cells (MSCs) derived from adult human tissues, including bone marrow (BM), adipose tissues (AT), and umbilical cord blood (CB) were conducted. AT-MSCs and CB-MSCs suppressed T-cell proliferation in mixed lymphocyte preparations as effectively as BM-MSCs, implying that MSCs have immunosuppressive effects as one of common characteristics of MSC. We showed that AT-MSCs and CB-MSCs enhanced the engraftment of HSCs as effectively as BM-MSCs in NOD/SCID mice, suggesting that AT-MSCs and CB-MSCs can be used as alternative stem cell sources for enhancing the engraftment and homing of HSCs. CB-MSCs derived from different donors showed different degrees of efficacy in enhancing the engraftment of HSCs. To investigate the critical factors of MSCs used for enhancing the HSC engraftment, we identified the difference in gene expressions according to MSCs’ heterogeneity and culture conditions using microarray analysis and real-time PCR in multiple donors of CB-MSC day 7 after plating at different cell densities. Culture conditions had greater effect than MSCs’ heterogeneity on the difference in the gene expression of the cells, though the difference in the gene expressions of CB-MSCs was identified among different donors. Especially, CXCR7 showed the significant difference in the gene expression according to culture conditions. When CXCR7 in MSCs plating at high cell density that increased the efficiency of the HSC engraftment more than MSCs plating at low cell density was inhibited, the HSC engraftment was not increased as much as MSC plating at high cell density. This result suggests that CXCR7, known as a chemokine receptor able to bind the chemokines CXCL12/SDF-1 and CXCL11, in MSCs could be the critical factor of MSCs for enhancing the HSC engraftment by MSCs. In conclusion, our results showed that BM-MSCs, AT-MSCs and CB-MSCs preferentially enhanced the engraftment of HSCs.

Thus, AT-MSCs and CB-MSCs could be an alternative source of stem cells for cotransplantation with HSCs in HSCT. Although each of the MSC populations exerted different influences on the engraftment of HSCs and the gene expression profiles, the difference in the gene expression of MSCs and the effect of MSCs for enhancing the HSC engraftment were predominantly observed in the culture conditions, implying that ex vivo expansion according to culture conditions can overcome MSC’s heterogeneity. Especially, the effect of MSCs plating at high density that enhanced the HSC engraftment may come from CXCR7. These results represent an important step toward preparing appropriate MSCs used for emerging as a thera- peutic strategy and understanding their cellular signal mechanisms of the HSC engraftments.

Poster Board Number: T-3325
FACTORS RELEASED BY HUMAN UMBILICAL CORD BLOOD-MESENCHYMAL STEM CELLS ENHANCE SURVIVAL OF HL-1 CARDIOMYOCYTES UNDER SIMULATED ISCHEMIA
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OBJECTIVES: Cardiac stem cell therapy may contribute to functional improvement of the infarcted heart, even in the absence of de novo formation of contractile cells. Saving ischemically damaged cardiomyocytes from cell death by paracrine survival stimuli is one suggested mechanism. Because such paracrine effects are difficult to quantify in vivo, we sought to investigate the protective stem cell activity in a conditioned medium - cell culture ischemia model. METHODS: Human umbilical cord blood-mesenchymal stem cells (CB-MSC) were expanded and conditioned culture medium was prepared. Cultivated murine HL-1 cardiomyocytes were subjected to combined oxygen glucose serum deprivation in CB-MSC-conditioned medium or control medium. After 5 hours treatment the total remaining cells as well as apoptotic cells converting the poly-caspase substrate SR-VAD-FMK were quantified using an automated high content imaging system. In addition, morphologi- cal changes of the nuclei connected to apoptosis were assessed. Via MTS assay metabolic activity was determined. Activation of kinases Akt and Erk1/2 and transcription factor Stat3 was investigated by western blot after 3 and 5 hours treatment. RESULTS: After 5 hours oxygen glucose serum deprivation the rate of detached dead cells was significantly lower in CB-MSC conditioned medium compared to control medium (29±3% vs. 39±7%). Furthermore, the fraction of non-apoptotic cells was increased in CB-MSC conditioned medium. Cell viability assessed via MTS assay was enhanced significantly by 23±7% relative to control. CB-MSC conditioned medium further lowered nuclear shrinking and fragmentation, the major nuclear morphological changes associated with apoptosis. Phosphoryla- tion of cell survival promoting kinases Akt and Erk1/2 was elevated when cells were kept in CB-MSC conditioned medium during oxygen glucose serum deprivation. Stat3 phosphorylation was enhanced significantly by factor 9±2 relative to control after 3 hours and declined to factor 6±3 after 5 hours. CONCLUSIONS: Factors released by human CB-MSCs protect cardiomyocyte-like HL-1 cells from the deleterious impact of oxygen glucose serum deprivation. This effect is associated with the activation of cell survival promot- ing kinases Akt and Erk1/2 and transcription factor Stat3. Studying the interaction between stem cells and ischemically damaged cardiomyocytes on a molecular level is indispensable to explain the beneficial effects of cardiac stem cell therapy. Our findings reinforce the thesis that paracrine mediated survival stimuli contribute to these effects.
Detailed Program and Abstracts — Thursday, June 14

**Poster Board Number: T-3326**

**THE SAFETY AND EFFICACY OF HUMAN BONE MARROW-DERIVED CLONAL MESENCHYAL STEM CELLS IN A RAT SPINAL CORD INJURY MODEL**

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Mesenchymal stem cells (MSCs) are multipotent and differentiate into a range of cell types and are being tested for their regenerative potential. However, most preclinical and clinical studies have used a mixed population of mononuclear cells, which has led to confused results in the outcomes of clinical trials. In this study, we aimed to evaluate the safety and efficacy of human bone marrow-derived clonal mesenchymal stem cells (hcMSCs), homogeneously isolated by using subfractionation culturing methods (SCM), in a rat spinal cord injury (SCI). Human bone marrow-derived primary (hpMSCs) and clonal mesenchymal stem cells (hcMSCs) were labeled with PKH26 before freezing, and then kept in a nitrogen tank. The rat SCI model was made with using a vascular clip at T9 level. One hour after injury, approximately 1.5×10^5 cells were gently washed three times with PBS then transplanted into the lesion site. Rats were divided into four groups of sham, SCI+PBS, SCI+hpMSCS and SCI+hcMSCS. Motor function test was performed during 4 weeks post injury by BBB score. Rats were sacrificed at 1 day, 1 week, 2 weeks and 4 weeks and spinal cords were removed for histological analysis. Our results showed that the transplantation of hpMSCs and hcMSCS had no significant adverse effect on inflammatory reaction, compared with that of the SCI+PBS groups. In addition, transplantation of hcMSCS improved the recovery of hind limb locomotor function and the expression level of neurofilament protein, and reduced the cavity size.

**Poster Board Number: T-3327**

**DESIGNING A CLINICAL PROGRAM FOR AUTOLOGOUS ADIPOSE DERIVED CELL THERAPIES IN CARDIAC PATIENTS**

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Developing a cell therapy product for commercial use is a novel and complicated process. Determining the appropriate infrastructure for each stage of product development and testing can be crucial to taking the product to the clinic. Establishing appropriate timelines and meeting budgets demands can be a challenge for start-up companies with limited funding. This session will discuss the Bioheart experience in taking cell therapy products to the clinic and the challenges to ensure patient safety and financial security in the path to commercialization. Bioheart has collaborated with many groups to bring these therapies to patients including the Ageless Regenerative Institute and Regenerative Medicine Institute. In a recent protocol focused on congestive heart failure, more than 10 patients have been successfully treated using adipose derived stem cells. The cells are delivered directly into the heart muscle using a catheter. These patients have demonstrated on average, an absolute improvement of 13 percentage points in ejection fraction and an increase of 100 meters in their 6 minute walk distance at their 6 month follow up. This means that the patient’s heart appears to be functioning better on echocardiogram. In addition, the patients have improved their exercise capacity which allows for a more active and normal lifestyle. This data is in agreement with the many years of pre-clinical animal studies that were completed by ARI and Bioheart demonstrating the safety and efficacy of this therapy including a study led by Keith March, MD, PhD, Director of the Vascular and Cardiac Center for Adult Stem Cell Therapy at the Indiana University. The adipose cells showed a tendency toward cardiomyocyte regeneration, prominent angiogenesis (growth of new blood vessels) and reduction in the infarction size. The prominent angiogenesis demonstrated by ADSCs, make them the perfect therapy for ischemic tissue.

