INTERNATIONAL 2017 SYMPOSIA

BASEL SWITZERLAND 2017

27 FEBRUARY – 1 MARCH

TRANSLATIONAL OPPORTUNITIES IN STEM CELL RESEARCH

PROGRAM GUIDE

ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

stem BANCC Stem Cells for Drug Discovery

Basel Stem Cell Network

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WELCOME

Dear Colleagues:

We would like to welcome you on behalf of the International Society for Stem Cell Research, StemBANCC and the Basel Stem Cell Network. The ISSCR, StemBANCC, and BSCN are proud to partner together to bring you *Translational Opportunities in Stem Cell Research*, a joint-conference of the ISSCR International Symposia 2017.

Basel is Switzerland's oldest university city and one of Europe's leading teaching and research locations. As Switzerland's most dynamic economic region and one of the most productive worldwide, Basel is a key location for the life sciences industry.

ISSCR, StemBANCC, and BSCN have created a diverse, engaging program that will explore latest translational opportunities in the stem cell field, cutting-edge technology helping to drive the research, and inspired perspectives on where the area of research is heading. Throughout the next few days, you will hear from leaders pushing the boundaries of stem cell science towards clinical applications and disease therapies.

Our supporters and exhibitors help make this meeting possible. We ask that you join us in thanking them for their support by exploring the newest tools on exhibit during the meeting and recognizing the supporters listed in our acknowledgements.

As always, we are grateful for your support – thank you for joining us. The measure of our meeting's success is not only the information you gain from listening to presentations, but also the connection and future collaborations that can be initiated. Our meeting is small enough to allow access to our speakers and leaders, so we encourage you to engage in conversation during breaks, lunches, or poster receptions.

On behalf of all of the organizers and people who made this event possible, we hope you find great value during the scientific sessions and in the new connections you are able to cultivate.

Sincerely,

Members of the Organizing Committee

Mark Burcin, F. *Hoffmann-La Roche Ltd., Switzerland*

Sally Cowley, *University of Oxford, United Kingdom*

Fiona Doetsch, *Biozentrum, University of Basel, Switzerland*

Andrew Elefanty, *Murdoch Childrens Research Institute, Australia*

Gordon Keller, *McEwen Centre for Regenerative Medicine Ontario Cancer Institute, Canada*

Jack Price, *King's College London, United Kingdom*

Timm Schroeder, *Swiss Federal Institute of Technology (ETH), Switzerland*

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ABOUT THE ISSCR

Mission Statement

The International Society for Stem Cell Research (ISSCR) is an independent, nonprofit organization established to promote and foster the exchange and dissemination of information and ideas relating to stem cells, to encourage the general field of research involving stem cells and to promote professional and public education in all areas of stem cell research and application.

History and Philosophy

With more than 4,100 members from over 60 countries, the ISSCR is the preeminent transnational, cross-disciplinary science-based organization dedicated to stem cell research.

Formed in 2002, the Society promotes global collaboration among talented and committed stem cell scientists and physicians, and plays a catalyzing role in the development of effective new medical treatments.

The Society brings together investigators who are engaged in both fundamental and applied research. Their investigations include the use of pluripotent stem cells and stem cells within adult organs and tissues to create applications in specific therapeutic settings.

The ISSCR represents academia and industry on a broad range of issues that affect the well-being of patients and their families, and strives to educate the public and government regulators on the basic principles of stem cell science and the realistic potential for new medical treatments and cures.

The leadership of the ISSCR is acutely aware of the responsibility the Society bears to promote the highest scientific and ethical standards, and is dedicated to integrity in the rigor and quality of the research community's scientific work, the public policy stands it takes on stem cell related issues, and the organization's relations with its key constituents and the public. Only such an abiding commitment to integrity can ensure that as the ISSCR grows, it will continue to serve a fair and trusted advocate by both its internal and external stakeholders.

Contact Us

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www.isscr.org
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- Wednesday, 1 March 14-15

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Welcome to the compact trade fair city of Basel

On behalf of the government of the Canton of Basel-Stadt, I would like to welcome you to the city of Basel. Its central location, first-rate transport connections and excellent infrastructure make Basel one of the most prominent trade fair and convention cities in Europe.

Hosting the BASELWORLD and Art Basel fairs, the city is home to the most prominent international clock and jewellery fair and contemporary art show. During your stay, you will see that our city has a lot more to offer. Not only is Basel the most dynamic economic region in Switzerland, it is also an international centre of research, science and education as well as one of the most modern event cities in Europe. On top of this, Basel is one of Europe's foremost centres of fine arts .

Cultural diversity manifests itself in every corner of the city, with internationally famous museums and spectacular special exhibitions of global renown vying for attention. Visitors have a choice of over 40 museums, among them Kunstmuseum Basel and Fondation Beyeler. Basel is also well worth a visit from an architectural perspective: Buildings designed by top international architects, such as Herzog & de Meuron, Richard Meier, Renzo Piano and Frank O. Gehry attract architecture buffs from all over the world. Theater Basel – Switzerland's biggest triple-genre venue –, numerous world-class orchestras and scores of international festivals all add to Basel's unique cultural atmosphere.

Basel offers an exceptionally high quality of life, which is appreciated all round. Uniquely situated in the border triangle, the cosmopolitan city's picturesque old centre, numerous green areas and recreational zones as well as diverse shopping facilities and lively restaurant scene offer a wide range of leisure activities. Comprehensive information and interesting articles on this subject are available on the Internet at www.basel.ch.

We hope that you will enjoy your stay and find enough time to discover our beautiful city. The complimentary 'mobility ticket' you received at the hotel allows you to travel on the entire public transport system.

I am confident that you will feel very welcome in our cosmopolitan city of culture and wish you a successful stay as well as wonderful impressions.

Yours sincerely,

Elisabeth Ackermann
President of the Government

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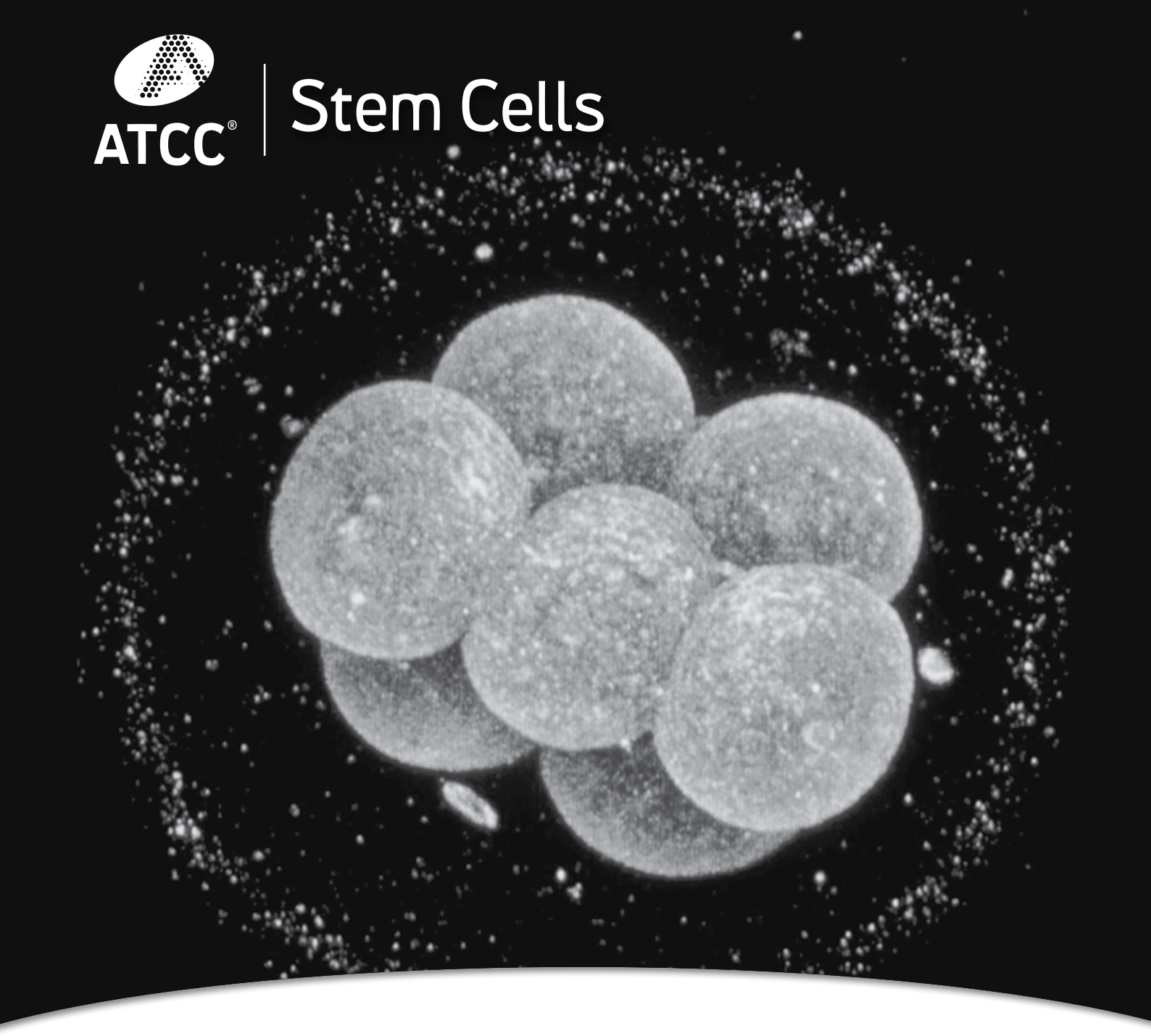

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Mouse embryo confocal image courtesy of Dr. David Becker

GENERAL INFORMATION

VENUE

The *Translational Opportunities in Stem Cell Research* International Symposium is located at the Congress Center Basel. All program sessions and activities are in the Hall 4 Building, Second Floor.

Registration and coat check are on Congress Center Basel, Hall 4 Building, Second Floor Foyer.

Program sessions take place in the Congress Center Basel, Hall 4 Building, Second Floor, Montreal Room.

Refreshment breaks, lunches, poster sessions, Welcome Reception and the Exhibit Hall are all located on Congress Center Basel, Hall 4 Building, Second Floor Foyer.

REGISTRATION AND BADGE PICKUP

Pick up your attendee name badge at the Congress Center Basel, Hall 4 Building, Second Floor, during posted hours at the registration desk.

Name badges are required for admission to all sessions, poster presentations, social events and the Exhibit Hall. Since the meeting badge serves as proof of participation, all attendees, speakers and exhibitors are required to wear their badges at all times during the International Symposium as well as the Welcome Reception and all other special events. Access to events may be refused if the meeting badge is not displayed.

Registration Desk and Badge Pickup Hours

MONDAY, 27 FEBRUARY 10:00 – 18:15
TUESDAY, 28 FEBRUARY 8:00 – 18:30
WEDNESDAY, 1 MARCH 8:00 –16:30

Should you misplace or lose your badge an administration charge of $20 USD will be imposed on any replacement badge. A drop box for badge recycling will be provided on the Second Level.

