JUNE 18, 2014

WEDNESDAY LATE BREAKING ABSTRACT POSTER PRESENTATIONS

6:30 PM- 7:30 PM ODD numbered posters presented
7:30 PM- 8:30 PM EVEN numbered posters presented

W-L-4002

USING HUMAN INDUCED PLURIPOTENT STEM CELLS TO MODEL PATIENT-SPECIFIC PRIMARY IMMUNODEFICIENCY DISEASES AND GAINING INSIGHT INTO HUMAN THYMOCYTE DEVELOPMENT

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There are over 150 recognized forms of human primary immunodeficiencies (PIDs), and an estimated 1 person in 40,000 carries a mutation in one allele. One of the most severe types of PID is severe combined immunodeficiency (SCID), and is characteristic of T- and B- lymphocyte function. Hematopoietic stem cell transplant (HSCT) is the most successful treatment, but requires a compatible HLA-match and early identification of the disease and transplant. The recombination activating gene 1 (RAG1) plays an important role in thymocyte development, thus hypomorphic mutations within this gene can result in a wide phenotypic range. Mutations that ablate RAG1 activity result in SCID and infections, while lowered RAG1 activity will shift the phenotype from increased risk of infections to autoimmunity-related burdens, with varying degrees of T cells present. Diseases range from the most severe to the least severe. These include Omenn syndrome, gamma-delta leaky SCID, leaky SCID, and granuloma, and correlate with RAG activity. By using induced pluripotent stem cells (iPSCs) derived from patients with PIDs (including SCID, Omenn syndrome, and leaky SCID), we have characterized T lineage differentiation in vitro and recapitulated the patient-specific blocks in development for each. Further, assessment of recombination in the derived T lineage cells suggests a distinct and restricted T cell repertoire. As a proof-of-principle for therapeutic treatment, we have stably expressed ectopic RAG1 with wild-type activity in several of the hypomorphic RAG1 iPSC lines and observed improved T lineage development. Together, our findings demonstrate that iPSCs can be used to model PID diseases and elucidate the effects that patient-specific hypomorphic muations have on T cell developmental. This helps to not only answer important questions about the precise effects that the loss of proteins such as RAG1 have in a human context, but also allow the assessment of therapeutic approaches in vitro.

W-L-4003

MODELING SELF-ORGANIZED PATTERN FORMATION AND GASTRULATION-LIKE EVENTS IN VITRO USING MICRO-PATTERNED CELL CULTURE

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Understanding how the cells of the embryo generate patterns is a central challenge in developmental biology but is a difficult question to address mechanistically in vivo. Here we exploit our recent demonstration that human embryonic stem cells confined to a circular colony with micropatterning technology differentiate in an ordered sequence to all three germ layers to explore aspects of gastrulation and self-organized cell patterning in vitro. We show that during differentiation, the entire top surface of the colony remains a continuous epithelium and that in a region expressing markers of the primitive streak, cells mimic many aspects of gastrulation in the embryo, including signaling events, an EMT transition, rearrangement of the actin cytoskeleton, and cell migration out of the epithelium. We also use this system to explore how signals generate patterns and demonstrate that self-organized pattern formation follows a two-step mechanism. In the first step, endogenous BMP inhibitors Chordin and Noggin confine the response to the exogenously supplied BMP to the colony border while in the second step, BMP induces a patterning system of Nodal and its inhibitors Lefty1 and Cer1 and the interaction between these determines the size of the mesendodermal territory. These results establish that many aspects of development including gastrulation movements and self-organized pattern formation can be studied quantitatively in vitro using micropatterned hESC culture.

W-L-4004

HUMAN INDUCED PLURIPOTENT STEM CELLS PREDICT BREAST CANCER PATIENTS' PREDILECTION TO DOXORUBICIN-INDUCED CARDIOTOXICITY

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Doxorubicin (Adriamycin) is an anthracycline chemotherapy agent effective in treating a wide range of malignancies with a well-established dose-response cardiotoxic side-effect that can induce heart failure. Even at relatively low doses of doxorubicin (cumulative doses of 200-250 mg/m2), the risk of cardiotoxicity is estimated at 7.8% to 8.8%. Doxorubicin-induced cardiotoxicity (DIC) can range from asymptomatic reductions in left ventricular ejection fraction (LVEF) to highly symptomatic (Class III to Class IV) heart failure. At present, it is not possible to predict which patients will be affected or to protect patients who will likely suffer this devastating side effect. Here we demonstrate that patient-specific human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) can recapitulate this predilection to doxorubicin-induced cardiotoxicity (DIC) at the single cell level. hiPSC lines were generated from eight female breast cancer patients, four of whom had experienced DIC and four who did not, and differentiated into cardiomyocytes. Optimal in vitro doses of doxorubicin were established using live cell imaging, immunofluorescence, and microarrays, highlighting cardiac developmental-related gene regulation as a cellular response to low levels of doxorubicin. Independent component analysis was used

to establish DIC-related modules of differential gene expression. We evaluated DIC in patient-derived hiPSC-CMs by examining cell viability, reactive oxygen species (ROS) production, metabolic function, apoptosis, DNA damage, and calcium handling. DIC patient-derived cells were consistently more sensitive to doxorubicin toxicity and exhibited significantly lower levels of mitochondria in the hiPSC-CMs. Mechanistically, we found that antioxidant pathway genes are differentially regulated in DIC patient-derived hiPSC-CMs. Our data indicate that hiPSC-CMs are a suitable platform for identifying and verifying the genetic basis for DIC, allowing the prediction of DIC susceptibility and modification to treatment regimen. Finally, the sensitivity of this platform may allow identification of therapeutic agents that protect against DIC.

W-L-4005

PODOCALYXIN PROMOTES BREAST CANCER TUMOR GROWTH AND METASTASIS BY ENHANCING TUMOR-INITIATING CELL PROPERTIES

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Podocalyxin is a CD34-related sialomucin important in regulating cell adhesion, migration, and polarity of hematopoietic progenitors, vascular endothelia and specialised kidney epithelial (podocytes). However, podocalyxin is also overexpressed on a subset of aggressive primary breast tumors with high risk of distant metastases and poor prognosis. We used two complementary methods to determine if podocalyxin has a direct functional role in breast cancer cell behaviour. First, we ectopically expressed podocalyxin in the human breast cancer cell line, MCF-7. Parental MCF-7 cells express low levels of endogenous podocalyxin and are minimally invasive and non-metastatic in immunodeficient mice. Forced expression of podocalyxin in MCF-7 caused apical membrane expansion, microvillus formation, loss of adhesion in monolayer culture, and enhanced tumorsphere formation. Next, using shRNA, we silenced podocalyxin expression in MDA.MB-231 cells; a basal-like breast cancer cell-line that expresses high levels of endogenous podocalyxin, exhibits poorly polarized monolayer and tumorsphere architecture *in vitro*, and forms "metastatic" lung tumors *in vivo*. We found that deletion of podocalyxin in MDA.MB-231 cells attenuated tumorsphere-forming efficiency. In addition, using mouse xeongraft models, we found that podocalyxin expression is critical for the progression of primary tumors and distant metastases to the lung, liver and bone marrow. Importantly, podocalyxin expression did not alter initial "seeding" of tumor cells but was instead, important for the more longterm establishment of tumor nodules. Together, both gain-of-function and loss-of-function experiments suggest a role for podocalyxin in tumor-initiating cell (TIC) function, primary tumor growth and metastasis.

To test whether targeting Podxl might be of therapeutic benefit, we developed a novel panel of monoclonal antibodies (mAbs) against tumor-expressed human podocalyxin. Although several candidate mAbs were highly selective for podocalyxin expressed on MDA.MB-231 cells, we found that systemic treatment of tumor-bearing mice with one of these (anti-PodoC1) inhibits both primary tumor

development and metastatic progression. This therapeutically important pre-clinical finding suggests that up-regulation of podocalyxin on breast cancer cells may be a key event in tumor progression and metastasis. Moreover, because podocalyxin expression is associated with metastases in many other epithelial cancers, podocalyxin-targeted therapies may have far-reaching applications.

W-L-4006

MOLECULAR DEFINITION OF THE DEVELOPING HUMAN STRIATUM

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The brain is the most complex of biological systems and our knowledge on the developmental mechanisms underlying its formation and organization have been based on studies in rodents. Here we systematically investigated gene expression changes that occur in the prenatal human striatum and cerebral cortex development during post-conceptional weeks 2 to 20. We identified tissue- and temporal-specific co-expression gene networks, differentially expressed genes and a minimal set of bimodal genes, including those encoding transcription factors, that distinguish striatal from neocortical identities. Unexpected differences from mouse striatal development were discovered. We monitored 35 determinants at protein level, revealing the regional domain expression, and their refinement, during striatum development. These results provide an unprecedented resource and opportunity to gain global understanding of how transcriptional processes converge to specify human striatal and neocortical neurons during development.

W-L-4007

GANGLIOSIDES AS A POTENTIAL NEW CLASS OF STEM CELL MARKERS: THE CASE OF GM1 AND GD3 IN MINI-PIG BONE MARROW MESENCHYMAL STEM CELLS

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In the search for specific markers of the differentiation of mini-pig bone marrow mesenchymal stem cells (mpBMCs) toward neuronal cells, we studied their glycosphingolipid pattern, with particular attention to gangliosides. mpBMCs contained GM3, GM1 and GD3 as major gangliosides. In order study, their distribution in mpBMCs and its possible change during the differentiation of neuronal cells. When mpBMCs were cultured under neural differentiation media contained BME/DMSO/BHA, most of mpBMCs acquired the distinctive morphological features like neural cells. In differentiated cells, expression of neural markers such as neural precursor marker (nestin), neuronal markers (β-tubulin, neurofilament-M) and astrocyte marker (GFAP) were further demonstrated by revered transcription-PCR, western blotting and immunofluorescence. Specifically, we find that a significant increase in GM1 and GD3 expression was observed during neural differentiation of mpBMCs. These results suggest that GM1 and GD3 may play a role in the neural differentiation process of mpBMCs. Acknowledgement: This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (PJ00999901)" RDA and the KRIBB Research Initiative program (KGM4251614).

W-L-4008

MESENCHYMAL STROMAL CELLS PROLONG LIFESPAN OF PROGERIA MOUSE MODEL.

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The accumulation of cellular damage, including DNA damage, contributes to aging and aging-related degenerative changes. Excision repair cross complementary group 1-xeroderma pigmentosum group F (ERCC1-XPF) is a structure-specific endonuclease that is required for multiple DNA repair pathways. Mutations affecting expression of ERCC1-XPF cause a severe progeroid syndrome in humans and in mice. We have used both natural aged mice and ERCC1-deficient mice as a model of accelerated aging to examine the effect of aging on adult stem cell function. We previously published that muscle derived stem/progenitor cells (MDSPCs) become dysfunctional with both natural and accelerated aging in regard to proliferation and differentiation. We now show that bone marrow MSCs (BM-MSCs) derived from naturally aged and progeroid Ercc1-/- mice rapidly become senescent with reduced ability to differentiate in culture compared to BM-MSC from young mice. We also demonstrated previously that intraperitoneal (IP) administration of 1 X 106 MDSPCs isolated from young wild-type mice into ERCC1-deficient mice confers significant lifespan extension. Here we examined the ability of BM-MSCs derived from young mice to extend lifespan. Our preliminary results indicate that BM-MSCs from young mice, subjected briefly to oxidative stress, also prolong lifespan in Ercc1-/- mice, similar to MDSPCs. In contrast, BM-MSCs not subjected to oxidative stress appear to have no effect on lifespan. Taken together, these results suggest that two types of adult stem cell populations, BM-MSCs and MDSPCs, from young mice and subjected to oxidative stress conditions extend lifespan following IP injection in a mouse model of human progeria. Furthermore, our results suggest that the effect on lifespan is most likely mediated through a

paracrine effect by factors secreted from young, but not old stem cell populations. Progress on identifying the paracrine factors able to extend lifespan in progeroid mice will be presented.

W-L-4009

ZINC FINGER PROTEIN 423 TARGETS HEDGEHOG SIGNALING GENE EXPRESSION IN ADULT NEURAL STEM CELLS, AND INTEGRATES SIGNALING PATHWAYS BY THE NICHE.

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Zinc finger protein 423 (Zfp423) is a 30 Kruppel like C2H2 zinc finger transcription factor which binds to EBF, BMP-responsive SMAD, and possibly Retinoic acid receptor proteins. In mice lacking Zfp423 (Zfp423n12/n12), adult neurogenic regions are malformed, including the ventricular (VZ) and subventricular (SVZ) zones lining the lateral ventricles and the subgranular zone of the dentate gyrus. We found significant accumulation of Sox2+, Pdgfra+ and DCX+ cells lining the LV, especially at the dorsal lateral wall and ventral tip, and in the anterior LV. Increased DCX+ signal is also detected at the Zfp423n12/n12 olfactory bulb. Adult neural stem cells (aNSCs) cultured from the VZ and SVZ of the Zfp423n12/n12 showed accelerated growth under both renewal and differentiation conditions, increased number of DCX+ neuroblasts after RA differentiation, but decreased neurite formation after either BMP or RA differentiation. In addition, Zfp423n12/n12 aNSCs were more susceptible to growth arrest by TgfB1. Whole genome expression analysis, supported by high-throughput microfluidic quantitative PCR confirmation, showed an altered timing of gene expression after RA or BMP differentiation, and implicates hedgehog signaling as the most significant Zfp423 target under renewal and RA or BMP differentiation conditions. Surprisingly, lack of Zfp423 inverted the relative expression levels of Gli2 and Gli3 transcription factors relative to wild type control cultures. Growth arrest related genes, such as Gas6 and Gadd45a are down regulated in Zfp423n12/n12, consistent with the overgrowth phenotype. Furthermore, Zfp423 alters the expression of Nrn1, Anxa3, Gria1, Igf2bp3 and Zic2. In conclusion, RA, BMP and hedgehog signals from the niche require Zfp423 to coordinate the appropriate early progenitor and stem cell response during neurogenesis.

W-L-4010

GENOMIC DELETION OF ARX IN HESCS REDUCES GLUCAGON AND INSULIN POSITIVE CELLS DURING PANCREATIC ENDOCRINE DIFFERENTIATION

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Human embryonic stem cells (hESCs) are capable of generating cells of all germ layers including endoderm-derived pancreatic beta-cells. Recently, in vitro differentiation protocols that promote the formation of hormone positive cells have been developed, which allow modeling of human pancreatic development. Using this hESC differentiation approach we examined the role of the transcription factor Aristaless-Related Homeobox (ARX) in human pancreatic endocrine cell development. The rationale that ARX plays a role in human pancreas development is based on tissue samples from patients with X-linked lissencephaly with abnormal genitalia (XLAG) which have null mutations in the ARX gene. The pancreatic islets of XLAG patients lack glucagon-positive alpha cells but retain insulin-positive beta cells and somatostatin-positive delta cells. To model human developmental ARX deficient pancreatic phenotypes, we have generated ARX knockout (ARX ko) hESCs using zinc-finger nuclease mediated targeted genomic editing and subjected them to pancreatic endocrine differentiation conditions. Following a stage-specific differentiation protocol aimed at forming pancreatic endocrine cells, ARX ko hESCs were compared to unmodified hESCs (WT). ARX ko cells efficiently formed CXCR4-positive definitive endoderm cells (WT: 94±1%, ARX ko: 95±1%), PDX1-positive foregut endoderm (WT: 91±3%, ARX ko: 94±3%), and NKX6.1-positive pancreatic progenitors (WT: 49±3%, ARX ko: 70±2%, $p<0.05$). Continued culture yielded similar fractions of pancreatic endocrine cells in both WT and ARX ko (24-28%). ARX ko endocrine subpopulations contained abundant somatostatin-positive cells (WT: 71±4%, ARX ko: 94±1%, $p<0.05$) with lower numbers of insulin positive cells (WT: 78±1%, ARX ko: 27±4%, $p<0.05$) and dramatically decreased numbers of glucagon-positive cells (WT: 40±8%, ARX ko: 4±1%, $p<0.05$). In addition to the formation of unihormonal somatostatin positive cells, differentiated ARX ko hESCs had elevated expression of PAX4, NKX2.2, ISL1, HHEX, PCSK1 and PCSK2 as well as reduced expression of PAX6 and IRX2 compared to WT cells.

Delivery of an adenoviral human ARX overexpression vector (AdARX) to ARX ko and WT hESC-derived pancreatic progenitors or late endocrine progenitors resulted in a 50-150 fold overexpression in both genotypes and differentiation stages. ARX ko cells treated with AdARX contained significantly more insulin positive cells (59±9% of the total number of endocrine cells) with no change in the number of glucagon or somatostatin positive cells. AdARX treated ARX ko cells also had a recovery of PAX6 expression and a reduction in the levels of PAX4, NKX2.2, and HHEX compared to nontransduced ARX ko cultures.

While XLAG and ARX ko hESCs endocrine populations both contain numerous somatostatin-positive cells and few glucagon-positive cells, XLAG pancreata contain many insulin-positive cells whereas ARX ko hESCs do not. This reduction of insulin in ARX ko cells points to key differences between XLAG samples and differentiated ARX ko cells that, during in vitro culture, ultimately favours somatostatin-positive cell formation with low expression of PAX6 and high expression of HHEX. Taken together the in vitro phenotype of hESC-derived ARX ko endocrine cells suggests that ARX is critical for the formation of glucagon positive lineages and that ARX and/or downstream targets such as PAX6 may be involved in the formation of hESC-derived insulin positive cells.

W-L-4011

VEGF MEDIATED CREATION OF A FUNCTIONAL HEMATOPOIETIC STEM CELL NICHE OUTSIDE OF THE BONE MARROW

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Hematopoietic stem cells (HSCs) may egress the BM upon injury and, at least transiently drive hematopoiesis in remote organs. It is unclear, however, whether extra-medullary microenvironments can function as niches and maintain HSCs properties long term. Here via conditional induction of transgenic VEGF, we generated anew functional splenic niches operating alongside non-compromised BM hematopoiesis. The process is associated with a marked increase in total HSCs number .Importantly, expanded HSCs operating from within the VEGF-induced spleen fully maintain long-term repopulation capabilities. Once generated in an SDF1 dependent process, the splenic niche continues functioning after VEGF withdrawal, as well as following transplantation onto lethally-irradiated mice. VEGF preconditioning amplifies mesenchymal stromal cells number and improves engraftment of exogenous HSCs in non-irradiated mice. Together, these findings demonstrate that VEGF as a single factor can generate extra HSC niches in a osteoblast-free microenvironment which may also be harnessed as a temporary HSCs refuge until the restoration of natural BM niches.

W-L-4012

HUMAN LGR5+ LIVER STEM CELLS RETAIN GENETIC STABILITY AFTER LONG TERM IN VITRO EXPANSION AND ALLOW DISEASE MODELING IN VITRO.

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Despite the enormous replication potential of the human liver, there are currently no culture systems available that sustain hepatocyte replication and/or function in vitro. iPS-derived hepatocytes have emerged as an alternative source of hepatocytes for biomedical applications. We have shown previously that single mouse Lgr5+ liver stem cells can be expanded as epithelial organoids in vitro and can be differentiated into functional hepatocytes in vitro and in vivo. We now describe conditions allowing long-term expansion of single adult human liver stem cells. The expanded cells are highly stable at the chromosome and structural level, while sustaining single base changes at a rate similar to that of the germ-line. In vitro, the cells can readily be converted into functional hepatocytes. Organoids from α1-antitrypsin deficiency patients mirror the in vivo pathology. Clonal long-term expansion of primary adult liver stem cells opens up experimental avenues for disease modeling, toxicology studies, regenerative medicine and gene therapy.

W-L-4013

HUMAN EMBRYONIC STEM CELLS AS TOOLS TO STUDY THE ONCOGENIC BASIS OF NEUROBLASTOMA

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Human embryonic stem cells (hESC) hold great promise for applications in regenerative medicine, disease modeling, and developmental biology studies because of their capacity of self-renewal and their developmental potential to generate various cell types. As such, disruptions to normal stem cell function can have devastating consequences and result in life-threatening diseases. Understanding how such diseases arise will afford novel insights into how we can prevent and treat those diseases.
Neuroblastoma is a common childhood malignant tumor of neural crest origin, arising in the sympathetic nervous system. Among the genetic alterations identified in neuroblastoma, amplification of the oncogene MYCN is strongly associated with highly malignant behavior and poor prognosis. We have successfully developed an efficient procedure for the rapid differentiation of hESC into human neural crest stem cells (NCSC) using PA6 coculture and HNK1 positive cell isolation. For the current study, we evaluated the consequences of MYCN over-expression in human embryonic stem cell-derived NCSC (hNCSC). Lentiviral mediated overexpression of MYCN in undifferentiated hNCSC was readily tolerated and promoted cell proliferation and self-renewal. MYCN overexpression induced hNCSC genes as well as the PcG proteins BMI-1 and EZH2 in hNCSC. In addition, MYCN regulates cell-cycle related genes, such as p16 and cyclin D1. Together these studies confirm that human NCSC tolerate expression of MYCN and ectopic expression of the oncogene initiates transition to neuroblastoma-like cells. Further phenotypic and molecular characterization both in vitro and in vivo are undergoing in the lab.
By creating novel hESC-based models to study the origin and biology of neuroblastoma, we aim to gain novel insights into this tumor that will aid in the development of novel therapy.

W-L-4014

THERAPEUTIC ANGIOGENESIS IN A MURINE MODEL OF LIMB ISCHEMIA BY RECOMBINANT PERIOSTIN AND ITS FASCICLIN I DOMAIN

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Periostin is an extracellular matrix protein implicated in various biological processes, including differentiation, adhesion, migration, invasion, and survival. Periostin is expressed in injured tissues, such as the heart with myocardial infarction, and promotes angiogenesis and tissue regeneration. However, the molecular mechanism associated with periostin-stimulated angiogenesis and tissue repair is still unclear. In order to clarify the role of periostin in neovascularization, we examined the effect of periostin in migration and angiogenic potential of human endothelial colony forming cells (ECFCs) in vitro and in an

ischemic limb animal model. Recombinant periostin protein stimulated migration and tube formation of ECFCs. In order to identify the functional domains of periostin implicated in angiogenesis, five fragments of periostin, including four repeating FAS-1 domains and a carboxyl terminal domain, were expressed in Escherichia coli and purified to homogeneity. Of the five different domains, stimulation of migration and tube formation of human ECFCs by the first FAS-1 domain was as potent as that stimulated by the whole periostin. Chemotactic migration of ECFCs induced by the full length and the first FAS-1 domain of periostin was abrogated by blocking antibodies against β3 and β5 integrins. Intramuscular injection of the full length and the first FAS-1 domain of periostin into the ischemic hindlimb of mice resulted in attenuation of severe limb loss and promotion of blood perfusion and neovascularization. In addition, intramuscular injection of the full length and the first FAS-1 domain of periostin promoted homing of intravenously administered ECFCs to the ischemic limb and ECFC-mediated vascular regeneration. These results suggest that the first FAS-1 domain is responsible for periostin-induced migration and angiogenesis and it can be used as a therapeutic tool for treatment of peripheral artery occlusive disease by stimulating homing of ECFCs.

W-L-4015

ROLE OF GANGLIOSIDES IN DOPAMINERGIC NEURAL DIFFERENTIATION OF HUMAN DENTAL PULP-DERIVED MESENCHYMAL STEM CELLS

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Gangliosides are ubiquitous components of the membranes of mammalian cells that are thought to play important roles in various cell functions such as cell-cell interaction, cell adhesion, cell differentiation, growth control, and signaling. This study investigated the possible role of gangliosides in dopaminergic neural differentiation. Then, in order to derive dopaminergic neuronal-like cells, hDPSCs were induced in vitro for 12 days by using a cocktail that included sonic hedgehog and fibroblast growth factors. The cells adapted a neuronal morphology and expressed the neuronal markers Map2 and βIII-tubulin. Reverse transcription-polymerase chain reaction analysis showed that the cells expressed dopamine-specific genes such as TH, Pitx-3, Nurr-1 and DAT. Immunofluorescence analysis showed that ganglioside biosynthesis was associated with the dopaminergic neural differentiation of hDPSCs. Specifically, GD3 and GT1b were express during dopaminergic neural differentiation. These results suggest that gangliosides may play a role in the dopaminergic neural differentiation process of hDPSCs.