**Poster Board Number: T-3328**

**CLINICAL OBSERVATION OF 8 CASES OF PATIENTS WITH JUVENILE RHEUMITOID ARTHRITIS TREATED BY AFFINITY UMBILICAL CORD MESENCHYAL STEM CELLS**

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Background: Juvenile rheumatoid arthritis (JRA) is a common rheumatic disease in childhood, which mainly characterizes chronic arthritis and relates all the body systems, also can cause the first factor for childhood mutilation and loss of sight. This disease can be divided whole boy type, multi-join type and hyp-join type, showing long time fever, rash, arthralgia, leukocytosis. The disease commonly involves childhood of 2-16 years, affecting their growth and development, and is very difficult to be cured. Objective: To observe the effect of using umbilical cord mesenchymal stem cell (U-MSC) to treat JRA and try to explore a new way for the disease treatment. Method: From March to November 2011, we collected 8 cases of JRA (according to the diagnostic standard of USA rheumatism society ) patients to our hospital, 5 males, 3 females , ages from 2 to 12 years, their courses of disease had been for 1 to 3 years. The patients had been repeatedly instructed to use hormone, NSAIDS, DMAIDS and other drugs for therapy without good effect. We tested the patients' immune index containing T cell subgroup, Treg, and phlegmonosis index(IL-6,INF-Υ,TNF-α,IL-17,TGF-β). We used to treat JAR could be a safe and prospective way , which may regulate immune disorder and make Treg up, make the inflammator factor down. And we also could reasonably decrease their medicine use. The laboratory index showed 5 of the 8 patients' Treg up-regulation, and the inflammatory factor showed 4 of the 8 patients' IL-6, TNF-a, IL-17 level descended after treatment for 1 week, but after 4 months the IL-17 level raised gradually up. While other 4 of the 8 patients' IL-6, TNF-a, IL-17 level still decreased. Conclusion: U-MSC used to treat JAR could be a safe and prospective way , which may regulate immune disorder and make Treg up, make the inflammation factor down.
**THERAPEUTIC APPLICATION OF HUMAN ADIPOSE STEM CELLS TO REPAIR CARDIAC DAMAGE AND DISEASE**

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Stem cell therapy offers the potential for enhanced restoration of the loss of heart function associated with cardiac disease and infarcts that affects millions of people each year. Adipose stem cells (ASC) in particular represent a bountiful and readily accessible source of autologous adult stem cells with demonstrated capacity for cardiac lineage differentiation. We have previously demonstrated that the hematopoietic stem cell (CD34+LinNeg) sub-population of human whole bone marrow is crucial for the regenerative effects following injection of bone-marrow derived samples into infarcted hearts in a murine model of cardiac ischemia. We now wish to assess whether re-programming stem cells towards the cardiogenic lineage prior to use in therapy could potentially further enhance tissue repair and the restoration of cardiac function. Since ASC are multipotent and more abundant and accessible than HSC, we have elected to use adipose-derived samples in the present study. Like bone marrow derived samples enriched in HSC, adipose derived samples enriched in ASC (CD90+CD105+CD45-CD31-) were observed to effect improvement in cardiac output (LVEF) and reduce scarring (mean percentage area fibrosis of total heart) following inter-cardiac transplantation into SCID mice directly after induced myocardial infarction. To assess whether re-directing ASC towards the cardiac lineage prior to transplantation may increase the efficacy of stem cell-based therapies to treat cardiac damage, we constructed a lentiviral vector engineered to constitutively express the re-programming factors Gata4, Mef2c, and Tbx5. We are able to efficiently transfect human ASC samples with this vector, and detect up-regulation of cardiogenic lineage markers 14 days post-transfection. These results lay the groundwork to test the relative capacity of ASC and re-programmed ASC to facilitate repair from myocardial infarcts.

**ENGINEERING OF A HUMAN DEATH-INDUCIBLE MESENCHYMAL STEM CELL LINE; TOWARDS STANDARDIZED AND SAFE CELLULAR THERAPY.**

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**Introduction**

Human bone marrow-derived Mesenchymal Stem/Stromal Cells (hMSCs) are promising candidates in regenerative medicine, potentially indicated for tissue repair, tumour targeting, and treatment of immune-related diseases. However, the high inter-donor variability, combined with the intrinsic heterogeneity of hMSC preparations and the lack of quality standards, limits a coherent exploitation of their therapeutic potential. If the generation of immortalized hMSCs could solve cell-source based problems, it also raises safety concerns regarding their stability / tumorigenicity. Towards the standardized and safe clinical use of hMSCs, the aim of this study is thus to generate hTERT-immortalized hMSCs including an inducible suicide system. The engineered cell line should conserve the properties of primary hMSCs, while being efficiently inducible toward apoptosis in vitro and in vivo. Materials & Methods Primary hMSCs were transduced at early passage with a lentivirus carrying hTERT. A retrovirus-mediated transduction allowed the integration of an inducible, modified apoptotic caspase 9 gene into the immortalized population. A clone having stably integrated the death-device was isolated and expanded. Cells were analyzed phenotypically, and characterized for their differentiation capacity (adipogenic, osteogenic, chondrogenic), tumorigenicity (RT-PCR analysis and in vivo study), and their immunogenicity. The killing efficiency of the death-device was assessed in vitro by Annexin-V/PI staining and in vivo by using a luciferase reporter system. In vivo studies were based on ectopic implantation in nude mice. Additional viral transductions (i.e., for VEGFα, BMP-2) were performed to validate the feasibility to introduce further functionalities in the engineered cell line. The stability of the transgenes was assessed at different passages and after each transformation. Results Fresh hMSCs were successfully immortalized (>280 doublings over more than 1 year) and stably transformed with an inducible-death device. The induction of the death system led to an efficient killing in vitro (>95%) and in vivo. Despite an extensive expansion and even following additional transductions, the generated clonal cell line was phenotypically identical to primary hMSCs. Cells could also differentiate toward osteoblastic, adipogenic and chondrogenic lineages with similar or increased efficiency when compared to their primary counterparts. Engineered hMSCs displayed no immunogenic properties (no allogenic CDB+ activation) and no tumorigenicity in vitro (normal RNA level of p53, p21, cMyc and Rb1 genes) and in vivo (absence of tumor development 3 months post implantation). Conclusion We engineered a stable hMSCs line maintaining typical functionalities and inducible into apoptosis upon demand. The cell line represents a standardized cell source for a variety of possible clinical indications with increased safety margins. In fact, cells can be extensively tested and screened prior to clinical use and potentially killed once they have elicited their function in vivo. The study opens the general perspective of using genetic tools not only to modify cellular function, but also to standardize and increase the safety of cellular therapy.

**AGING CAUSES ACTIVATION OF UROKINASE SYSTEM AND MMPs IN HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS**

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**Introduction.** Mesenchymal stem cells, including adipose-derived mesenchymal stem cells (ADSCs) actively participate in tissue repair and regeneration due to their differentiation capacities as well as abilities to stimulate the growth of blood vessels and nerves. ADSCs produce a wide range of growth factors, which stimulate migration and surviving of endothelial cells and axons. These cells also produce matrix proteases, including metalloproteinases (MMPs) and urokinase, which mediate extracellular matrix remodeling and proteolytic activation of growth factors. Aging is associated with impaired tissue repair, increased extracellular matrix to cells ratio as well as changes in the composition of extracellular matrix. We previously demonstrated that ability of ADSCs to stimulate blood vessel growth decreases with age due to deteriorated secretion of angiogenic growth factors. Age-associated changes in proteases secretion by ADSCs was not known, therefore the aim of our study was to access how aging affects urokinase system and MMPs production by human ADSCs. Methods. ADSCs were isolated.
from subcutaneous fat tissue samples collected during surgical procedures from 61 patients (aged 2.5 to 82 years old, mean age 56.6±15.6 years). Cells were characterized as MSCs according to their immunophenotype and differentiation capability. Telomere length as well as urokinase (uPA), its receptor (uPAR), plasminogen activator inhibitor 1 (PAI-1), MMP2, MMP9 expression in cultured ADSC were assessed using real-time PCR. uPAR surface expression was estimated by flow cytometry. Content of pro- and active forms of MMP2 and MMP9 were determined by zymography. Level of PAI-1 secreted by ADSCs was accessed in conditioned medium by ELISA. Results. Expectedly, ADSCs from aged patients had shorter telomeres (correlation between relative telomere length and age r = -0.56, p<0.01). Cells of elder patients had elevated uPAR and PAI-1 gene expression (correlation between expression level and age r=0.44, p=0.001 and r=0.46, p=0.01, correspondingly) as well as uPAR surface expression (r=0.65, p=0.01). These changes also inversely correlated with telomere length in ADSCs (r=-0.35, p<0.04 for uPAR mRNA and r=-0.54, p=0.01 for PAI-1 mRNA). We found that ADSC with shorter telomeres produced significantly more PAI-1 to conditioned medium (r=-0.41, p=0.02). Content of pro- forms of MMP2 and MMP9 was higher in ADSC obtained from patients older than 60 years compared to younger patients (p<0.05) and we observed similar tendency for active forms of MMP2 and MMP9, but it was not statistically significant. Conclusions. Our data suggest that aging is associated with up-regulation of urokinase system and MMPs in ADSC. Elevated proteolytic activity of ADSCs from elder patients can reflect cells adaptation to the changes in extracellular matrix during aging and can also act as a compensatory mechanism, providing more efficient activation of fewer amounts of secreted growth factors.