NAVIGATING CONGRESS CENTER BASEL

The International Symposium's program sessions and events will take place in the areas noted below:

Hall 4 Building, Second Floor, Foyer

- Registration Desk and Badge Pickup
- Exhibit Hall
- Poster Hall
- Refreshment Break Stations
- Lunch Stations
- Welcome Reception and Tuesday Reception

Hall 4 Building, Second Floor, Montreal Room

- Program sessions

Hall 4 Building, Second Floor, Rio Room

- Speaker Lounge

INTERNET ACCESS

Complimentary access to the internet is available within the Congress Center Basel during the International Symposium thanks to our sponsor BD Switzerland Sàrl.

The network name is ISSCRBasel. To log in, enter UserID 2187556369 and Password 2048.

As a courtesy to speakers, please be sure to silence any mobile phones and devices and refrain from using the internet during sessions. Please note that the bandwidth of this connection might be limiting.

MOBILE APP

Downloading the Basel 2017 International Symposium web-based mobile app is easy! Simply enter **http://bit.ly/2kk71hr** into your phone or tablet's browser to automatically access the mobile app. Or if you have a QR reader in your mobile device, scan the QR to the right to view the mobile app.

GENERAL INFORMATION

INTERACTIVE ATTENDEE DIRECTORY

The interactive attendee directory makes it easy to connect with fellow attendees during the symposium. This tool is available to all registrants who selected "Yes" during registration.

- Browse through the list of attendees
- Connect with speakers and other leaders in stem cell science
- Set up one-on-one meetings with researchers like yourself
- Schedule a meeting with one of the exhibitors to explore their products and lab supplies
- Search after the conference for attendees you've met

Log in to the interactive attendee directory at **https://www.etouches.com/esocial/204589** using the same email and password you used to register for this International Symposium.

HELP DESK

Please visit the registration counter on the Second Floor with any questions you may have.

RECORDING POLICY

Still photography, video and/or audio taping of the sessions, presentations and posters at the International Symposium is strictly prohibited. Intent to communicate or disseminate results or discussion presented at the meeting is prohibited until the start of each individual presentation. Thank you for your cooperation.

SPECIAL EVENTS

Refreshment Breaks

Enjoy refreshment breaks on Second Floor Foyer, Congress Center Basel. Complimentary coffee and tea will be served during the following days and times:

MONDAY, 27 FEBRUARY 15:00 – 15:30

TUESDAY, 28 FEBRUARY 10:00 – 10:30 AND 16:00 – 16:30

WEDNESDAY, 1 MARCH 10:30 – 11:00

Lunch Hours and Poster Sessions

Refresh midday with a complimentary lunch during scheduled poster presentations on the Second Floor Foyer, Congress Center Basel. Complimentary lunch will be served during the following days and times:

TUESDAY, 28 FEBRUARY 12:00 – 14:30

WEDNESDAY, 1 MARCH 12:00 – 14:30

Welcome Reception

The Welcome Reception will take place on **Monday, 27 February from 16:45 – 18:15** on the Second Floor Foyer, Congress Center Basel. Stay after the plenary session concludes and enjoy a "Taste of Switzerland" with complimentary beer and wine as well as light traditional snacks.

Networking Reception

A networking reception is scheduled for **Tuesday, 28 February from 17:30 – 18:30** on Second Floor Foyer, Congress Center Basel. Complimentary beer and wine as well as light snacks will be provided.

Exhibit Hall

The Exhibit Hall features 28 leading suppliers and vendors. Walk through the hall on Second Floor, Congress Center Basel and support the Exhibitors who help make this Symposium possible.

POSTERS & EXHIBITS

POSTER SET-UP

The posters will be accessible throughout the duration of the International Symposium. Please place posters in their designated location on the Second Floor, Congress Center Basel on **MONDAY, 27 FEBRUARY from 12:00 – 17:00**. Poster adhesive tape will be provided.

POSTER TAKEDOWN

Poster presenters are responsible for their own posters. Presenters will need to take down their posters on **WEDNESDAY, 1 MARCH from 14:30 – 17:00**.

Posters that have not been removed by **WEDNESDAY, 1 MARCH at 17:00** will be discarded by the organizer.

POSTER PRESENTATIONS

Poster presenters are required to be present at their posters during scheduled poster presentations. All poster presentations are scheduled during lunch breaks.

ODD-NUMBERED POSTERS
TUESDAY, 28 FEBRUARY FROM 12:30 – 14:00

EVEN-NUMBERED POSTERS
WEDNESDAY, 1 MARCH FROM 12:30 – 14:00

Don't forget to participate in the "VIP" (Very Important Poster) contest! Attendees are encouraged to place stickers on their favorite posters and help decide the winner of the "VIP" contest. The poster with the most stickers will be declared the winner.

EXHIBIT HALL

The Exhibit Hall will be open throughout the International Symposium. Feel free to walk through the stands and discuss solutions with all exhibitors onsite. Exhibits will be located on the Second Floor, Congress Center Basel.

Exhibit Hall Hours

MONDAY, 27 FEBRUARY	**12:30 – 18:15**
TUESDAY, 28 FEBRUARY	**8:30 – 18:30**
WEDNESDAY, 1 MARCH	**8:30 – 14:30**

While in the Exhibit Hall, please visit the City of Basel stand to learn more about attractions and events to enjoy during your stay.

Basel
basel.ch

ORGANIZERS & REVIEWERS

ORGANIZING COMMITTEE

Marc Burcin
F. Hoffmann-La Roche Ltd, Switzerland

Sally Cowley
University of Oxford, UK

Fiona Doetsch
Biozentrum, University of Basel, Switzerland

Andrew Elefanty
Murdoch Children's Research Institute, Australia

Gordon Keller
McEwen Centre for Regenerative Medicine, University Health Network, Canada

Jack Price
Kings College London, UK

Timm Schroeder
Swiss Federal Institute of Technology (ETH) Zürich, Switzerland

ABSTRACT REVIEWERS

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University of Basel, Switzerland

Martin Graf
F. Hoffmann-La Roche, Switzerland

Danwei Huangfu
Memorial Sloan Kettering Cancer Center, U.S.

Paul Jennings
Medical University Innsbruck, Austria

Darrell Kotton
Boston University School of Medicine, U.S.

Prisca Liberali
Friedrich Miescher Institute for Biomedical Research, Switzerland

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Martin Pera
University of Melbourne, Australia

Timm Schroeder
ETH Zürich, Switzerland

Verdon Taylor
University of Basel, Switzerland

Richard Wade-Martins
University of Oxford, United Kingdom

Caleb Webber
University of Oxford, United Kingdom

Robert Zweigerdt
Hannover Medical School, Germany

MONDAY, 27 FEBRUARY

13:00 - 14:00 OPENING KEYNOTE SESSION

13:00 - 13:15 **Welcome Remarks:**
Nancy Witty, *ISSCR CEO*
Timm Schroeder, *Basel Stem Cell Network*

13:15 - 14:00 **Keynote Address:**
Magdalena Götz, *Helmholtz Zentrum* München, Germany
NEURONAL REPLACEMENT AFTER INJURY - TURNING GLIA INTO NEURONS

14:00 - 15:00 MODELING TISSUE DEVELOPMENT

Chair: **Timm Schroeder**, *Swiss Federal Institute of Technology (ETH) Zürich, Switzerland*

14:00 - 14:30 **Fiona Doetsch**, *University of Basel, Biozentrum, Switzerland*
REGULATION OF STEM CELLS IN THE ADULT MAMMALIAN BRAIN

14:30 - 14:45 **Evangelos Kiskinis**, *Northwestern University Feinberg School of Medicine, U.S.*
DISSECTING DNA METHYLATION DYNAMICS DURING THE DEVELOPMENT AND FUNCTION OF THE HUMAN SPINAL CORD

14:45 - 15:00 **Ernest Arenas**, *Karolinska Institutet, Sweden*
MOLECULAR MODELING AT A SINGLE CELL LEVEL OF HUMAN MIDBRAIN DOPAMINERGIC NEURON DEVELOPMENT IN VIVO AND IN STEM CELLS

PROGRAM SCHEDULE

15:00 - 15:30 COFFEE BREAK

15:30 - 16:45 MODELING TISSUE DEVELOPMENT

Chair: **Timm Schroeder**, *Swiss Federal Institute of Technology (ETH) Zürich, Switzerland*

15:30 - 16:00 **Darrell Kotton**, *Boston University School of Medicine, U.S.*
ENDODERMAL DIFFERENTIATION OF PLURIPOTENT STEM CELLS TO UNDERSTAND DEVELOPMENT AND REGENERATE IN VIVO ORGAN FUNCTION

16:00 - 16:15 **Nicole Dubois**, *Mount Sinai Hospital, U.S.*
FOXA2 MARKS A VENTRICULAR PROGENITOR POPULATION DURING HEART DEVELOPMENT

16:15 - 16:45 **Olivier Pourquié**, *Harvard University/Brigham and Women's Hospital, U.S.*
MAKING SKELETAL MUSCLE IN VITRO TO STUDY MUSCULAR DYSTROPHIES

16:45 - 18:15 WELCOME RECEPTION

TUESDAY, 28 FEBRUARY

09:00 - 10:30 CELLULAR DISEASE MODELS I

Chair: **Martin Pera**, *University of Melbourne, Australia*

9:00 - 9:30
Caleb Webber, *University of Oxford, United Kingdom*
STEM CELL MODELS FOR NEURODEGENERATIVE DISEASES: NEW THERAPEUTICS, SINGLE CELL APPROACHES AND CAVEATS

9:30 - 9:45
Sovan Sarkar, *University of Birmingham, United Kingdom*
THERAPEUTIC MODULATION OF AUTOPHAGY IN HUMAN INDUCED PLURIPOTENT STEM CELL-BASED MODEL OF A NEURODEGENERATIVE LIPID STORAGE DISORDER

9:45 - 10:00
Jerome Mertens, *The Salk Institute for Biological Studies, U.S.*
AGE-EQUIVALENT INDUCED NEURONS AND REJUVENATED IPSC-DERIVED NEURONS TO STUDY SPORADIC ALZHEIMER'S DISEASE IN THE CONTEXT OF HUMAN AGING

10:00 - 10:15
Annie Kathuria, *King's College London, United Kingdom*
IPSC DERIVED HYPOTHALAMIC NEURONS FROM AUTISTIC INDIVIDUALS SHOW MORPHOGENETIC ABNORMALITIES DURING EARLY DEVELOPMENT

10:15 - 10:30
An Verheyen, *Janssen Pharmaceutica NV, Belgium*
MODELING FTDP-17 LINKED TAUOPATHIES AND ALZHEIMER'S DISEASE WITH GENETICALLY MODIFIED HUMAN IPSC

10:30 - 11:00 COFFEE BREAK

11:00 - 12:00 CELLULAR DISEASE MODELS I

Chair: **Martin Pera**, *University of Melbourne, Australia*

11:00 - 11:30
Anja Nitzche, *Pfizer, United Kingdom*
MODELLING THE PHARMACOLOGICAL AND CLINICAL RESPONSE IN PRIMARY ERYTHROMELALGIA USING INDUCED PLURIPOTENT STEM CELLS