W-L-4016

ADENYLYL CYCLASE-ASSOCIATED PROTEIN 1 IS A RECEPTOR FOR HUMAN RESISTIN AND MEDIATES INFLAMMATORY ACTIONS OF HUMAN MONOCYTES

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Human resistin is a cytokine that induces low-grade inflammation by stimulating monocytes. Resistin-mediated chronic inflammation can lead to obesity, atherosclerosis, and other cardiometabolic diseases. Nevertheless, the receptor for human resistin has not been clarified. Here, we identified adenylyl cyclase-associated protein 1 (CAP1) as a functional receptor for human resistin and clarified its intracellular signaling pathway to modulate inflammatory action of monocytes. We found that human resistin directly binds to CAP1 in monocytes and upregulates cyclic AMP (cAMP) concentration, protein kinase A (PKA) activity, and NF-κB-related transcription of inflammatory cytokines. Overexpression of CAP1 in monocytes enhanced the resistin-induced increased activity of the cAMP-dependent signaling. Moreover, CAP1-overexpressed monocytes aggravated adipose tissue inflammation in transgenic mice that express human resistin from their monocytes. In contrast, suppression of CAP1 expression abrogated the resistin-mediated inflammatory activity both in vitro and in vivo. Therefore, CAP1 is the bona fide receptor for resistin leading to inflammation in humans.

W-L-4017

CRITICAL ROLE OF ENDOTHELIN RECEPTOR SIGNALING IN FOLLICULAR MELANOCYTE STEM CELLS IN ADULT SKIN

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Melanocyte stem cells (McSCs) reside in the hair follicle bulge where they are essential for hair pigmentation and have the potential to also regulate epidermal pigmentation. A better understanding of the molecular mechanisms that govern these stem cells holds broad implications in pigmentation disorders including gray hair and vitiligo. In this study, we investigated the role of Endothelin (Edn) signaling in adult McSCs. Previous studies suggested that Edn promotes melanocyte proliferation in vitro. In addition, we previously reported that at the onset of anagen phase, Edn1 is upregulated in follicular epithelial cells surrounding McSCs that express endothelin receptor B (EdnrB). To elucidate the role of Edn signaling in McSCs in vivo, we genetically overexpressed Edn1 throughout the basal layer of the epidermis during the hair cycle of the adult mice. We found that Edn1 overexpression promotes McSC

proliferation and differentiation during anagen. We previously reported that McSCs can be induced to migrate upwards, exiting the follicular niche to generate epidermal melanocytes in response to skin injury or UVB irradiation. In addition to the role of Edn1 during normal homeostasis, we show that Edn1 overexpression in the epidermis promotes the upward migration of McSCs toward the epidermal surface following skin injury or UVB irradiation. Conversely, loss of EdnrB in melanocytes failed to properly express differentiation markers including tyrosinase, which are critical for melanogenesis. These defects were manifested grossly by hair graying in mutant mice. Collectively, our study demonstrates that Edn/Ednrb signaling is a key regulator of McSCs during regeneration of hair melanocytes as well as epidermal melanocytes, revealing a potential novel approach for treating skin pigmentation disorders.

W-L-4018

MAINTENANCE AND MASS DIFFERENTIATION OF HUMAN IPSCS INTO CARDIOMYOCYTES IN A 3D ENVIRONMENT USING ALGINATE

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Derivation of pluripotent cells from somatic counterparts has brought tremendous hope for the cure and treatment of many different diseases, especially in case of transplantation. Furthermore differentiated cells derived from these pluripotent cells are excellent tools for developing drugs and testing disease. However the numbers of differentiated cells are not enough for downstream applications. A great deal of research has been carried out over the past few years on efficient methods to derive and further differentiate induced pluripotent stem cells (iPSCs). Yet still mass amounts of differentiated cells are required for transplantation or building artificial organs. Furthermore conventional two dimensional differentiation methods limit cell interaction which may render the cells unsuitable for further applications. Considering these factors, we looked into growth, maintenance and differentiation of iPSCs in a rotating wall vessel bioreactor (RWVB) using alginate beads. Cells maintained in alginate possess unique characteristics due to improved cell-cell interaction and enhanced cell growth in a 3D environment which resembles tissue growth in the body. Furthermore due to the low physiological fluid shear conditions, the RWVB provides greater cell growth. These characteristics help provide mass amounts of cells ready for following applications. Our human iPSCs were initially grown and expanded in a 2D culture and subsequently characterized to verify pluripotency. Cells indicated high percentage of pluripotency restricted markers like TRA-1-81 and TRA-1-60. Cells were then encapsulated into alginate beads and grown in a rotating wall vessel bioreactor. We chose 1.1% alginate to construct the beads as this concentration had proven optimum for ESC growth in previous experiments. Cells indicated viability and growth for upto four weeks. Furthermore we tested for pluripotency characteristics after different time points. Data indicated that cells maintained pluriptotency for up to four weeks with high survival rate. iPSCs were then differentiated into cardiomyocytes inside the bioreactor to produce large scale supply of cardiomyocytes for further applications as described. Live/dead staining of cells demonstrated that significant number of cells remain live after differentiation.

W-L-4019

SET7 AT THE INTERSECTION BETWEEN HIPPO/YAP AND WNT/BETA-CATENIN SIGNALLING IN INTESTINAL REGENERATION AND CANCER

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Methylation of non-histone proteins is emerging as a regulatory mechanism that controls protein function. We have demonstrated that the lysine methyltransferase Set7 (Setd7) is required for the methylation and proper function of Yes-associated protein (Yap), a member of the Hippo signaling pathway. Mice with an intestinal epithelial cell (IEC)-specific deletion of Set7 (Setd7ΔIEC mice) display increased IEC proliferation and turnover, which corresponded to increased expression of Yap-dependent target genes. Mechanistically we identified that methylation of K494 of Yap is important for cytoplasmic retention, an important regulatory mechanism in the Hippo pathway. Recently, several studies have identified important crosstalk between the Hippo/Yap and Wnt/β-catenin pathways during intestinal regeneration and tumorigenesis. We found that Set7 is expressed in intestinal stem cells (ISCs) and Setd7ΔIEC mice have impaired intestinal regeneration following damage, associated with decreased expression of Wnt/β-catenin target genes including the ISC marker Lgr5. Consistent with impaired Wnt/β-catenin signaling, deletion of Set7 in tumour-prone mice that carry the Apc truncation mutation (ApcMin/+ mice) abrogated spontaneous tumour development. Further, we show that Set7 interacts directly with β-catenin and is required for its nuclear accumulation following Wnt signaling. Taken together, our results identify methylation-dependent checkpoints in both the Hippo/Yap and Wnt/β-catenin pathways that regulate the subcellular localization of key proteins and identify Set7 as a novel regulator of ISC function.

W-L-4020

CYTOTOXIC EFFECT OF COPAIBA OIL IN STEM CELLS

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Copaiba oil is produced by exudation from the trunks of trees belonging to the genus Copaifera. The traditional properties attributed to copaiba oil is antimicrobial, anti-inflammatory, anti-tetanus, antitumor, anti-blennorrhea and as a urinary antiseptic. In addition, it has been used for the treatment of bronchitis, skin diseases, ulcers and syphilis, as well as for healing wounds. Considering the several biological

activities of copaiba oil, it is interesting to study its influence on cell proliferation and differentiation. This study aimed to evaluate the effect of copaiba oil on mesenchymal stem cell proliferation and its in vitro cytotoxicity. The cells obtained from the pulp of deciduous exfoliated teeth (SHEDs) were characterized in passage 7 and 7x104 cells were placed in 96-well culture plates. After confluence, the SHEDs were treated with copaiba oil (Copaifera officinalis) at concentrations of 0.5, 20, 110, 140, 200, 350, 500 and 1,000 µg/mL, dissolved in DMSO (1%) of the culture medium, DMEM. The control was the culture medium with 1% DMSO. After 24 hours, cell viability was assessed by MTT ([3-(4,5-dimethylthiazol-2-yl)-2,4-diphenyltetrazolium bromide]) assay. Cytotoxicity was measured in the culture supernatant using lactate dehydrogenase (LDH) delivery assay. For the MTT assays, cell viability for the Copaiba oil groups was compared with the control, the absorbance of which for MTT assay was considered 100% In the LDH test, the treatment of the cells with Triton X-100 at 2% for 10 minutes was used as the control for cell death. The control value for LDH was 83.8 U/L. The lowest concentrations used (0.5, 20 and 110 µg/mL) did not differ from the control for the MTT (120.3, 91.4 and 74.1% of control, respectively) and for the LDH (103.5, 91.8 and 87.8 U/L, respectively) assays. . The concentration of 140 µg/mL promoted statistical significance concerning the decrease in cell viability (46.7%, with $p = 0.030$) and the increase in cytotoxicity (177.4 U/L, with $p = 0.024$), in comparison with the control. At concentrations of 200, 350, 500 and 1,000 µg/mL, the cells did not proliferate when compared to the control (2.5, 2.5, 2.0 and 2.5%, respectively, with $p = 0.000$). The results of the cytotoxicity testshowed statistical difference in relation to the control and the higher concentrations tested, with values of 347.8, 359.8, 329.0 and 342.0 U/L for 200, 350, 500 and 1,000 µg/mL, respectively, with $p = 0.000$. The results of the MTT and LDH tests showed statistical equivalence. The concentration of 140 µg/mL was the marker for establishing the statistical difference between the lowest and the highest concentrations. It is suggested that copaiba oil in high concentrations, 140, 200, 350, 500 and 1,000 µg/mL, may have affected the functioning of the cells, which in turn may have led to their death. In conclusion, low concentrations of copaiba oil were not cytotoxic; however, there was no evidence that the use of copaiba oil improves cell viability. On the other hand, it can be concluded that increased concentrations of copaiba oil promoted a decrease in cell viability and an increase of (cytotoxicity in the stem cells. Although copaiba oil is widely used in traditional medicine, caution must be taken for medical application due to its cytotoxicity; however, the oil components should be studied for their possibility of antitumor potential.

W-L-4021

PHYSICOCHEMICAL AND BIOLOGICAL ANALYSES OF SCAFFOLDS WITH DEXAMETHASONE CULTIVATED WITH HUMAN STEM CELLS

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A variety of biomaterials are undergoing studies for application in tissue engineering, among which are poly (lactic-co-glycolic) acid (PLGA) scaffolds, produced by the electrospinning technique. Through this technique it is possible to add different bioactive molecules during the production process of the biomaterial, such as dexamethasone, which is characterized by its ability to promote the differentiation of mesenchymal stem cells to osteogenic lineage. This study aimed to test PLGA scaffolds with different quantities of dexamethasone (Darsheng Trade & Tech Development, USA - 100% pure). Seven groups of scaffolds were produced in the following proportions: 1 part dexamethasone to 20 parts PLGA, which was identified as 1:20. When 1 part dexamethasone was used with 10 parts PLGA, it was identified as 1:10. Following this, 1:4, 1:2, 3:4 and 1:1, respectively. Therefore, the studied groups were: A: PLGA scaffold without dexamethasone as control; B: 1:20; C: 1:10; D: 1:4; E: 1:2; F: 3:4 and G: 1:1. All the scaffolds were evaluated for their physicochemical characteristics and their interaction with MSCs from deciduous teeth. The amount of dexamethasone incorporated in the scaffolds, detected by UV light, showed a good rate of dexamethasone incorporation for all the types of scaffolds: B: 26.70%; C: 46.70%; D: 26.70%; E: 53.30%; F: 40%; G: 46.70%. The scanning electron microscope images showed fibers distributed in a random manner in all the scaffolds with a large number of interconnected pores. Except for group B, the average of the fiber diameter increased with the increase of dexamethasone incorporation: A: 601 ± 123; B: 432 ± 27; C: 1086 ± 78; D: 735 ± 35; E: 1,094 ± 41; F: 1,396 ± 156; G: 997 ± 26. With the increase of dexamethasone incorporation, the thickness of the scaffolds also increased A: 105.7 ± 37.8; B: 98 ± 34.8; C: 115.7 ± 10.3; D: 113.3 ± 32.1; E: 119.0 ± 35.5; F: 134.3 ± 42.7; G: 142.3 ± 13.6. The incorporation of dexamethasone did not change the hydrophobicity of the scaffolds, which was measured by contact angle analysis: A: 127.8 ± 2.67; B: 129.7 ± 2.25; C: 125.3 ± 0.61; D: 124.8 ± 0.43; E: 129.8 ± 2.59; F: 131 ± 1.08; G: 132 ± 1.4. With the increase of dexamethasone incorporation, the elastic modulus (Young's modulus) of the scaffolds decreased: A: 53.76 ± 16.56; B: 50.56 ± 13.37; C: 38.04 ± 7.89; D: 34.22 ± 9.34; E: 31.05 ± 3.94; F: 21.54 ± 5.21; G: 19.04 ± 2.19. In relation to the cell interaction, through the MTT assay, it could be observed that cell viability on day 3 and 7 increased with the amount of dexamethasone incorporated (E, F and G), in comparison with the scaffolds with lower amount or zero dexamethasone (A, B, C, D). This fact was inverted on day 14 and 21, where, with the increase of dexamethasone incorporation, cell viability decreased. These preliminary results suggest that the produced scaffolds, with the exception of Young's modulus, did not modify their morphological and mechanical characteristics. More importantly, when submitted to contact with the cells, the scaffolds showed satisfactory results regarding the maintenance of cell viability. One of the possible reasons for a decrease in cell viability may be due to modification of the cellular machinery, such as the process of differentiation. In relation to this issue, more studies are required. Therefore, regardless of the concentration of dexamethasone, the scaffolds with dexamethasone appear to be a promising tool for application in tissue engineering.

W-L-4022

INFUSION OF SHEDS OR TRANSPLANT WITH SCAFFOLDS AFTER EXPERIMENTAL SPINAL CORD INJURY - A FUNCTIONAL COMPARISON

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Spinal cord injury (SCI) is a disabling traumatic condition and available therapeutic approaches are poorly effective. In the search for new treatments, stem cell transplants have been studied with a view to minimizing spinal cord injury in animal models. Stem cells from human exfoliated deciduous teeth (SHEDs) are considered a feasible source of stem/progenitor cells with the ability of self-renewal and the promotion of tissue regeneration. The present study was designed to investigate the effectiveness of different transplant methods of SHEDs transplant, via direct injection or in culture with random nanofiber scaffolds, followed by the evaluation of functional recovery after spinal cord contusion in rats. For this purpose, scaffolds were produced with poly (lactic-co-glycolic) acid (PLGA) in 1,1,1,3,3,3-hexafluoro-2-propanol using the electrospinning (ES) technique to act as a support for the SHEDs. The animals were previously anesthetized and the laminectomy was performed between T9 and T10. After the laminectomy at the 9th thoracic vertebral level, a 10 g weight was dropped from a height of 25 mm to produce the injury by the use of the NYU Impactor. SHEDs were implanted into the injury site 1 hour after injury, at a concentration of 3X10e5 cells diluted in 10µl PBS (phosphate buffered saline) or 1.5X10e5 cells cultivated with the scaffolds. Twenty-nine male Wistar rats were distributed into 5 groups: sham (n=4), SCI+vehicle (PBS) (n=8), SCI+Scaffold (n=6), SCI+Scaffold+SHEDs (n=7) and SCI+SHEDs (n=4). The functional deficits and functional recovery were evaluated during the locomotor activity in the open field using the Basso, Beatie, and Bresnahan (BBB) scale. Preliminary locomotor results demonstrated that the SHEDs group exhibited an increase in BBB scores in the second week in comparison with the vehicle group; an effect which remained in the second week. The Sham group remained different from the other groups from the second day. SCI+Scaffold, SCI+Scaffold+SHEDs and the SCI+Vehicle group were not different, showing only the spontaneous recovery. The transplant of the SHEDs with the scaffolds in the acute phase was not effective in promoting functional recovery after spinal cord injury. It is probable that the scaffolds, when transplanted in the acute phase of spinal cord injury, do not increase the capacity of regeneration and, as a consequence, reduce the efficacy of the SHEDs, impairing the development of locomotor recovery. However, acute transplantation of the SHEDs by infusion was effective in promoting functional recovery after spinal cord injury in comparison with the other groups, showing the potential use of SHEDs for partial spinal cord regeneration.

W-L-4023

ANALYSIS OF ANGIOGENIC ACTIVITY OF HUMAN NEONATAL FORESKIN STEM/STROMAL CELLS IN THE CHICK EMBRYO CHORIOALLANTOIC MEMBRANE.

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Human neonatal foreskin stromal cells (hNSSCs) can be isolated from new born male infant foreskin. In the past 10 years, several studies have confirmed that hNSSCs have multiplex potential to differentiate into adipocytes, osteoblasts, neural cells, schwann-like cells, smooth muscle cells and hepatocytes. Recently, we confirmed hNSSCs have a prospective to involve in angiogenesis and a capability to differentiate into endothelial-like cells in vitro, but their angiogenic ability in in vivo environment are not well known.The multilineage differentiation potential of hNSSCs was assessed through differentiation in to adipocytes, osteoblasts and endothelial-like cells, using respective inducer materials which were subsequently confirmed using immunfluorescence, cyotchemistry and qRT-PCR. hNSSCs revealed highest migration potential in the naive condition and well responded for in vitro wound healing scratch assay. In in vivo, when hNSSCs were seeded onto chick chorioallantoic membranes (CAM), human von Willebrand factor, CD31, smooth muscle actin and factor XIIIa positive cells were observed to engraft in the chick endothelium. CAMs transplanted with differentiated hNSSCs had a greater number of regular vessels containing human cells and more incorporated cells per vessel compared to CAMs transplanted with undifferentiated hNSSCs. The naive hNSSCs were more inclined towards to skin lineage differentiation such as epidermis besides irregular vasculature as it was confirmed by CD1a, CK5/6, CK19, FXIIIa and S-100. These findings imply a potential angiogenic role for hNSSCs, not only in the induced condition but also in the naive circumstance; overall angiogenesis was enhanced more by the differentiated cells. These results suggest that hNSSCs may contribute to blood vessel formation.
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W-L-4024

INDUCTION OF CYTOKERATIN 3 EXPRESSION IN IMMORTALIZED HUMAN ORAL MUCOSAL EPITHELIAL CELLS BY THE TRANSDUCTION OF PAX6.

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Purpose: To induce the expression of corneal epithelium-specific cytokeratin 3 (K3) in immortalized human oral mucosal epithelial cells (OKF6/TERT-1 cells) using lentiviral transduction of PAX6. Methods: OKF6/TERT-1 cells were transduced with two types of lentiviruses, each carrying one of the two variants of PAX6 (PAX6 variant 1 and 2). The cells were cultured in modified keratinocyte serum-free medium (K-sfm) for 3 days after transduction and were cultured in keratinocyte conditioned medium

(KCM) with 3T3 feeder cells for another 11 days to stratify them. The gene expressions were examined with quantitative reverse transcription PCR (qRT-PCR) and immunofluorescence imaging on day 3 and day 14. Results:OKF6/TERT-1 cells had no expression of K3 without transduction of PAX6 (Gene expression compared to GAPDH was 0.01±0.00). OKF6/TERT-1 cells expressed K3 on day 3 only when they were transduced with PAX6 variant 1 (Gene expression compared to GAPDH was 7.31±0.66, $P<0.05$). Mucin 16 (MUC16) was also up-regulated by the transduction of PAX6 variant 1 ($P<0.05$). Cytokeratin 13 (K13) was down-regulated by the transduction of both PAX6 variants ($P<0.05$). Expression of cytokeratin 12 (K12), CRYAA and RPE65 were not induced by the transduction of both PAX6 variants. The Expression of PAX6 and K3 disappeared after the stratification of OKF6/TERT-1 cells. Conclusions:Corneal epithelium-specific K3 was successfully induced by the transduction of PAX6 variant 1. The present data also suggest that two variants of PAX6 have differential role in the corneal epithelium- specific phenotype.

W-L-4025

PROSPECTIVE IDENTIFICATION AND ISOLATION OF FUNCTIONALLY DISTINCT POPULATIONS OF NEURAL STEM CELLS AND NEUROSPHERE-INITIATING CELLS IN THE ADULT FOREBRAIN

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Neurosphere formation has been widely used as a surrogate for neural stem cell (NSC) function but the relationship between neurosphere-initiating cells (NICs) and NSCs remains unclear. We prospectively identified, and isolated by flow cytometry, adult mouse lateral ventricle subventricular zone (SVZ) NICs as Glast^{mid}EGFR^{high}PlexinB2^{high}CD24$^{-/low}$O4/PSA-NCAM$^{-/low}$Ter119/CD45^{-} (GEPCOT) cells. These cells were highly mitotic in vivo and short-lived based on *Ascl1*^{CreERT2} and *Dlx1*^{CreERT2} fate-mapping. In contrast, a quiescent population of pre-GEPCOT cells that expressed higher levels of Glast and lower levels of EGFR and PlexinB2 could not form neurospheres but expressed the stem cell markers *Glast-CreER*^{T}, *GFAP-CreER*^{T2}, *Sox2*^{CreERT2}, and *Gli1*^{CreERT2} and was long-lived in vivo. While GEPCOT NICs were ablated by temozolomide, pre-GEPCOT cells survived and repopulated the SVZ with NICs and neuroblasts. Fetal deletion of the polycomb protein *Bmi-1* using *Nestin-Cre* depleted pre-GEPCOT and GEPCOT cells but adult *Bmi-1* deletion using *Nestin-CreER*^{T2} only depleted GEPCOT NICs, demonstrating that mitotically active GEPCOT NICs depend more acutely on Bmi-1 than quiescent pre-GEPCOT NSCs. Our data functionally and phenotypically distinguish quiescent NSCs from NICs and make it possible to study their properties in vivo.

W-L-4026

TRANSPLANTATION OF HUMAN DOPAMINERGIC NEURONS DIFFERENTIATED FROM UMBILICAL CORD BLOOD-DERIVED INDUCED PLURIPOTENT STEM CELLS INTO THE RAT STRIATUM

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Human induced pluripotent stem cells (hiPSCs) are promising sources for regenerative therapies like the replacement of dopaminergic neurons in Parkinson`s disease (PD). They offer an unlimited cell source that can be standardized and optimized to produce applicable cell populations to gain maximal functional recovery. In the present study human cord blood-derived iPSCs (hCBiPSCs) were differentiated into dopaminergic neurons utilizing inhibition of transforming growth factor-β and bone morphogenetic protein signaling by the small molecules dorsomorphin and SB 431542 for neural induction. Differentiated hCBiPSCs stained positive for dopaminergic neuron markers, exhibited voltage-gated ion currents, were able to fire action potentials and displayed synaptic activity indicating synapse formation. We investigated the in vivo survival and differentiation of in vitro predifferentiated hCBiPSCs after intrastriatal transplantation in healthy rats and in the unilateral 6-OHDA-lesioned rat model of PD. Grafted hCBiPSC-derived cells survived, however, only half of the grafts revealed several TH+ dopaminergic cells with fiber outgrowth and reinnervation of the lesioned striatum.

W-L-4027

COMPARISON OF FILTRATION AND CENTRIFUGATION FOR CRYOPROTECTANT REMOVAL FROM THAWED CELL SUSPENSIONS

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The current protocols for initiating stem cell culture often require the removal of cryoprotectant agents (CPA) before inoculation. These protocols are manual and open processes which are prone to variability and contamination. With the new FDA guidelines on cell therapy (CT) manufacturing the industry is under pressure to close as many cell processing steps as possible during these manufacturing processes. We have thus identified the need for a closed system for CPA removal before culture initiation. In this work we have designed and tested a small scale (1 mL processing volume) filtration system which can operate in a closed system for CPA removal of freshly thawed cell suspensions. This novel filtration device was compared to a standard manual, open centrifugation process. Our results show that the novel filtration system's performance is comparable to the control centrifugation process in terms of cell recovery, DMSO wash and proliferation potential post thawing. The entire automated process was

performed in less than 30 minutes. With this work we have shown that a filtration system for the washing of small volumes of cell suspensions is a valid alternative to centrifugation.