**Poster Board Number: T-3332**

**HUMAN NAÏVE UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELLS ARE EFFECTIVE FOR TREATMENT AS WELL AS PREVENTION OF GVHD IN NSG MOUSE MODEL**

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Recently, the studies about GVHD prevention and treatment using mesenchymal stem cells are proceeding but the therapeutic effects are still controversial. Therefore, this study was designed to address that human naïve umbilical cord blood-derived mesenchymal stem cells (UCB-MSC) has effect on treatment as well as prevention in xenogeneic GVHD model using NSG mouse. To investigate the prevention effect of UCB-MSC, 5x10⁵ naïve UCB-MSC were transplant ed via tail vein with single (day 0) or repeat dose (day 0, 3, 6). After 60 days, while the survival rate of UCB-MSC single injection group was 12.5%, UCB-MSC repeat injection improved the survival rate up to 62.5%. To observe the therapeutic effect of UCB-MSC, they were transplanted via tail vein with single (day 18) or repeat dose (day 18, 21, 24) after GVHD onset. Naïve UCB-MSC increased the survival rate up to 50% with single injection. After observing until 60 days, we analyzed plasma of all live mice to investigate immune suppressive molecules secreted from UCB-MSC. In this analysis, we could detect PGE2, TGF-β1 and IL-10 and their levels were significantly increased compared to control group that was not administered UCB-MSC. In addition, suppressor T cells increased 44.78% in repeat injection of preventing group and 40.75% in single injection of treating group. Taken together, our data confirmed that human naïve UCB-MSC is effective at treating as well as preventing GVHD by secreting immune suppressive molecules and increasing suppressor T cells. This study as a clinically applicable model suggests that repeat injection is needed for GVHD prevention but the therapeutic effect is enough with single injection.

**Poster Board Number: T-3333**

**SOLUBLE CCL5 FROM BM-MSCS IN THE BRAINS OF AD MICE WITH AB DEPOSITION**

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Microglia have the ability to eliminate amyloid β (Aβ) by a cell-specific phagocytic mechanism, and bone marrow (BM) stem cells have shown a beneficial effect through endogenous microglia activation in the brains of AD mice. However, the mechanisms underlying BM-induced activation of microglia have not been resolved. We show that BM-derived mesenchymal stem cells (MSCs) induced the migration of microglia when exposed to Aβ in vitro. Cytokine array analysis of the BM-MSC media obtained after stimulation by Aβ further revealed elevated release of the chemoattractant factor, CCL5. The CCL5 was increased when BM-MSCs were transplanted into the brains of Aβ-deposited AD mice, but not normal mice. Interestingly, alternative activation of microglia was associated with elevated CCL5 expression. Furthermore, by generating a chimeric mouse, we ascertained that the activated microglia resulted from endogenous BM cells that were recruited into the brain by CCL5. Additionally, we observed that neprilysin (NEP) and IL-4 derived from the alternative microglia were associated with a reduction in Aβ deposition and memory impairment in AD mice. These results suggest that the recruitment of the alternative microglia into the brain is driven by CCL5 secretion from the transplanted BM-MSCs, which itself is induced by Aβ deposition in the AD brain. This work was supported by the grants for the Bio & Medical Technology Development Program (2010-0020234) and Basic Science Research Program (2010-0003949, 2010-0009421) funded by the National Research Foundation (NRF) of the Ministry of Education, Science and Technology, Republic of Korea.

**Poster Board Number: T-3334**

**PROSPECTS OF USING MOUSE BONE MARROW MESENCHYMAL STEM CELLS OVEREXPRESSING VEGF IN A MURINE DOUBLE TRANSGENIC MODEL OF ALZHEIMER’S DISEASE**

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Objectives: Our proposal is to investigate the effect of intracerebral transplantation of mouse bone marrow mesenchymal stem cells overexpressing VEGF (MSC-VEGF), an important angiogenic and neurotrophic factor, on the vasculature and neurodegeneration in double transgenic model of Alzheimer’s disease (2xTg-AD) with APPswe/PS1dE9 mutation. Since the Aβ peptide inhibits angiogenesis and promotes the degeneration of blood capillaries, our hypothesis is that mesenchymal stem cells overexpressing VEGF (MSC-VEGF), when transplanted into the brain, might have a potential to increase the vascularization and angiogenesis rate in 2xTg-AD animals. Methods: Bone marrow mesenchymal stem cells from 6 weeks old C57BL6/Egfp mice were transplanted with up-
VEGF vector, constructed with the promoter, enhancer and intron 1 of cytomegalovirus to express human VEGF165. Transfection was performed using electroporation and expanded at the 14th passage. Transgenic 2xTg-AD animals at 6, 9 and 12 months old were transplanted with MSC-VEGF and MSC. To the control groups were administered saline solution. Forty days later, the animals were tested for Open Field, Social Recognition and Plus-maze Discriminative Avoidance behavioral tasks, to access locomotion, novelty exploration, learning and memory. At the end of the behavioral tests, the animals were perfused and their brains were analyzed for vascularization, neurogenesis, cortical atrophy, neuroprotection and clearance of Aβ, by immunohistochemistry and immunofluorescence for CD31, DCX, NeuN, Iba-1, GFAP and Aβ6E10, respectively. Results: Transplantation of MSC-VEGF promoted neovascularization in 2xTg-AD animals, possibly by a paracrine effect. MSC-VEGF treatment recovered the innate interest in the novelty exploration in 12 months old 2xTg-AD animals, improved social recognition memory in 6 months 2xTg-AD animals and sustained learning activity at 9 months of age. Both MSC and MSC-VEGF treatment were able to improve motor deficits and recover long-term memory in 9 and 12 months old transgenic animals. There was retention of long-term memory, independent of the genotype, age and treatment. Conclusions: The beneficial effect of MSC-VEGF transplantation is related to the paracrine action of factors, mainly VEGF, which favored the neovascularization and the proliferation, viability and migration of endothelial cells. Thus, this modulation ultimately promoted improvement in cognitive deficits of 2xTg-AD animals. In addition to contribute to the understanding of the participation of bone marrow stem cells in angiogenic mechanisms in the brain of 2xTg-AD transgenic animals, this study has important therapeutic implications on angiogenesis damage, present in neurodegenerative diseases, promoted by Alzheimer’s disease.