11:30 - 11:45
Filip Roudnicky, *F. Hoffmann-La Roche Ltd, Switzerland*
MODELING OF SEVERE METABOLIC GENETIC DISEASES IN VITRO USING GENOME EDITING AND HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION TO VASCULAR ENDOTHELIAL CELLS

11:45 - 12:00
Nicola Beer, *University of Oxford, United Kingdom*
INTERROGATING THE FULL SLC30A8 ALLELIC SPECTRUM IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ISLET-LIKE CELLS

PROGRAM SCHEDULE

12:00 - 14:30 LUNCH AND POSTER VIEWING

12:30 - 14:00 POSTER SESSION I

(ODD NUMBERED POSTERS PRESENTED)

14:30 - 16:00 EMERGING TECHNOLOGIES

Chair: **Nissim Benvenisty**, *Hebrew University, Israel*

14:30 - 15:00 **Matthias Lutolf**, École *Polytechnique Fédérale de Lausanne, Switzerland*
ENGINEERING IN VITRO ORGANOGENESIS

15:00 - 15:15 **Andrew Cohen**, *Drexel University, U.S.*
CELLPHONES TO SUPERCOMPUTERS: HARDWARE AND SOFTWARE FOR INTEGRATED ANALYSIS AND VISUALIZATION OF 5-D MICROSCOPY MOVIES OF PROLIFERATING CELLS

15:15 - 15:30 **Daniel Coutu**, *Swiss Federal Institute of Technology (ETH) Zürich, Switzerland*
QUANTITATIVE MULTIDIMENSIONAL TISSUE CYTOMETRY OF MURINE BONE AND MARROW STEM CELLS

15:30 - 16:00 **Colin Akerman**, *University of Oxford, United Kingdom*
INTEGRATING OPTOGENETIC TECHNOLOGIES INTO HUMAN INDUCED PLURIPOTENT STEM CELL RESEARCH

16:00 - 16:30 COFFEE BREAK

16:30 - 17:30 DRUG DISCOVERY I

Chair: **Mark Burcin**, *F. Hoffmann-La Roche Ltd, Basel, Switzerland*

16:30 - 17:00 **Martin Pera**, *University of Melbourne, Australia*
INHIBITION OF DYRK1A DISRUPTS NEURAL LINEAGE SPECIFICATION IN HUMAN PLURIPOTENT STEM CELLS

17:00 - 17:30 **Kevin Eggan**, *Harvard University, U.S.*
PITFALLS AND PROMISE OF USING HUMAN STEM CELL FOR STUDYING DISEASE

17:30 - 18:30 RECEPTION

WEDNESDAY, 1 MARCH

09:00 - 10:30 CELLULAR DISEASE MODELS II

Chair: **Olivier Pourquié**, *Harvard University/Brigham and Women's Hospital, U.S.*

9:00 - 9:30
Danwei Huangfu, *Memorial Sloan Kettering Cancer Center, U.S.*
HUMAN PANCREAS DEVELOPMENT AND DISEASE THROUGH THE LENS OF PLURIPOTENT STEM CELLS

9:30 - 9:45
Daniel Tornero, *Lund University, Sweden*
RECONSTRUCTION OF A FUNCTIONAL THALAMO-CORTICAL PATHWAY IN STROKE-INJURED RAT BRAIN USING HUMAN SKIN-DERIVED CELLS

9:45 - 10:00
Stefania Fedele, *University of Basel, Switzerland*
IN VITRO MODELING OF GLUCOCEREBROSIDASE (GBA) FUNCTION IN PARKINSON´S DISEASE

10:00 - 10:30
Ludovic Vallier, *Cambridge Stem Cell Institute and Wellcome Trust Sanger Institute, United Kingdom*
STEM CELLS TO MODEL AND TO STUDY LIVER DISEASES

10:30 - 11:00 COFFEE BREAK

11:00 - 12:00 CELLULAR DISEASE MODELS II

Chair: **Olivier Pourquié**, *Harvard University/Brigham and Women's Hospital, U.S.*

11:00 - 11:30
Pura Muñoz-Cánoves, *ICREA, Pompeu Fabra University and CNIC, Spain*
ROLE OF PROTEOSTASIS IN STEMNESS

11:30 - 11:45
Carlo Cusulin, *F. Hoffmann-La Roche Ltd, Switzerland*
THE ROLE OF Aβ IN INDUCING AN ALZHEIMER'S DISEASE SPECIFIC TRANSCRIPTIONAL PROFILE IN IPSC-DERIVED NEURONS

11:45 - 12:00
Agnes Maillet, *A*STAR, Singapore*
ANALYZING THE FUNCTIONAL IMPACT OF GENETIC VARIANTS ON DOXORUBICIN-INDUCED CARDIOTOXICITY USING GENOME EDITING TECHNOLOGY

PROGRAM SCHEDULE

12:00 - 14:30 LUNCH AND POSTER VIEWING

12:30 - 14:00 POSTER SESSION II

(EVEN NUMBERED POSTERS PRESENTED)

14:30 - 15:35 DRUG DISCOVERY II

Chair: **Melissa Thomas**, *Eli Lilly and Company, U.S*

14:30 - 15:00 **Martin Graf**, *F. Hoffmann-La Roche Ltd, Innovation Center Basel, Basel, Switzerland*
HUMAN STEM CELLS MODELS FOR DRUG DISCOVERY

15:00 - 15:15 **Brent Ryan**, *University of Oxford, United Kingdom*
ESTABLISHING PHENOTYPIC SCREENING ASSAYS IN IPSC-DERIVED DOPAMINERGIC NEURONS FROM PARKINSON'S DISEASE PATIENTS

15:15 - 15:45 **Viktor Lakics**, *AbbVie Deutschland GmbH and Co. KG, Germany*
INTRA- AND INTER-LABORATORY REPRODUCIBILITY OF A CELLULAR DISEASE PHENOTYPE AND A LONG-TERM DIFFERENTIATION PROTOCOL FOR HUMAN IPSC-DERIVED NEURONS: A MULTI-SITE, MULTI-OMICS STUDY

15:45 - 16:40 CLOSING KEYNOTE SESSION

15:45 - 16:30 **Keynote Address:**
Lorenz Studer, *Memorial Sloan Kettering Cancer Center, U.S.*

16:30 – 16:40 **Closing Remarks:**
Jack Price, StemBANCC

SPONSORS & EXHIBITORS

EXHIBITORS

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Alpha MED Scientific develops the most sensitive micro-electrode array (MEA) technology for in-vitro electrophysiology. The MED64 family of systems includes MEAs ideal for stem cell researchers who need the accuracy that only the most sensitive MEAs can provide. Our newest product, the MED64 Presto, combines the sensitivity of the MED64 in a multi-well, 384 electrode format for higher throughput electrophysiology.

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Axiogenesis is a leading provider of commercial-grade in vitro differentiated cells derived from human induced pluripotent stem cells (iPSCs), along with validated assays, which enable drug development. Assays for each cell type have been developed for advanced drug discovery, safety pharmacology / in vitro toxicology, and disease / tissue modeling. Axiogenesis' innovative and proprietary cell types and assays accelerate and improve drug candidate selection, lower costs and thus increase overall drug development efficiency. Key products include Cor.4U cardiomyocytes and fibroblasts, Peri.4U peripheral neurons, CNS.4U central nervous system cells, Dopa.4U dopaminergic neurons, and Astro.4U astrocytes. Products in development encompass iPSC-derived sensory neurons, skeletal muscle cells, as well as matured cardiomyocytes.

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SPEAKER ABSTRACTS

MONDAY, 27 FEBRUARY, 2017

OPENING KEYNOTE

NEURONAL REPLACEMENT AFTER INJURY - TURNING GLIA INTO NEURONS

Götz, Magdalena[1], Grade, Sofia[2], Mattugini, Nicola[3]
[1]Institute of Stem Cell Research, Helmholtz Zentrum Muenchen, Neuherberg/Munich, Germany, [2]Helmholtz Center Munich, Munich, Germany, [3]Ludwig-Maximilians University, Munich, Germany

A key question for neuronal replacement therapies is to which extent new neurons that can be either locally generated or transplanted are adequately integrated into the neuronal circuitry of brain regions that do not integrate new neurons normally as adults. I will present our recent data that brain-wide adequate connectivity and fully functional integration into the visual cortex can indeed be achieved for young neurons derived from late embryonic cerebral cortex transplanted after a mild injury (Falkner, Grade et al., 2016). I will now present unpublished data about the influence of the environment on this integration by comparing results in an invasive traumatic injury model and in the absence of any injury. I will then talk about what we know about these environmental factors and the mechanisms of scar formation before turning to the approach using direct neuronal reprogramming to replace degenerated neurons.

MODELING TISSUE DEVELOPMENT

REGULATION OF STEM CELLS IN THE ADULT MAMMALIAN BRAIN

Doetsch, Fiona
Biozentrum, University of Basel, Switzerland

Specialized niches support the life-long maintenance and function of tissue-specific stem cells. Adult neural stem cells in the ventricular-subventricular zone (V-SVZ) generate neurons as well as glia throughout life. V-SVZ stem cells are found at the interface between the cerebrospinal fluid, which flows through the brain ventricles, and a planar vascular plexus. Quiescent V-SVZ stem cells dynamically integrate intrinsic and extrinsic signals to become activated to divide and generate progeny. However little is known about the niche signals that mediate their behaviour. We show that the lateral ventricle choroid plexus (LVCP), which floats in the brain ventricles, is not only the primary producer of cerebrospinal fluid and important for homeostatic support functions, but is also a key compartment of the V-SVZ stem cell niche. The LVCP secretes diverse factors that differentially affect adult stem cells and their progeny. Importantly, the functional effect of the LVCP secretome changes throughout the lifespan, with neural stem cells being especially sensitive to age-related changes. I will also discuss the results of a small molecule screen we performed to identify activators of quiescent adult neural stem cells. Together these provide insight into novel regulators of adult neural stem cell dynamics and heterogeneity.

DISSECTING DNA METHYLATION DYNAMICS DURING THE DEVELOPMENT AND FUNCTION OF THE HUMAN SPINAL CORD

Kiskinis, Evangelos[1], Ziller, Michael J.[2], Ortega, Juan[1], Quinlan, Katharina[1], Meissner, Alexander[3]
[1]Northwestern University Feinberg School of Medicine, Chicago, U.S., [2]Max Planck Institute of Psychiatry, Munich, Germany, [3]Harvard University, Cambridge, U.S.