W-L-4028
ALCOHOL DISRUPTS LIVER STEM CELL DIFFERENTIATION

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Treatment of alcoholic cirrhosis continues to be a major challenge to our health care system. Liver stem/progenitor cells (LSPCs) in normal individuals possess an extensive capacity for proliferation and differentiation into functional liver cells. These tissue-specific precursors play an important role in the process of liver injury repair. Excessive alcohol consumption impairs host ability to repair liver injury, but the underlying mechanism is poorly understood. We hypothesized that alcohol impairs LSPC differentiation program. To test this hypothesis, primary human liver stem cells (HL1-1 cells) were cultured initially in proliferation medium to achieve approximately 80% confluence and then in differentiation medium containing different concentrations (0, 50, and 100 mM) of ethanol. Morphological changes of cells during differentiation were examined with phase contrast microscopy. Cell expression of differentiation markers at gene and protein levels was determined by real-time RT-PCR and flow cytometry, respectively. Ethanol exposure induced morphological change of HL1-1 cells toward a myofibroblast-like phenotype. Ethanol exposure significantly reduced the number of cells expressing E-cadherin (35.1% reduction when cultured with 100 mM ethanol, $p<0.05$). In contrast, ethanol exposure increased the number of cells expressing collagen I protein (5.5 and 8.7 fold of the control value in 50 and 100 mM ethanol groups, respectively, $p<0.05$) during differentiation. Ethanol also stimulated Snail repressor gene expression (3.2 fold of the control value in 100 mM ethanol group, $p<0.05$) and alpha-smooth muscle actin gene expression (2.1 fold of the control value in 100 mM ethanol group, $p<0.05$) by HL1-1 cells. These results indicate that alcohol disrupts molecular signaling regulation in LSPCs, switching the track of LSPC differentiation from their original direction toward a hepatocyte phenotype to the generation of a myofibroblast-like phenotype. This change of LSPC differentiation track may serve as a mechanism for alcohol-promoted fibrogenesis in the injured liver, which may eventually lead to liver cirrhosis.

W-L-4029
CALRETICULIN PROMOTES OSTEOGENESIS AND INHIBITS CHONDROGENESIS: A CRUCIAL ROLE IN RUNX2 NUCLEAR TRANSLOCATION

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Calreticulin is an endoplasmic reticulum (ER)-resident protein with calcium buffering and chaperoning functions. In the present study, we demonstrate a novel regulatory role of calreticulin in the commitment and differentiation of mouse embryonic stem (ES) cells to the osteogenic and chondrogenic lineage. Osteogenesis was diminished in calreticulin knock-out cells compared to wild-type cells. Interestingly, chondrogenesis was enhanced in the absence of calreticulin. In the calreticulin-null cells, the nuclear translocation of the critical transcription factors involved in skeletal development, the runt-domain related transcription factor 2 (Runx2) and Osterix (Osx), were impaired. Their localization was affected by changes in intracellular calcium levels. Further investigation of the signaling pathway downstream of calreticulin revealed that calcineurin, a calcium-sensitive phosphatase, affected the localization of Runx2 and OSX. The mechanism in which calreticulin regulates the location of Runx2 seems to be via the signal transducer and activator transcription factor 1 (STAT1). It is widely known that Runx2 has an important role in chondrocyte maturation, which in turn is necessary for osteoblast development. In the absence of calreticulin, with its nuclear translocation impaired, Runx2 cannot properly carry out its roles in skeletogenesis. This accounts for the arrested osteoblast development and the higher abundance of chondrocyte-like cells in calreticulin-null cell cultures.

W-L-4030

FUNCTIONAL FIDELITY AND EPIGENETIC CHARACTERIZATION OF MODULAR GENETIC CIRCUITS IN MESCS

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Progress in stem cell engineering could be accelerated through the ability to deliver multiple genes in a single plasmid to the desired genomic locus. The integrated transgenic material must have high functional fidelity, defined as robust and un-silenced gene expression, with minimal inter-genetic unit interference. This enables tunable exogenous control over inducible transgene units. To this end, we constructed a modular library of gene circuits to identify configurations that demonstrate prolonged circuit function. This library compared simple single-gene and polycistronic transcription units, both constitutively and exogenously-inducible, with more complex regulatory circuits expressing multiple transcription units with varying configurational and regulatory complexity. We aimed to elucidate points at which possible functional fidelity issues (epigenetic silencing and promoter interference) manifested. Particularly in response to increased promoter number and type, circuit size, and transcription unit reading frame orientation.

W-L-4031

PROGRAMMED APPLICATION OF TGF-BETA3 AND RAC1 INHIBITOR NSC23766 COMMITTED HYALINE CARTILAGE DIFFERENTIATION OF ADIPOSE-DERIVED STEM CELLS (ADSCS) FOR OSTEOCHONDRAL DEFECT REPAIR

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Objective: Hyaline cartilage differentiation is always the challenge on the application of stem cells for joint repair. TGFs and BMPs can initiate cartilage differentiation but often leads to hypertrophy and calcification which is related to abnormal rac1 activity. In this study, we developed a strategy of programmed application of TGFβ3 and *Rac1* inhibitor NSC23766 to commit the hyaline cartilage differentiation of ADSCs for joint cartilage repair. Methods: ADSCs were isolated and cultured in micromass and pallete culture model to evaluate the chondrogenic and hypertrophic differentiation. The function of rac1 was investigated with constitutively active Rac1 mutant (CA-Rac1) and dominant negative Rac1 mutant (DN-Rac1). The efficacy of ADSCs with programmed application of TGFβ3 and rac1 inhibitor for cartilage reparation was studied in a rat osteochondral defect model. Results: The results showed that TGFβ3 promoted ADSCs chondro-lineage differentiation and NSC23766 prevented ADSCs derived chondrocytes from hypertrophy *in vitro*. Combination of ADSCs, TGFβ3 and NSC23766 promoted the quality of osteochondral defect repair in rat with much less chondrocytes hypertrophy and significantly higher ICRS macroscopic and microscopic score. Conclusion: The findings illustrated that programmed applicaiton of TGFβ3 and Rac1 inhibitor NSC23766 can commit ADSCs into chondro-lineage differentiation and improve the efficacy of ADSCs for cartilage defect repair. It suggests a promising stem cell based strategy for articular cartilage repair.

W-L-4032

INTEGRIN-BETA 1 PROMOTES SURVIVAL OF ADIPOSE TISSUE-DERIVED STEM CELLS IN THE INFARCTED HEARTS THROUGH EXTRACELLULAR SIGNAL-REGULATED KINASE

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Background: Adipose tissue-derived stem cells (ASCs) are a promising cell source for repairing damaged myocardium. However, reported improvements of cardiac function have been generally modest partly due to low survival of injected ASCs in hostile ischemic hearts (e.g., oxidative stress), with the underlying mechanisms remaining largely unknown. Integrin-β1 belongs to a family of receptors for many extracellular matrix proteins. This study was to investigate the roles of integrin-β1 in the survival ASCs of using an *in vivo* murine model of myocardial infarction and an *in vitro* cell culture system.
Methods: ASCs were isolated from four 8-week-old BALB/c mice, pooled together, and labeled with luciferase used for tracking viability of injected ASCs in live mice. Integrin-β1 expression in ASCs was eliminated using lentivirus carrying small hairpin RNA ($ASC^{integrin\text{-}\beta1\text{-}}$) or overexpressed by lentiviral transduction ($ASC^{integrin\text{-}\beta1+}$). For *in vivo* study, mice (n=3 mice/group) were subjected to a permanent

occlusion of left anterior descending coronary artery. Control ASC ($ASC^{control}$), $ASC^{integrin\text{-}\beta1+}$, or $ASC^{integrin\text{-}\beta1-}$ were injected into the border zone of infarcted hearts. Survival of injected ASCs expressing luciferase in live mice was imaged using Bioluminescent Imaging System. For *in vitro* study, ASCs were treated with 10 mM H_2O_2 and/or extracellular signal-regulated kinase (ERK) inhibitor PD98059 for 2 h. Cell viability was measured by lactate dehydrogenase (LDH) release, an indicator of cell membrane damage. Results: The *in vivo* results showed that injected ASCs survived in the infarcted hearts for 7 days and there were more viable ASCs in $ASC^{integrin\text{-}\beta1+}$-injected hearts than in $ASC^{control}$-injected hearts. However, a significant decrease of ASCs was observed in $ASC^{integrin\text{-}\beta1-}$-injected mice, indicating that integrin-β1 is involved in the survival of injected ASCs in ischemic hearts and overexpression of integrin-β1 enhances ASCs to resist hostile myocardial environment. The following *in vitro* data confirmed the *in vivo* findings. Upon H_2O_2 exposure, overexpression of integrin-β1 in ASCs significantly decreased LDH release compared with other groups while integrin-β1 knockout increased LDH release from ASC. In addition, PD98059 significantly inhibited LDH release from $ASC^{control}$ but not from $ASC^{integrin\text{-}\beta1-}$, suggesting that ERK pathway participates in the integrin-β1-mediated ASC survival. Conclusions: Integrin-β1 promotes survival of adipose tissue-derived stem cells in infarcted hearts through extracellular signal-regulated kinase. This finding may help to guide the design of novel therapies for improving ASC-mediated repair capacity by increasing the ability of ASCs to persist in ischemic hearts.

W-L-4033

MOLECULAR BASIS OF MOUSE EPIBLAST STEM CELLS SELF-RENEWAL MEDIATED BY WNT/BETA-CATENIN

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Maintenance of mouse epiblast stem cells (mEpiSCs) in vitro requires supplementation of both basic fibroblast growth factor (bFGF) and activin A (ActA). In a recent study, we have discovered that mEpiSCs self-renewal can be achieved without either bFGF or ActA by modulating Wnt/β-catenin signaling. The stabilization and retention of β-catenin in cytoplasm, by either a combination of small molecules (CHIR99021 and IWR-1) or various genetic modifications to β-catenin, could maintain a long-term self-renewal of mEpiSCs. To look into the molecular mechanism underlying such phenomenon, we firstly investigated the role of TGFβ/Smad signaling in β-catenin-mediated mEpiSCs self-renewal. Phosphorylation of Smad2 is detected in mEpiSCs cultured in both bFGF/ActA and CHIR99021/IWR-1 conditions. When treated with A83-01, a TGFβRI inhibitor, mEpiSCs in both bFGF/ActA and CHIR99021/IWR-1 conditions cannot self-renew. We also studied the effect of Taz, a transcription co-activator whose activation and turnover are closely linked to β-catenin, on mEpiSCs self-renewal. Ectopic expression of Taz and activation of its target genes in mEpiSCs induced rapid differentiation. Conversely, reduction of Taz level in mEpiSCs promoted cell proliferation. Taken together, these results could help in better understanding the role of Wnt/β-catenin signaling in the regulation of mEpiSCs fate.

W-L-4034

GENERATING A DATABASE OF HUMAN STEM CELLS AND THEIR DIFFERENTIATED DERIVATIVES: A PLATFORM FOR IN VITRO MODELING OF HUMAN DEVELOPMENT LINEAGE DIFFERENTIATION AND DISEASE MODELS

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RNAseq, miRseq, and array-based DNA methylation assays have been used to characterize and compare the relative differentiation potentials of a collection of human pluripotent stem cells. Human embryonic stem cells (hESC) and a variety of induced pluripotent stem cells (iPSC) made from a variety of different sources and reprogramming methods were subjected to differentiation protocols designed to generate endoderm, mesoderm, ectoderm, as well as embryoid bodies. Integrative analyses identified miR and mRNA gene expression modules and DNA methylation profile changes, whose differential activation, repression, and regulatory interactions could be associated with each state and state-transition. Unexpectedly, the pluripotent stem cell collection exhibited strong differences in their relative ability to undergo one differentiation versus another. For example, some cell lines were strongly biased in their ability to produce ectodermal versus mesodermal derivatives. mRNA transcriptome profile differences among pluripotent cell lines were much less significant and offered little power to predict polarized versus balanced differentiative potential. However, stem cell state-specific DNA methylation and miR expression pattern differences were of a larger magnitude and their correlations with lineage specific differentiation regulatory networks demonstrated some predictive power for alternative or differential differentiation potential. Taken together, we demonstrate that these data can be used to understand early developmental differentiation events and regulatory pathways and improve our ability to use human pluripotential stem cells to model disease. To maximize the utility of the present stage of these data, we have populated a suite of bioinformatics tools and several web accessible data analysis resources suitable for exploration, discovery, and user dataset comparisons to improve of understanding and modeling of early developmental pathways and regulatory mechanisms responsible for generating alternatively differentiated states.

W-L-4035

REPROGRAMMING IN VIVO PRODUCES TERATOMAS AND iPSCS WITH TOTIPOTENCY FEATURES

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The ability to reprogram differentiated cells into induced pluripotent stem cells (iPS cells) has considerably improved our current understanding of cellular plasticity and has also helped to paving the way towards regenerative medicine. However, little was known about whether or not *in vivo* reprogramming is feasible and if so, what type of cells are generated *in vivo* and what are their implications in the organism. We have generated a "reprogrammable" transgenic mouse strain that ubiquitously express the so-called Yamanaka factors upon treatment with doxycycline. We have shown that transitory induction of the four Yamanaka factors in mice, for just one week, is able to induce dedifferentiation and pluripotency in a variety of tissues and to various degrees. Mice that had activated the reprogramming factors developed multiple teratomas, which is indicative of complete reprogramming events *in vivo*. Indeed, reprogrammable mice present circulating iPS cells in the blood and, at the transcriptomic level, these *in vivo* generated iPS cells are closer to embryonic stem cells (ES cells) than standard *in vitro* generated iPS cells. Moreover, *in vivo* iPS cells efficiently contribute to the trophectoderm lineage and generate embryo-like structures that express embryonic and extraembryonic markers, suggesting that they achieve a more plastic or primitive state than ES cells. Our results could be relevant for the development of future applications of reprogramming in regenerative medicine.

W-L-4036

MIR-137 CONTROLS PROLIFERATION AND DIFFERENTIATION OF HUMAN ADIPOSE TISSUE STROMAL CELLS

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Demonstrating the molecular mechanisms that human adipose tissue-derived mesenchymal stem cell (hADSC) differentiation and proliferation could develop hADSC-based cell therapy. In this study, we confirmed the roles of microRNA-137 (miR-137) on hADSC proliferation and adipogenic differentiation. We showed that overexpression of miR-137 inhibited both hADSC proliferation and adipogenic differentiation. Overexpression of miR-137 downregulated protein and mRNA levels of Cdc42, a predicted target of miR-137. In contrast, inhibition of miR-137 with 2'O methyl antisense RNA increased proliferation and adipogenic differentiation in hADSC. Luciferase reporter activity of the construct in which the miR-137 target site from the Cdc42 3'UTR was lower in miR-137-transfected hADSC than in control miRNA-transfected hADSC. RNA interference-mediated downregulation of Cdc42 in hADSC inhibited their proliferation and adipogenic differentiation. Our results indicate that miR-137 regulates hADSC adipogenic differentiation and proliferation by directly targeting Cdc42. These findings improve our knowledge of the molecular mechanisms governing hADSC differentiation and proliferation.

JUNE 19, 2014

THURSDAY LATE BREAKING ABSTRACT POSTER PRESENTATIONS

6:00 PM - 7:00 PM ODD numbered posters presented
7:00 PM - 8:00 PM EVEN numbered posters presented

T-L-4001

LONZA L7™: A HPSC CULTURE SYSTEM FOR RESEARCH AND FUTURE CLINICAL APPLICATIONS

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Defined and feeder-independent cell culture systems have provided a superior platform for reproducibility and standardization of human pluripotent stem cell (hPSC)-based research. As the field advances towards potential clinical applications involving hPSC derived cell progenies, it is mandatory that hPSC culture systems meet clinical regulatory compliance. To address this need, Lonza has developed a robust xeno-free, feeder-free and defined system that could be translated to GMP and clinical grade manufacturing. L7™ hPSC Culture System is a culture platform (medium, matrix, passaging solution and cryosolution) that supports every-other-day feeding of hPSCs. Aim: To evaluate Lonza's L7™ hPSC Culture System for maintenance and expansion of human ES cells and iPS cells. Methods: A hESC line (*NKX2-5*$^{eGFP/w}$ hESCs) and two hiPSC lines (LQTS2-hiPSC1.4, DF-hiPSC4) were maintained in L7™ hPSC Medium and passaged every 5-7 days using L7^{TM} hPSC Passaging Solution. Dissociated hPSC colonies were seeded on L7^{TM} hPSC Matrix. Molecular characterization for pluripotency was performed using immunostaining and FACS analysis. The differentiation potential of hPSCs into the three primary germ lineages was characterized through embryoid bodies *in vitro*. Results: Human PSCs were maintained using the L7™ hPSC Culture System for 5 passages. Cellular expansion was approximately 7-10 fold due to high attachment rates when using the L7™ hPSC Passaging Solution and L7™ hPSC Matrix (>90%). Morphologically, hPSCs were tightly packed having a high nucleus to cytoplasmic ratio. Moreover, the efficiency of spontaneous differentiation was significantly reduced when compared to conventional hPSC culture systems. After 5 passages, all hPSC lines revealed nuclear localization of Oct-4 and membrane localization of pluripotent surface antigens. FACS analysis revealed >90% of hPSCs were positive for pluripotency markers (Oct-4, SSEA4, Tra-1-60 and Tra-1-81). These results confirm that the L7™ hPSC Culture System provides rapid and exponential expansion of hPSCs while maintaining pluripotent characteristics. Conclusions: The L7™ hPSC Culture System manufactured by Lonza, provides an easy

and efficient method to expand and culture hPSCs in a regulatory compliant environment for hPSC-based regenerative medicine applications.

T-L-4002
DEVELOPMENT OF A NOVEL XENO-FREE MEDIUM FOR FEEDER-FREE CULTURE OF HUMAN STEM CELLS

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Culture of human ES cells and iPS cells has attracted a lot of interest due to the applications of stem cells in both drug screening as well as regenerative medicine. Most researchers co-cultivate human ES or iPS cells on mouse derived MEF feeder cells. However, presence of the feeder cells could affect the application of the resulting stem cell to other areas of research. Moreover, the current conventional culture medium may contain animal-derived serum; this may increase the risk trans-species infection from implantation of stem cells or stem-cell derived materials. Such issues could cause a setback in the clinical application of stem cell research. In order to solve such issues, we have developed a new culture medium, ReproXF, which does not contain any animal-derived components. Culture with ReproXF allows researchers to cultivate iPS and ES cells under feeder-free conditions without compromising the quality. Here, we demonstrate that iPS cells cultivated using ReproXF showed alkaline phosphatase activity. Furthermore, these iPS cells showed strong expression of the pluripotency markers OCT3/4, NANOG, SSEA-1, TRA1-60 and TRA1-80 by both immunostaining and flow cytometry. Also, from the result of immunostaining, we have confirmed that the karyotype of these iPS cells is normal. In addition, we also confirmed that iPS cells cultivated by ReproXF possess the ability to differentiate into neurons and cardiomyocytes. Taken together, these data show that ReproXF medium not only allows iPS cells to remain in the undifferentiated, pluripotent state, but also to retain the ability to differentiate under standard conditions.
In conclusion, we believe that ReproXF medium will allow researchers to produce large quantities of high quality of human ES or iPS cells for use regenerative medicine and basic research.

T-L-4003
A FLUORESCENT PROBE TARGETING DNA METHYLTRANSFERASE 3B TO TRACK PLURIPOTENCY WITHIN LIVE IPS CELLS

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A significant challenge in stem cell research is unambiguous detection of pluripotency in a population of reprogrammed iPS cells. Typically, multiple genetic markers are assessed to test whether cells are fully reprogrammed and pluripotent. For iPS cells, the changes in DNA methylation patterns have also been used as a marker for successful reprogramming in iPS cells. Previous embryonic stem cell and iPS cell studies have revealed that DNA methyltransferase 3b (Dnmt3b) is a significantly expressed nuclear

protein that is positively correlated to pluripotency, and is responsible for a majority of de novo DNA methylation in those cells. We hypothesize that monitoring Dnmt3b levels in iPS cells may lead to a powerful marker of pluripotency. Furthermore, a non-destructive monitoring of Dnmt3b levels in live iPS cells would allow the capturing of dynamic changes that occur in the enzyme level under different treatments. To this end, we designed and synthesized a fluorescent probe, which is both membrane and nuclear permeable, based on the Dnmt3b selective inhibitor nanaomycin A. Within a model cell line which expresses high levels of Dnmt3b, the fluorescent probe was found to a) localize in nucleus, b) co-localized with a Dnmt3b specific antibody, c) correlate to the amount of Dnmt3b antibody staining, and d) work in both lightly fixed and live cells. The fluorescent compound is now being used with pre-established pluripotent iPS cell lines to quantify the fraction of cells expressing high levels of Dnmt3b. The results suggest that the Dnmt3b fluorescent probe can be used in parallel with other biomarker measurements to increase the robustness of pluripotency analysis.

T-L-4004
MODELING ANESTHETIC-INDUCED DEVELOPMENTAL NEUROTOXICITY USING HUMAN EMBRYONIC STEM CELL-DERIVED DEVELOPING NEURONS

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Introduction: Growing evidence has demonstrated that prolonged exposure of developing animals during rapid synaptogenesis to general anesthetics (e.g., ketamine) induced widespread neurotoxicity followed by long-term memory and learning abnormalities. However, there is no direct clinical evidence showing any such effect at any dose due to the lack of appropriate human model, raising serious concerns about the safety of anesthetic use in pregnant women and young children. We have developed an *in vitro* model of human embryonic stem cell (hESC)-derived neurons so that we can, under control conditions and during intense neuronal growth, test the effects of anesthetics, underlying mechanisms, and prevention strategies. We hypothesized that ketamine, an antagonist of N-methyl-D-aspartate receptor (NMDAR), induced toxicity in hESC-derived developing neurons and that this toxicity occurred through NMDAR-mediated deregulation of intracellular calcium, reactive oxygen species (ROS), and mitochondrial fission pathways. Methods: Neurons were derived from hESCs through a four step differentiation protocol, and stained with antibodies against immature neuron marker (doublecortin) and NMDAR. The presence of functional NMDAR in differentiated neurons was confirmed by whole cell patch-clamp recording. Following acute ketamine and NMDA exposure, the changes of $[Ca^{2+}]_c$ was analyzed by Fura-2 imaging. Two-week-old neurons were then treated with ketamine (20 and 100 μM) for 24 h with or without the ROS scavenger Trolox. Cell apoptosis, autophagy, ROS, mitochondrial fission were then analyzed. All fluorescence imaging was done with confocal microscopy. Protein expression was analyzed using Western blot. Autophagy-related gene expression was assessed using qRT-PCR. Results: Differentiated neurons not only expressed immature neuron marker but also had functional NMDAR. NMDA enhanced $[Ca^{2+}]_c$ while ketamine attenuated the NMDA-induced $[Ca^{2+}]_c$ increase. Washout of ketamine allowed $[Ca^{2+}]_c$ recovery to control level. In the ketamine-treated culture (100 μM, 24 h), autophagosomes were found in almost every cell occupying majority of cytosol volume. The presence of autophagy was further confirmed by the increased conversion of LC3βI to LC3βII (an autophagy-specific marker). Of 84

autopohgy-related genes analyzed, 53 genes were upregulated and 9 genes were downregulated. In addition, the significant increases of ROS production, neuronapoptosis, and mitochondrial fission were detected in the ketamine-treated culture. Importantly, Trolox significantly decreased ROS production and attenuated ketamine-induced apoptosis. Conclusions: 1) These data for the first time demonstrate that ketamine induces both autophagy and apoptosis in stem cell-derived human neurons possibly through NMDAR-mediated deregulation of intracellular calcium, ROS, and mitochondrial pathways. 2) Neuroapoptosis during brain development has been considered as the major toxicity from general anesthetics. It is possible that in addition to apoptosis, autophagy contributes to anesthetic-induced learning abnormalities. 3) Stem cell-associated neurogenesis system may provide a simple and promising *in vitro* human model for rapidly screening anesthetic neurotoxicity and studying the underlying mechanisms as well as prevention strategies to avoid this toxicity.