Poster Board Number: T-3335
INTERLEUKIN-17 DRIVEN PD-L1 EXPRESSION ON MOUSE MESENCHYMAL STEM CELLS AMELIORATE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS DEVELOPMENT
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Recent studies indicated that in vivo administration of mesenchymal stem cells (MSCs) could significantly ameliorate the pathogenesis of experimental autoimmune encephalomyelitis (EAE), however, the mechanism by which MSCs exert their therapeutic effect remains elusive. Since IFNγ, TNFα and IL-17A are reported to be crucial in the development of EAE, we stimulated MSCs with different combinations of these three cytokines. We found that PD-L1, a B7 family molecule with the ability to inhibit the function of T cells, was induced upon IFNγ and TNFα stimulation. Interestingly, when IL-17A was added, PD-L1 expression was further increased by several folds. This synergistic induction of PD-L1 in MSCs was abolished by blocking or depleting inducible nitric oxide synthase (iNOS) which is known to play a critical role in MSC-mediated immunosuppression. Moreover, addition of nitric oxide (NO) donor into iNOS deficient MSCs could restore the synergistic effect. Although administration of MSCs could ameliorate EAE in mice, co-administration of antibodies to PD-L1 with MSCs was found to abolish the therapeutic effect of MSCs. The effect of anti-PD-L1 is exerted through MSCs since anti-PD-L1 alone showed no effect. The key role of PD-L1 in MSC-based EAE therapy was also confirmed by the observation that iNOS deficient MSCs are not as effective as wild type MSCs in EAE mice. Therefore, PD-L1 expression is indispensable for MSCs-based therapy of EAE.
inflammation and reactive species production play a pivotal role in the pathogenesis of DR. These noxes are associated with the loss of pericytes and neuronal cells in the early stage of DR and may also be the driving force in the continued insult to the retina during the latter stage of the disease. Nowadays, there is no medical intervention that could prevent or delay the onset of DR. All therapeutic options involve highly invasive techniques and are applicable only in advanced stages of the disease. Therefore, the generation of less invasive and more effective strategies to prevent or delay the onset of DR is needed. Stem cell-based therapy represents an emerging therapeutic approach by which vascular and neuronal damage may be treated. Among that, multipotent mesenchymal stromal cells (MSCs) are highly attractive since they have been used to regenerate damage tissues and treat inflammation related diseases. Taking into account the mechanisms that trigger the initial steps of DR, the administration of MSCs could have favorable effects because they might (i) differentiate into pericytes and neuronal cells, replacing the dead ones; (ii) be neuro and vascular protective by the secretion of trophic factors; (iii) reduce oxidative damage by the scavenging of reactive oxygen species; and (iv) reduce inflammation by the secretion of anti-inflammatory cytokines. Therefore, the aim of this work was to evaluate the direct contribution of systemically administered MSCs in the natural evolution of DR. For this, mice with type 1 diabetes (T1D), in which we have previously proved that the administration of MSCs have no impact in blood glucose level, received 0.5x10^6 MSCs at a time in which the pro-damage mechanisms are present but retinal histology is still preserved. First, we studied -by flow cytometry- the biodistribution of donor MSCs in mice with T1D. For this, donor cells were isolated from isogenic mice that express GFP (MSCsGFP). Three months post-administration, MSCsGFP were mainly found in the eyes of T1D mice, and in minor degree in other organs severely damaged during the disease like heart, kidney and pancreas. Second, we analyzed -by histology- the presence of structural changes in the retina. Whereas untreated T1DM mice exhibit structural alterations (including thickening in the IPL, INL and ONL retinal layers, and reduction in cell nuclei in the GCL retinal layer) in MSC-treated T1D mouse retinas were architecturally organized as in normal mice. Finally, we assessed -by qRT-PCR- the level of molecular marker characteristic of DR including (i) markers of thickening of the vascular basement membrane; (ii) markers of inflammation; and (iii) markers of angiogenesis. Irrespective if T1D mice received or not MSCs, these markers were overexpressed in the retina in comparison with normal mice. Overall, our results show that in T1D mice systemically administered MSCs mainly home into the eyes and prevent the structural damage of the retina. Additional experiments are in course to clarify the mechanisms associated to the observed therapeutic effect.

**Poster Board Number: T-3338**

**ADIPOSE- DERIVED STEM CELLS TRANSPLANTATION WITH LOSARTAN TREATMENT ENHANCE MUSCLE REGENERATION IN INJURED MDX MICE**

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Duchenne muscular dystrophy (DMD) is a recessive X-linked form of muscular dystrophy, which results in muscle degeneration, difficulty walking, breathing, and death. Muscle-derived stem cells (MDSCs), myoblasts, hematopoietic stem cells, adipose-derived stem cells (ASCs), and induced pluripotent stem cells (iPSCs) have been used as a source for stem cell therapy in the treatment of DMD. Specifically, ASCs as a material for stem cell therapy have a variety of advantages. For successful ASC transplantation, it is very important to make a good stem cell niche. Losartan is one of the drugs that may improve the stem cell niche, by attenuating the transforming growth factor-β (TGF-β) signaling pathway. The ASC transplantation with losartan treatment can be an effective method to treat DMD. Therefore, the aim of this study was to demonstrate whether the ASCs transplantation with losartan treatment in injured-mdx mice improves muscle regeneration. Gastrocnemius and soleus muscles of mdx mice were lacerated, and then, mouse ASCs were locally transplanted to the injured muscles of the mdx mice that were treated with or without oral losartan. ASCs transplanted into injured muscles of mdx mice directly differentiated in myofibers and dystrophin expression in the ASCs was identified both in vivo and in vitro. Remarkable muscle regeneration and inhibition of muscle fibrosis were also observed in the ASCs and losartan combined treatment group. Consequently, the present study shows that the stem cell therapy using ASCs with losartan improved muscle regeneration in injured-mdx mice.

**Poster Board Number: T-3339**

**AUTOLOGOUS DENTAL PULP STEM CELLS IN REGENERATION OF DEFECT EXPERIMENTALLY CREATED IN CANINE PERIODONTAL TISSUE**

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Until now, the bone regenerative capacity of dental pulp stem cells (DPSCs) has been investigated, however no investigation has evaluated DPSCs in the regeneration of periodontium that consisted of cementum, periodontal ligament (PDL) and supporting bone. In this study, we investigated the effect of DPSCs on regeneration of periodontal tissue defect experimentally-created in canine model. Canine maxillary premolar were cut around the root-enamel boundary, the pulp tissues were gently collected and then subjected to enzymatic digestion. The digest was then plated and propagated until homogenous fibroblastic cells dominated the culture. In parallel surgically-created mesial three-walled periodontal defects with ligature-induced periodontitis were produced bilaterally in the first lower premolar teeth of ten mongrel canines. Simultaneously, DPSCs were derived from maxillary premolar teeth of the same animals. Four weeks after creation of the periodontitis model, on one side, autologous passaged-3 DPSCs combined with Bio-oss were implanted as the test group. On the other side, only Bio-oss was implanted as a control. Eight weeks after surgery, regeneration of the periodontal defects was evaluated both histologically and histomorphometrically in terms of bone,PDL and cement formation. The pulp stem cells appeared fibroblastic in morphology throughout the cultivation period. They possessed differentiation potential into bone, cartilage and adipose cell lineages. The majority of cells tended to express CD90 and CD44 surface...
intracarotid injection of adipose tissue-derived platelet-derived growth factor receptor β-positive cells in canine model: feasibility of cell delivery under mannitol-induced blood-brain barrier opening

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Chu, Kon, Lee, Soon-Tae, Kim, Jeong-Min, Youn, Sung-Won, Lee, Jong-Young, Park, Dong-Kyu, Kim, Soyun, Kim, Manho, Lee, Sang Kun, Han, Moon-Hee, Roh, Jae-Kyu

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PDGFβ-positive pericytes is a multipotent mesodermal stem cell, which can be easily isolated from adipose tissue-derived stromal cells. The purpose of this study is to evaluate the safety and feasibility of intracarotid injection of adipose tissue-derived PDGFβ-positive (AT-PDGFRβ+) cells in canine model after mannitol-induced transient blood-brain barrier (BBB) opening. The canine subcutaneous adipose tissue for normal canine was prepared after the step of acquisition, magnetic sorting, transfection by lenti-GFP and transfection by transfection. The stable proliferation of AT-PDGFRβ+ was achieved at the condition of DMEM / F12 + 5%FBS + N2 supplement without O2 saturation. On blood test, the leukocyte count, serum calcium, serum potassium and total protein level is decreased at immediate post-infusion as compared to baseline, but the other complete blood count, electrolyte, liver and renal batteries are within normal range including CRP. On neurological scales, all six dogs showed intact motor and consciousness. The brain MRI of 1 day and 7 days showed small sized infarct in two dogs. The incidence of small sized infarct was 50% (additional) in the protocol 1, and 25% (transient) in the protocol 2. In conclusion, the intracarotid infusion of AT-PDGFRβ+ cells under preparation of hyperosmolar mannitol is a feasible and safe method for the cell delivery.
LOW SERUM CULTURED ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS AMELIORATE RAT MODEL WITH ZYOMSAN INDUCED SEVERE PERITONITIS