DNA methylation is a well-studied, yet still incompletely understood component of the multi-layered epigenome. The somatic DNA methylation landscape is established early in development, but remains highly dynamic within focal regions that tend to overlap with gene regulatory elements. The functional significance of these dynamic changes remains unresolved. The de novo DNA methyltransferases DNMT3A and DNMT3B are enzymes that methylate previously unmethylated DNA and exhibit a dynamic expression pattern in the central nervous system (CNS). Evidence from mouse models suggests they play diverse and critical roles during neural development and function. However, besides gross developmental phenotypes, little is known about their specific roles and molecular targets in the CNS and specifically in the development and function of the spinal cord. Here we utilized a powerful human embryonic stem cell (ESC) differentiation model for the generation of spinal cord neuronal subtypes including motor neurons (MNs), in combination with genetic mutations in the de novo DNA methylation machinery to dissect the role of DNA methylation in directing somatic cell fate. We specifically employed CRISPR/Cas9 gene editing strategies and established DNMT3A/DNMT3B and double 3A/3B knockout ESC lines and genetically matched wild-type control cells in combination with RNA sequencing and whole genome bisulfite DNA sequencing. Our quantitative and high-resolution molecular analysis allowed us to pinpoint the specific targets of de novo DNA methylation. We then employed functional, electrophysiological and optogenetic approaches to investigate the consequences of epigenetic perturbations during the

SPEAKER ABSTRACTS

development of spinal cord subtypes. We find that DNA methylation dynamics play a critical role in early neuralization and spinal cord fate commitment, and specifically impair motor neuron development. We also observe that specific DNA methylation pattern defects lead to decreased dendritic arborization and altered electrophysiological properties in motor neurons. Our work provides a specific list of epigenetically deregulated targets and a mechanistic link between de novo DNA methylation, cellular differentiation and human neuronal function.

Funding Source: Les Turner ALS Foundation, Target ALS Foundation, Muscular Dystrophy Association

MOLECULAR MODELING AT A SINGLE CELL LEVEL OF HUMAN MIDBRAIN DOPAMINERGIC NEURON DEVELOPMENT IN VIVO AND IN STEM CELLS

La Manno, Gioele[1], Gyllborg, Daniel[1], Villaescusa, Carlos J.[1], Salto, Carmen[1], Nishimura, Kaneyasu[1], Codeluppi, Simone[1], Zeisel, Amit[1], Toledo, Enrique M.[2], Borm, Lars[1], Stott, Simon RW[2], Lönneberg, Peter[1], Falk, Anna[3], Bruestle, Oliver[4], Barker, Roger[2], Linnarsson, Sten[1], **Arenas, Ernest**[1]

[1]Karolinska Institutet, Department of Medical Biochemistry and Biophysics, Stockholm, Sweden, [2]John van Geest Centre for Brain Repair, University of Cambridge, United Kingdom, [3]Karolinska Institutet, Neuroscience, Stockholm, Sweden, [4]LIFE & BRAIN Center, University of Bonn, Bonn, Germany

Most of our current knowledge of the molecular mechanisms controlling neural development comes from studies performed in species other than human or in human stem cells in vitro. Very little is thus known about the molecular players actually regulating human neural development in vivo. This knowledge is particularly important for the development of stem cell-based replacement therapies or in vitro disease modeling of neurodegenerative diseases such as Parkinson's disease, where midbrain dopaminergic neurons degenerate. In order to gain a better understanding of human midbrain development in vivo, we performed single-cell RNA-sequencing. This method allows de novo discovery of new cell types and the identification of uniquely expressed genes in each cell type. Our study identifies 27 different cell types in the developing human ventral midbrain from week 6-11, including unexpected cells such as 5 types of ventricular zone progenitors, 5 radial glia cell types and 3 types of embryonic dopaminergic neurons. This comprehensive reference dataset was subsequently used to assess the content and quality of human pluripotent stem cells as well as human neuroepithelial stem cell differentiated into dopaminergic neurons with GSK3β inhibitors and Shh. Single-cell RNA-seq analysis revealed a variety of stem cell-derived cell types corresponding to those found during midbrain development in vivo. A machine learning tool was used to rigorously and quantitatively compare the transcriptomes of each cell type in vitro and in vivo. Endogenous midbrain cells showed distinct, unambiguous identities characterized by stable states and rapid transitions to the next cell type within a lineage. Instead, pluripotent or neuroepithelial stem cells differentiated in a continuous manner, without sharp transitions, giving rise to many intermediate cell states not captured in vivo, but also high quality endpoint dopaminergic cells. We thus conclude that human stem cell differentiation protocols and the cell types obtained in vitro can be further improved. In sum, we think that the type of analysis used here can be used as a general strategy to identify ways to improve the differentiation of stem cells into specific cell types and to carefully assess the quality and safety of stem cell preparations prior to clinical application.

ENDODERMAL DIFFERENTIATION OF PLURIPOTENT STEM CELLS TO UNDERSTAND DEVELOPMENT AND REGENERATE IN VIVO ORGAN FUNCTION

Kotton, Darrell
Boston University School of Medicine, Boston, U.S.

Regeneration of in vivo organ function using pluripotent stem cell (PSC)-derived cells is dependent on the production of functional cells typically prepared in vitro through techniques, such as "directed differentiation." For example, the directed differentiation of pluripotent stem cells in culture into endodermal lineages typically relies on recapitulation of a sequence of developmental milestones known to occur during embryonic organogenesis of the desired cell or tissue type. However, this approach is limited when the mechanisms regulating in vivo organogenesis are unknown. This presentation will demonstrate the use of pluripotent stem cells to decipher the minimal pathways regulating specification of two endodermal organ primordia, the lung and thyroid, and the validation that these same pathways are evolutionarily conserved in vivo during embryonic organogenesis in mouse foreguts. Importantly, these signaling pathways can be utilized to generate lung and thyroid progenitors from normal mouse and human PSCs as well as from patient-specific iPS cells that carry genetic mutations responsible for disease. Finally, the regenerative functional potential of these cells is demonstrated through reconstitution of in vivo thyroid function in hypothyroid mouse recipients following transplantation of purified Nkx2-1+ thyroid progenitors derived from mouse pluripotent stem cells.

SPEAKER ABSTRACTS

FOXA2 MARKS A VENTRICULAR PROGENITOR POPULATION DURING HEART DEVELOPMENT

Dubois, Nicole[1], Bardot, Evan[2], Calderon, Damelys[2], Santoriello, Francis[2], Songyan, Han[2], Cheung, Kakit [2], Jadhav, Bharati [2], Burtscher, Ingo [3], Artap, Stanley [4], Jain, Rajan [4], Epstein, Jonathan [4], Lickert, Heiko [3], Gouon-Evans, Valerie [2], Sharp, Andrew J.[2]

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The identification of cardiac progenitor populations, such as the first and second heart fields, has fundamentally improved the understanding of cardiac development and disease. These progenitor populations contribute to the developing heart in a distinct spatial and temporal manner. However, they do not have chamber-specific differentiation potential, and the mechanisms that direct atrial versus ventricular specification remain largely unknown. Here we have identified a new progenitor population that gives rise to both ventricular chambers of the differentiated heart, but not the atria. We further determined that this progenitor population is specified during gastrulation when it transiently expresses Foxa2, a gene not previously implicated in cardiac development. Transcriptional analysis of Foxa2+ and Foxa2- progeny at different stages of cardiac development (cardiac mesoderm, cardiac crescent stage) reveals gene regulatory networks associated with Foxa2+ and Foxa2- cardiac progenitor populations, and potentially implicated in atrial-ventricular specification and differentiation. To demonstrate a functional relevance for Foxa2+ progenitor cells we performed competitive chimera experiments and genetic loss of function studies. Our results show that Foxa2-deficient cells largely fail to contribute to the ventricular chambers, and that abrogating key regulators of cardiac development in ventricular progenitors results in severely impaired heart development. Lastly, we translated our findings to the pluripotent stem cell (PSC) differentiation system with the goal to generate chamber-specific cell types. Using multiple reporter systems we identified isolated and characterized an analogous Foxa2+ cardiac mesoderm population in vitro. We determined the pathways that are involved in the generation of human FOXA2+ cardiac mesoderm, and show that FOXA2+ cells preferentially differentiate to ventricular cardiomyocytes in vitro. Together, these findings provide new insights into the developmental origin of ventricular and atrial cardiovascular cells, and may lead to the establishment of new strategies for generating chamber-specific cell types from PSCs for regenerative medicine and disease modeling approaches (Nature Communications, in press).

MAKING SKELETAL MUSCLE IN VITRO TO STUDY MUSCULAR DYSTROPHIES

Pourquié, Olivier
Department of Genetics, Harvard Medical School, Department of Pathology, Brigham and Women's Hospital, Harvard Stem Cell Institute, Boston, Massachusetts, U.S.

Progress toward finding a cure for muscle diseases has been slow because of the absence of relevant cellular models and the lack of a reliable source of muscle progenitors for biomedical investigation. We have developed an optimized serum-free differentiation protocol to efficiently produce striated, millimeter-long muscle fibers together with satellite-like cells from human pluripotent stem cells (ES/iPS) in vitro. By mimicking key signaling events leading to muscle formation in the embryo, in particular the dual modulation of Wnt and bone morphogenetic protein (BMP) pathway signaling, this directed differentiation protocol avoids the requirement for genetic modifications or cell sorting. Beyond the study of myogenesis, this differentiation method offers an attractive platform for the development of relevant in vitro models of muscle dystrophies and drug screening strategies, as well as providing a source of cells for tissue engineering and cell therapy approaches. We have introduced DMD-like mutations in human wild type iPS lines thus allowing to compare the effect of the mutations to the parental line of the same genetic background. We will discuss our results on the cellular mechanisms underlying Duchenne Muscular Dystrophy (DMD) obtained using such in vitro models of isogenic iPS cells differentiated to muscle cells.

SPEAKER ABSTRACTS

TUESDAY, 28 FEBRUARY, 2017

CELLULAR DISEASE MODELS I

STEM CELL MODELS FOR NEURODEGENERATIVE DISEASES: NEW THERAPEUTICS, SINGLE CELL APPROACHES AND CAVEATS

Webber, Caleb
University of Oxford, Oxford, United Kingdom

Patient-derived neuronal cell models hold much promise for the understanding of neurodegenerative disease. However, significant challenges remain which vary according to the disorder and neuronal cell type model. Here, I will first demonstrate the consequences of cellular heterogeneity to molecular comparisons and the importance of comparing equivalent cell populations. I will then illustrate how we are using dopaminergic neuron models in the Oxford Parkinson's Disease Centre to successfully identify candidate therapeutics with encouraging results. Finally, I will present findings from our single-cell efforts to elucidate the unfolding disease-associated processes being modelled.