T-L-4005

MICE WITH HUMAN IMMUNE SYSTEMS SERVE AS MODELS FOR HUMAN NEURAL STEM CELL TRANSPLANT ACCEPTANCE

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Utilization of human embryonic stem cells (ESCs) for transplantation therapy presents the problem of immune rejection of donor hESC derivatives. While tissue rejection can be managed by chronic immunosuppressive regimes, these drugs carry severe side effects including infections and malignancies. Our work directly addresses this important regenerative medicine issue by investigating human leukocyte antigens (HLA). Because mouse and human immune systems fundamentally differ, it has not been possible to model effects of human stem cell transplants in a mouse system, nor have we been able to translate mouse-to-mouse transplantation results into the clinic. To overcome these limitations, we have established cutting-edge mouse models with human immune systems. These models rely on immunocompromised mice as recipients for human umbilical cord blood-derived hematopoietic stem cells. These mice go on to develop a human immune system, complete with HLAs, and have been used to engraft hESC-derived neural progenitors that are either HLA matched or mismatched. We have been able to generate mice with > 50% reconstitution with human immune cells, including T and B lymphocytes, which are central cellular players in host vs. graft rejection. Additionally, we have analyzed expression levels of HLA antigens on hESC-derived neural progenitors, and successfully HLA haplotyped cord blood samples and hESC lines. Neural progenitors derived from these cell lines have been transplanted into brains of recipient humanized mice. Immunohistochemical analysis of these brains has demonstrated a human immune reaction against neural progenitors with mismatched HLA haplotypes. This immune reaction is generally more confined to mice with massive brain immune cell recruitment and activation of microglia and peripheral macrophages of host origin. The use of these next-generation humanized mouse models is expected to allow us to determine the role(s) of HLAs in human neural stem cell transplant tolerance.

T-L-4006

TREATMENT OF TYPE 2 DIABETES IN IMMUNODEFICIENT MICE WITH MACRO-ENCAPSULATED INSULIN-SECRETING CELLS DERIVED FROM HUMAN EMBRYONIC STEM CELLS.

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Type 2 diabetes accounts for ~90% of all cases of diabetes, yet these patients are not currently eligible for islet transplantation due, in part, to insufficient donor tissue. Human embryonic stem cells (hESCs) are a potential alternative to cadaveric islets for treating patients with diabetes. We previously reported that hESC-derived pancreatic progenitor cells reversed hyperglycemia in a rodent model of type 1 diabetes, but their capacity to treat type 2 diabetes has not been examined. The current study aimed to determine: a) the effect of high fat diets (HFDs) on the maturation of hESC-derived progenitor cells in vivo, and b) if hESC-derived insulin-producing cells were capable of reversing hyperglycemia in a mouse model of type 2 diabetes. Immunodeficient SCID-beige mice were placed on one of four diet regimens (n=11/group): 10% kcal fat, 45% kcal fat, 60% kcal fat or a "western" diet. Mice in all HFD groups rapidly developed diet-induced obesity, impaired glucose homeostasis, insulin resistance and dyslipidemia. After 50 days on the diets, mice received TheracyteTM devices containing hESC-derived pancreatic progenitor cells (n=7/group) or sham surgery (n=4/group). Progenitor cells effectively developed into glucose-responsive insulin-secreting cells irrespective of diet regimen and there were no significant differences in human C-peptide secretion or graft composition between groups. Therefore, exposure to HFDs did not impact the maturation of hESC-derived progenitor cells in vivo. Notably, at 20 - 24 weeks post-transplant, HFD mice had significantly improved glucose tolerance and HbA1C levels compared to mice that received sham surgery, suggesting that the hESC-derived insulin-producing cells were capable of treating diet-induced dysglycemia in a mouse model of type 2 diabetes.

T-L-4007

EPIGENETIC MODULATORS RESTORE CONSTITUTIVE AND LIVER-SPECIFIC REPORTER EXPRESSION IN MOUSE LIVER PROGENITOR CELLS

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Cell lines with reporter constructs are useful for tracing such cells in vivo following transplant or for determining their lineage fate in vivo and in vitro. To better understand the biology of liver progenitor cells (LPCs) we generated cell lines from an actin-EGFP mouse and a TAT GRE lacZ mouse respectively. LPCs from the actin-EGFP mouse facilitate cell tracing following transplant as the reporter is constitutively expressed. LPCs from the TAT GRE lacZ mouse express beta-galactosidase under the control of a promoter that is not only liver specific, but expressed only in mature hepatocytes. Therefore differentiation and maturation of LPCs derived from fetal TAT GRE lacZ mice that do not express beta-

galactosidase can be simply documented and quantified by lacZ expression. We found that the utility of such LPC lines is severely limited by down-regulation of the transgene following extended culture. In this study, we show that epigenetic mechanisms are responsible for suppressing expression of the respective transgenes and the demethylating agent 5-azacytidine and the acetylating agent sodium butyrate will restore expression of the respective transgenes.

T-L-4008
THE TUMOR SUPPRESSOR MULE/HUWE1/ARF-BP1 IS AN ESSENTIAL REGULATOR OF PANETH CELLS AND THE STEM CELL NICHE IN THE INTESTINE

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Stem cells give rise to a specialized daughter cell that in turn provide essential niche signals to sustain them. Here we show that the conditional deletion of the E3 ubiquitin ligase Mule/Huwe1/Arf-BP1 (Mule) in the intestinal epithelium of APCmin mice promotes the growth of a large number of adenomas that are populated by paneth and stem cells. This is also evident in tumours that spontaneously arise in aged Mule knockout mice. Deletion of Mule in the gut results in hyperactive Wnt signaling and thus increased proliferation. Mule plays an important role in fine tuning this pathway by its regulation of cMyc and potentially other components. Genetically the increased proliferation can be exclusively rescued by concomitant deletion of cMyc. We conclude that Mule is an important tumor suppressor in the gut that utilizes distinct modes to regulate the number and positioning of paneth cells and thus “the stem cell niche”.

T-L-4009
NEURAL COMPETENT CELLS OF ADULT HUMAN DERMIS BELONG TO THE SCHWANN LINEAGE

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Resident neural precursor cells (NPCs) have been reported for a number of adult tissues. Understanding their physiological function or, alternatively, their activation after tissue damage or in vitro manipulation remains an unsolved issue. Here we investigated the source of human dermal NPCs in the adult. By following an unbiased, comprehensive approach of cell surface marker screening, cell separation, transcriptomic characterization and in vivo fate analyses, we found that p75NTR+ precursors of human foreskin can be ascribed to the Schwann (CD56+) and perivascular (CD56-) cell lineages. Moreover, neural differentiation potential was restricted to the p75NTR+CD56+ Schwann cells and correlated with

Sox2 expression levels. Loss and gain of function experiments demonstrated that Sox2 levels dictate neural competence in dermal precursors and thus Sox2 is a major determinant of cell fate also in this system. Double positive NPCs were similarly obtained from human cardiospheres, indicating that this phenomenon might be widespread and underlie stromal NPCs previously described in diverse tissues.

T-L-4010
KERATIN 15 (KRT15) ANNOTATES A UNIQUE TRANSIT-AMPLIFYING POPULATION IN THE ESOPHAGEAL SQUAMOUS EPITHELIUM

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Background: The esophagus comprises a squamous stratified epithelium marked by equilibrium between proliferation and differentiation. Stem cells and progenitor cells reside within the proliferating basal cell compartment. We have identified a potential esophageal stem cell population localized in the basal compartment that is CD34+ with self-renewal capacity and gives rise to all differentiated lineages in the suprabasal and superficial compartments of the epithelium (Kalabis et al. JCI 2008). We now determine that keratin 15 (KRT15) annotates a transit-amplifying population emerging from the basal cell compartment. AIMS & METHODS: Herein, we investigated the role of the hair follicle stem cell marker, KRT15, in the murine esophageal epithelium. We determined the expression and localization of KRT15. Next, we performed lineage tracing using a RU486-inducible Cre recombinase under control of KRT15 promoter mice (*KRT15-crePR1*) crossed with Rosa Reporter mice ($Rosa^{mTomato/mGFP}$). Cre recombination was induced by daily RU486 administration for 5 days and mice were sacrificed 1, 3, 5, 7 and 10 days following recombination. GFP staining was then performed to visualize KRT15 labeled cells. RESULTS: We demonstrated that KRT15 is located predominantly in the basal compartment of the esophagus epithelium. KRT15 expression is correlated inversely with KRT13 expression, which is associated with differentiated cells, suggesting that KRT15 expression is restricted to undifferentiated cells. Furthermore, Ki67+ cells express KRT15, but KRT15 expression is not restricted to KI67+ cells, suggesting that KRT15 expression is not dependent exclusively upon cellular proliferation. We also found that KRT15 expression is decreased by 11.7 fold in the stem cell side-population and by 9.6 fold in CD34+ cells. In the mouse esophagus, BrdU-retaining basal cells show positive staining for KRT15 but at a lesser extent than surrounding cells. We next performed lineage tracing experiments. Interestingly, at 1, 3 and 5 days following Cre recombination, we observed an increasing number of GFP+ cells in the esophagi of $KRT15\text{-}crePR1;Rosa^{mTomato/mGFP}$ mice. Ten days following Cre recombination, GFP+ cells were still detected in the mouse esophagus. For every time point studied, GFP+ cells were visualized in the basal and the suprabasal compartments. However, even at 10 days, GFP+ cells are not found in the entire esophagus suggesting that KRT15+ cells cannot give rise to all cells located in the mouse esophagus. CONCLUSION: KRT15 may represent a novel transit-amplifying cell marker in the mouse esophageal epithelium. This new discovery suggests that esophageal basal cells have divergent capacities to give rise to suprabasal cells, and there is a stem cell/transit-amplifying cell system.
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T-L-4011

A NOVEL ANTI-APOPTOTIC HORMONE IN HUMAN EMBRYONIC STEM CELLS PROMOTES ENDOGENOUS AKT SIGNALING AND SELF-RENEWAL

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The self-renewal and pluripotency of human embryonic stem cells (hESCs) are dependent on a cocktail of exogenously supplemented growth factors such as insulin or insulin-growth factor (IGF). However, few of these factors, including insulin/IGF are produced endogenously by hESCs. Here, we report a novel peptide hormone that is secreted by hESCs and required for their growth and maintenance through activation of the PI3K/AKT pathway. Strikingly, this recombinant peptide is sufficient to replace insulin in hESC media, and exerts potent anti-apoptotic and growth promoting effects on hESCs. In contrast, depletion of this peptide by shRNA leads to increased apoptosis, reduced AKT phosphorylation and increased sensitivity to insulin deprivation. We propose that this novel peptide is produced endogenously by hESCs and signals in an autocrine fashion through an as-yet-identified receptor to a mediate hESC growth and maintenance.

T-L-4012

PRECISE GENE CORRECTION OF DUCHENNE MUSCULAR DYSTROPHY IPS CELLS USING TALENS AND CRISPR/CAS9

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Duchenne Muscular Dystrophy (DMD) is a severe muscle degeneration disease caused by the loss-of-function mutations in Dystrophin gene on X chromosome. Exon skipping to modulate mRNA splicing patterns using antisense oligonucleotide is a promising approach currently tested in clinical trials, however, the effect of antisense oligos is transient. Recently, targeted genome editing using engineered nucleases, such as TALENs and CRISPR/Cas9, have been demonstrated to be effective and efficient to modify the target region of the genome in many organism.
In this study, we aim to restore the mutated dystrophin protein in the patient-derived induced pluripotent stem (iPS) cells by engineered nucleases. We derived iPS cells from a DMD patient who lacks the expression of Dystrophin protein due to one base pair frame shift. To restore the reading frame of dystrophin protein, we devised two approaches. One is to induce a small deletion or insertion to modulate protein reading frame. Another is to knock-in the deleted exon with a repair template.
To compare the targeting efficiency and off-target risk between TALEN and CRISPR, we constructed

several nucleases to target dystrophin gene. We demonstrate high recombination efficiencies in the both systems. We confirmed the successful correction of the Dystrophin reading frame by Sanger sequence for frame-shift clones, and by PCR and Southern blotting for the exon 44 knocked-in clones. To assess the risk of off-target mutagenesis, we looked at the candidate off-target sites by T7 Endonuclease I assay and also examined the whole genome protein-coding regions by exome sequencing. So far, we observed no severe off-target mutagenesis introduced by either TALEN or CRISPR treatment. Finally, we successfully detected the expression of dystrophin protein in skeletal muscle cells differentiated from the genetically corrected iPSCs. We expect our "genomic surgery" approaches using TALEN or CRISPR in patient-derived iPSCs will facilitate cell therapies for DMD.

T-L-4013
RELEASABLE MAGNETIC PARTICLES FOR HIGH RECOVERY PURIFICATION OF RARE STEM CELLS

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The muti-potent and regenerative capabilities of stem and progenitor cells are active areas of research. Purification of stem cell culture systems details a need to separate differentiated cell populations from un-differentiated stem clusters where if not performed, the heterogeneous populations would lead to teratoma formation within animal engraftment models. Many cell specific purification systems use an approach known as Magnet-activated cell sorting a widely utilized method of interest in clinical medicine as well as basic biological science. In this technique, antibody-functionalized magnetic microbeads are utilized to bind to certain cell types in a heterogeneous suspension enabling subsequent separation via magnetic fields. Separation can be accomplished by positive selection, where the cell type of interest is magnetically tagged and isolated, or by negative selection, where non-target cell types are tagged and extracted. A major gap in magnetic cell sorting technology, particular relevant to the positive selection capture method, is the inability to detach the magnetic tag without significant loss of viability, phenotypic identity, and recovery. Such detachment capability is of critical importance in stem cell biology and regenerative medicine, where certain cell types are manipulated under highly defined conditions and may be ultimately implanted in vivo. In addition, many of the current magnetic based approaches suffer from poor separation recovery leading to the need to pool multiple samples to obtain proper plating efficiency. We have designed a releasable magnetic microbead platform using a readily dissolvable hydrogel capable of highly specific antibody-mediated adhesion to target stem cells and easy detachment of magnetic particles. We have demonstrated a 15-fold increase in recovery against current magnetic platforms for CD34+ endothelial progenitor cells (EPCs) from unpurified human buffy coat. The release cells were cultured for over 7 days and exhibited native morphology and function. The significance of this approach lies in its simplicity and scalability, which may be useful considerations in areas such as reprogramming, where well-defined cell subsets must be routinely obtained in quantity, or mechanistic studies where for example small quantities of defined subsets must be obtained reproducibly and rapidly to form co-cultures and/or incorporate mechanical stimulation. The novel platform will provide the clinical and biomedical research community with the unique ability to selectively label and separate target cell populations from

heterogeneous suspensions without the adverse consequence of retained magnetic beads on target cells while retaining high target stem cell purity and recovery.

T-L-4014
THE ACTIVATION OF CXCR2 BY ACETYLATED PRO-GLY-PRO HAS THERAPEUTIC EFFECTS IN ISCHEMIC LIMB

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Many therapeutic approaches to treat ischemic diseases using endothelial progenitor cells (EPCs) have been developed. EPCs can integrate into blood vessels and stimulate neovascularization of the ischemic limbs and hearts. Therefore, mobilization and recruitment of bone marrow-derived endothelial progenitor cells are critical for ischemia-induced neovascularization. The exact signaling, however, involved in the homing of EPCs to sites of endothelial injury remains to be understood.
Chemokine receptor 2 (CXCR2), a receptor of interleukin 8 (IL-8), mediates neutrophil migration to the site of inflammation. The angiogenic effects of IL-8 in intestinal vascular endothelial cells are mediated by this receptor. Our hypothesis is that CXCR2 is involved in the regulating growth and survival of endothelial cell and EPCs through the mechanism similar to IL-8-regulated angiogenesis.
We explored the role of CXCR2 in angiogenesis and tissue regeneration by using Acetylated Pro-Gly-Pro (Ac-PGP), which is the endogenous degradation product of extracellular collagen and binds to CXCR2. Ac-PGP stimulated chemotactic migration, tube formation ability of human EPCs in vitro. The blockade of CXCR2 abrogated Ac-PGP-induced migration and tube formation of EPCs. Intramuscular injection of Ac-PGP into the ischemic hindlimb resulted in the attenuation of the severe limb loss and the stimulation of blood perfusion and angiogenesis in the ischemic limb. CXCR2 knockout mice showed the attenuation in Ac-PGP-induced in vivo neovascularization and ischemic limb salvage. These results suggest that Ac-PGP has therapeutic effects by stimulating neovascularization through CXCR2-dependent mechanism.

T-L-4015
IDENTIFICATION OF TISSUE INHIBITOR OF METALLOPROTEINASE-1 AS A NOVEL CHEMOATTRACTANT MOLECULE FOR HUMAN NEURAL STEM CELLS

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We recently reported that human neural stem cells (hNSCs) have the interesting characteristic of migration toward an intracranial glioma. However, the molecules and mechanisms responsible for tumor tropism are unclear. In this study, we used microarray and proteomics analyses to identify a novel chemoattractant molecule, tissue inhibitor of metalloproteinase-1 (TIMP-1), secreted from human brain tumor tissues. We demonstrate that TIMP-1 significantly enhances hNSC adhesion and migration in a cell culture system. These effects were critically dependent on CD63, as short hairpin RNA-mediated ablation of CD63 expression attenuated the response. TIMP-1 significantly increased the number of focal adhesions (FAs) and cytoskeletal reorganization for cell migration in hNSCs, whereas knockdown of CD63 resulted in decreased hNSC spreading, FAs, and migration, even after TIMP-1 treatment. In addition, TIMP-1 binding to CD63 activated [[Unsupported Character - Symbol Font ]]1 integrin-mediated signaling through Akt and FAK phosphorylation, leading to pattern changes in distribution of vinculin and F-actin. Furthermore, inactivation of [[Unsupported Character - Symbol Font ]]1 integrin by use of a blocking antibody, or inhibition of phosphoinositide 3-kinase (PI3K) signaling impaired the migration of hNSCs toward TIMP-1. Collectively, our results underline TIMP-1 as a novel and effective key regulator of CD63 and [[Unsupported Character - Symbol Font ]]1 integrin-mediated signaling, which regulates hNSC adhesion and migration.

T-L-4016

INHIBITION OF AUTOPHAGY AS A NEW MEANS OF IMPROVING CHEMOTHERAPY EFFICIENCY IN TRIPLE-NEGATIVE BREAST CANCERS

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The triple-negative breast cancer (TN BC) subtype is the most aggressive form of invasive BC. Despite intensive efforts to improve BC treatments, patients with TN BC continue to exhibit poor survival, with half developing resistance to chemotherapy. Here we identify autophagy as a key mechanism in the progression of TN tumors and in their acquisition of chemoresistance. We demonstrate that LC3B, a protein involved in autophagosome formation, is a reliable marker of poor prognosis in TN BC, validating this prognostic value at both the mRNA and protein levels in several independent cohorts. We also show that LC3B has no prognostic value for other BC subtypes (luminal or HER2 BC), thus revealing a specific impact of autophagy on TN tumors. We further demonstrate that autophagy is not only activated in the TN BC cells, but also essential for their proliferation, their invasive properties, and the growth of TN BC tumors in vivo. Interestingly, the activity of transcriptional co-activator YAP1 (Yes-associated protein) is regulated by the autophagy process and we identify YAP1 as a key actor in the autophagy-dependent proliferative and invasive properties of TN BC. Finally, using a patient-derived TN tumor transplanted into mice, we show that autophagy allows human TN BC to evade chemotherapy. Overall, our data identify LC3B as a new prognostic marker for TN BC and the inhibition of autophagy as a promising therapeutic strategy for TN BC patients.

T-L-4017

CDR3, A FABP7 BINDING SMALL MOLECULE FLUORESCENT PROBE FOR NEURAL STEM CELL ISOLATION

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Neural stem cell isolation methods are limited due to the lack of well-defined cell surface markers and tools to detect intracellular markers. To date most methods depend on the labeling of extracellular markers using antibodies, with intracellular markers remaining inaccessible in live cells. Fluorescent small molecules are efficient tools for intracellular labelling in live cells. Through the high throughput screening of an in-house diversity oriented fluorescence library, we have identified a novel fluorescent chemical probe CDr3 which is selective for neural stem cell by binding to the intracellular protein FABP7 (Fatty Acid Binding Protein-7, also known as brain lipid binding protein).
We have successfully applied CDr3 for the isolation of high FABP7-expressing cells from embryonic and adult mouse brains. Cell sorting viability was a critical factor in achieving significant enrichment numbers. The isolated cells form neurospheres in culture, express markers of neural stem cell and differentiate into neurons, astrocytes and oligodendrocytes. Characterization of cells sorted with Aldefluor and other antibodies showed that the cells isolated by CDr3 exhibit a phenotype distinct from the cells sorted by conventional methods. FABP7 labeling with CDr3 represents a novel method for rapid isolation of neural stem cells based on the expression of a single intracellular marker.

T-L-4018

THE TRANSCRIPTION FACTOR MOHAWK PROMOTES THE TENDON DIFFERENTIATION VIA ACTIVATING TGFBETA SIGNALING PATHWAY IN MESENCHYMAL STEM CELLS

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Transcription factors (TFs) play a central role in lineage specific differentiation of stem cells, however, the key TFs in teno-lineage differentiation of mesenchymal stem cells (MSCs) are poorly understood. Here, we investigated the role of the TF Mohawk (Mkx) in tendon differentiation. Here, we found that Mkx expression level was dramatically lower in tendinopathy than in normal tendon tissue and Mkx is highly expressed in tendon progenitor cells comparing with other types of stem cells. Importantly, forced Mkx expression strikingly promoted tenogenesis of MSCs by tenogenic genes activation, even more effectively than Scleraxis (Scx), an important TF in tendon. Moreover, we found Scx was regulated by Mkx through TGFbeta signaling and identifed Tgfb2 as a direct downstream target of Mkx. Finally, the application of Mkx-MSC cell-sheets promoted the formation of tendon-like tissues in a mouse model of

Achilles tendon defect. Taken together, our studies show an unrecognized role of Mkx in promoting tendon differentiation of MSCs by inducing Scx through TGFbeta signaling pathway and by inducing tenogenic genes expression.

T-L-4019
DIRECTED DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO LUNG PROGENITORS

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Chronic respiratory diseases are a major cause of mortality and morbidity worldwide. Hundreds of millions of people suffer every day from chronic respiratory diseases. According to the latest World Health Organization (WHO) estimates, currently over 300 million people have chronic respiratory diseases. Total deaths from chronic respiratory diseases are projected to increase by more than 30% in the next 10 years without interventions. Adult stem cells are still ill defined in the lung. In addition, the natural repair capacity provided by endogenous adult epithelial stem/progenitor cells is often insufficient and appears to diminish with age. During embryonic development of the lung, several evidences showed that the distal-most epithelial cells (Sox9+Id2+) are a multipotent progenitor cell population. As the lung branches, self-renewing progenitors are found within the epithelial budding tips and generate the full-repertoire of respiratory cell types. Human Pluripotent Stem Cells (hPSCs)-derived lung cells hold future promise of regenera¬tive medicine approaches to treat serious lung diseases. We have established an efficient method for in vitro directed differentiation of hPSCs into embryonic distal lung progenitors by using a stepwise protocol mimicking lung embryogenesis. Current data and ongoing work will be discussed here.