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Background: Peritoneum dialysis (PD) is one important therapy for end-stage kidney disease (ESKD). However, patients cannot continue PD therapy for an extended period because of ultra filtration (UF) failure, infection, or other complications. One of the reasons for UF failure is submesothelial fibrosis after peritonitis. Above all, fun- gal peritonitis is a major problem as the fibrosis can progress rapidly and it may evolve into encapsulating peritoneal sclerosis (EPS) even after the peritoneal dialysis catheter has been removed. We have developed an original culture system for low serum cultured adipose-derived mesenchymal stromal cells (LASCs). We have also reported the therapeutic potential of LASCs, especially immuno- modulatory effect, in various animal models. In this study, we used rat peritonitis model induced by zymosan administration after peritoneal scrape accompanied complement activation and attempted to treat the model by LASCs. Methods: The fungal peritonitis was in- duced in the rat model by administrating zymosan daily for 5 days after scraping the peritoneum mechanically. The rats were divided into two groups; LASC (L group) or vehicle (V group) administr- ation intra peritoneally with PD solution (1.5% glucose, neutral liquid) every day. On day 5, rats were euthanized and the peritoneums were harvested, then the thickness of the peritoneum, the infiltra- tion of inflammatory cells, and the deposition of the complement were compared between the groups. To trace LASCs injected into peritoneum cavity, CFSE labeled LASCs were administered to the zymosan model, and then assessed by immunofluorescent stain- ing. For the in vitro study, primary cultured mesothelial cells (MCs) were co-cultured with LASCs directly or indirectly in trans-well, and we observed the interaction. Results: On day 5, microscopic find- ings in L group had less plaques and less edematous. Histologically, the thickness of the peritoneum, the infiltration of inflammatory cells and the deposition of complements (both C3 and membrane attack complex (MAC)) in L group was significantly more reduced than those in V group. In addition, the mesothelial cell layer on the peritoneal surface in L group recovered more quickly compared with that in V group. Also, the layer in L group showed complement regulatory factors (CD55, Crry, CD59) clearly. In the tracing study, LASCs were laid mainly on the surface of peritoneum along with the recovered MCs. For the in vitro study, MCs directly co-cultured with LASCs proliferated earlier than those cultured without LASCs. The effect was also observed when the MCs were separated from LASCs by the trans-well insert. Conclusions: Administration of LASCs into the peritoneal cavity suppressed the inflammation of peritonitis induced by zymosan, and the mesothelial cell layer in L group recovered earlier than that in V group. This data suggests that LASCs have the multiple effects on peritoneal damage. In the future, LASC therapy may be useful for treating peritoneal injury during fungal infection of the peritoneum.

STEMLNESS AND STEM CELL RELATED GENE EXPRESSION PROFILE OF THE WOUND ENVIRONMENT IN PATIENTS WITH CHRONIC WOUND

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The role of endogenous stem cells in tissue repair has been explored in clinical and pre-clinical studies. Mesenchymal stem cells (MSCs) enhance tissue repair by up regulation of genes that modulate excessive inflammation and by providing an environment which enhance the proliferation and differentiation of tissue-endogenous stem/progenitor cells. For efficient and targeted therapy of the chronic wound, its niche must be explored to identify key cellular and molecular factors involved in the process. However, it has not been known if chronic non-healing patients show different profile in the expression of stem cell markers, stem cell related genes, and signaling molecules compared to patients who progress to healing. In the present study, we assessed stemness markers and MSC specific and related gene expression profile in patients with chronic non-healing wound. During debridement, a routine wound care procedure of removal of tissue from in and around the wound to optimize wound healing, tissue samples was collected mRNA extracted, and a standard quantitative RT-PCR along with customized stem cell PCR arrays were conducted. We found that the stemness genes such as SOX2, POU5F1, WNT3A and TERT were down regulated up to seven fold in patients with non-healing wound. In particular, MSC specific markers and associated genes such as BGLAP, NGFR, EGF, HNF1A, IFNG and BGLEP were down regulated four to seven fold in comparison to patients with a healing wound. We have additional evidence showing that the stem cell signaling factor Wnt3a is down regulated in impaired-healing wounds in an animal model which correlate to our clinical findings. Defining the repertoire of stem cell and stem cell related signals expressed in patients with chronic non-healing wounds will help define the environmental niche of non-healing wounds relative to normal healing. These data will contribute in identifying target cells, genes and/or molecules to promote effective treatment of chronic non-healing wounds.

REAL TIME BIODISTRIBUTION DETERMINATION OF NONCULTURED ADIPOSE-DERIVED REGENERATIVE CELLS AFTER INTRAVASCULAR INJECTION INTO A HEALTHY SYNGENEIC HOST ANIMAL

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Understanding the temporal and anatomical patterns of biodistribution by a cellular therapeutic is critical for establishing its safety profile. Biodistribution is dependent on many different variables such as cell type and origin, health status of the recipient, and route of delivery. Therefore, where and for how long the cells will reside is likely unique for each combination of variables. Establishing an understanding of how the cells behave when delivered into a normal, healthy recipient provides a baseline for comparison and helps address one of the key cell therapy safety questions. “What happens if I inject the cells into someone who does not need them?” Materials
After mincing fat pads, adipose was treated with Celase® reagent to deliver at three different doses (2.5, 4 and 6 x 10^6 cells per animal) by tail vein injection. ADRCs were isolated from inguinal fat pads on tissue-culture polystyrene were used as the control. Comparing to monolayer culture, spheroid-derived ASCs shared similar distribution and retention may be compared in various injury and disease models.

RESULTS

The distribution pattern of ADRCs was similar regardless of dose. Upon tail vein injection ADRCs were found within the lungs and spleen by two hours. By day 3 cells were observed still primarily within the lungs and spleen but significant relocation to the mandibular and parotid lymph nodes had occurred. No signal activity was observed in the brain, heart or other major organ systems. Peak luciferase intensity occurred between initial the time point of 2 hours post-injection and seven days, depending on the animal, but did not correlate strongly with dose in the range delivered. By day 21, only background signal was detectable. Conclusion This study reports the results of the first ever GLP-level study of ADRC biodistribution. Murine ADRCs home into the spleen, lymphatic system after initially being filtered out by the pulmonary capillary bed in healthy, non-injured, syngeneic animals. Both the pattern and temporal retention of the cells is consistent with a normal physiological response to syngeneic cells and is consistent with the conclusion that vascular delivery of these cells is safe. Importantly, these data provide a baseline to which variations in cell distribution and retention may be compared in various injury and disease models.