Funding Source: IMI StemBANCC Monument Trust/ Parkinson's Disease UK Oxford University

THERAPEUTIC MODULATION OF AUTOPHAGY IN HUMAN INDUCED PLURIPOTENT STEM CELL-BASED MODEL OF A NEURODEGENERATIVE LIPID STORAGE DISORDER

Sarkar, Sovan[1], Jaenisch, Rudolf[2]
[1]*University of Birmingham, UK, Birmingham, United Kingdom,* [2]*Whitehead Institute for Biomedical Research, Cambridge, U.S.*

Autophagy dysfunction has been implicated in the accumulation of misfolded proteins and in cellular toxicity in diverse human diseases, whereas upregulation of this process is beneficial in various transgenic mouse models including a number of neurodegenerative disorders. However, the role of autophagy in the context of lysosomal lipid storage disorders associated with neurodegeneration is not clear. We have demonstrated defective autophagic flux in Niemann-Pick type C1 (NPC1) disease characterized with cholesterol accumulation in the late endosomal/ lysosomal compartments, where the maturation of autophagosomes was impaired due to defective amphisome formation caused by failure in the SNARE machinery. Compromised autophagy was shown in the liver and cerebellum of Npc1 mutant mice, as well as in neuronal and hepatic cells derived from NPC1 patient-specific induced pluripotent stem cells (iPSCs). These NPC1 iPSC-derived disease-affected cells also exhibited a cell death phenotype in the absence of any external stressors; thus providing a screening platform for therapeutic evaluation of drugs in these human cell types. TALEN-mediated correction of the disease-causing mutation in NPC1 iPSCs restored functional autophagy in the isogenic line, suggesting a role of the wild-type NPC1 protein in mediating this process. Of potential therapeutic relevance is that upregulation of autophagy in NPC1 disease restored autophagic flux and cargo clearance through a bypass mechanism independent of amphisome formation. Through chemical screens, we identified small molecule autophagy inducers that were effective in rescuing the autophagy defect and improving cell viability in NPC1 iPSC-derived neuronal and hepatic cells. However, exposure of NPC1 mutant cells to HP-β-cyclodextrin, a cholesterol-lowering agent currently being tested in patients, had an undesirable side-effect in further impeding autophagy. Nonetheless, PEG-lipid micelles effectively enabled cholesterol efflux without overtly compromising autophagy and cell viability; thus could be used as a combinatorial treatment strategy with autophagy inducers. Our study points to investigating the role and manipulation of autophagy in other iPSC-based models of lysosomal lipid storage and neurodegenerative disorders.

Funding Source: NIH, NNPD Foundation, Whitehead Institute (RJ), Birmingham Fellowship, Wellcome Trust (SS)

AGE-EQUIVALENT INDUCED NEURONS AND REJUVENATED IPSC-DERIVED NEURONS TO STUDY SPORADIC ALZHEIMER'S DISEASE IN THE CONTEXT OF HUMAN AGING

Mertens, Jerome, Gage, Fred H.
The Salk Institute for Biological Studies, San Diego, U.S.

Everybody ages. Unfortunately, this banal fact represents a huge health threat for our aging societies as old age is the preeminent risk factor for many human diseases with Alzheimer's disease (AD) leading the way. With the very rare exception of aggressive inherited versions of the disease, AD exclusively affects old people, while young and middle-aged people never get affected. Thus, biological aging plays a key role in the development of AD in humans. However, our current understanding of the cell biological manifestation of old age, and how it makes our brain cells more vulnerable to disease is surprisingly sparse. Current iPSC technologies allow us to study AD directly in human neurons that can be generated directly from patients' skin cells. However, the involved iPSC reprogramming

SPEAKER ABSTRACTS

process results in rejuvenation of the cells and thus yields cells that resemble 'baby' neurons rather than age-equivalent, or 'old' neurons. Here, we have established that the direct conversion of patient fibroblasts into induced neurons (iNs) allows circumventing the rejuvenating reprogramming process and results in cultures of age-equivalent neurons that show aging signatures at the transcriptional, nuclear pore and other levels. To study the involvement of cellular aging in AD pathogenesis, we took advantage of combing both technologies to generate both, 'old' iNs as well as 'baby' iPSC-iNs from a large set AD patients and healthy control donors. Using whole transcriptome RNA-Seq analysis of purified neurons, we identify transcriptional signatures that are specific for AD in patient-specific neurons, and test which of those are exclusively visible in 'old' iN cells, and which ones are also present in rejuvenated iPSC-iNs. This strategy allows to study age-dependent mechanisms in AD, with the goal to identify pathways that can be targeted to prevent pathogenesis.

IPSC DERIVED HYPOTHALAMIC NEURONS FROM AUTISTIC INDIVIDUALS SHOW MORPHOGENETIC ABNORMALITIES DURING EARLY DEVELOPMENT

Kathuria, Annie, Srivastava, Deepak, Price, Jack
King's College London, United Kingdom

Autism spectrum disorder is a neurodevelopment disorder that affects 1 in 68 children. There are very few human studies that describe the cellular pathophysiology behind this disease. In this study, we use human induced pluripotent stem cells as a way to understand the molecular underpinnings of ASD. iPSC lines were generated from hair root biopsies from three categories: autistic patients carrying deletions in synaptic genes (NRXN1 and SHANK3), non-syndromic patients, and healthy individuals. These lines were then differentiated into two different types of neurons- cortical and hypothalamic. Analysis of the hypothalamic neurons generated from the SHANK3 individuals, reported morphogenetic phenotypes: the cells developed differently from the controls, they were smaller and had longer neurites. Whilst the cortical neurons from the SHANK3 individuals reported no change in cellular morphology. This suggests that SHANK3 plays a different role in different regions of the brain. Furthermore, analysis of the hypothalamic neurons generated from the sporadic ASD individuals also showed a decrease in cell size when compared to the controls. The phenotypes observed in the neurons derived from iPSCs appear to be associated with autism. Thus, suggesting that we can not only track the pathophysiology of ASD in-vitro but also this can be used for future drug screening.

Funding Source: EU-AIMS and StemBANCC

MODELING FTDP-17 LINKED TAUOPATHIES AND ALZHEIMER'S DISEASE WITH GENETICALLY MODIFIED HUMAN IPSC

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Tauopathies like Alzheimer's disease (AD) and frontotemporal dementia (FTD) are among the most common forms of dementia currently lacking any cure. Development of an effective therapy remains challenging, partly due to the lack of predictive in vitro translational models. Over the past few years, induced pluripotent stem cells (iPSC) have been used to model various human disorders and have been shown to serve as useful translational in vitro tools. We have recently developed a more translational human tau aggregation model using AAV-induced expression of mutant P301L tau in human iPSC-derived cortical neurons. In the course of FTD, this mutation leads to aggregation of tau into neurofibrillary tangles, ultimately leading to cell death. Robust tau aggregation was triggered with K18 fibrils (tau 4-repeat domain) within a short time frame and this aggregation could be quantified with HTS-suitable AlphaLISA technology1. We employed this model for screening a small library of compounds. The first results of this screen will be discussed. Additionally, we also developed a tauopathy model by introducing the pathogenic P301S mutation in exon 10 of the MAPT gene using Zinc finger technology. To ensure the inclusion of tau exon 10 containing the P301S mutation, an additional intronic IVS10+16 mutation was introduced (P301S/IVS10+16; double mutant; DM). Both mutations have been linked with FTD. As a control, single mutant (SM) iPSC (IVS10+16) were generated, expressing wild type 4R and 3R tau. Transcriptomics, proteomics and functional assays were used to characterize these lines. All clones were pluripotent with a normal karyotype and the presence of the P301S and IVS10+16 mutations was confirmed. After differentiation into neurons, SM and DM neurons expressed both 3R and 4R tau while control neurons mainly expressed 3R tau. Live cell calcium imaging revealed a higher bursting frequency in DM compared to SM neurons and tau aggregation could be induced after extended culturing of DM neurons and addition of K18. Overall, these genetically modified iPSC-derived tau aggregation models reveal several phenotypes observed in tauopathies and may serve as valuable translational in vitro tools for drug discovery.

Funding Source Support by IMI Joint Undertaking (grant agreement n°115439), resources of which are composed of financial contribution from the European Union›s Seventh Framework Programme (FP7/2007-2013) and EFPIA companies in kind contribution

SPEAKER ABSTRACTS

MODELLING THE PHARMACOLOGICAL AND CLINICAL RESPONSE IN PRIMARY ERYTHROMELALGIA USING INDUCED PLURIPOTENT STEM CELLS

Nitzsche, Anja[1], Cao, Lishuang[1], McDonnell, Aoibhinn[1], Alexandrou, Aristos[1], Saintot, Pierre-Philippe[1], Loucif, Alexandre L.[1], Brown, Adam R.[1], Young, Gareth[1], Mis, Malgorzata[2], Randall, Andrew[2], Gutteridge, Alex[1], Waxman, Stephen G.[3], McKernan, Ruth M.[1], Whiting, Paul[1], Stevens, Edward B.[1], Bilsland, James[1]

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Patient derived induced pluripotent stem cells (iPSC) largely recapitulate disease pathophysiology and hold great promise for novel drug target validation. The technology has significant potential enabling novel therapeutics testing on individual patients and their cognate iPSC-derived cells, to better predict efficacy. However, there are few clear examples of a candidate therapeutic response in patient iPSC disease models actually correlating to an effect in the clinic, particularly for chronic neuropathic pain. Inherited Erythromelalgia (EM) is associated with gain-of-function mutations in the peripheral nerve sodium channel subunit Nav1.7 largely triggered by heat. In this study, the response of a potent small molecule to specifically inhibit the channel was investigated. Five EM patients, with unique mutations and well documented clinical history, received the channel inhibitor. There was an overall statistically significant treatment response, in a range correlating to a mechanistic effect described by the individual mutations. IPSC generated from these patients together with those from four healthy donors were differentiated into sensory neurons. Electrophysiological characterisation demonstrated spontaneously firing neurons, increased excitability and aberrant response to heat in the patient neurons. Careful analysis revealed a range of hyper-excitability linked to individual mutations, an effect normalised with the Nav1.7 inhibitor. EM is a complex clinical pain phenotype and no direct cellular to clinical response relationship was discernible, though a correlation of the mildest to most severe clinical phenotype did correspond with the preclinical hyper-excitability in cognate iPSC sensory neurons. This bench to beside approach is a proof-of-concept to illustrate the translational power of iPSC technology. IPSC derived sensory neurons enable recapitulation of sensory nerve fibre dysfunction in vitro, validation of the Nav1.7 channel as pivotal in the underlying disease mechanism and support the characterisation of a novel selective channel blocker as a putative treatment option. Future studies will verify if iPSC derived sensory neurons can be suitable for predicting treatment response in larger patient cohorts in a personalised medicine approach.

Funding Source: Pfizer Ltd.