T-L-4020
VARIABILITY IN SUBJECTIVE REVIEW OF UMBILICAL CORD BLOOD COLONY FORMING UNIT ASSAY

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Introduction: Successful use of progenitor cells in human umbilical cord blood (UCB) for cell therapy is dependent on the presence of colony forming stem and progenitor cells. Colony forming unit (CFU) assay are used to measure the biological potential of the hematopoietic stem cells from UCB samples. CFU assays are evaluated by trained technicians that manually count the number of colonies formed. The goal

of this study was to quantitatively evaluate the reproducibility of current manual counting methods based on subjective classification of burst-forming unit-erythroid (BFU-E), colony forming unit granulocyte,macrophage (CFU-GM), and colony forming unit- granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM). Methods: Fresh cord blood-derived cells and reconstituted frozen cells from 10 cord blood samples were cultured in duplicate under standard conditions in ungridded 35 mm well-plates (20 fresh,20 frozen). Color images of the entire plate were acquired using a Leica DMI3000 inverted microscope equipped with a Marzhauser motorized stage and QImaging QICAM camera. Color images were acquired with a 2.5X objective (1.9 microns/pixel) and combined into a single full field-of-view image for review. Three expert reviewers reviewed the images on a digital display and manually outlined the three types of CFUs in each image. The reviewers' outlines were then combined and the percent agreement for each colony type was calculated. Results: The mean(s.d.) number of colonies per plate were 96(22) and 58(31) for fresh and frozen samples, respectively. The mean percentage of colonies/plate where all three reviewers agreed was 77.1%(8.7) and 78.0%(8.4) for fresh and frozen samples, respectively. Reviewer agreement based on colony type in the fresh samples was 40.3%(9.3), 24.9%(12.7), and 5.8%(6.8) for BFU-Es, CFU-GMs, and CFU-GEMMs, respectively. Reviewer agreement for colony type in the frozen samples was 55.4%(11.4), 40.0%(17.3), and 3.4%(7.1) for BFU-Es, CFU-GMs, and CFU-GEMMs, respectively. Visual inspection of the combined reviewer maps indicated considerable variability in the size and extent of colonies manually identified by the reviewers. Conclusions: The results of this study illustrate the inherent variability observed in the subjective review of CFU assays. Although reviewers agreed on CFUs 77-78% of the time, their ability to agree on the progenitor type was significantly less for all colony types. This indicates the need for more rigorous, objective and standardized methods for characterizing hematopoietic colony formation in UCB samples.

T-L-4021

MYOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS BY THE TRANSDUCTION OF MYOD PROTEIN FUSED WITH CELL-PENETRATING AND pH-SENSITIVE FUSOGENIC PEPTIDES

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MyoD, a basic helix-loop-helix transcription factor, plays key roles for muscle differentiation. Forced expression of MyoD triggers myogenesis in various cell lines including mesenchymal stem cells (MSCs). Instead of viral gene delivery method having a high risk of the genome alteration, we developed a safe method for muscle differentiation using the engineered MyoD protein (MyoD-IT). It was designed to contain the TAT peptide for cell penetration and the membrane-disrupting INF7 peptide, which is an improved version of the HA2 peptide derived from influenza. MyoD-IT showed greatly improved nuclear targeting ability through an efficient endosomal escape induced by the pH-sensitive membrane disruption of the INF7 peptide. By applying MyoD-IT to a culture, human MSCs were efficiently differentiated into long spindle-shaped myogenic cells expressing myosin heavy chains. Moreover, these cells differentiated by an application of MyoD-IT fused to myotube with high efficiency through co-culturing with mouse C2C12 myoblasts. Because internalized proteins can be degraded in cells without altering the genome, the myogenic differentiation of MSCs using MyoD-IT can be a safe and clinically applicable method.

T-L-4022

PHYSICOCHEMICAL PROPERTIES AND BIOLOGICAL EVALUATION OF PDLLA/PLLALMA SCAFFOLDS FOR TISSUE ENGINEERING

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The chemical features of polyester scaffolds used in tissue engineering are crucial for the suitable affinity and adhesion of the cells over the surface of the material. These interactions can be improved by the presence of biomolecules, such as fibronectin, vitronectin, laminin and collagen, through specific bio-recognizing mechanisms. Today, the most common method for functionalizing polyesters scaffolds with the biomolecules quoted above, involves the partial hydrolysis of their surface with a basic/acid solution. This strategy leads to the cleavage of the ester bonds of the polymer and generating free carboxylic groups, which are essential for the attachment of the biomolecules using chemical reagents. To avoid the necessity of the hydrolysis stage, poly(L-latic acid-co-L-malic acid) (PLLALMA) was used, which is a low molecular weight polymer containing many free carboxylic groups in the chain. This was blended with a high molecular weight poly(DL-lactide) (PDLLA) to construct micro/nanofiber scaffolds by electrospinning. The ratio between two polymers varied from zero to 30% (m/m) of PLLALMA content. The images obtained from scanning electron microscopy analysis (SEM) showed the nonwoven fibers distributed randomly and without beads. The mean diameter (μm) of the fibers calculated from the SEM images were 2.40 ± 0.67 (100% PDLLA), 0.93 ± 0.37 (10% PLLALMA), 0.72 ± 0.29 (20% PLLALMA) and 0.73 ± 0.21 (30% PLLALMA) showing that as the PLLALMA content in the blend increases, the diameter of the fibers decreases. Contact angle analyses were performed in order to determine if there were changes in the hydrophobic nature of the PDLLA scaffolds, due to the presence of the PLLALMA. The 100 % PDLLA scaffold showed an initial contact angle of 129.5° ± 1.6, while the scaffolds containing 10% and 20% of PLLALMA showed angles of 134.8° ± 2.1 and 134.7° ± 1.9, respectively. The contact angle for the matrix containing 30% of PLLALMA could not be measured because the water droplet was completely absorbed by the material. As the matrix containing 30% of PLLALMA is the one with the higher content of this polymer in the blend, it was submitted to a cell adhesion assay using DAPI reagent to evaluate the behavior of the stem cells with regard to these scaffolds. Mesenchymal stem cells from deciduous teeth were used in the experiments and three groups were compared: the cells cultivated in the well of a polystyrene plate (control), a matrix composed of just PDLLA polymer and the scaffold containing 30% PLLALMA. The number of adhered cells in the three groups was 143.55 ± 41.26, 143.33 ± 130.63 and 134.44 ± 64.11, respectively. There was no statistical difference among the groups (p= 0.99), which means that the cells adhered to the scaffold containing the PLLALMA as well as the standard polystyrene plates used in cell cultures. Therefore, the PLLALMA polymer did not change the adhesion properties of the scaffold, besides being a good alternative for introducing other specific biomolecules to increase stem cell growth over the matrix.

T-L-4023

ANALYSIS OF MOUSE EMBRYONIC STEM CELL CULTURE IN THREE-DIMENSIONAL SCAFFOLDS

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Undifferentiated mouse embryonic stem cells (mESCs) are typically expanded and maintained on systems that usually involve 2-dimensional (2-D) substrates, which fail to provide the complex 3-dimensional (3-D) microenvironments that these cells are used to during embryonic development. The aim of this study has been to evaluate the mESC viability and capability of growth in the inner core of 3-D scaffolds. The mESC line used, derived from C57Bl/6 mouse embryos, was isolated and characterized in the laboratory. The mESC were cultured in DMEM high glucose, supplemented with 10% fetal calf serum, 50 IU/mL penicillin and 50 µg/mL streptomycin, recombinant mouse leukemia inhibitory factor (LIF - 1,000 IU/ml), 1 mM sodium piruvate, 2 mM L-glutamine, 0.1 mM b-mercaptoethanol and 0.1 mM non-essential amino acids, kept at 5% CO2 and 37°C in humidified atmosphere. To produce the scaffolds, the polymer poly(D,L-lactide-co-glycolide) (75:25) was dissolved in dichloromethane:ethanol (8:2) hexafluoro-2-propanol to create a 20% (w/v) solution. The experimental set-up to conduct the electrospinning included a high voltage of 14 kV, a collector to needle distance of 20 cm and a constant flow rate of 3.0 mL/h. Some scaffolds were hydrolyzed with NaOH (hydrophilic group). The scaffolds of microfibers were characterized with a scanning electron microscope (SEM) model EVO50 (Carl Zeiss). The average diameter and mean deviation were calculated after 150 measurements for each sample using the software ImageJ. The three groups evaluated were mESCs cultured in hydrophilic scaffolds, mESCS cultured in hydrophobic scaffolds (both fixed in 24 well plates) and the control group of mESCs cultured in a 24 well plate coated with gelatin (0.1%). A total of 3x105 cells/well were seeded and the evaluations were performed after 2 and 7 days of culture. Cell viability was accessed by MTT test and the capability for cell growth in the inner core of the 3-D scaffolds was accessed by phalloidin-rhodamine and DAPI dye followed by histological section of the scaffolds. The statistical analyses were performed according to the test for Multiple Comparisons and Tukey post test. The significance level considered was 5%. The data were analyzed by SPSS16 software. The SEM images showed that the fibers from the hydrophilic scaffold presented an average diameter of 3.593 (range between 1.14 - 6.34) micrometers and the range of the hydrophobic scaffolds was 3.664 (range between 1.51 - 4.98) micrometers. Comparing days 2 and 7, the control group at day 7 had more viable cells in comparison with the hydrophilic ($p=0.001$) and hydrophobic ($p=0.000$) groups. However it is important to mentioned that between the scaffold groups,

the mESCs cultured in the hydrophilic scaffolds presented the highest viability during the period analyzed when compared with the hydrophobic (p=0.006) group. The phalloidin and DAPI dye showed that even when the mESCs were cultured for 7 days in the scaffolds they were not capable of growing in the inner core of the 3-D scaffolds. According to the data presented, it is possible to culture mESCs in scaffolds hydrophilic rather than hydrophobic. However, the cells are not able to reach the core of the scaffold. It is important that this problem be overcome in order to enable the use of the whole 3-D scaffold structure and to make it an efficient device for the growth of stem cells and their ensuing use for tissue engineering.

T-L-4024

EVALUATION OF ELECTROSPUN MATRICES FOR THE CO-CULTIVATION OF MESENCHYMAL STEM CELLS AND SKIN KERATINOCYTES

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The regeneration of skin is an important field for tissue engineering. Currently available treatments are insufficient to prevent scar formation and promote healing of the patient, especially in large burns and chronic wounds. Due to the great need for skin substitutes with the ability of regenerating large amounts of skin, as well as the lack of an ideal replacement, the current study has aimed to produce a cutaneous substitute with a PDLLA polymer as a biomaterial. These, in turn, must be able to serve as a suitable support for cellular growth for the period of time required for tissue regeneration. For this purpose, scaffolds were constructed by the electrospinning technique and divided into 3 groups: 1) PDLLA matrices, 2) PDLLA/NaOH, which were PDLLA scaffolds hydrolyzed with a solution of NaOH 0.75M and 3) PDLLA/Lam, also hydrolyzed with NaOH and in which the protein laminin was linked by covalent binding. They were all constructed with 2 different fiber diameters, with the smallest at the top of the scaffold. These scaffolds were characterized by morphology and fiber diameter and their hydrophilicity or hydrophobicity features. Mesenchymal stem cells were then seeded onto the bottom of the scaffold and, after 24 hours, skin keratinocytes were seeded on the other side. This procedure was performed in all the groups. The groups were evaluated for cell adhesion on the day of the seeding and on days 7, 14 and 21 for viability with WST-8 assay. From day 7, the scaffolds were submitted to an air/liquid system of culture. As a result, the scaffolds presented well formed fibers which were randomly distributed. The treatment of the matrices with NaOH for 15 minutes did not substantially affect the structure of the fibers, but it was enough to hydrophilize the surface of the biomaterials, which is necessary for laminin linkage. The fiber diameter for all the groups was 4.58 µm for the largest fibers and 574 nm for the smallest. The pore size of the scaffolds obtained were approximately 27.5 µm and 3.44 µm, respectively, for the largest and smallest fibers. The linkage of the laminin was confirmed by immunofluorescence assay. For the biological analysis, cell adhesion was greater in the PDLLA/Lam

scaffolds with absorbance of 2.268± 0.494, in comparison with 1.264±0.473 for the control (PDLLA scaffold) and 1.159±0.120 for the PDLLA/NaOH scaffold. On day 7 of the viability analysis, the absorbance for the PDLLA scaffold was 1.148±0.411, the PDLLA/NaOH group was 1.380±0.501 and the PDLLA/Lam was 1.990±0.255. On day 14, the absorbance for groups 1, 2 and 3 were 1.032±0.169, 0.755±0.016, and 1.636±0.313, respectively. On day 21, the results were 2.204±0.317, 1.437±0.024, 2.811±0.477 respectively for groups 1, 2, and 3. In general, in terms of the biological analysis, the PDLLA/Lam group showed the best results for cell adhesion and viability tests. Histological analysis is being processed for greater understanding of the behavior of the cells interacted within the scaffolds. In conclusion, the PDLLA scaffolds, mainly the PDLLA/Lam groups, showed good results for the co-cultivation of the cells, with good cell adhesion and the presence of viable cells. These biomaterials were capable of providing support for the growth of the cells, which was observed by the increase in the absorbance over time. Therefore, although histological analysis is still in progress, these scaffolds promise to be suitable biomaterials for use in tissue engineering.

T-L-4025

DISEASE MODELING OF LATE ONSET POMPE DISEASE SPECIFIC IPS CELLS

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Pompe disease is an inherited neuromuscular disorder caused by a deficiency of acid-glucosidase-alpha (GAA). The clinical symptoms of Pompe disease include progressive weakness, respiratory failure and ventricular hypertrophy. Based on clinical phenotype, Pompe disease is classified to infantile form and late-onset form. Hypertrophic cardiomyopathy is usually remarkable in infantile Pompe disease. Even in late-onset Pompe disease, cardiovascular complications including cardiac hypertrophy, and arrhythmia were clinically important, however cardiac involvements are less often and milder than infantile form. Enzyme replacement therapy has been shown to ameliorate these symptoms. Gene therapy using lentivirus- or adenovirus-associated vectors is another possible treatment strategy. Patient/disease-specific iPS cells have been used for disease modeling, drug screening and cell therapy. Huang et al have already reported infantile Pompe disease iPSC and successfully differentiated into cardiomyocyte like cells. (Huang et al Hum Mol Genet. 2011) We tried to generate late-onset Pompe disease iPSCs and differentiated into cardiomyocyte for disease modeling. In addition, we attempted to generate gene-corrected Pompe disease iPS cell by lentiviral gene transfer.
GAA was cloned into cDNA expressing third-generation lentiviral vectors (CS2-EF1α-GAA). To assess the transfection efficacy, Venus, an YFP variant protein, was also cloned into the vector (CS2-EF1α-Venus). Then, we transfected lentiviral vectors containing GAA and Venus into control iPSC (TkDA3-4, healthy donor) at MOI 10, 50 and 100 to determine the optimized titer for gene correction. Expressions of GAA and Venus in iPSC were observed in dose dependent manner.
Pompe disease iPSCs (HPS0175, 0176, 0177) were generated from late-onset Pompe disease patient fibroblast reprogrammed by Sendai Virus. Control iPSC (HPS0223) was also generated from healthy donor by Sendai Virus. Both healthy control (HPS0223) and Pompe disease iPSCs (HPS0175, 0176, 0177) had similar pluripotency characters shown by immunohistochemistry and RT-PCR.
Robust cardiomyocyte differentiation was conducted according to directed differentiation protocol using GSK-3 inhibitor and Wnt inhibitor. Beating cardiomyocyte was observed 10 days after the differentiation both healthy control (HPS0223) and Pompe disease iPSC (HPS0175). Cardiomyocyte derived from

Pompe disease iPSC demonstrated disease specific hallmarks, such as massive glycogen accumulation and lysosomal enlargement.
It is suggested that even in late-onset Pompe disease iPSC, glycogen accumulation and lysosomal enlargement remain after the cardiomyocyte differentiation. Our results are compatible with previous clinical study showing cardiac hypertrophy sometimes seen in late-onset Pompe disease. Pathological change in differentiated cardiomyocyte might be an explanation of the cardiovascular complications in late-onset Pompe disease. We would like to generate gene-corrected iPSCs and differentiate into cardiomyocyte to evaluate the efficacy of gene transfer.

T-L-4026

EARLY SORTING OF FULLY REPROGRAMMED HUMAN IPS CELLS WITH NOVEL CELL SURFACE GLYCAN MARKERS HALVES LEAD TIME TO BONA FIDE IPS CELLS AND ENABLES MASS PRODUCTION OF HIGH QUALITY IPS CELL LINES

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We have developed a robust technique for isolation of iPS cells and generation of new iPS cell lines using surface marker based isolation of reprogrammed cells by fluorescence assisted cell sorting (FACS). We identified type 1 Lewis glycans as novel markers of pluripotent stem cells by direct chemical characterization of cell surface glycoprotein glycans and glycosphingolipids as well as screening of a panel of anti-glycan monoclonal antibodies and lectins. Molecular characterization of the glycans and their biosynthesis in pluripotent stem cells and differentiated cells revealed that they are sensitive markers of pluripotency. Live cell imaging with anti-type 1 Lewis glycan antibodies during reprogramming of human fibroblasts showed that marker-positive cells emerged early in the process and could be identified based on their surface marker expression before they started to form the characteristic stem cell colonies. Based on this knowledge, we started developing a method for efficient isolation of new iPS cell lines using FACS isolation of the reprogrammed cells. With specific combination of antibodies against terminal and extended type 1 Lewis glycans, we were able to purify the pluripotent cells from any contaminating cells in one FACS step. Subsequent expansion of the cells led to a pure iPS cell line in less than half the time compared to traditional approach of cell line maturation by repeated passaging and culturing. After 2-3 passages, there was enough cell mass for both cell line banking and characterization. In addition to reduced time from somatic cell sample to iPS cell line (down to 4-5 weeks compared to at least 10 weeks without sorting), the benefits of the method included lowered need for sample cells, transfection vectors, media, plates, coating substrate, incubator space, and expert time for passaging the cells. Further, the new method removed the need to manually select and dissect the visually identified colonies for propagation. Instead, the whole process could be performed by pipetting the reagents and cells from one multi-well plate to another, enabling automation at every step. We are currently pursuing development of a fully automated cell line generation system based on integration of cell sorter and culture robot.

T-L-4027

PHENOTYPING POLYGENIC ALZHEIMER'S DISEASE WITH PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

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The burden with neurodegenerative diseases is escalating significantly worldwide and is currently over 13%. Therefore it is pertinent to understand the underlying mechanism of disease development in an effort to identify molecular targets for therapeutic intervention. Induced pluripotent stem cell (iPSC) technology offers opportunity now to recapitulate the disease process in the Petri dish with cells derived from patients. The generation of iPSC offers a unique opportunity to develop disease-relevant neurons in large numbers and assemble them as mini brain to study the disease process. We have generated iPSC from a cohort of Alzheimer's disease (AD) patients, characterised and generated forebrain cortical neurons to ascertain if relevant phenotypic expression can be used to predict AD in the Petri dish. Our comprehensive microarray data at the pluripotent state from sporadic AD patients demonstrate that 293 genes were differentially expressed compared to normal samples. In particular the following genes were significantly different ; MAPK10 - mitogen-activated protein kinase (2.5 fold change) that may be implicated in the regulation of the beta-amyloid precursor protein/APP signalling during neuronal differentiation by phosphorylating APP formation, PIK3R1 - phosphoinositide-3-kinase, regulatory subunit 1 (alpha) (1.6 folds change) may be involved in insulin resistance and GPX2 -glutathione peroxidase 2 (-1.7 fold change)- could possibly explain the increased susceptibility to oxidative stress. These data conform with our in vitro phenotype data where we have shown that AD-specific iPSC clones were more susceptible to oxidated stress. The APOE genotype is relevant in sporadic forms of AD patients; APOE 4/4 more susceptible. Our recent data on APOE protein expression in iPSC-derived neurons indicated an over expression of recently identified protective APOE-25(BG) in APOE 3/3 patients compared to those with APOE4/4 genotype. This is an important correlation and validation of in vitro AD model based on patient-derived iPSC technology.

T-L-4028

ROBUST REGENERATIVE RESPONSES FOLLOWING THE PARTIAL ABLATION OF MYELINATING SCHWANN CELLS IN ADULT MICE

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Myelinating Schwann cells (SCs) are specialised glial cells responsible for producing the myelin that ensheaths axons enabling salutatory conduction and providing neuroprotective and trophic support. Ablation of SCs during development has revealed their pivotal role in establishing normal nerve function. The intricate role of Schwann cells in maintaining axonal integrity and function following Schwann cell

death in adult life remains largely unexplored. We have generated MBP-DTR transgenic mice that express the diphtheria toxin receptor (DTR) under the control of the myelin basic protein (MBP) promoter. MBP-DTR mice express DTR in SCs and oligodendrocytes rendering both populations selectively sensitive to DT-mediated apoptosis. In MBP-DTR mice but not wild-type controls, DT challenge (10ug/kg, single i.p. injection) induced apoptosis of SCs, as revealed by colocalisation of TUNEL and S100b or Sox-10. Clinically, MBP-DTR+DT mice exhibited reduced motor coordination and hind limb weakness that peaked ~25 days post-DT, and was followed by rapid clinical recovery within ~2-3 days. MBP-DTR+DT mice developed sporadic demyelination, axonal mitochondria accumulation, and redistribution/disappearance of ion channels and cell adhesion molecules by 25 days post-DT. Ex-vivo electrophysiological assessment of sciatic nerves indicated a transient reduction in compound action potential amplitude that correlated with clinical deficit and recovery. We investigated whether remyelination might account for clinical recovery, MBP-DTR+DT and WT+DT mice were administered the thymidine analogue BrdU (0.8mg/ml via drinking water) for 7 days prior to perfusion fixation on days 7, 14, 21, 28 and 35 days following DT challenge. In the sciatic nerve at day 21, 28 and 35, MBP-DTR+DT mice exhibited an increase in proliferative cells compared to WT+DT mice. At these time-points, BrdU-positive cells were immunoreactive for Sox10, indicating Schwann cells enter the cell cycle after DT induced ablation of mature Schwann cells. Electron microscopy revealed evidence of remyelination at 28 and 35 days post-DT as indicated by an increase in g-ratios.
Our data indicate that targeted apoptosis of myelinating Schwann cells induced rapid functional recovery following targeted demyelination that correlated with robust Schwann cell proliferation and remyelination. Further dissection of regenerative mechanisms will identify the critical mechanisms responsible for functional recovery in this model.

T-L-4029
EXAMINING THE POTENTIAL OF EMBRYONIC STEM CELLS TO UNDERGO BROWN ADIPOGENESIS WITHIN 3D ALGINATE HYDROGEL MICROSTRANDS

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The ability of brown adipocytes to expend energy as heat makes them promising for obesity therapies. However, brown adipocytes have proven to be difficult to transplant *in* vivo due to instability of the cells once injected, creating a need for an encapsulation model to better understand the role of brown adipocytes in preventing and treating obesity. There is great potential in using pluripotent stem cells for this type of cell therapy, with emphasis on directed differentiation. One component that contributes greatly to the differentiation of stem cells is their microenvironment or niche. *In vivo*, the microenvironment of cells is three-dimensional making it is necessary to fabricate a microenvironment of similar dimension *in vitro.* Another aspect of the microenvironment that must be taken into account during fabrication is its fluidity. A prominent example of a material that can take a three-dimensional shape and maintain fluidity is the hydrogel. Here we present data that a hydrogel-microstrand microenvironment with a liquefied core can facilitate the differentiation of mouse embryonic stem cells (mESCs) into adipocytes. These microstrands were fabricated using a simple approach, which involved the dispensing of an alginate-cell solution into calcium chloride through a capillary silica tip. The self-

assembly behaviors and growth of mESCs in alginate microstrands were studied and compared to the growth of brown preadipocytes as a positive control. The cells within the microstrands were then subjected to directed differentiation into mature brown adipocytes over the course of three to four weeks. After careful execution, brown adipocyte differentiation within the microstrands was confirmed by oil red O staining and immunocytochemistry for the adipocyte-specific markers perilipin and peroxisome proliferator-activated receptor γ (PPARγ), and brown adipocyte-specific uncoupling protein 1 (UCP-1), followed by observation under the Nikon Eclipse 80i fluorescence microscope and Leica SP5 confocal microscope, and qPCR analysis of a set of brown adipocyte-specific genes. Preliminary functional analysis of brown adipocytes for the cells within the hydrogel microstrands was done using the Seahorse XF24[3] metabolic analyzer. These initial results suggest that cells within the microstrands display a higher overall oxygen consumption rate than undifferentiated cells. Further characterization of these microstrands will include optimization of the functional analysis and quantification of brown adipocyte gene expression. In summary, we fabricated three-dimensional alginate-microstrand constructs for mESC self-assembly and directly differentiated the mESCs into brown adipocytes with high efficiency. This shows significant promise in fabrication of a novel system that can direct stem cell differentiation into brown adipocytes for understanding brown adipogenesis and its implication in obesity and diabetes, leading to future clinical applications.