Poster Board Number: T-3345

ENHANCEMENT OF CUTANEOUS WOUND HEALING BY HUMAN ADIPOSE-DERIVED STEM CELLS: THE EFFECT OF SHORT TERM SPHEROID FORMATION

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Adipose-derived stem cells (ASCs) represent an important source of mesenchymal stem cells because abundant autologous cells can be easily obtained via liposuction procedures. ASCs are commonly cultured as monolayers using conventional tissue culture techniques. These methods have been proved adequate, but several reports have demonstrated a loss of the replicative ability, colony-forming efficiency, and differentiation capacity with time in culture. In this study, we tested the hypothesis that ASCs experiencing short-term spheroid formation exhibit enhanced therapeutic potentials, which can accelerate cutaneous wound healing in a nude mouse model. The ASCs were passaged 2 times and plated onto chitosan films for spheroid formation. After 7 days, the spheroids were dissociated and replated on tissue-culture polystyrene. Culture media was refreshed every 2-3 days. ASCs were cultured for 7 more days and then harvested for experiments. ASCs that were constantly cultured on tissue-culture polystyrene were used as the control. Comparing to monolayer culture, spheroid-derived ASCs shared similar distribution of cell surface markers, and their adipogenic and osteogenic differentiation capabilities were comparable. Lactate dehydrogenase assay revealed less cytotoxicity of spheroid-derived ASCs under a serum starvation condition. Moreover, spheroid-derived ASCs exhibited higher expansion efficiency and colony-forming activity. Importantly, we demonstrated that spheroid-derived ASCs exhibited significant upregulation of CXCR4, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and pluripotency marker genes (Sox-2, Oct-4 and Nanog). The differences in protein expression were further confirmed by western blot analysis. Spheroid-derived ASCs also showed significant more migration in an in vitro wound healing assay and a transwell migration assay, but the enhanced migration could be inhibited by a CXCR4-specific peptide antagonist (AMD3100). The enhanced expression of matrix metalloproteinases (MMP-9 and MMP-13) in spheroid-derived ASCs could also be inhibited by AMD3100. By application of the expanded ASCs in a dorsal cutaneous wound model of nude mice, we further showed a faster wound healing in the group that received spheroid-derived ASCs at postoperative day 10. In the cutaneous wounds that received spheroid-derived ASCs, histology showed a significantly thicker epidermal layer and immunohistochemistry of human nuclear antigen demonstrated more cellular retention of ASC. Moreover, significantly more cells labeled with the proliferative marker Ki-67 were noted in the group of spheroid-derived ASCs. The results presented here indicated that ASCs can be activated nonchemically by short-term spheroid formation to express CXCR4, HGF, VEGF, and pluripotent markers. The upregulation of CXCR4 in the spheroid-derived ASCs further increases MMP-9 and MMP-13 expression, resulting in enhanced migration of these cells. The interaction between stromal-derived factor-1 and CXCR4 plays an important role in the homing of mesenchymal stem cells to the site of injury. In our animal study, we observed significantly more cellular engraftment of spheroid-derived ASCs in the cutaneous wound tissue with accelerated wound healing comparing to monolayer-cultured ASCs. Therefore, short-term spheroid formation of ASCs on chitosan films can increase their therapeutic potentials for wound healing.

Poster Board Number: T-3346

CONSTRUCTION OF LARGE AMOUNTS CULTURE SYSTEM BY AN AUTOMATED CELL PROCESSING MACHINE FOR THE CELL TRANSPLANTATION

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Recently, practical applications of pluripotent and/or multipotent stem cell for various diseases are proceeding exponentially. Several clinical trials has been reported a significant improvement of patient prognosis and quality of life using autologous cells. While they will widespread as the general health care, manufacturing cellular products remains to be solved. We are conducting a clinical trials using autologous cardiac stem cells (CSCs) from patients with ischemic heart disease (AutoLogous Human CArdiac-Derived Stem Cell to Treat Ischemic cArdiomyopathy, ALCADIA). The phase I trial is approaching to complete, and the phase II multicenter trial is under a plan. In order to perform the phase II trial effectively, it's necessary to develop a system to cultivate stem or progenitor cells with a given quality and high throughput. To solve this problem, we focused on the robotics of automated and high throughput culture system (AutoCulture) made by Kawasaki Heavy Industries. AutoCulture can change the medium, centrifuge, split, and record the morphology, and has already succeeded in culturing the cell lines such as HeLa cell, and can cultivate the maximum 29 types of cell lines at the same time. However, it has not been reported stem
or progenitor cells have been cultivated for clinical application by a automated robotics in Japan. Therefore, we have examined and compared the growth rate and the characteristics of CSCs cultivated by automated cell processing machine and the manual handling culture. In this study, there were no significant difference of CSCs growth rates between the AutoCulture (Population Doubling: PD+6 to 7 in 2 weeks) and the manual handling culture (PD+6 to 7 in 2 weeks).

Poster Board Number: T-3347

INFLAMMATORY STATUS DETERMINES THE EFFECTIVENESS OF MESENCHYMAL STEM CELL THERAPY OF ADVANCED LIVER CIRRHOSIS

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Mesenchymal stem cells (MSCs) have been shown to be effective in the treatment of inflammatory disorders only in some patients. To investigate the factors that determine the effectiveness of MSC therapy, we established the mouse model of severe liver cirrhosis with carbon tetrachloride and found that MSCs were highly effective in reversing serum levels of total bilirubin, albumin, aspartate aminotransferase and alanine aminotransferase as well as liver fibrin deposition. Since our previous studies have shown that the immune modulatory effects of MSCs are elicited by inflammatory cytokines, we treated the cirrhotic mice with dexamethasone prior to the administration of MSCs. We found that such pretreatment dramatically eliminated the effects of MSCs, revealing a critical role of inflammation in the curative effects of MSCs. Interestingly, in clinical settings, among the liver cirrhosis patients transplanted with MSCs, those belonged to groups with Child-Pugh score B and C, especially patients with acute inflammation, exhibited improved outcome of their conditions. Our findings demonstrate that the inflammatory status determines the effectiveness of MSCs in treating liver cirrhosis and provides a novel strategy for patient selection and treatment protocol designs for mesenchymal stem cell therapy.

Poster Board Number: T-3348

LOW SERUM CULTURED ADIPOSE-DERIVED MESENCHYMAL STEM CELLS AMELIORATE CRESCENTIC GLOMERULONEPHRITIS BY FUNCTIONAL POLARIZATION OF MACROPHAGES INTO IMMUNOREGULATORY M2 PHENOTYPE

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Introduction: Glomerular crescents determine renal prognosis in patients with rapidly progressive glomerulonephritis, however efficient therapeutic approach to regulate crescent formation has not emerged since diverse leukocyte subsets contribute to the lesion. We have reported that adipose tissue-derived stem cells (ASC) promoted regeneration in a rat model of acute kidney injury. More recently, we have shown that ASC more strongly modulate T-cell immune reaction than bone marrow derived mesenchymal stem cells (BM-MSC). In the present study, we examined the renal protective effects of ASC focusing on their immunomodulatory properties in anti-glomerular basement membrane glomerulonephritis (anti-GBM GN) leading severe crescentic formation. Methods: Necrotizing crescentic glomerulonephritis was induced in WKY rats by intraperitoneal injection of anti-GBM mAb, which bore close resemblance to nephritis in human Goodpasture disease. Renal function and histology were assessed in animals treated with ASC or BM-MSC. To evaluate ASC-driven functional M2 polarization in macrophage, we cultured peritoneal macrophages with ASCs or BM-MSC. Results: Intravenous injection of ASC significantly prevented renal dysfunction and proteinuria in diseased animals. The score of crescent formation was significantly decreased in ASC group compared to control group. Interestingly, infiltration of M2 macrophages in glomeruli was increased only in ASC group despite comparable number of infiltrated macrophages to control group. IL-10 concentration in renal cortex from diseased rat was higher in ASC group than in control group. In vitro co-culture system clearly demonstrated that ASC, but not BM-MSC, directly turned macrophage into M2 phenotype. Moreover, these effects of ASC were more prominent in low serum cultured ASC (LASC) than high serum cultured ASC (HAS). Administered ASCs were scattered in multiple organs and number of ASC in diseased glomeruli was 1.5/glomerulus/cross section without any difference between HAS and LASC, but this would be efficient since individual LASC could polarize around 200 macrophages to M2 phenotype in vitro. These results collectively suggest that LASC recruited into diseased glomerulus make stronger effect on macrophages for protection of glomerular injury and crescent formation than HAS. Conclusion: ASC exerted profound immunoregulatory properties especially on macrophages and ameliorated glomerular injury in rat anti-GBM glomerulonephritis model. In human immunosuppressive therapy such as corticosteroid and cyclophosphamide remains the prevailing therapy for CGN, but this is often restricted for their adverse effect such as infection and agent cytotoxicity. Therefore, LASC administration would be desirable therapeutic approach to improve prognosis of anti-GBM GN patients.