MODELING OF SEVERE METABOLIC GENETIC DISEASES IN VITRO USING GENOME EDITING AND HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION TO VASCULAR ENDOTHELIAL CELLS

Roudnicky, Filip[1], Lan, Yanjun[2], Zhang, David Jitao[2], Dernick, Gregor[2], Staempfli, Andreas[2], Bordag, Natalie [3], Wagner Golbs, Antje [3], Christensen, Klaus [2], Graf, Martin [2], Meyer, Claas[2], Cowan, Chad[4], Patsch, Christoph[2]

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The use of human pluripotent stem cells (hPSCs) for in vitro disease-modeling is limited by the lack of robust and efficient protocols for the differentiation of relevant adult cell types. Recently, we have reported a method to generate vascular endothelial cells from hPSCs (Patsch et al. Nat Cell Biol. 2015). This novel and robust protocol in conjunction with use of programmed nucleases allowed us to model severe metabolic genetic disease in vitro. Vascular endothelium is considered to have an important role in development of metabolic insulin resistance. Therefore we have focused on two rare genetic diseases, showing insulin resistance, with mutations in AKT2: loss of function mutation or dominant active mutation (Glu17Lys (E17K)). Using TALENs we have previously generated an allelic series of isogenic hPSC lines with wild-type AKT2, knockout of AKT2, or a single AKT2E17K allele (Ding et al. 2013). We have successfully differentiated those hPSC lines to vascular endothelial cells in vitro. We have evaluated the RNA expression, metabolome and secretome of these lines and identified an increase in inflammation in knockout of AKT2 and AKT2E17K lines. Using leukocyte adhesion assay we could show an increase in leukocyte binding to AKT2 and AKT2E17K lines compared to wild-type AKT2. AKT2E17K line showed striking increase in secretion of inflammatory mediators: IL-6 (5-fold), IL-8 (2.2-fold), MCP-1 (2.8-fold) and PAI-1 (1.8-fold) compared to wild-type AKT2. These results could suggest that vascular dysfunction due dysregulated insulin signaling may contribute to coronary artery disease. Our further studies will be designed to elucidate this connection.

SPEAKER ABSTRACTS

INTERROGATING THE FULL SLC30A8 ALLELIC SPECTRUM IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ISLET-LIKE CELLS

Beer, Nicola Louise[1], Abaitua, Fernando[1], Champon, Benoite[1], Thomsen, Soren K.[1], Gaulton, Kyle[2], Thurner, Matthias[1], van de Bunt, Martijn[1], Kramer, Philipp[1], Moralli, Daniela[1], Hastoy, Benoit[1], Honore, Christian[3], Webber, Jonathan[1], Green, Cath[1], Gloyn, Anna L.[1], Davies, Ben[1], McCarthy, Mark[1]

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Using the full allelic spectrum to improve our understanding of type 2 diabetes (T2D) pathogenesis has great value in the design of future therapeutics. The gene encoding the islet-specific zinc transporter-8 (ZnT8; gene SLC30A8) harbours both common and rare coding variants associated with T2D susceptibility: the common W325R-SLC30A8 variant increases T2D risk (OR=1.14; p=4.8×10−23), whilst the rare predicted-protein truncating variant R138X-SLC30A8 variant reduces T2D risk (OR=0.47; p=0.0067). ZnT8 localises to insulin secretory granules where it controls granular Zn2+ influx, and insulin crystallisation and packaging within the beta-cell. Moreover, co-secreted Zn2+ also inhibits glucose-stimulated insulin secretion. The influence of common and rare SLC30A8-alleles on islet function remains to be established. Previous characterisation shows species-specific (rodent versus human) differences in ZnT8 activity and glucose homeostasis. We aimed to investigate the mechanisms influencing T2D susceptibility for rare and common coding SLC30A8-alleles in human islet-like cells. Using CRISPR-Cas9, we introduced the T2D risk R325-SLC30A8 and T2D-protective X138-SLC30A8 alleles into human induced pluripotent stem cells (hiPSCs). Heterozygous SLC30A8 variant iPSC lines were subjected to genomic and pluripotency quality control checks, before undergoing directed in vitro endocrine pancreas-lineage differentiation. Flow cytometry analysis showed all lines yielded ~30-50% insulin and NKX6.1 double-positive cells, with SLC30A8 induction confirmed in this same cell population via qRT-PCR. The effect of these variants on allele-specific SLC30A8 transcription, and the consequence of these mutations on the function of human islet-like cells, is being explored. This combination of iPSC genome editing and in vitro differentiation uniquely facilitates the study of T2D-associated SLC30A8 variants on human islet cell function, which has direct impact upon future drug design.

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EMERGING TECHNOLOGIES

ENGINEERING IN VITRO ORGANOGENESIS

Lutolf, Matthias P., Gjorevski, Nikolce, Ranga, Adrian
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The earliest steps of development are characterized by cellular reorganization and differentiation within a three-dimensional (3D) microenvironment. This 3D context allows for a complex spatial interplay between biochemical and mechanical signals, and governs important cellular rearrangements leading to morphogenesis. In vitro approaches have attempted to recapitulate key features of these processes, and it has now become possible to generate an increasing variety of self-organizing multicellular tissue constructs termed organoids. While important aspects of the 3D in vivo organization have been recreated in these organoid systems, such studies have been exclusively performed in animal-derived matrices whose properties cannot be readily modulated. As such, the uncharacterized interactions between cells and this extracellular matrix have proven to be a major challenge to understanding the underlying regulatory mechanisms governing morphogenesis. In this talk, I will highlight recent efforts in my lab to employ tunable synthetic hydrogels in order to disentangle the contributions of biochemical and mechanical effectors of the microenvironment in the specification of stem cell fate and self-organization.

CELLPHONES TO SUPERCOMPUTERS: HARDWARE AND SOFTWARE FOR INTEGRATED ANALYSIS AND VISUALIZATION OF 5-D MICROSCOPY MOVIES OF PROLIFERATING CELLS

Cohen, Andrew, Winter, Mark, Wait, Eric, Mankowski, Walter
Drexel University, Philadelphia, U.S.

Live cell and tissue microscopy captures 5-D images of proliferating human cells throughout processes of disease and development. These 5-D images are movies, with two or three spatial dimensions, plus multiple imaging channels plus time. Optical microscopes are improving dramatically, capturing ever higher spatial and temporal resolutions with increased tissue penetration and decreased phototoxicity. The biology also continues to improve, with new approaches based on induced pluripotency for obtaining cells, and new 3-D culturing techniques for organoids and tissue engineering. A single movie can contain over 1 terabyte of image data, with each experiment containing dozens, or even hundreds of such movies. Computational tools are required to explore and analyze this data. While

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there is still no "one size fits all" approach to working with these movies, there has been much progress towards broadly applicable solutions. Computational image analysis for live cell microscopy starts with automated algorithms for segmentation, tracking and lineaging. Segmentation delineates individual cells in each image frame. Tracking establishes temporal correspondences among segmentation results. Lineaging identifies parent-daughter relationships for dividing cells. These algorithms are continually improving, but still require human visualization and validation. Where current approaches rely on 2-D projections or slices, effective visualization requires true 3-D rendering that adaptively uses available hardware from workstation to laptops, cell phones and tablets, etc. Validation works in combination with the visualization, making any errors in the automated processing easy to identify and quick to correct. A new set of freely available software tools designed for integrated visualization and analysis called LEVER 5-D will be described. LEVER 5-D combines a cell-type and imaging-modality specific segmentation with application independent tracking, lineaging, visualization and validation. A related program called CloneView 5-D makes the LEVER analysis together with the image data interactively available on any web-connected device. Together, these programs provide a new open source/open data approach to measuring 5-D images of proliferating cells and extracting meaningful knowledge.

QUANTITATIVE MULTIDIMENSIONAL TISSUE CYTOMETRY OF MURINE BONE AND MARROW STEM CELLS

Coutu, Daniel L., Kunz, Leo, Kokkaliaris, Konstantinos D., Schroeder, Timm
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Bone and bone marrow harbor a dual stem cell system where hematopoietic stem cells (HSCs) self-renew and differentiate to produce all blood and immune cells and skeletal or mesenchymal stem cells (MSCs) produce bone, cartilage and the marrow stroma responsible to support hematopoiesis. The molecular phenotype of HSCs is well characterised but the microenvironments or niches where they self-renew and differentiate is less well understood. The cells known as MSCs on the other hand are still poorly characterised and comprise heterogeneous cell subpopulations. Hence, the molecular and cellular architecture of bone marrow is in large part unknown, due primarily to technical difficulties in imaging bone and marrow tissues. We here describe our efforts to develop methods allowing the identification of MSCs in vivo and their lineage hierarchy using multidimensional FACS analysis, bioinformatics tools, 3D multicolor imaging and lineage tracing techniques. Flow cytometry on over 20 putative MSC markers is used to identify skeletal cell subpopulations. These are then mapped in bone sections using up to 8 colors immunofluorescence and deep (700mm) confocal imaging. Genetic labeling and lineage tracing is then used to decipher the lineage hierarchy between these populations. Combined with quantitative 3D image analysis, we also show how our novel imaging technique is useful to analyse the complex microenvironments of the bone marrow niches where HSCs self-renew and their progeny differentiate.

INTEGRATING OPTOGENETIC TECHNOLOGIES INTO HUMAN INDUCED PLURIPOTENT STEM CELL RESEARCH

Akerman, Colin J.
University of Oxford, United Kingdom

Optogenetic technologies typically involve the use of light-sensitive proteins to control the flux of ions in order to modulate the behaviour of cells. This approach has had its greatest impact in the field of neuroscience, where it has been used to selectively increase or decrease the electrical excitability of neurons in a spatially and temporally controlled manner. However, the fact that light can be used to move key ion species across the plasma membrane affords new opportunities to use these technologies as ion modulatory tools in non-excitable cell types. I will describe two examples where my group are using optogenetic strategies to investigate ion-sensitive processes in cell types derived from human induced pluripotent stem cells (iPS cells). The first example involves the use of light-activated ion pump proteins to regulate the proliferative behaviour of neural stem cells derived from human iPS cells. Through optical manipulation of intracellular ion concentration, we are able to control the cell cycle dynamics of neural progenitor cells. The second example involves the use of light-activated ion channel proteins to control the behaviour of astrocytes. This approach is enabling us to investigate specific signalling mechanisms that operate between astrocytes and neurons in a human co-culture system.

Funding Source: ARUK, IMI STEMBANCC

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DRUG DISCOVERY I

INHIBITION OF DYRK1A DISRUPTS NEURAL LINEAGE SPECIFICATION IN HUMAN PLURIPOTENT STEM CELLS

Pera, Martin F.[1], Bellmaine, Stephanie F.[1], Ovchinnikov, Dmitry A.[2], Mallanack, David T.[3], Wolvetang, Ernst J.[2], Elefanty, Andrew G.[4], Stanley, Edouard G.[4], Williams, Spencer J.[5]

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Genetic analysis has revealed that the dual specificity protein kinase DYRK1A has multiple roles in the development of the central nervous system. Increased DYRK1A gene dosage, such as occurs in Down syndrome, is known to affect neural progenitor cell differentiation, while haploinsufficiency of DYRK1A is associated with severe microcephaly. Using a set of known and newly synthesized DYRK1A inhibitors, along with CRISPR-mediated gene activation, we show here that chemical inhibition of DYRK1A interferes with neural specification of human pluripotent stem cells, a process equating to the earliest stage of human brain development. Specifically, DYRK1A inhibition insulates the self-renewing subpopulation of human pluripotent stem cells from powerful signals that drive neural induction. Our results suggest a novel mechanism for the disruptive effects of the absence or haploinsufficiency of DYRK1A on early mammalian development, and reveal a requirement for DYRK1A in the acquisition of competence for differentiation in human pluripotent stem cells.