T-L-4030
EZH2 IS REQUIRED FOR NEURAL CREST-DERIVED CARTILAGE AND BONE FORMATION

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The emergence of craniofacial skeletal elements, in particular jaws, was a crucial step in the evolution of higher vertebrates. Most facial bones and cartilage are generated during embryonic development by cranial neural crest cells, while an osteochondrogenic fate is suppressed in more posterior neural crest cells. Key players in this process are Hox genes, which suppress osteochondrogenesis in posterior neural crest derivatives. How this specific pattern of osteochondrogenic competence is achieved remains to be elucidated. Here we demonstrate that Hox gene expression and osteochondrogenesis are controlled by epigenetic mechanisms. Ezh2, a component of the polycomb repressive complex 2 (PRC2) catalyzes trimethylation of lysine 27 in histone 3 (H3K27me3), thereby functioning as transcriptional repressor of target genes. Conditional inactivation of Ezh2 does not interfere with localization of neural crest cells to their target structures, neural development, cell cycle progression, and cell survival. However, loss of Ezh2 results in massive de-repression of Hox genes in neural crest cells usually devoid of Hox gene expression. Accordingly, craniofacial bone and cartilage formation is fully prevented in Ezh2 conditional knock-out mice. Our data indicate that the capacity of craniofacial skeleton formation in higher

vertebrates is crucially dependent on epigenetic regulation that keeps in check inhibitors of an osteochondrogenic differentiation program.

T-L-4031

THE EXPRESSION OF RIBBON SYNAPSE-SPECIFIC PROTEINS IN HESC-DERIVED PHOTORECEPTOR PRECURSOR CELLS IS ENHANCED BY HESC-DERIVED RPE AND THE SYNAPTOGENIC FACTOR TSP-1 IN AN EX-VIVO RETINAL EXPLANT MODEL

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Retinal degenerations such as age-related macular degeneration and retinitis pigmentosa are common and major causes of blindness in developed countries. Transplantation of photoreceptor precursor cells (PPCs) derived from human embryonic stem cells (hESCs) is a promising and widely applicable approach for the treatment of these blinding conditions. However, the factors that promote PPC engraftment are largely unknown. To facilitate optimization of PPC transplantation, we developed a novel *ex-vivo* system and will focus on determining the factors that enhance PPC functional integration.
Harvested neural retinas from adult rats were dissected into equal-sized quadrants and placed directly on different retinal pigment epithelium (RPE) cell lines (RPE-J, ARPE19 and hESC derived RPE). At different time points overall tissue microstructure and outer nuclear layer (ONL) thickness were assessed to determine the type of RPE that supported the healthiest retinal explant. To mimic PPC transplantation to the subretinal space, hESC-derived PPCs were inserted between the neural explant and underlying RPE and their functional integration was assessed by determining the proportion of PPCs expressing the ribbon synapse-specific proteins Bassoon and Ribeye. Finally, we examined PPC integration efficiency in the presence of the synaptogenic factor thrombospondin-1 (TSP-1).
Explants co-cultured with hESC-derived RPE maintained normal gross morphology and viability for up to two weeks, whereas the explants cultured on ARPE19 and RPE-J died by the end of the first week. Furthermore, the proportion of PPCs expressing Bassoon and Ribeye was significantly higher when co-cultured with hESC-derived RPE than when co-cultured with ARPE19 (20% vs. 6%). Noticeably, in the presence of TSP-1 the proportion of Bassoon positive PPCs co-cultured with hESC-derived RPE increased to ~30%. A model system that includes retinal explants, co-cultured with hESC-derived RPE, can be used to screen and define factors that influence the integration efficiency of transplanted PPCs. Identifying factors that enhance integration of PPCs into retinal explants, such as TSP-1, in an *ex-vivo* system can then be validated in *in-vivo* models of retinal degeneration.

T-L-4032

FUNCTIONAL TISSUE ENGINEERED CORNEAL ENDOTHELIUM DERIVED FROM MONKEY IPS CELLS

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Corneal endothelial dysfunction remains a common indication for keratoplasty, accounting for half of the total number of such procedures. Keratoplasty using donor cornea includes problems such as immunological rejection. To bypass these problems, we examined application of induced pluripotent stem cells (iPSCs) to engineer artificial corneal endothelium. Corneal endothelium as well as corneal stroma originates from cranial neural crest cells (NCCs). On the basis of these findings, we first tried to differentiate human and cynomolgus monkey iPSCs into neural crest cells (iPS-NCCs). Subsequently, the iPS-NCCs were cultured in the specific endothelium-inducing medium. After one week of culture, monolayer of IPSCs-derivd tissue engineered corneal endothelium (iTECE), which shows hexagonal mosaic pattern mimicking the human corneal endothelium was obtained. We next measured the pump function attributable to Na,K-ATPase activity using an Ussing chamber. As a result, significantly higher Na,K-ATPase pump activity of both human and monkey iTECE sheet compared to that of cultured human corneal endothelial cell line (B4G12) sheet was observed. Finally, we transplanted iTECE sheets into rabbit cornea to evaluate in vivo pump function of iTECE. The iTECE transplanted rabbit cornea maintained transparency and usual corneal thickness, whereas control corneas without TECE showed marked edema and increased corneal thickness. Thus, we successfully produced iTECE from human and monkey iPSCs, which has sufficient pump function to maintain corneal thickness *in vitro* and *in vivo*. To apply iTECE to regenerativemedicine of corneal endothelial cells, Further long-term *in vivo* evaluation of TECE transplantation is required.

T-L-4033

SMALL MOLECULE APPROACH TO PRODUCE NEURAL CELLS FROM ADULT STEM CELLS

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Mesenchymal StemCells (MSCs) can be easily isolated and expanded from various postnatal and adult tissues, such as bone marrow, adipose tissue, hair follicles. So, donors or the patients themselves can provide sufficient cell sources in the clinical application. Many neural induction protocols have been reported, such as co-culturing with neural cells, exposed to neurotrophic factors or cytokines, or transfection genes like Noggin and Notch transcription factors. However, a safer, easier and more efficient procedure is required for clinical application. Recently, it has been demonstrated natural and synthetic small molecules are useful chemical tools for controlling and manipulating cell fate. We used

DNA methyltransferase inhibitor and histone deacetylase inhibitor to reprogram MSCs to pluripotency, and elevated the intracellular level of cAMP to direct MSCs to differentiate into neuronal-like cells. To test our hypothesis, MSCs were collected from bone marrow or hair follicles derived from different species, such as rat, cat and human. Immunocytochemisty results showed more than 70% cells differentiate into neuronal-like cells, which is confirmed by real-time PCR and westen blot. We also detected neurotrophins and dopamine secretion by ELISA, and apparent K+ and Na+ current were recorded by whole cell patch-clamp.

T-L-4034

THE ROLE OF CELL PROLIFERATION IN HUMAN ADIPOGENESIS

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It has been widely accepted that mitotic clonal expansion/cellular proliferation is a prerequisite for adipogenesis using 3T3L1 as an in vitro cellular model. However, it is not clear whether this also holds true for human adipogenesis. Using adipose tissue derived human mesenchymal stem cells (hMSCs) as an in vitro model for adipogenic differentiation, we identified through microarray analysis a list of 8 cell cycle regulators whose expression was universally down-regulated in response to the treatment of adipogenic inducing media (AIM, composed of DEX/Insulin/IBMX), which were further confirmed by RT-PCR across different time points post AIM induction (12, 24, 36, 48, 72 and 96h). Cell proliferation assay using the Click-iT EdU Alexa Fluor Imaging kit demonstrated that cell proliferation was restricted to the first 48hrs of AIM induction and ceased completely subsequently, whereas cell proliferation continues to oscillate in response to each media change at 48hr intervals in cells treated with control media or DEX containing media. Reduced cell proliferation in response to AIM would predict reduced total cell number, which was confirmed by Resazurin assay at 72hrs, 7D and 12D post induction in comparison to control media or DEX treatment, and cell death was validated not to be a contributing factor. To address whether cell proliferation observed during the first 48hrs of AIM treatment was a prerequisite for adipogenic initiation, cell proliferation was either inhibited using siRNAs targeting specific cell cycle genes, or enhanced by supplementing exogenous growth factor bFGF in a time course-dependent manner. In both scenarios, AIM media was prepared using heat-inactivated fetal bovine serum to inactivate existing growth factors within the serum. Expression knock-down of CDK1 at the initiation of AIM treatment consistently promoted greater adipogenic differentiation by increasing the percentage of total fat cells as compared to siControl treated or untransfected cells. Stimulation of cell proliferation by supplementing exogenous bFGF significantly promoted adipogenesis only when applied during the first 24hr of AIM induction. Application of bFGF for 1 or 2 days at later time windows of AIM induction significantly inhibited adipogenesis, even though total cell numbers were still significantly increased. Our results so far imply that cell proliferation is not a prerequisite for human adipogenesis. However, cell proliferation stimulation can be beneficial for adipogenesis during a highly restricted time window, likely before the onset of adipogenic cell fate determination by increasing the population of cells capable of committing to adipogenic cell fate. Our study revealed a dynamic time-dependent role of cell proliferation during human adipogenesis.

T-L-4036

EVALUATION OF THE EXPRESSION AND REGULATION OF STEM CELL PLURIPOTENCY AND SELF-RENEWAL MAINTENANCE GENES.

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Stem cells have two important properties; their pluripotency and self-renewal. There are different genes and transcription factors involved in the maintenance of these properties. Oct4, Nanog and Sox2 have been identified as the core regulatory transcription factors of these properties. The hypothesis of this study is that there are other genes involved in the regulation of stemness. Therefore, we aim to mine these potentially novel stem cell regulatory genes from our laboratory's existing Affymetrix microarray data. Bioinformatics and functional analyses were performed to identify genes with best fit to pluripotency. Differentiation experiments were undertaken on these genes to monitor their expressional changes. YY1AP1, a transcription regulatory gene, was selected due to its consistent down-regulation during the differentiation of human embryonic stem cells. This gene was knocked down in hUES1 stem cells and gene expression of differentiation markers was analysed. Knocked down cells were differentiated by EB formation. Evaluation of protein interactions was undertaken through western blotting and immunoprecipitation experiments. Results suggesting that the knock down of YY1AP1 has a significant effect on the expression of pluripotency markers, its binding partner YY1 and specific lineage markers will be presented. It will also be shown that YY1AP1 and YY1 directly interact at the protein level and that knocking YY1AP1 leads to a down regulation YY1 and YY1AP1 proteins.

JUNE 20, 2014

FRIDAY LATE BREAKING ABSTRACT POSTER PRESENTATIONS

6:00 PM - 7:00 PM ODD numbered posters presented
7:00 PM - 8:00 PM EVEN numbered posters presented

F-L-4001

CHROMOSOME LOOP STRUCTURES THAT AUGMENT CONTROL OF ES CELL IDENTITY

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The pluripotent state of embryonic stem cells (ESCs) is produced by active transcription of cell identity genes and repression of genes encoding lineage-specifying developmental regulators. Recent studies reveal that super-enhancers drive expression of key pluripotency genes, and chromosome structure regulators such as cohesin are highly enriched in these regulatory domains. Here we integrate ESC cohesin ChIA-PET data with other genomic data to identify DNA loops associated with cohesin genome-wide. We find that super-enhancers loop to key pluripotency genes, as expected. More remarkably, these super-enhancers and their target genes generally occur within large DNA loops that are bounded by pairs of interacting CTCF sites, which are co-occupied by cohesin. In contrast, most other active genes are not found in such loops. A large fraction of the enhancer-associated transcription apparatus is located within these large DNA loops. Two lines of evidence argue that the large CTCF-bounded loop constrains super-enhancer function to sites within the loop. The ChIA-PET data indicate that DNA interactions within the loops tend to be restricted to sites within the loop. Furthermore, CRISPR-mediated deletion of CTCF boundaries alters expression of both super-enhancer target genes and neighboring genes that normally reside outside the loop. We discuss these and additional insights into the local chromosome structures involved in regulation of active pluripotency genes and silent polycomb-occupied genes.

F-L-4002

MENDING REPROGRAMMING AND HIGH THROUGHPUT METHODOLOGIES: IMPROVED METHODS FOR IPSC DERIVATION AND FUNCTIONAL CHARACTERIZATION

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Current methods to derive induced pluripotent stem cell (iPSC) lines from human dermal fibroblasts by viral infection rely on complex protocols. Once these cells are specified to cardiomyocytes or neural subtypes further functional measures are required. Optical mapping has its unique advantages as it gives high spatial and temporal resolution which allows capturing of highly complex phenomena like ventricular fibrillation in a much more precise and detailed manner as well as the ability to capture calcium transient's and action potential simultaneously. One major factor contributing to the time required to derive lines is the ability of researchers to identify fully reprogrammed unique candidate clones from a mixed cell population containing transformed or partially reprogrammed cells and fibroblasts at an early time point post infection. Failure to select high quality colonies early in the derivation process results in cell lines that require increased maintenance and unreliable experimental outcomes. Here, we describe an improved method for the derivation of iPSC lines using fluorescence activated cell sorting (FACS) to isolate single cells expressing the combination of $CD13^{NEG}SSEA4^{POS}Tra\text{-}1\text{-}60^{POS}$ cell surface markers between 7-10 days post infection. This technique prospectively isolates fully reprogrammed iPSCs, and depletes both parental and "contaminating" partially reprogrammed fibroblasts, substantially reducing the time and reagents required to generate iPSC lines. iPSC lines derived under this technology produced more unique and stable clones following retroviral infection than manual picking methods, expressed common markers of pluripotency at later passages, and possessed spontaneous differentiation potential in vitro and in vivo. FACS derivation produced iPSC lines had a normal karyotype and matched the parental DNA fingerprint. To demonstrate the suitability of FACS for high throughput iPSC generation as well as the functional characterization required for cardiomyocyte specification, we derived 228 individual iPSC lines from a variety of 76 tissue sources that ranged in diseases from Cardiovascular disorders, neurological disorders and diabetes using either integrating (retroviral) or non-integrating (Sendai virus) reprogramming vectors and performed extensive characterization on a subset of those lines. Here we applied optical mapping on different cardiac preparations to study cardiac pathophysiology and drug action. This demonstrates feasibility of creating highly pure iPSCs for cellular therapeutics, drug screening, and understanding human disease phenotypes.

F-L-4003

UNDERSTANDING THE MECHANISMS OF DIRECT CARDIAC REPROGRAMMING

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We previously reported that the introduction of three cardiac developmental transcription factors, Gata4, Mef2c and Tbx5 (GMT) is sufficient to induce mouse fibroblasts to adopt a cardiomyocyte-like phenotype in vitro and in vivo. Although direct reprogramming of fibroblasts to induced cardiomyocyte-like cells (iCMs) is a promising new approach to cardiac muscle generation and heart repair, the

underlying molecular mechanisms that orchestrate this process are unknown. Here we track transcriptional changes in gene expression and interrogate direct interactions of GMT with the genome over the course of cardiac reprogramming in vitro. To characterize transcriptional changes in gene expression during reprogramming, GMT were overexpressed in cardiac fibroblasts isolated from transgenic mice containing the cardiac αMHC-GFP reporter. iCMs were isolated by fluorescence activated cell sorting of GFP+ cells at multiple different time points throughout cardiac reprogramming. Whole transcriptome analyses revealed both transient and persistent patterns of gene expression throughout the processes of cardiac reprogramming. To identify direct DNA targets of GMT, we constructed a synthetically tagged GMT retroviral systems that generated iCMs similar to those generated using the non-synthetically tagged retroviral system. Chromatin immunoprecipitation of tagged GMT revealed striking patterns of GMT:DNA occupancy over time. Together, these studies characterize molecular changes that occur during reprogramming and provide a framework for further mechanistic studies.

F-L-4004
REVERSAL OF BETA CELL DE-DIFFERENTIATION BY A SMALL MOLECULE INHIBITOR OF THE TGF-BETA PATHWAY

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Dysfunction or death of pancreatic β cells underlies both types of diabetes. This functional decline begins with β cell stress and de-differentiation. Current drugs for T2D lower blood glucose levels, but they do not directly alleviate β cell stress nor prevent, let alone reverse, β cell de-differentiation. We show here that Urocortin 3 (Ucn3), a marker for mature β cells, is down-regulated in the early stages of T2D in mice and when β cells are stressed in vitro. Using an insulin expression-coupled lineage tracer, with Ucn3 as a reporter for the mature β cell state, we screen for factors that reverse β cell de-differentiation. We find that a small molecule inhibitor of the TGFβ pathway protects cells from the loss of key β cell transcription factors and restores a mature β cell identity even after exposure to prolonged and severe diabetes. We suggest that screening for compounds that inhibit TGFβ signaling specifically in β cells may yield new drugs that, in combination with traditional blood-glucose lowering medicines, will delay, prevent or even restore the loss of healthy, mature β cell function in T2D patients.

F-L-4005
DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS TO PURKINJE NEURONS

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It remains a challenge to differentiate human induced pluripotent stem cells (iPSCs) or embryonic stem (ES) cells to Purkinje cells, one of the largest types of neurons in brain. In this study, we derived iPSCs from human skin biopsies and first differentiate them to Purkinje progenitors, by adding Fgf2 and insulin to the embryoid bodies (EBs) in a time sensitive manner, which activates the endogenous production of Wnt1 and Fgf8 from EBs that further patterned the cells towards a midbrain-hindbrain-boundary tissue identity. Neph3-positive Purkinje progenitors were sorted out by using flow cytometer and cultured either alone or with granule cell precursors, in a 2-dimentional or 3-dimentional environment. However, the Purkinje progenitors failed to mature further under the above conditions. By co-culturing the progenitors with murine cerebellar slices, we observed mature Purkinje-like cells with right morphology and marker expression, which yet showed no appropriate membrane properties. Neph3-posotive cells could survive transplantation into the cerebellum of newborn immunodeficient mice and differentiate to L7- and Calbindin-positive neurons. Obtaining mature human Purkinje cells in vitro will have significant implications in studying the mechanisms of SCAs and other cerebellar diseases.

F-L-4006
IN VITRO STUDY FOR THE TOXICITY OF SILVER NANOPARTICLES IN RAT TENDON-DERIVED STEM CELLS

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Tendon injuries have become more common in sports activities and workplace. Tendon-derived stem cells (TDSCs), which have been recently identified in human, mice and rat tendons, seem to have a great potential for tendon engineering because of their stem cell characteristics and tissue-specific differentiation properties. To allow the stem cells to grow in a 3D microenvironment in vivo, a 3D scaffold is needed. The material used in synthesizing this scaffold has posed a challenge in tendon repair until the rising of silver-nanoparticles (AgNPs). Nowadays, AgNPs have been used for a wide range of biomedical applications owing to its potent anti-bacterial activity, anti-inflammatory effect and the ability to accelerate wound healing. Because of their attractive properties, AgNPs are suggested to be the material used to fabricate scaffold in tendon engineering, which allows the TDSCs to grow on it. But before combining AgNPs and TDSCs in tendon engineering, their interaction should be investigated for their incorporation into a successful nanoscaffold. Thus, to determine the risk of the use of AgNPs, we studied how AgNPs affect rat TDSCs' viability, proliferation and tenogenic differentiation.
TDSCs were treated with different doses of AgNPs for 1 to 14 days. Finally, cellular uptake of the AgNPs was evaluated. After 24 hours, decline of cell viability of TDSCs was observed at IC50 of 60 μg/ml of AgNPs. Even though lower dosage of AgNPs was used, cytotoxicity and inhibitory effect of proliferation occurred dose-dependently. For tenogenic differentiation, there was a reduction on expression level of tenogenic marker such as tenomodulin (TNMD) and scleraxis (SCX), content of collagen synthesis and even numbers of TDSCs. In summary, our data demonstrated that AgNPs have an inhibitory effect on the proliferation and tenogenic differentiation of TDSCs. High dose ($>IC_{50}$) of AgNPs induced acute cytotoxic effect in terms of the viability and apoptosis induction in TDSCs. Therefore, AgNPs may not be a compatible scaffold material in tendon engineering despite its outstanding advantages. In the future, we should further clarify the apoptotic mechanism induced by AgNPs in order to contribute to the regulation of AgNPs in nanomedicine.

F-L-4007

AN ENHANCED AND EFFICIENT CELLULAR REPROGRAMMING METHOD FOR GENERATING HUMAN INDUCED NEURONS

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Recent advances in somatic cell reprogramming have illustrated the malleability of the somatic epigenome, particularly through demonstrations of reprogramming of adult mouse and human fibroblasts and blood cells to induced pluripotent stem (iPS) cells. More recent studies showed direct lineage reprogramming of human fibroblasts to cardiomyocytes and induced neurons (iNs) under defined conditions. However, human cells appear to be less plastic and have a higher epigenetic hurdle for reprogramming to iNs. As such, the same three factors that readily convert mouse fibroblasts to iNs are not sufficient to convert human fibroblasts to iNs. Here we show that SH2B adaptor protein 1β (SH2B1) enhances neurite outgrowth of iNs reprogrammed from human fibroblasts as early as Day 14, when combined with miR124, BRN2 and MYT1L (IBM) under defined condition. Further, SH2B1 can increase the efficiency of conversion to 15-20%. These SH2B1-enhanced (S-IBM) iNs showed canonical neuronal morphology, expressed multiple neuronal markers, such as TuJ1, NeuN and synapsin, and functional proteins for neurotransmitter release, including GABA, vGluT2 and tyrosine hydroxylase. Importantly, SH2B1 accelerated mature process of functional neurons and exhibited action potentials as early as Day14. Without SH2B1, the IBM iNs do not begin to exhibit weak action potentials until Day 21. We then tested whether S-IBM iNs neuronal networks were functionally capable of generating Ca2+ transients. Cells were preloaded with the calcium indicator Fura2-AM, and the neurons were earmarked using GFP fluorescence. Ca2+ transients detected in S-IBM iNs showed the changes in Fura2-AM signal over time after glutamate stimulation. Our data demonstrate that SH2B1 can enhance neurite outgrowth and accelerate the maturation of human iNs under defined conditions. We will test if the S-IBM method will allow us to convert peripheral blood mononuclear cells to iNs. This approach will facilitate the application of iNs in regenerative medicine, drug screening, and in vitro disease modeling.

F-L-4008

TERNARY COMPLEX AS NON-VIRAL VECTOR OF PLASMID DNA FOR THE MODULATION OF HUMAN MESENCHYMAL STEM CELL

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In this study, we have developed a safe and effective non-viral vector for gene delivery into human mesenchymal stem cell (hMSC). We were prepared anionic charged ternary-complex of plasmid DNA using branched polyethyleneimine and hyaluronic acid(HA). HA as anionic polymer is biocompatible polymer and component of extra cellular matrix throughout body and is able to bind the CD44 receptor

specifically on the surface of hMSC membrane. Ternary complexes were prepared by electrostatic self-assembly. And we characterized the physicochemical properties of ternary complex and confirmed the CD44 targeting effect of ternary complex. These results suggest that anionic charged ternary-complexes have potential for effective non-viral vector for gene delivery into hMSC.