Poster Board Number: T-3349

ASTROGLIAL ACTIVATION BY ENVIRONMENTAL ENRICHMENT SYNERGISTICALLY IMPROVES FUNCTIONAL RECOVERY BY TRANSPLANTED MESENCHYMAL STEM CELLS

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We investigated the effects of environmental enrichment (EE) on the function of transplanted mesenchymal stem cells (MSCs) and the combined effect of EE and MSC transplantation on neurobehavioral function in an animal model of chronic hypoxic-ischemic (HI) brain injury. HI brain damage was induced in 7-day-old mice by unilateral carotid artery ligation and exposure to hypoxia (8% O2 for 90 min). At 6 weeks of age, the mice were randomly injected with either MSCs (1×105 cells) or PBS into the striatum and were randomly assigned to either EE or standard cages (SC), comprising MSC-EE (n=18), MSC-SC (n=19), PBS-EE (n=12), PBS-SC (n=17), and untreated controls (n=23). Rotarod, forelimb-use asymmetry, and grip strength tests were performed to evaluate neurobehavioral function. The fate of transplanted cells and the levels of endogenous neurogenesis, astroglial activation and paracrine factors were also assessed. As a result, EE and MSC transplantation synergistically improved rotarod latency at constant speed of 48 rpm (156.8±18.1 sec) and at accelerating speed (168.5±15.1 sec) 8 weeks after treatment (p<0.05). Forelimb-use asymmetry and grip strength were also improved compared to those of the other group (p<0.05), whereas ASC transplantation causes only hemiplegic grip strength recovery. MSC-EE mice also showed a higher GFAP+ cell density than the other groups at 2 weeks post-treatment (F = 18.186, p<0.05); GFAP+ cell densities were MSC-EE (12.5 ± 1.2 %), MSC-SC (8.2 ± 0.7 %), PBS-EE (5.7 ± 0.5 %), and PBS-SC (4.9 ± 0.6 %). At 8 weeks after treatment, MSC-EE (5.5 ± 0.7 %) still showed a sig-
Safeguarding the Babies: Exploring Placental Cells as a Future Treatment for Critical Radiation Injury

Inhalation of radionuclides or exposure to high levels of radiation can cause serious damage to the body, particularly in the lungs and heart. However, the immune response that follows the radiation can also cause harm to the body's defense mechanisms, making it difficult to treat radiation-induced injuries.

One promising approach is the use of placental cells as a treatment option. Placental cells are stem cells that are easily available and can be used as an allogeneic cell therapy. They are simple to collect and can be stored as a product "off the shelf," making them a readily available option for treatment.

The current study investigated the use of placental cells to mitigate the effects of systemic radiation. The cells were injected into C3H mice, a genetically defined strain that is sensitive to radiation. The mice were exposed to a total body lethal radiation dose of 750 rads. The results showed that a single injection of placental cells after radiation exposure led to a significant increase in GFAP+ cell density without any adverse effects.

The study also showed that the combination of EE and MSCs do not increase the detrimental effects of radiation. This suggests that the use of placental cells in radiation therapy is safe and effective.

Conclusion: The use of placental cells as a treatment for radiation injury shows promise as a safe and effective option. Further research is needed to determine the optimal dosage and timing of injection for optimal results.
Detailed Program and Abstracts — Thursday, June 14

C was depleted or provided in drinking water *ad libitum* considering experimental groups and allogenic adipose-derived stem cells were locally transplanted to the injured lesion. Vitamin C supplied mice exhibited more complete regeneration of damaged muscle than those depleted vitamin C in both gross and microscopic observations. Carbonylated proteins, which are the end-products of oxidative stress, were detected potently in vitamin C-depleted mice and the positivity was more potent in the presence of transplanted stem cells. Moreover, the serum total vitamin C level and ascorbic acid (reduced form) to dehydroascorbic acid (oxidized form) ratio also were decreased in the ASC transplanted groups. Collectively, these data can be considered as proof of vitamin C utilization by cells in vivo. In conclusion, we demonstrated that vitamin C increases the viability of transplanted stem cells by diminishing the oxidative and inflammatory stress, and thereby it can positively affect muscle regeneration in the case of adipose-derived stem cell transplantation and might be used as a therapeutic agent supplied in cell transplantation.

**Poster Board Number: T-3353**

**ENHANCED HOMING, PERMEABILITY, AND RETENTION OF BONE MESENCHYMAL STEM CELLS (MSC) USING PULSED FOCUSED ULTRASOUND: IMPLICATIONS FOR TARGETING STEM CELL DELIVERY**

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Introduction: Therapeutic bone marrow stem cells (BMSC, also known as mesenchymal stem cell, MSC) are capable of homing to sites of inflammation and ischemia following intravascular injection, but are often limited by inefficient tropism to pathological sites. Directed homing and tissue integration of BMSCs may be key to improving their therapeutic applications. Therefore the development of a non-destructive, non-invasive technique that would release chemoattractants resulting in BMSCs localization within or at the periphery of pathology would be valuable for cellular therapies. Pulsed focused ultrasound (pFUS) is an emergent noninvasive modality that non-destructively emphasizes the non-thermal effects of FUS (i.e., acoustic cavitation and radiation forces). These non-thermal mechanisms increase tissue permeability and enhance drug or gene delivery, while inducing only minor and transient morphological changes within the treated region. Relatively little is known about the cellular and molecular biological effects of pFUS exposures beyond the structural changes that result in vascular leakage. Cellular and molecular biology of tissues can be dramatically altered by mechanical force and stress through the process of mechanotransduction (i.e. tissue cytokine and chemokine expression in response to pFUS mechanical forces).

In this study, we examined the bioeffects of pFUS in murine muscle and kidney tissue and found that pFUS exposures induced local expression of chemoattractant factors (cytokines, growth factors, integrins) and investigated the utility of pFUS to direct BMSC homing in vivo. ([Unsuppported Character - ]

Methods: Balb/c or C3H mice were intra-muscularly administered 106 human BMSC 2 hr post-pFUS. Mice with renal disease received cisplatin (15 mg/kg) induced acute tubular necrosis (ATN) 24 hr before pFUS. At various time points, tissue was subjected molecular analyses for cytokine and growth factors, histology, or immunofluorescence for detection of BMSC. Results: pFUS significantly increased local cytokines (i.e., IL1β, IL-2, IL-3, IL-5, IL-6, IL-10, IL-17, IFNγ, MCP-1, GMSCF, and RANTES) in pFUS-treated tissue that declined to contralateral levels by 3 days post-pFUS. Elevated cytokines were accompanied by increased expression of growth factors (i.e., VEGF, FGF, PIGF, HGF SDF-1) and ICAM and VCAM usually associated with active BMSC homing. Following pFUS, significantly greater numbers (5-10x) of human BMSC migrated into pFUS-treated tissues compared to controls. Enhanced homing, permeability, and retention (EHPR) of BMSCs were significantly increased (10x) by repeated doses of BMSC coupled with repeated pFUS exposures in muscle without damage to tissue. pFUS coupled with BMSC infusion in the ATN model resulted in significantly greater numbers of stem cells in treated kidney compared to control and was also found to decrease the peak BUN and Creatinine level on day five post cisplatinum injection compared to control mice or mice that received only BMSC. Conclusions: The ability to couple pFUS with cell infusions, both during the innate pathological inflammation or after it has resolved, greatly increases the flexibility of stem cell-based therapies. pFUS may provide the temporal and spatial control over directing stem cells to sites of pathology thereby potentially improving treatment of pathologies or regenerative medicine.

**Poster Board Number: T-3354**

**INJECTABLE BIOMATRIX FOR ARTHROSCOPIC SURGERY IN DEGENERATIVE CARTILAGE**

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Abrasion, microfracture and osteochondral graft are examples of methods for treating osteoarthritis. However, these procedures are invasive, requiring open surgery. Herein, we have developed an injectable biomatrix (IBM) which can be applied on damaged or degenerative cartilage. We have also demonstrated the efficacy of the IBM with umbilical cord-derived mesenchymal stem cells (UC-MSCs) on damaged rabbit cartilage. IBM was composed of 2% hyaluronic acid derivative and 3% type I collagen isolated from human umbilical cord at a ratio of 1:2. UC-MSCs within the IBM were successfully differentiated into chondrocytes in vitro and in vivo conditions through transplanting to the subcutaneous layer of nude mice. For efficacy test, UC-MSCs within IBM were injected into the injured cartilage of adult New Zealand white rabbits. After 12 weeks of transplantation, histological analysis showed the regeneration of cartilage on the damaged site. In conclusion, our study shows that IBM is promising for the treatment of degenerative osteoarthritis via arthroscopic procedures.