Funding Source: Australian Research Council; National Health and Medical Research Council; University of Melbourne

PITFALLS AND PROMISE OF USING HUMAN STEM CELL FOR STUDYING DISEASE

Eggan, Kevin
Harvard University, Cambridge, U.S.

The discovery of human ES and iPS cells now makes it principally possible to produce a limitless quantity of diverse cell types for the study of disease. I will describe both success stories and challenges encountered during our efforts to implement these strategies in the context of studying developmental and degenerative disorders of the nervous system.

WEDNESDAY, 1 MARCH, 2017

CELLULAR DISEASE MODELS II

HUMAN PANCREAS DEVELOPMENT AND DISEASE THROUGH THE LENS OF PLURIPOTENT STEM CELLS

Huangfu, Danwei
Memorial Sloan Kettering Cancer Center, New York, U.S.

My laboratory is interested in understanding pancreatic development and disease through applying genetic approaches in human pluripotent stem cells (hPSCs), including embryonic and induced pluripotent stem cells (hESCs and hiPSCs). Combining CRISPR/Cas-mediated gene editing and stem cell technologies, our reverse genetics approach has revealed the roles of key transcription factors pancreatic development and diabetes. Using the forward genetics approach, we performed the first genome-wide knockout screen for identification of developmental regulators. We identified novel genes that regulate the formation of definitive endoderm cells, which give rise to most pancreatic cells among other cells in the respiratory and digestive tract. Together our findings establish the use of hPSCs as a genetic model system for studying congenital defects and diabetes.

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RECONSTRUCTION OF A FUNCTIONAL THALAMO-CORTICAL PATHWAY IN STROKE-INJURED RAT BRAIN USING HUMAN SKIN-DERIVED CELLS

Tornero, Daniel, Lindvall, Olle, Kokaia, Zaal
Lund University, Lund, Sweden

Intracerebral transplantation of stem cells or their progeny leads to improved function in animal models of stroke through different mechanisms such as modulation of inflammation, trophic action and stimulation of plasticity. Whether graft-derived neurons replace those neurons which have died, receive functional synaptic inputs from the recipient's brain, integrate into host neural circuitry, and thereby contribute to recovery is virtually unknown. We now show using electron microscopy and rabies

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virus-based trans-synaptic tracing that human induced pluripotent stem cell (iPSC)-derived cortical neurons, when implanted in stroke-injured rat cortex, receive direct afferent monosynaptic connections from the same brain structures, including cortex and thalamic nuclei, which normally project to cortical neurons located at the implantation site. We demonstrate by electrophysiological in vivo recordings that these afferent inputs are functional, and that the grafted neurons respond to mechanical stimulation of nose and paw. We confirm the functionality of the synapses on the grafted cells using optogenetic stimulation of afferent axons from host thalamic neurons. The reestablishment of functional monosynaptic connections from a normal pattern of multiple brain areas indicates that human iPSC-derived cortical neurons can participate in the reconstruction of injured neuronal circuitries such as the thalamo-cortical pathway. Our findings demonstrate the capacity of stem cell-derived neurons, and specifically human iPSC-derived cortical neurons, to replace those neurons which have died and restore specific functional synaptic connections in the stroke-injured cerebral cortex. Although only representing a first and early step in a clinical translation, our study illustrates the potential of functional restoration by cortical neuronal replacement as a future therapeutic approach for stroke and other neurodegenerative diseases affecting cerebral cortex.

IN VITRO MODELING OF GLUCOCEREBROSIDASE (GBA) FUNCTION IN PARKINSON´S DISEASE

Fedele, Stefania[1], Gündner, Anna Lisa [2], Zhang , Jitao David [2], Ruf, Iris[2], Müller, Stephan[2], Certa, Ulrich[2], Graf, Martin[2], Jagasia, Ravi[2], Collo, Ginetta[3], Taylor, Verdon[3]
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Mutations in the GBA gene (glucocerebrosidase), which encodes the lysosomal enzyme acid-β-glucocerebrosidase (GCase), are known to cause Gaucher disease (GD), the most common lysosomal storage disorder. In recent years, it has been reported that GBA mutations represent the strongest common genetic risk factor for Parkinson´s disease (PD). However, the specific contribution of mutant GBA to the pathogenesis of PD remains unknown. Here we analyze the role of GBA in dopaminergic (DA) neurons derived from genetically engineered human embryonic stem cells (GBA+/+ and GBA-/- hESCs). We described for the first time aberrant DA neuronal phenotypes resulting from loss of GCase activity and these can be rescued by recombinant GCase enzyme treatment. To identify neuron-specific pathways affected by GCase deficiency we performed exome-sequencing analysis on DA neuron cultures derived from mutant GBA-/- hESCs. Importantly, the aberrant phenotypes found in GBA-/- hESC-derived DA cultures were recapitulated in DA neurons derived from PD patient-derived induced pluripotent stem cells carrying the heterozygous GBA-N370S mutation (GBA-N370S hiPSCs). Our results indicate a central role for the lysosomal GCase enzyme in the control of DA neuron differentiation. I will show our results analyzing the molecular pathway downstream of aberrant GCase activity in Parkinson´s disease.

STEM CELLS TO MODEL AND TO STUDY LIVER DISEASES

Vallier, Ludovic[1], Sampaziotis, Fotis[2], Morell, Carola[2], Rimland, Casey[2], Tilson, Samantha[3], Saeb-Persy, Kourosh[4]
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End-stage liver disease is one of the most common cause of mortality in modern countries and can only be treated by liver transplantation, which is strongly limited by the lack of organ donors and the side effects of immunosuppressive treatment. Therefore, the development of new therapeutics against liver diseases represents a global health challenge. However, the lack of relevant, physiological and high through put in vitro system to model liver disease represents a major challenge. Indeed, current models consist in cell cultures, precision slice biopsies, and whole organ perfusion systems or animal models. 2D culture systems lack functional cell-to-cell interactions and anatomical structures needed to reveal mechanistic details of

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disease involved in fibrosis, while perfusion and biopsy based models cannot persist long enough to accurately model fibrotic progression. Finally, animal models while essential often lack the physiological relevance to human diseases. For all the reasons, the use of human pluripotent stem cells and more recently of primary liver organoids provide a unique opportunity to model liver disease in vitro. Indeed, these stem cell can grow indefinitely in vitro while maintaining the capacity to differentiate into the main functional cell type of the liver: hepatocytes and cholangiocytes. The resulting cells can then be grown in 3D culture system mimicking the architecture of the liver to uncover the deepest molecular mechanisms directing disease onset. Here, we will describe our latest progress in developing such culture systems not only to model but also to study liver diseases of genetic origin and induced by chronic injury.

Funding Source: This research is funded by the ERC grant Relieve IMDs, the NC3Rs, the Wellcome Trust and MRC.

ROLE OF PROTEOSTASIS IN STEMNESS

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Skeletal muscle has a remarkable capacity to regenerate by virtue of its resident Pax7-expressing stem cells (satellite cells), which are normally quiescent in the adult. Upon injury, quiescent satellite cells activate and proliferate, to subsequently differentiate and form new myofibers or self-renew to restore the quiescent satellite cell pool. Through a combination of global gene expression/bioinformatics and molecular/cellular assays, we found that resting satellite cells have basal autophagy activity, which is required to maintain the quiescent state. Impaired autophagy in old satellite cells provokes loss of proteostasis and oxidative stress. We will discuss the consequences of the autophagy failure in the regenerative potential of muscle stem cells, particularly in the context of aging.

THE ROLE OF ABETA IN INDUCING AN ALZHEIMER'S DISEASE SPECIFIC TRANSCRIPTIONAL PROFILE IN IPSC-DERIVED NEURONS

Cusulin, Carlo, Christensen, Klaus, Graf, Martin, Burcin, Mark, Baumann, Karlheinz, Patsch, Christoph
Hoffmann-La Roche, Basel, Switzerland

Alzheimer's disease (AD) is a neurodegenerative disorder, characterized by β-amyloid deposits, intracellular Tau aggregates, neuronal death and dementia. The disease is prevalently sporadic, but the hereditary cases provided a great insight into its etiology. In the familial forms, mutations typically involve genes encoding amyloid precursor protein (APP) or presenilin, the catalytic component of the γ-secretase complex. iPSCs derived from these patients can be differentiated in neurons that show abnormal production of the Aβ peptides. Such cellular system offers the possibility to investigate the role of these peptides in the pathological process. We used potent and specific compounds that are able to block or alter the production of amyloid peptide to investigate the role of Aβ in downstream cellular functions. The compounds belong to three different classes, β-secretase inhibitors (BACEi), γ-secreatse inhibitors (GSI) and modulators (GSM), and act in different ways on the processing of β-amyloid. We first verified that these compounds were able to reduce the production of Aβ40 and/or Aβ42 in both control and patient derived neurons. All three compounds were very effective, with IC50s in the nanomolar range. Subsequently, we studied the effect of compound treatment on the expression of Tau and its phosphorylation. As previously described, high doses of GSI led to the accumulation of Tau, which was dependent on the accumulation of the c-terminus of APP. This effect was not observed when cells were treated with the other compound classes. Finally, to fully understand the role of amyloid peptides in the cellular function, we performed RNA-sequencing on the patient-derived neurons that were treated with the BACEi, GSM or GSI at doses that would substantially reduce the production of Aβ42. As expected, GSI treatment induced the most substantial transcriptional changes, because of the Notch-related effects. The comparison of gene expression after treatment with compounds allowed us to search for common pathways that are downstream of Aβ and likely to be relevant in the pathological processes in AD. While the relevance of β-amyloid production in the context of neuronal death needs to be elucidated further, our model provides a robust platform to investigate the ongoing pathological processes of AD in vitro.

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ANALYZING THE FUNCTIONAL IMPACT OF GENETIC VARIANTS ON DOXORUBICIN-INDUCED CARDIOTOXICITY USING GENOME EDITING TECHNOLOGY

Maillet, Agnes, Tan, Kim , Brunham, Liam
*A*STAR, Singapore, Singapore*

Pharmacogenomics has recently allowed the identification of important genetic markers that predispose to specific adverse drug reactions (ADRs), such as cisplatin-induced ototoxicity, statin-induced myopathy or anthracycline-induced cardiotoxicity. However, identification of DNA variants associated with specific drug response phenotypes, in general, cannot prove that a given DNA variant is causally related to the phenotype in question and laboratory-based functional assessments are necessary. In the past decade, human pluripotent stem cells (hPSC) have emerged as powerful tools to study human phenotypes in physiologically relevant models. Recently, our group has used hPSC-derived cardiomyocytes (hPSC-CMs) to describe the characteristics of doxorubicin-induced cardiotoxicity (DIC). We found that doxorubicin causes dose-dependent increases in apoptotic and necrotic cell death, reactive oxygen species production, mitochondrial dysfunction and increased intracellular calcium concentration. We characterized genome-wide changes in gene expression caused by doxorubicin using RNA-seq, as well as electrophysiological abnormalities caused by doxorubicin with multi-electrode array technology. Finally, we showed that disruption of TOP2B, a gene implicated in DIC in mouse studies, significantly reduces the sensitivity of hPSC-CMs to doxorubicin-induced cell death. This cellular model system allows the study of specific pharmacogenetic variants associated with DIC, by introducing these variants into hPSCSs using CRISPR/Cas9 and evaluating the difference in response to doxorubicin in mutant cells compared to wild-type cells.