F-L-4009

THE EFFECT OF OXYGENATED MYCOLIC ACID COMPOSITION ON MACROPHAGE CELL LIPIDIC VACUOLES FUNCTION DERIVED FROM MURINE BONE MARROW STEM CELLS

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The oxygenated mycolic acids from M. Bovis have a high antigenicity, these compounds are formed by α-alkyl-β-hydroxy fatty acids found in mycobacteria, and constitute a large part of the architecture of the cell wall mycobacterium, corroborating in pathogenic strains (M. tuberculosis) in contrast with α-mycolic acids. However, previous reports has been showed that these compounds (keto-mycolic acids) are involved in the formation of lipid vacuoles in macrophages, a process involved in the latency of pathogenic mycobacteria such as M. tuberculosis and M. avium. In other hand, the activation of the immune system requires that presence of an incipient infection first be detected this essential step is carried out by tissue macrophages, which alert innate immunity, these sentinel cell types are derived from circulating precursors, knows as blood monocytes. Briefly we isolate blood monocytes from mice bone marrow; primary culture was realized and 2x106 cells/ml of adherents progenitors was planted in culture dishes and proliferated into homogenous population of bone marrow-derived macrophages in presence of 10ng/ml of oxigenated mycolic acids from M. bovis (AN5 strain), assays of phagocytic activity were performed used fluorescent labeled zymosan A bioparticles and opsonizing reagent with a multiplicity of infection of 10, the phagocytosis was stoped adding ice-cold PBS. The characterization of these progenitors cells was made with Mac-1 and 4/80 surface antigen expression and the RNA extracting was realized according to the previous reports. The results obtained from the present work will be expand our understanding of these oxygenated mycolic acids may play an important role in the latency of the phagocyte in early stage of development.

F-L-4010

CLINICAL-GRADE HUMAN PLATELET LYSATE PRODUCED AT INDUSTRIAL SCALE FOR ISOLATION, EXPANSION, AND CRYOPRESERVATION OF MESENCHYMAL STROMAL CELLS

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The use of fetal bovine serum (FBS) for clinical manufacturing of stem cell products poses risks including the potential for viral and prion transmission and the possibility of adverse immunological reactions. Human platelet lysate, a cell-free formulation of growth factors and cytokines produced from donated platelet units, has emerged as a viable, xenogeneic-free alternative. Traditionally, human platelet lysate has been prepared by individual laboratories in small batches using protocols that differ in the number of platelet units pooled, the processing of platelets, and the requirement for heparin. These differences can significantly impact stem cell growth, morphology, and multipotency. To address this issue, we have developed a highly standardized, industrial-scale production process for human platelet lysate using good manufacturing practices (GMP). Our PLUS™ human platelet lysate is produced using platelet units obtained from AABB-accredited blood banks and has no requirement for heparin. All lots of PLUS™ have tested negative for bacterial, fungal, and mycoplasma contamination and have met accepted endotoxin criteria. Growth factor levels have been consistent across all lots with average concentrations of 9,939±682, 641±257, 3,175±309 and 156±18 pg/mL for PDGF-BB, VEGF, EGF, and bFGF, respectively. Our focus for this study was to assess the capacity of PLUS™ human platelet lysate to serve as a media supplement for the isolation, ex vivo expansion, and cryopreservation of bone marrow mesenchymal stromal cells (MSCs). To perform these experiments, we obtained unprocessed bone marrow from multiple donors. We tested three different concentrations of PLUS™ (2.5%, 5%, and 10% v/v) and compared these to both 10% FBS as well as to a commercially available serum-free medium. We utilized a colony forming unit-fibroblast (CFU-f) assay to assess the impact of media composition on MSC isolation (via plastic adherence) and found that PLUS™ was comparable to FBS in terms of the number of colonies obtained, but yielded colonies with larger numbers of cells. We cultured these cells over multiple passages and observed a significantly higher expansion rate at each passage with PLUS™ supplementation. We assessed PLUS™-cultured cells at each passage by flow cytometry and found them to be consistent with an MSC phenotype by ISCT guidelines (positive for CD105, CD73, CD90; negative for CD45, CD34, CD14, CD20). At each passage, we also cryopreserved cells using various concentrations of PLUS™ or FBS with dimethyl sulfoxide (DMSO) as well as commercially available serum-free freezing solutions. Results with PLUS™ showed a high cell viability (>90%) and comparable proliferation post-thaw. These studies demonstrate that our industrial-scale manufacturing process for PLUS™ human platelet lysate yields a consistent product that can replace FBS in important steps involved in the production of MSCs for clinical use.

F-L-4011

HUMAN INTESTINAL ORGANOIDS DERIVED FROM PLURIPOTENT STEM CELLS

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A rapidly proliferating population of adult stem and progenitor cells drive self-renewal and differentiation in the intestinal epithelium. Organoid cultures of murine intestine have shown that genome editing can be used to study a dynamic intestinal epithelium in vitro. There are currently two general methods for the derivation of human intestinal organoids from primary tissue or hPSCs. Here we derive long-term human intestinal organoid cultures from hPSCs by a new method using an endogenous LGR5-GFP reporter. We found these tissues respond to the canonical signals for differentiation and self-renewal and share a highly similar transcriptional profile to biopsy-derived organoids. This new method for the generation of genetically modified intestinal epithelium provides platform to study human pathophysiology in an otherwise isogenetic background.

F-L-4013
MIRGATOR V3 A MICRORNA PORTAL FOR DEEP SEQUENCING EXPRESSION PROFILING AND MRNA TARGETING

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microRNA have an important role in regulating stem cell self-renewal and differentiation by repressing the translation of selected mRNAs in stem cells and differentiating daughter cells. Deep sequencing has become the principal technique in cataloging of miRNA repertoire and generating expression profiles in an unbiased manner. Here, we describe the miRGator v3.0 update that compiled the deep sequencing miRNA data available in public and implemented several novel tools to facilitate exploration of massive data. The miR-seq browser supports users to examine short read alignment with the secondary structure and read count information available in concurrent windows. Features such as sequence editing, sorting, ordering, import and export of user data, would be of great utility for studying iso-miRs, miRNA editing and modifications. Coexpression analysis of miRNA and target mRNAs, based on miRNA-seq and RNA-seq data from the same sample, is visualized in the heat-map and network views where users can investigate the inverse correlation of gene expression and target relations, compiled from various databases of predicted and validated targets. By keeping datasets and analytic tools up-to-date, miRGator, available at http://mirgator.kobic.re.kr, should continue to serve as an integrated resource for biogenesis and functional investigation of miRNAs.

F-L-4014
ALL OPTICAL ELECTROPHYSIOLOGY IDENTIFIES EXCITABILITY DEFICITS IN ALS MOTOR NEURONS

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Cell reprogramming technologies have created unprecedented opportunities for studying patient-specific human cell types that were previously inaccessible. Many studies have used iPSC-based and direct conversion methods to model neurological and neurodegenerative diseases, effectively describing disease-related phenotypes in multiple neuronal subtypes. However, investigating functional electrophysiological deficits in now widely available patient neurons has been limited by currently available techniques. Patch clamp is laborious and has not been amenable to a high-throughput format, while multi-electrode array (MEA) systems only gather population-level data.
Here, we describe a novel all-optical electrophysiology method, called Optopatch, based on simultaneous optical voltage imaging and optogenetic actuation. Voltage imaging occurs through a novel genetically encoded fluorescent voltage indicator, called QuasAr2. Optogenetic actuation occurs through a novel blue light-activated channelrhodopsin, called CheRiff. Custom optics and software allow simultaneous stimulation and recording from large numbers of single cells embedded in a complex network. Optopatch enables rapid electrophysiology measurements from hundreds of cells per day with a high signal-to-noise ratio. We developed Optopatch protocols to measure spontaneous and induced activity to characterize cell autonomous excitability and action potential waveforms.
We applied Optopatch assays to stem cell-derived motor neurons. We tested the technology with a previously validated model of ALS with a mutation in the SOD1 gene and an isogenic control cell line generated by genetically correcting the disease-causing mutation. More than 300 individual neurons were measured across both disease and control cells, in multiple differentiations. Each trial showed an increased excitability of the ALS motor neurons at low levels of stimulation, consistent with published MEA and patch clamp data. Interestingly, under strong stimulation, the ALS motor neurons fired fewer action potentials. Tests of several drugs, including retigabine, showed a reversal the ALS excitability phenotype, demonstrating that the Optopatch platform can be used in pharmacological screens. The ease of acquiring Optopatch data creates an opportunity to measure electrophysiology of neuronal subtypes from many models of neurological disease.

F-L-4015

THE EUROPEAN HUMAN PLURIPOTENT STEM CELL REGISTRY - A GLOBAL DATA REPOSITORY OF VALIDATED HPSC

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The increase in research activities and the development of applications of human pluripotent stem cells (hPSC), including human embryonic and induced pluripotent stem cells (hESC, hiPSC), triggered the generation of thousands of new cell lines. These lines are increasingly generated, collected and stored in large centralized banks worldwide. However, there is no centralized hub to search for and compare all the different cell lines, which makes it difficult to locate a cell of interest or search for compare cells located

in different banks. In addition, it is challenging to gather relevant ethical and scientific information for the cells, partially because of a lack of standards but also because this information is not comprehensively available. The European human pluripotent stem cell registry (hESCreg) provides a centralized platform to collect and validate hPSC ethical and scientific data. Founded in 2007 as a registry focused on hESC, hESCreg expanded its scope to include all hPSC-lines with the goal to serve the community as a central database, support harmonization, standardization and develop norms for the conduct of registration, data access and nomenclature. The scope and features of this public and open database and portal will be described. The registry will be re-launched with improved accessibility, registration, search and browse options at the 12th ISSCR-meeting.

F-L-4016

KLF4 PROMOTES SELF-RENEWAL LEUKEMIC STEM CELLS WHILE INHIBITING NORMAL HEMATOPOIETIC STEM CELLS

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Tyrosine kinase inhibitors (TKIs) are the standard treatment for eradicating BCR-ABL+ progenitor cells in chromic myeloid leukemia (CML); however, disease relapses upon drug discontinuation because TKIs do not effectively eliminate leukemic stem cells (LSC). The development of novel strategies aimed at eradicating LSC without harming normal hematopoietic stem cells (HSC) is essential for the cure of CML patients. The success of LSC-directed therapy depends on the identification of novel molecular pathways that selectively regulate LSC function. The Krüppel-like factor 4 (KLF4) is a bi-functional transcription factor that can either activate or repress gene transcription acting as an oncogene or a tumor suppressor depending on the cellular context. Analysis of published dataset from chronic phase CML patients revealed elevated level of KLF4 in highly enriched LSC compared to progenitor cells indicating that KLF4 might be implicated in LSC regulation. To study the role of KLF4 in regulation of LSC function, we used a CML mouse model combining somatic deletion of Klf4 and retroviral transduction and transplantation of HSC. Mice receiving BCR-ABL-transduced wild type HSC developed and succumbed to CML. In contrast, mice transplanted with BCR-ABL-transduced Klf4Δ/Δ HSC showed an initial expansion of myeloid leukemia cells followed by a progressive loss of myeloid leukemia cells resulting in overall increased survival. The inability to maintain CML in the absence of KLF4 was caused by attrition of LSC in the bone marrow and the spleen. Furthermore, deletion of KLF4 impaired the ability of LSC to recapitulate leukemia in secondary recipients suggesting a loss of self-renewal. In contrast to LSC, KLF4 deletion led to increased self-renewal of HSC assessed by serial competitive transplantation. To identify KLF4 target genes involved in LSC self-renewal, we performed a comparative gene expression microarray analysis using wild type and Klf4Δ/Δ LSC purified by cell sorting from leukemic mice. Analysis of gene expression revealed significant downregulation of known pathways of LSC self-renewal (i.e. Alox5, Wnt5b, Wnt7b, Numb) and upregulation of inhibitors of these pathways (i.e. NLK, Itch, Msi2h). We also identified genes involved in cell growth and mitosis with potential function as novel regulators of self-renewal cell divisions in LSC. These studies demonstrate that KLF4 deletion impairs self-renewal of LSC while improving self-renewal of HSC and thus KLF4 emerges as a novel target to eradicate LSC without altering normal hematopoiesis.

F-L-4017

BMP-INDUCED GENOME-WIDE LOW EPIGENETIC STATE OF HISTONE METHYLATION MARKS LINKS HAIR FOLLICLE STEM CELL QUIESCENCE WITH A STATE OF HIGH GENOME PLASTICITY

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Adult stem cells display higher potential or plasticity than their more differentiated progeny and in many tissues they are largely quiescent throughout an animal's life in many tissues including hair follicle (HF). Previously, we demonstrated that mouse hair follicle stem cells (HFSCs) that exit the niche during the quiescent phase of adult HF homeostasis undergo reversible fate transitions between stem and early progenitor cell states, but upon initiation of cell divisions outside the niche they commit to differentiation without self-renewing the stem cell pool. Remaining HFSCs replenish the pool (self-renew) during the succeeding proliferative phase of hair homeostasis by few divisions symmetrical relative to their neighboring niche. These data demonstrate that in HF, quiescence immediately precedes definitive cell fate decisions, and suggest that tissue stem cells might be at more plastic state when quiescent than when proliferating. Indeed, we find that dedifferentiation induced by the expression of four pluripotency factors in adult skin epithelium occurs more readily in quiescent than in proliferative HFSCs. Furthermore, H2B-GFP fluorescence recovery after photobleaching (FRAP) experiments in different HF cell types within fresh skin explants or in cell culture, unraveled a more dynamic chromatin state in quiescent than in proliferative HFSCs. Finally, we find that both in cell culture and in normal adult skin, BMP signals that establish and maintain HFSC quiescence simultaneously induce changes in mRNA levels of several histone modifying enzymes, and corresponding genome-wide erasure of histone methylation marks that associate with both transcriptional repression and activation in the HFSCs. High chromatin dynamics, low levels of several important histone methyl marks, and high genome plasticity, were previously found to characterize cultured embryonic stem cells. Here we show that this association can also occur in adult tissue stem cells during normal homeostasis. Moreover, our data in HF suggest that the same signaling pathways responsible for tissue stem cell quiescence, also promote chromatin states that augment genome plasticity, thus coupling regulation of two fundamental characteristics of adult tissue stem cells.

F-L-4018

STRIATAL ASTROCYTES PRODUCE NEUROBLASTS AFTER STROKE

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In the adult mouse brain, neurogenesis is limited to two anatomical structures, one of which is the subventricular zone of the lateral ventricles. In response to stroke, neural stem cells in the subventricular zone 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 subventricular zone; previous studies have shown that parenchymal astrocytes can acquire in-vitro neurosphere-forming capacity after brain lesions. It is not known whether parenchymal astrocytes can display such neurogenic potential also in vivo. Here, we used Connexin-30-CreER transgenic mice or a Cre-expressing adenovirus to fate-map striatal astrocytes after experimental stroke. We will present data providing evidence for stroke-induced local neuroblast production from parenchymal astrocytes in vivo and show that Notch signaling is an important regulator of this process. Our findings highlight a previously unknown level of plasticity in the adult brain and suggest a possible new target for therapeutic

F-L-4019

THE ADMINISTRATION OF CELL-FREE HEMOGLOBIN PROMOTES HEMATOPOIETIC RECOVERY IN SUB-LETHALLY IRRADIATED MICE.

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Hemoglobin (Hb) is one of the most abundant proteins of the human body serving a vital function in oxygen transport. In addition to oxygen transport, hemoglobin is thought to have other important and wide-ranging effects. There have been several reports of the effect of hemoglobin administration on hematopoiesis in humans. The first described infusion of hemoglobin in humans in 1947 was observed to increase hemoglobin production and accelerate red blood cell production. CD163, the hemoglobin-haptoglobin receptor has been found on a subset of hematopoietic progenitors (HPCs) in humans (Matthews et al., 2006) and is a potential mediator of this response. In a mouse model of bone marrow injury induced by sub-lethal irradiation, we demonstrated that administration of purified native human Hb enhanced hematopoietic recovery. C57BL/6 mice were first subjected to a single dose of 700 cGy of irradiation on the start of the study (day 0). As a result of injury to the mammalian bone marrow at this dose of radiation, there is a substantial drop in all blood cell counts over several weeks. Although intended as a non-lethal dose of radiation, death can result from hemorrhage or infection. On days 1 and 2 post-irradiation, mice were administered either ~2 mg of a purified cell-free 20 mg/mL Hb solution or an equal volume of saline. R&D grade hemoglobin suitable for in vivo administration was purified from human red blood cells according to an established proprietary scaled-down GMP purification process for the preparation of products designed for human administration. Blood samples were taken from one-half of each group on day 4-post irradiation and from the other half on day 6. At 30 days post-irradiation, the following was recorded: complete blood count, body weights and clinical behavior. When compared to the saline control, the administration of Hb produced significantly higher red blood cell count, total Hb (i.e., endogenous red cell Hb) and hematocrit on days 4, 6 and 30 post-irradiation. Hb-treated animals also

had a significantly higher lymphocyte count at day 30 post-irradiation. The administration of hemoglobin did not have any adverse effect on body weight or general behavior of the animals. Taken together, these data show that the administration of cell-free, native Hb can promote hematopoiesis after irradiation by potentially targeting murine multi-potential HPCs as evidenced by the increases in multi-lineage cell production. This may be of clinical relevance in the treatment of radiological-induced bone marrow injury, or in the promotion of bone marrow re-engraftment after myelo-ablative therapy.

F-L-4020

THE EFFECT OF AGING AND ATHEROSCLEROSIS ON HEMATOPOIETIC STEM CELL FUNCTION IN APOE-/- MICE

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Background: A temporal decline in tissue stem cell functionality may be a key component of aging, especially when cardiovascular disease is associated. Despite the self-renewal capacity of stem cells, a number of studies suggest that the ability to successfully regenerate tissue is gradually lost with aging. Therefore, the aim of this study was to evaluate how aging and atherosclerosis affect bone marrow hematopoietic stem cell (HSC) function in mice.

Methods: Male mice (n= 24) were divided into C57 and apoE-/- (2, 12 and 24-month-old) groups. Mice were euthanized and bone marrow was flushed out of tibias and femurs, mononuclear cells (MNC) were placed on culture. Hematopoietic stem cells (HSC) were obtained by immunomagnetic depletion of non-adherent cells. Briefly, HSC were incubated with Lineage antibody cocktail (CD3e, CD11b, CD45R/B220, LY-6G and LY-6C, TER-119/Erythroid) conjugated with magnetic beads and subjected to the BD IMag magnetic column. HSCLin- were stained with antibody against Sca-1 (stem cell antigen), CD 90.2, CD 133, CD 117 or matched isotype control. Oxidative stress was assessed by 2,7-dichlorofluorescein (DCF) by flow cytometer. For apoptosis detection, HSC were resuspended in Binding Buffer and incubated Annexin V-FITC and propidium iodide (PI). Plasma inflammation markers, interleukin-6 (IL-6) and monocyte chemoattractant protein-1(MCP-1), were detected using cytometry bead array assay. Also, advanced oxidation protein products (AOPP) were measured in plasma by spectrophotometric assay. A FACSCanto II cytometer was used for the flow cytometric analysis. Data were acquired and analyzed using BD FACSDiva, FCAP Array and FCS Express 4 softwares. Lipid profile and aortic lipid deposition were also analyzed. Data are mean±SEM. Statistical analysis was performed with two way ANOVA test a p-value less than 0.05 was considered statistically significant.

Results: Aging and atherosclerosis increased ROS levels and apoptosis in HSC. Peroxide levels were markedly augmented in aged mice (C57-2m: 1964 ± 201, C57-12m: 11572 ± 449, C57-24m: 43858 ± 1789; apoE-2m: 1979 ± 316, apoE-12m: 17104 ± 465, apoE-24m: 50348 ± 2345 MFI, $p<0,05$). Apoptosis was also increased with aging and atherosclerosis (C57-2m: 5.2 ± 0.8, C57-12m: 18.6 ± 2.5, C57-24m: 33.4 ± 3.1; apoE-2m: 9.5 ± 2.1, apoE-12m: 26.9 ± 3.2, apoE-24m: 42.7 ± 3.6 %, $p<0,05$). Atherosclerotic adult and elderly animals showed higher levels of IL-6 (C57-2m: 276 ± 36; C57-12m: 328 ± 6,8; C57-24m: 426 ± 57,3; apoE-2m: 302 ± 9,5; apoE-12m: 452 ± 7,1, apoE-24m: 798 ± 110 pg/mL, $p<0,05$) and

MCP-1 (C57-2m: 325 ± 23, C57-12m: 337 ± 15, C57-24m: 358 ± 27; apoE-2m: 354 ± 26; apoE-12m: 492 ± 23, apoE-24m: 623 ± 86 pg/mL, p<0,05). Atherosclerosis, but not aging, augmented AOPP levels and aortic lipid deposition.
Conclusion: The present study provides evidence that aging and atherosclerosis can cause oxidant stress and impair HSC function. Such findings could lead to new insights into the mechanisms by which aging and atherosclerosis can affect immunity and inflammation.

F-L-4021

EXPRESSION OF MUCINS AND GLUTATHIONE-S-TRANSFERASES IN DENTAL STEM CELLS ISOLATED FROM HUMAN THIRD MOLAR: CYTOCHEMICAL STUDY

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Introduction. As oral tissues are highly exposed to external environment there must be strong defense mechanisms to keep teeth and periodontal tissues healthy. Mucins form a protective layer for mucous membranes, which may include periodontal tissues. Marked protection against harmful substances have also detoxification enzymes, such as different glutathione S-transferases (GSTs).
Objectives. This study objectives were to determine if and how mucins (MUC1, MUC3, MUC4) and glutathione-S-transferases (GSTM1, GSTT1, GSTP) are being expressed in dental pulp stem cells (DPSCs) and periodontal ligament stem cells (PDLSCs) in the course of growth and differentiation.
Materials and Methods. The original normal human adult periodontal ligament stem cells (PDLSC) and dental pulp stem cells (DPSC) cultures were used, obtained from extracted intact third molar. The periodontal and dental pulp tissues were removed and cultivated in Neurobasal A medium supplemented with growth factors and gentamycin. The cells were stored in cell bank. For analysis the cells were either propagated in Neurobasal A medium supplemented with 10% of FCS or differentiated on the cover-glasses in the same medium without FCS but with supplement B27, retinoid acid and cAMP. The cells were cultured in CO2 incubator at 37oC for 1-21 days. Cells were fixed with 4% (or 8%) PFA and, if necessary, permeabilized with Tritone-X100. Expression of MUC1-alpha chain, MUC1-β-chain, MUC3, MUC4, and GSTM1, GSTT1, GSTP proteins was brought out by immunofluorescence staining using original mouse monoclonal antibodies. Specific staining was visualized with Alexa Flour conjugated secondary antibodies, cell nuclei were counterstained with DAPI and examined using fluorescence microscopy. Results and Conclusions: Both mucins and glutathione-S-transferases were expressed in PDLSCs and DPSCs cultures. However, the expression intensity was different in both types of cells during propagation and differentiation. The expression of MUC1 and MUC4 was the strongest among mucins, and GSTP and GSTT1 among glutathione-S-transferases. In general, the expression of mucins and GSTs was less intensive in DPSCs compared with PDLSCs.The stronger expression of mucins and GSTs in PDLSC-s can be explained by higher need to protect the periodontal cells against continuous exposure to pathogens in oral cavity compared to DPSCs, which have more isolated position in pulp. PDLSC and DPSC cultures may be good in vitro models to study pathogenetic role of external harmful factors in oral cavity tissues.

F-L-4022

DYNAMICAL-SYSTEMS ANALYSIS OF GENE EXPRESSION MODEL SIMULATION AND STATISTICAL ANALYSIS OF MICROARRAY DATA

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Embryonic stem cells (ESCs) have the ability to differentiate into all the types of somatic cells, that is, pluripotency. Pluripotent transcription factors (TFs) such as Oct4 and Nanog are activated in pluripotent state and are associated with self-renewal and proliferation. Expression level of pluripotent TFs gradually decrease through the course of cell differentiation. Inversely, differentiation TFs such as Gata6 increase their expression level, which prevent the expression of pluripotent TFs, and thus cells are differentiated. The interaction between these TFs consist of function modules for pluripotency and differentiation. These modules constitute basic components in the TF regulatory network, which govern the protein expression dynamics, whereas cell differentiation is interpreted as the cell-state transition by the dynamics. In addition, it is suggested that epigenetic modification such as DNA methylation and histone modification further solidifies the cellular state transition, by altering chromatin structure to generate transcriptionally active and inactive regions, which controls TFs activity. Indeed, this epigenetic modification is suggested to be an obstacle in reprogramming by induced pluripotent stem cells (iPSCs) to make a cellular state back to pluripotent state. However, the inter-relationship between the expression dynamics and the epigenetic modification is not fully uncovered.