**Poster Board Number: T-3355**

**PRECLINICAL AND CLINICAL TRIALS OF BONE-MARROW MESENCHYMAL STROMAL CELL IN AMYOTROPHIC LATERAL SCLEROSIS**

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by motor neuron loss. Although the underlying cause of the disease remains unclear, a variety of pathogenic mechanisms have been proposed. Despite promising preclinical studies showing the modification of the disease progression, most trials have failed to demonstrate any significant improvement in outcome. Therefore, stem cell therapy has been proposed as an alternative therapy for ALS. In animal model of ALS (transgenic SOD1mice), we evaluated the dose-dependent effects of human bone marrow mesenchymal stem cells (hMSCs)
obtained from an ALS patient (ALS-hMSCs) on SOD1 mice via intrathecal injection and showed the practical possibility of it for hMSCs. We transplanted different doses (1 × 10⁴, 2 × 10⁵, and 1 × 10⁶) of ALS-hMSCs into the cisterna magna and performed clinical observations including symptom onset and survival time, and locomotor performance using the rotated test. A cell dose of 1 × 10⁵ cells significantly prolonged life span and delayed the decline of motor performance. Most injected hMSCs distributed in the ventricular system and subarachnoid space and some migrated into the brain and spinal cord. These data suggested that intrathecal injection with an optimized cell number might be the practical potential route for stem cell therapy in ALS. With animal data, we evaluate efficacy and safety of Autologous Bone Marrow-derived Mesenchymal Stem Cell treatment in patients with ALS. After a lead-in period for 3 months, 22 patients were treated with MSCs twice at an interval of 1 month. After initial MSCs injection, all patients were followed up for 3 months and their disease course, clinical characteristics were assessed. Disease status of patients were analyzed with ALS functional rating scale-revised (ALSFRS-R) for primary outcome measure, and additional clinical findings after treatment were all collected for secondary outcome measure and safety. Age and disease-duration matched patients with ALS were selected as a control group. During the follow-up period, MSCs treatment yielded a significant lesser change of ALSFRS-R score, compared to control group (1.54 vs 3.56, p<0.01). Moreover, the slope of decline of ALSFRS-R was significantly lower during the follow-up period, compared to the lead-in period in MSCs treatment group (2.68 vs 1.54, p=0.04), whereas the slopes during the two periods were not different in the control group (3.15 vs 3.56, p=0.37). MSCs treatment was well tolerated except for occurrences of transient headache, low back pain, and myalgia. Our results suggest that intrathecal MSCs injection can slow disease progression and might be used as a disease modifying modality as an alternative treatment choice in patients with ALS

Poster Board Number: T-3356

MESENCHYMAL STEM CELL EXOSOME: A NOVEL STEM CELL-BASED THERAPY

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Mesenchymal stem cells (MSCs) are the cells of choice for regenerative medicine with more than 100 registered clinical trials evaluating their efficacy in 2010. The main allure of MSCs lie in their protective effects on an amazingly wide spectrum of tissue injury, ease of isolation from ethically palatable adult tissue sources, a large ex vivo expansion capacity, as well as demonstrated multipotency and immunosuppressive activity. It has been increasingly observed that the therapeutic efficacy of MSC therapy is not dependent on the engraftment of MSCs at the site of injury or differentiation capability of the transplanted MSC. To reconcile the discrepancy between these observations and the therapeutic effects of MSC, it was postulated that transplanted MSC secrete trophic mediators that reduce tissue injury and enhance repair. Consistent with this, we demonstrated that MSCs secrete membrane vesicles known as exosomes and these exosomes reduce myocardial ischemia/ reperfusion injury in a mouse model. The use of secreted exosomes and not MSCs eliminates the need for transplantation of viable replicating cells and thus mitigates many of the associated safety concerns and limitations. MSC exosome contains protein and RNA. Proteomic analysis of the exosome proteome revealed a diverse array of proteins distributed over a wide range of biochemical and cellular processes, and thus a large potential to intervene in a wide range of cellular activities. This large potential could provide a mechanistic underpinning for the therapeutic effects of MSC on a wide spectrum of complex diseases. Therefore exosomes represent a novel stem cell-based therapy.

Poster Board Number: T-3357

ASSESSMENT OF THE SAFETY TRANSPLANTATION OF ALLOGENIC MESENCHYMAL STROMAL CELLS OF BONE MARROW IN PATIENTS INFLAMMATORY BOWEL DISEASES: 4 YEARS OBSERVATION.

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Patients suffering from long-term Inflammatory Bowel Diseases (IBD) are at increased risk for cancer of the intestine (7-10 times more frequently than in the general population of people). IBD patients who are suffering from more than 10 years, the probability of cancer is increasing annually at 0.5%. Cell therapy is a medical technology a high degree of risk. In addition to invasive methods for early diagnosis of cancer, determination of tumor markers can identify groups at increased risk of cancer. Aim. To evaluate the safety of transplantation of allogeneic mesenchymal stromal cells (MSCs) of bone marrow in the treatment of patients with Ulcerative Colitis (UC) and Crohn’s disease (CD). Materials and methods. In the Department of Pathology bowel 86 patients with UC and CD in 2008-2012 years a behavioral system transplantation of allogeneic MSCs. 68 patients has a fore - year observation period. Patients underwent endoscopic control study of colon with multiple ladder biopsy. In the serum of patients determines the levels of tumor markers CA 242, CA 19-9 and REA as well as transforming growth factor-1β (TGF-1β) using test-systems company “Protein contour” (St. Petersburg), Orgentec (Germany ) Evroimmun (France).

Results. In 31 patients with UC for 12 months of observation, a decrease of endoscopic activity index from 8,3±0,26 to 0,76±0,2 points. In 8 patients with UC of 12 (66.7%) recorded the clinical and morphological remission. Baseline tumor marker CA 242 to MSC transplantation was 5,26±0,2 U/ml, the level of tumor marker CA 19-9 - 5,03±0,18 U/ml. After 6 months the level of tumor marker CA 242 was 4,93±0,17 U/ml, CA 19-9 - 4,83±0,16 U/ml after 12 months - 4,8±0,17 U/ml, 4,96±0,16 U/ml, respectively. After 24 months the level of tumor marker CA 242 was 4,85±0,2 U/ml, CA 19-9 - 5,25±0,22 U/ml. After 48 months the level of tumor marker CA 242 was 4,95±0,2 U/ml, CA 19-9 - 5,65±0,2 U/ml, REA - 6,25±0,25 U/ml. The level of TGF-1β before MSC transplantation was 73,4±16,2 pg/ml, rising to 1 week after injection to 792,5±50,5 pg/ml at 4 weeks after transplantation of MSCs level of TGF-1β was - 448,6±76,1 pg/ml. These levels of TGF-1β (less than 1500 pg/ml) are safe in terms of tumor development (malignancy) and maintenance of tumor growth or metastasis. During 24-46 months (42,3±0,8) of observation for patients who carried MSC transplantation, in neither case has been registered increase of tumor markers of over 10 U/ml and TGF-1β levels above 1500 pg/ml, as well as malignant transformation of IBD according to endoscopic examinations. Conclusion. Transplantation of allogeneic mesenchymal stromal cells of bone marrow did not lead to the development of tumor process and is a safe method of biological therapy of IBD.
MESENCHYMAL STEM CELLS DELIVER SYNTHETIC MICRORNA MIMICS TO GLIOMA CELLS AND GLIOMA STEM CELLS AND INHIBIT CELL MIGRATION AND SELF-RENEWAL

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Malignant glioma are the most common and aggressive primary brain tumors with poor prognosis and median survival of 12-14 months. Limitations to therapy include the distinctly infiltrative nature of the tumors and the high resistance to radio- and chemotherapeutic of residual tumor cells and glioma stem cells (GSC). MicroRNAs are emerging as potential cancer therapeutics; however, their clinical use is hindered by a lack of effective delivery mechanisms to tumor sites. Mesenchymal stem cells (MSCs) have been recently shown to migrate to experimental glioma and to exert anti-tumor effects by delivering cytotoxic compounds. Here, we examined the ability of MSCs derived from bone marrow, adipose tissue, placenta and umbilical cord to deliver synthetic microRNA mimics to neighboring glioma cells and glioma stem cells (GSCs). For these studies we examined the delivery of miR-124 and miR-145 mimics since both glioma cells and GSCs express very low levels of these microRNAs. Using fluorescent labeled miRNA mimics and in situ hybridization, we demonstrated that the MSCs examined delivered miR-124 and miR-145 mimics to co-cultured glioma cells and GSCs via gap junction-dependent and independent processes. Moreover, the transferred miR-124 mimic significantly decreased the expression of the target gene SCP-1 and its 3'UTR tagged to luciferase. The delivered miRNA mimics also decreased the migration of glioma cells and the self-renewal of GSCs. These results suggest that MSCs can functionally deliver synthetic miRNA mimics to glioma cells and GSCs and may provide an efficient route of therapeutic miRNA delivery in vivo.
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