We first constructed a simple dynamical-systems model with TF regulatory network that consists of the pluripotency and differentiation modules. As a result of extensive simulations, our model is found to show differentiation from a stem cell state, in which all TFs' expression levels shows oscillation. This oscillation in the expression levels in each cell gradually desynchronize through the cell division, and later, few cells start to differentiate, where the original oscillation is lost. In a differentiated state, differentiated TFs are activated while pluripotent TFs are silent. This differentiation is characterized by introducing the entropy of diversity in expressions, which shows transient increase in the stem cell and later decrease through the differentiation. The epigenetic fixation of differentiation is also studied by inducing the feedback pattern from gene expression to epigene pattern.

We then analyzed microarray data differentiating from ESCs to neuron and hepatocyte. Calculating the entropy of diversity in expressions, we found that the entropy during the course of neural differentiation transiently increased and then decreased. This result supports our simulation result. In addition, we calculated ratio of TFs expression in all gene expression. The ratio of TFs expression in neuron was higher than that in ESCs and iPSCs. Inversely, the ratio in hepatocyte was lower than that in ESCs and iPSCs. It is expected that epigenetic regulation adjusts the entropy and ratio of TFs expression. We plan to conduct the more detailed analysis and construct a better fitted model.

F-L-4023

ANTISENSE-MEDIATED EXON SKIPPING:A THERAPEUTIC STRATEGY FOR TITIN-BASED DILATED CARDIOMYOPATHY

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Truncating mutations in the giant sarcomeric protein Titin (TTN) are a major cause for inherited forms of dilated cardiomyopathy (DCM), a heart disease characterized by ventricular dilatation, systolic dysfunction, and progressive heart failure. To date, there are no specific treatment options for DCM patients but heart transplantation. Here, we show the beneficial potential of reframing TTN transcripts by antisense oligonucleotide (AON)-mediated exon skipping in human and murine models of DCM carrying a previously identified autosomal-dominant frameshift mutation in TTN exon 326. Correction of TTN reading frame in patient-specific cardiomyocytes derived from induced pluripotent stem cells (iPSCs) rescued defective sarcomere assembly and stability and normalized sarcomeric protein expression. The impact of skipping the mutated TTN exon 326 on myocardial function was further evaluated in Ttn knock-in mice. AON-treatment improved sarcomere formation and contractile performance in homozygous embryos and prevented the development of the DCM phenotype in heterozygous animals. These results demonstrate that disruption of the TTN reading frame due to a truncating DCM mutation can be restored by exon skipping in both patient cardiomyocytes in vitro and mouse heart in vivo, indicating RNA-based strategies as a potential treatment option for DCM patients.

F-L-4024

GENERATION OF ISOGENIC KNOCK-OUT AND KNOCK-IN IPSC LINES VIA RAPID GENOME EDITING TECHNIQUES

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Human induced pluripotent stem cell (iPSC) technology offers the benefits of a cell line coupled with the advantage of using human primary cells. However this requires standardization of iPSC generation and genetic modification as well as the ability to obtain large numbers of differentiated cells in an assay ready format. In the current study, we utilize cutting-edge genome editing technologies, (Zinc Finger Nuclease (ZFN) and Transcription activator-like effector nuclease (TALEN), to efficiently target desired gene-of-desired in human iPSC, and generate a novel panel of iPSC lines related to neurodegenerative disorders. These include 1) single and double knock-outs of disease-related genes; 2) isogenic reporter lines related to a specific disease by knock-in and/or safe harbor strategy, and 3) multiple targeted lines by combination of above. We will present functional characterization of representative lines including 1) ubiquitous reporter lines in safe harbors (e.g., on chr 13 or 19) in which the reporter (luciferase or GFP) is constitutively expressed; 2) lineage-specific knock-in lines targeting genes related to neural lineage (e.g.,

GFAP for astrocyte reporter and MAP2 for neuron reporter) using either fluorescent and luciferase genes as reporters, 3) knock-out of PD, AD, ALS, and autism -related genes in an isogenic background. The development and implementation of these novel disease-specific isogenic iPSC lines will create a more efficient disease-specific process underpinned by biological mechanism in a patient- and disease- specific manner rather than by trial-and-error.

F-L-4025

NEW APPROACHES TO PRECLINICAL DRUG DISCOVERY AND DEVELOPMENT: SURVEY RESULTS ON USE OF HUMAN STEM CELL DERIVED CARDIOMYOCYTES

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Stem cell applications represent an innovation in in vitro science with the potential to provide translational data. The development of human stem cell derived cardiomyocytes (hSC-CM) and applied technologies holds great potential for guiding preclinical cardiac safety screening and investigation efforts. However it is still under development how hSC-CM and applied technologies can be best used to evaluate various aspects of cardiac risks. It is important to understand present practices and provide framework for future development and directions.

ILSI-Health and Environmental Sciences Institute (HESI), a consortium with representatives from pharmaceutical companies, academia, and regulatory agencies, formed an hSC-CM working group in 2013. The working group designed and launched a survey in February 2014 to understand current uses of hSC-CM in drug discovery and development as well as its strengths and challenges.

The 30 question survey received 32 responses from 27 organizations (84% response rate). Seventy-three percent (73%) of respondents have evaluated hSC-CM's utility while 40% have implemented hSC-CM into their discovery or toxicology programs. MEA and impedance were the most used technologies to interrogate hSC-CM to detect cardiac risks. For perceived barriers regarding hSC-CM use, cell maturation compared to adult ventricular myocytes (66%), expense (59%), and reproducibility (55%) ranked the highest.

The survey results will both guide the future development of hSC-CM as an alternative drug discovery and development platform and support the FDA Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative, an initiative to provide a more robust and efficient preclinical evaluation of cardiac safety liabilities and promote drug discovery.

F-L-4026

CHARACTERIZATION OF NON INVASIVE SOURCES OF ADULT STEM CELLS OBTAINED FROM CLEFT LIP AND PALATE PATIENTS

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Bone reconstitution in craniofacial diseases, which affect about 2-3% of newborns, has been the focus of intensive researches for bone tissue engineering. The possibility of using mesenchymal stem cells (MSCs) for regenerative medicine has opened a new field of investigation aiming to find the best sources for the isolation of multipotent stem cells, in particular through non-invasive procedures. Therefore, in order to identify a non-invasive alternative source of stem cells with osteogenic potential without conferring morbidity to the bone donor area, we have used dental pulp (DP) obtained from deciduous teeth of Cleft Lip and Palate (CLP) patients to make bone tissue engineering. We also used orbicular oris muscle (OOM) and levator palatine muscle (LPM) fragments, which are regularly discarded during surgery repair (cheiloplasty and palatoplasty) of CLP patients, to make bone tissue engineering. We obtained cells from DP, OOM and LPM fragments, of CLP patients using previously described pre-plating technique. Through flow cytometry analysis, these cells were mainly positively marked for five mesenchymal stem cell antigens (CD29, CD90, CD105, CD73, CD166), and negative for hematopoietic and endothelial cell markers (CD45, CD34, CD31). After induction under appropriate cell culture conditions, these mesenchymal cells obtained from DP, OOM and LPM were capable to undergo chondrogenic, adipogenic, osteogenic and skeletal muscle cell differentiation, as evidenced by immunohistochemistry. We also demonstrated that these cells together with a biomaterial lead to bone tissue reconstitution in animal model. In conclusion, we showed that cells from OOM, LPM and DP have phenotypic and behavior characteristics similar to other adult stem cells, both in vitro and in vivo. Our findings suggest that these tissues (OOM, LPM, and DP) represent promising sources of stem cells to be used in alveolar bone grafting treatment (bone tissue engineering), particularly in young CLP patients. These results open new avenues to perform the treatment to these patients without morbidity and pain for them.

F-L-4027

IMMUNOCHARACTERIZATION OF EQUINE ADIPOSE-DERIVED STEM CELL BY QUANTITATIVE IMAGE FLOW CYTOMETRY

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Adult stem cells have been investigated with a particular interest for therapeutic use in the field of regenerative medicine. Equine adipose-derived mesenchymal stem cells (Eq adMSCs) are a potential cell source for autologous cell therapy due to their capacity to self-renew and differentiate in specialized cell types. They are known to modulate oxidative stress, secrete various cytokines as well as growth factors.

As a result of these attributes, Eq adMSCs possess immunomodulatory, angiogenic, anti-inflammatory and anti-apoptotic properties. At the International Society for Cellular Therapy meetings (2013) human mesenchymal stem cells were defined as aplastic-adherent cells expressing specific surface markers, including: CD105, CD90, CD73 or CD44 and CD29. Although similar definitions have been published for animal stem cells, no consensus has been reached for the identification and characterization of adipose-derived stem cells for veterinary applications. Therefore the aim of this study was to characterize Eq adMSCs by evaluating the immunophenotypic properties and the presence of intra-cytoplasmic proteins responsible for their non-differentiation stage. The information would then be used to establish references for stem cell immunocharacterization and application in equine specie. Eq adMSCs were obtained from the subcutaneous fat tissue of 18 adult horses. Briefly, fat tissue was minced, washed in PBS Buffer and digested for 30 min in a solution containing 1 mg/ml collagenase I and 0.1 mg/ml BSA Fr V (Sigma-Aldrich). The resulting cell suspension was filtered sequentially through a 100 µm and a 40 µm cell strainer (BD). Collagenase I was then inactivated by a double volume of PBS. The cells were centrifuged for 10min at 300 x g and re-suspended in D-MEM, low glucose containing 10% FBS and antibiotics (Invitrogen), plated at a density of 2.5 × 10E4 cells/cm2 and cultured for 8 days in 5% CO2 at 38.5C. After 24hrs, non-adherent cells were washed off and the Eq adMSCs were cultured until 90% confluence was obtained. The Eq adMSCs were then detached with trypsin, first passaged until 90% confluence, and cryopreserved after detachment in a freezing medium consisting of DMEM containing 10% FBS and 5% DMSO (Sigma-Aldrich). At passage 1, a cell sample from each horse was evaluated for immunophenotypic characterization. The following mesenchymal stem cell surface markers were evaluated: (CD29-RD1, CD44-FITC, and CD105-AF648), haematopoietic (CD34-FITC) as well as cytoplasmic proteins markers (SOX2-AF488 and OCT3/4-AF488) by quantitative imaging multicolor flow cytometry (ImageStream MK II - Amnis-Millipore). At the same passage, as an additional validation, osteogenic differentiation was induced. After 20 days Alizarin O Red was used to detect extracellular calcium deposition. Following multiplex quantitative image flow cytometry analysis, isolated equine adipose-derived stem cells of first passage were found to be CD44 (95.21±10.4%), CD29 (99.00±0.93%), CD105 (97.37±2.90%), SOX2 (79.10±25.84%) and OCT3/4 (79.82±14.99%) positive and CD34 (0.37±0.13%) negative. All the 18 horse cultures differentiated in osteogenic tissue confirming the efficiency of our purification process. These results for the first time demonstrated the presence of the ICTS main specific markers in Eq adMSC and validate the efficiency of our purification protocol.These information will be use to further improve our knowledge of Eq adMSC in this species.

F-L-4028
CELL COMPETITION MONITORS THE FITNESS OF EMBRYONIC STEM CELLS AT THE ONSET OF DIFFERENTIATION.

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Ensuring that mutations are not propagated within stem cell populations is key to proper development and homeostasis. This is especially relevant when it comes to pluripotent cells that not only can give rise to all lineages, including the germ line, but are also of great importance to regenerative medicine. Cell competition is a type of cell-cell interaction where the coexistence of two cell populations with different metabolic properties, signalling abilities or growth rates results in the growth of the stronger population at

the expense of the weaker one. Although primarily studied in Drosophila, we have found that cell competition monitors cellular fitness at the onset of embryonic stem cell differentiation. We observe that in mouse embryonic stem cells, and in the early post-implantation mouse embryo, defective cells are eliminated by wild-type cells. Interestingly this process is triggered specifically at the onset of differentiation. Furthermore, concurrent with the elimination of defective cells, wild type cells undergo compensatory proliferation ensuring in this way homeostasis of the overall cell population. Our work has also uncovered that key to the elimination of unfit epiblast cells is the establishment of differential expression levels of the proto-oncogene c-Myc, and that during this process those cells with lower c-Myc expression are eliminated in an apoptotic dependent manner. Here we will discuss the mechanisms that regulate the sensing of relative fitness levels and the establishment of differential levels of c-Myc expression. Importantly our studies demonstrate that during embryonic development cellular fitness is not only a cell intrinsic property, but is also a relative measure that is dependent on the fitness levels of surrounding cells. By allowing the recognition and elimination of abnormal cells, cell competition provides a mechanism that could control cellular fitness in a wide variety of settings, including stem cell

F-L-4029
DEFINING A MODEL FOR MITOCHONDRIAL FUNCTION IN MESC DIFFERENTIATION

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Differentiating embryonic stem cells (ESCs) undergo mitochondrial maturation leading to a switch from a system dependent upon glycolysis to a reliance on oxidative phosphorylation. This switch acts as a signal to the cell to decrease the rate of proliferation and initiate differentiation into downstream cell lineages, a critical step in embryogenesis and a potential target for disruption by xenobiotic compounds. We investigated the sensitivity of this metabolic shift to interruption of oxidative phosphorylation at various points of the mitochondrial electron transport chain in a differentiating mouse ESC model system. The impacts of exposures to chemical inhibitors of electron transport complexes were assessed using in-cell Western analysis of the differentiation biomarker, Goosecoid (GSC), a transcription factor highly expressed during embryonic gastrulation. Additional fluorescent bioassays were used to monitor cell number, mitochondria number and cell death. The effect on cell proliferation by inhibitor exposure varied depending on the complex tested. We found inhibitors of complex I led to a 91% decrease in cell number, which was > complex V (69% decrease) ≈ complex IV (66% decrease) > complex III (25% decrease) ≈ complex II (21% decrease). The pattern of altered differentiation as measured by GSC was similar (complex I inhibitor, 97%; complexes IV and V, ~50%; and little effect in complexes II and III, 12 and <1%, respectively). In contrast, inhibitors of electron transport at complexes I, IV and V showed increased number of mitochondria/cell in the surviving cell population. Mitochondria/cell decreased following inhibition of complexes II and III (~56%). These data will be combined with other measures of mitochondrial function to help define a mitochondrial adverse outcome pathway, to expand the application of the mESC developmental model and to aid in developing *in silico* models for toxicity testing. (*This abstract does not necessarily reflect USEPA policy*).

F-L-4030

STEM CELL SELF-RENEWAL AND DIFFERENTIATION IS CONTROLLED BY AN INTRICATE RNA DECAY

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Despite recent advances identifying regulatory pathways controlling the self-renewal and differentiation of stem cells, these mechanisms remain poorly understood. Here, we provide evidence that a highly conserved RNA degradation pathway called Nonsense-Mediated RNA Decay (NMD) exerts a crucial role in this decision. Originally discovered as a quality-control pathway that targets aberrant transcripts for decay, NMD is now known to degrade a subset of normal mRNAs, regulating ~10% of the normal transcriptome. This raises the possibility that NMD regulates normal biological processes and is, therefore, itself subject to regulation. In support of this, we previously reported the identification of a neurally expressed microRNA, miR-128, that represses NMD (Bruno *et al. Mol Cell*, 2011; 42: 500). miR-128 is sufficient to induce differentiation of mNSCs and upregulates transcripts involved in neural processes, the majority of which contain NMD-inducing features. This raised the possibility that miR-128 promotes neural differentiation by repressing NMD. Here we report evidence that miR-128 and other neurally expressed microRNAs operate in a circuit with NMD to control stem cell self-renewal and differentiation. miR-128 promotes differentiation by repressing the central NMD factor, UPF1, which otherwise promotes stem cell proliferation and inhibits differentiation by targeting mRNAs encoding pro-differentiation and proliferation-inhibitory factors. In a negative-feedback circuit, NMD represses miR-128 expression, thus preventing miR-128's pro-differentiation effects and promoting the pluripotent state. In response to differentiation cues, miR-128 is induced and NMD is consequently downregulated, thereby permitting differentiation. We also identified two other neurally expressed microRNAs, miR-9 and miR-124, that regulate the NMD factor, UPF3B. UPF3B controls a branch of NMD that targets a subset of transcripts degraded by the entire NMD pathway, suggesting that miR-9 and -124 can more precisely regulate transcripts involved in stem cell pluripotency and differentiation. We conclude that a highly conserved post-transcriptional circuit revolving around microRNAs and NMD acts as a switch that responds to external inputs to stably maintain the pluripotent state or induce differentiation.

F-L-4031

OVERCOMING CELLULAR IMMUNE RESPONSE IN REPROGRAMMING NON-HUMAN PRIMATE CELLS WITH MRNA

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Messenger RNA reprogramming holds advantages over other integration-free reprogramming methods in speed, efficiency, and chromosomal stability. We have previously optimized our mRNA reprogramming method that resulted in obtaining human iPSCs in just a week using feeder-free conditions. However, this powerful method has never been successfully applied to cells from any species other than human. B18R

protein, a soluble, extracellular decoy receptor for Type I interferons naturally expressed by Vaccinia virus to blunt immune responses in human cells was critical during the repeated transfection of synthetic mRNAs into human fibroblasts. B18R is species-specific and does not effectively help non-human cell reprogramming.
In order to create large animal disease models, we aimed to reprogram non-human primate fibroblasts using our human mRNA cocktail. By producing mRNAs in an improved process to reduce immune-generating RNA species, and by establishing a more tunable reprogramming medium instead of the commonly used Pluriton from Stemgent, we were able to efficiently reprogram monkey cells in just over a week, also under feeder-free conditions. The resulting iPSCs have several interesting properties compared to human iPSCs, and we are conducting various experiments to further characterize them.
To our knowledge this is the first time that non-human cells were reprogrammed into iPSCs using only mRNA molecules. Equipped with these new tools, we can now create truly integration-free iPSCs from many other species and compare them in intro disease modeling as well as in vivo testing.

F-L-4032

THE RELATIONSHIP BETWEEN ACCOUNTABILITY PRESSURES AND MOTIVATIONS FOR FUNDING HUMAN EMBRYONIC STEM CELL RESEARCH - AN INTERNATIONAL COMPARATIVE STUDY FOCUSED ON PHILANTHROPISTS

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Purpose due to the harsh funding circumstances during the first decade of the millennia, private research funders facing other accountability pressures than public agencies took a lead in funding hESC research. Organizational theory indicates that resource provision as well as geographic location will effect motivations for funding hESC research. Focusing on independently wealthy philanthropists, this study investigates how the extent of accountability pressures experienced by funders in different institutional environments influenced their motivations for funding hESC research. The study compares philanthropists to fundraising dependent nonprofits, public agencies, and corporations across three different institutional environments.
Methods: The data was collected in 2011/2012 in direct interviews with funders of hESC research, N=86, in Sweden, California, and South Korea, three countries having different tax systems, legislation, and normative climate when it comes to hESC research. The sampling was based on a systematic analysis of all hESC publications in PubMed coming out of Sweden, California and South Korea, and all funders were listed and categorized. The interviews had a survey part using pre-validated scales as well as a structural qualitative component. The survey was analyzed in one-way between-groups ANOVA with planned comparisons. The qualitative data was coded following a structured interview format.
Results: The ANOVA reveals that philanthropists experienced lower accountability pressures than public agencies, corporations, and fundraising nonprofits. Differing from other research funders, the ANOVA also shows that philanthropists did not primarily fund hESC research motivated by its clinical or commercial promise. Instead, the qualitative data indicates that philanthropists' motivations for funding hESC research were more diverse and differed between countries. In California, the main motivation was

complementing US federal funds, in Sweden it was scientific progress, and in South Korea philanthropists were closely aligned with commercial interests and focused on clinical applications. Conclusion: The extent of accountability pressures experienced by hESC research funders seems to have some influence on their motivations for funding hESC. Public agencies, corporations, and fundraising nonprofits experienced higher accountability pressures than philanthropists and were to a higher extent motivated by the clinical and commercial promise of hESC. Facing lower accountability pressures, philanthropists seemed to be influenced more by the national institutional environment than by the clinical potential of the research. In Sweden, philanthropists tried to mimic public research funders, whereas in California they strived to complement the NIH. South Korean philanthropists were not distinguishable from other types funders in their country in terms of their motivations, which can potentially be explained by the centralized institutional environment.

F-L-4033
LMX1A ENHANCES THE THERAPEUTIC EFFECT OF INDUCED NEURAL STEM CELLS IN A PARKINSON'S DISEASE MOUSE MODEL

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In our previous work, we have converted somatic cells directly into neural stem cell - like cells, namely induced neural stem cells (iNSCs). In the present study, we continued to test whether iNSCs have therapeutic effects when transplanted into a mouse Parkinson’s disease (PD) model. Given the central role of Lmx1a in the specification of dopaminergic neurons, we also introduced Lmx1a into iNSCs under a Nestin enhancer. Under differentiation conditions in vitro, iNSC-Lmx1a versus iNSC-GFP gave rise to a greater yield of dopaminergic neurons and secreted a higher level of dopamine. When engrafted into mouse PD models, iNSC-GFP reduced ipsilateral rotations of the mice, and iNSC-Lmx1a did so even further. Eleven weeks after transplantation, few iNSCs survived. The improved motor performance in iNSC-Lmx1a group correlated with a greater tyrosine hydroxylase (TH) signal abundance in the lesioned area of striatum, suggesting that iNSCs may have worked through a non-autonomous manner to enhance the functions of remaining endogenous dopaminergic neurons in brain.

F-L-4034
CHEMICAL IDENTIFICATION FOR MOLECULES THAT PROMOTE THE MATURATION OF HUMAN IPS DERIVED HEPATOCYTE

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High-throughput chemical screening is a widely used strategy in searching of molecules that play important roles in biological pathways. Chemical libraries that contain thousands of small molecular

weight compounds are now available. However, to perform high-throughput screenings, large numbers of cells are required, which might be a barrier particularly when human derived materials are involved. Lines of evidence have shown that ES and iPS cells recapitulate normal developmental processes, and provide attractive cell sources for the high-throughput screening. Here, we established a high-throughput screening system to find molecules that promote hepatic differentiation of human iPS cells. We differentiated human iPS cells into the hepatic lineage for 19 days, and then challenged with chemicals. We screened a chemical library, consisting of over 1000 clinically-used biological active pharmaceutical drugs. Based on immunochemical analysis with two hepatic markers, Albumin (ALB) and Alpha-fetoprotein (AFP), we searched for candidate chemicals that increase ALB and decrease AFP. We successfully identified the anti- estrogen drug, Tamoxifen, together with several other drugs as candidate drugs that potentiate hepatic maturation. The result that Tamoxifen potentiated hepatic maturation was consistent with the normal developmental phenomenon that hepatocytes become mature after birth, upon estrogen withdrawal. Since estrogen acts as a transcriptional regulator, we then examined its effects on the other candidate targets. We found that the target molecules were expressed within a certain time window during differentiation and were negatively regulated by Tamoxifen. The drug inhibition of this molecule could also show hepatic maturation with dose dependency.

We then checked whether the candidate drugs potentiated hepatic maturation of human iPS cell, by measuring the p450 enzymatic activity of CYP3A4, and ICG (Indo-cyanine-green) -uptake tests as an indicator for detoxification. The cells treated with candidate drugs showed a larger ICG-positive area than those in the control group. Luciferase assays were performed to evaluate p450 activity. Cells added with candidate drugs showed higher levels of luminescence.

In conclusion, we established the chemical screening system and obtained the candidate drug that potentiate the maturation of human iPS derived hepatocyte. And we believe that our human iPS-cell-based screening strategy enable us to determine the remaining unknown mechanisms during development.

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