DRUG DISCOVERY II

HUMAN STEM CELLS MODELS FOR DRUG DISCOVERY

Graf, Martin
F. Hoffmann- La Roche Ltd., Basel, Switzerland

At Hoffmann-La Roche, in 2009, only 3 years after Shinya Yamanaka's groundbreaking publication, we started to work with human pluripotent stem cells. Our goal is to develop relevant cellular in vitro models to test drug candidates. Towards this, we have implemented several protocols to differentiate cell lineages such as neurons, endothelial cells, smooth muscle cells, adipocytes, cardiomyocytes, hepatocytes, retinal pigment epithelium and podocytes. I will present case studies to show how we apply stem cell technologies to drug discovery. For us collaborations have been crucial to enable this work – only if we have access to the know-how of the scientific community we can be successful. Along these lines we have started in 2012 together with 34 partners a large IMI project (Innovative Medicines Initiative) the StemBANCC consortium. 11 Pharmaceutical companies and 23 academic institutions are working closely together to generate iPSCs from 500 patients and to use these cells for in vitro disease modelling. We have recruited patients in the area of peripheral nervous system disorders, central nervous system disorders, diabetes and patients with adverse toxicological effects. StemBANCC has become a rich source of patient iPS lines and even more important a network of scientist that closely work together.

ESTABLISHING PHENOTYPIC SCREENING ASSAYS IN IPSC-DERIVED DOPAMINERGIC NEURONS FROM PARKINSON'S DISEASE PATIENTS

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A number of cellular and biochemical deficits have been demonstrated to be involved in genetic forms of Parkinson's disease (PD). These include progressive aggregation of the pre-synaptic protein α-synuclein, mitochondrial dysfunction and perturbation of the autophagic/lysosomal pathway. To study these phenotypes in disease-relevant models, we have generated iPSC lines from over 100 individuals with genetic and sporadic forms of PD from which, we are able to differentiate these iPSC into dopaminergic neuronal

SPEAKER ABSTRACTS

cultures. As an exemplar, we have studied phenotypes in iPSC-derived dopaminergic neuronal cultures differentiated from PD patients with mutations in the gene encoding the lysosomal enzyme GBA. Phenotypes have been identified using a combination of –omics and targeted approaches. GBA patients demonstrate a 50 % decrease in GBA activity, in line with previous reports of heterozygous mutations in N370S carriers. We have also shown that GBA patients demonstrate a 2.5 fold increase in LC3 lipidation and a 3 fold increase in LAMP1 levels, indicating deficits in the autophagosome/lysosomal system. Furthermore, we observed increased α-synuclein release in patients with GBA mutations as measured by MesoScale Discovery assay. Additionally, mass-spectrometry has been carried out on iPSC lines and pathway analyses were performed using Ingenuity Pathway Analysis software to identify novel phenotypes in GBA lines. These phenotypes have been used as the basis of several screens including a dual-phenotypic assay assessing both in α-synuclein release and GBA activity. To this end, maturation of dopaminergic neurons was successfully miniaturised into 384-well plates for screening purposes. Small molecule libraries have been screened and potential modulators of these phenotypes identified. These data demonstrate a possible route for translation of iPSC phenotypes into medium-throughput screening and lead compound identification.

Funding Source: This research was supported by Parkinson's UK Monument Trust Award and StemBANCC Innovative Medicines Initiative Joint Undertaking n° 115439 from the European Union's Seventh Framework Programme (FP7/2007-2013)

INTRA- AND INTER-LABORATORY REPRODUCIBILITY OF A CELLULAR DISEASE PHENOTYPE AND A LONG-TERM DIFFERENTIATION PROTOCOL FOR HUMAN IPSC-DERIVED NEURONS: A MULTI-SITE, MULTI-OMICS STUDY

Lakics, Viktor[1], STEMBANCC Consortium Partners[2]
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Large public-private consortia offer excellent opportunities to design unique studies, performed in multiple laboratories to approach scientific questions otherwise difficult to address. In the STEMBANCC IMI consortium, we have completed a multi-site, multi-omics experiment to assess the intra- and inter-laboratory reproducibility of a long-term neuronal differentiation protocol for human induced pluripotent stem cells (hiPSCs) and a previously published disease phenotype. To determine whether robust differences between two iPSC lines were discernible, cortical neuronal differentiation of a healthy control and a monogenic Alzheimer's disease patient-derived hiPSC line (Presenilin-1 mutant) were induced multiple times in five different laboratories and samples were collected at two time points for unbiased transcriptomic and proteomic analyses. To confirm the reproducibility of a disease phenotype, the ratios of secreted beta-amyloid peptides were also measured. Intra-laboratory reproducibility of omics readouts was acceptable and excellent cross-site reproducibility of the previously identified disease phenotype was demonstrated. However, omics datasets from the five laboratories displayed large variation, rendering specific differences between the two differentiated hiPSC lines indistinguishable from each other in a combined dataset. Despite this significant inter-laboratory variation, we have demonstrated that using appropriate computational approaches and leveraging the power from collaborative datasets, the unwanted variance in omics data can be identified, making the detection of specific differences between hiPSC-derived neurons from different genetic backgrounds possible.

Funding Source: This project was supported by StemBANCC funding from the Innovative Medicines Initiative Joint Undertaking (Grant No: 115439); resources from the EU '7th Framework Program (FP7/2007e2013) ' and EFPIA companies in kind contribution.

CLOSING KEYNOTE II

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Abstract not available at time of printing.

POSTER ABSTRACTS

ODD NUMBERED POSTERS WILL BE PRESENTED TUESDAY, 28 FEBRUARY 12:30 TO 14:00

POSTER SESSION I

Note: Abstracts for Poster Session II (even-numbered posters) can be found on page 69

CELLULAR DISEASE MODELS

P001

THE ROLE OF LRRK2 IN HUMAN IPSC-DERIVED MACROPHAGES

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Mounting evidence suggests that immune dysregulation contributes to neurodegeneration in Parkinson's Disease (PD). Mutations in leucine rich repeat kinase 2 (LRRK2) are one of the most common genetic cause of PD, yet its function is still far from clear. Recent evidence implicates LRRK2 in immune function. LRRK2 protein is expressed abundantly in immune cell subsets, particularly myeloid cells, and its variants are risk factors for inflammatory bowel disease and leprosy. We have used macrophages derived from genetically tractable human induced Pluripotent Stem Cells (hiPSCs) to explore the bona fide function of endogenously-expressed LRRK2 in the most authentic cellular system available. We have generated hiPSC lines from LRRK2 mutation-carrying PD patients, age-matched controls, and have also made CRISPR-Cas9-edited hiPSC lines: LRRK2 knock out (KO), isogenically corrected G2019S mutation, and mCherry-tagged LRRK2. These hiPSC lines are then differentiated into macrophages, using a scalable, defined protocol developed in our lab. Physiological interactors of LRRK2 have been identified in hiPSC-macrophages by co-immunoprecipitation (co-IP) followed by mass spectrometry, using LRRK2 KO macrophages as a negative control. Immunostaining and confocal imaging analysis further reveals that LRRK2 is recruited to phagocytosed bioparticles, strongly suggesting its involvement in phagocytosis, which is a key function of macrophages and of their brain-resident counterparts, microglia. We are using an hiPSC-neuron-microglia co-culture model developed in our lab to extend these results to microglia.

Funding Source

We acknowledge financial support from the Wellcome Trust (WTISSF121302), the Oxford Martin School (LC0910-004), the Oxford Parkinson's Disease Centre (OPDC) and StemBANCC.

P007

MODELLING NEUROPSYCHIATRIC DISEASES USING PATIENT DERIVED IPSC

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Over the past few years iPSC have become valuable tools for the in-vitro modeling of neuropsychiatric diseases. Starting with the neuronal differentiation of iPSC from schizophrenia (SZ) patients, multiple groups reported disease associated phenotypes to proof this concept. Whereas some of these studies focused on neurodevelopmental aspects of the disease others described aberrant neuronal activity which is a common observation in SZ patient-based neuroimaging studies. In the present study, we differentiated iPSC derived from healthy donors and those affected with idiopathic SZ towards neuronal forebrain lineage. After around 8 weeks of total differentiation we observed first mature responses in terms of unprovoked spiking and burst-firing during multi-electrode array recordings (MEA, 24- and 96-well). The neurons also showed sensitivity to the application of neuromodulators such as bicuculline and NBQX. For the first time we also demonstrated homeostatic synaptic scaling of iPSC-derived neurons in terms of synaptic up- and downscaling in response to MK801 (NMDA receptor antagonist) or 4-AP (potassium channel blocker) incubation respectively. We then screened for disease-associated pathophysiologies using MEA and calcium imaging techniques (FLIPR, 384-well). In line with previous reports of genetic SZ iPSC models and patient-based studies we can conclude hypoactive and hypoexcitable forebrain neurons in our SZ lines. To identify underlying mechanisms involved in the altered firing behavior we performed next generation RNA sequencing and found several deregulated genes that play a pivotal role in neuronal excitability and neuronal firing probabilities. To provide more mechanistic proof for the hypoactivity phenotype, the neurons will be analysed by high content imaging to unravel potential deficits at pre- or postsynaptic sites. Based upon these insights we currently are designing approaches to rescue the pathophysiological phenotypes using tool compounds and siRNA technique.

POSTER ABSTRACTS

P009

NEURAL STEM CELL ASSOCIATED DEFECTS IN LIPID METABOLISM AND HUMAN COGNITIVE IMPAIRMENT

Bowers, Megan[1], Liang, Tong[1], Kovacs, Werner[2], Rohrl, Clemens[3], Gonzalez, Daniel[1], Cramb, Kaitlyn[2], Semenkovich, Clay F.[4], Jessberger, Sebastian[1]

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Adult hippocampal neurogenesis regulates specific types of learning and memory in rodents. Although adult neurogenesis persists throughout life in the hippocampus of humans as well, it remains controversial whether this process plays a significant role in human cognition. Fatty acid synthase (FASN), a key enzyme for de novo lipogenesis, is a critical regulator of adult neurogenesis in the mouse and is required for spatial learning and memory in a mouse model of running-induced adult neurogenesis. Underscoring the relevance of these findings to humans, a point mutation in FASN (FASN; R1819W) was identified in individuals with non-syndromic cognitive impairment, suggesting an intriguing hypothesis that impaired neurogenesis may contribute to the cognitive deficits observed in these individuals. To address this hypothesis FASN R1812W knock-in mice and human embryonic stem cell (hESC) lines with biallelic FASN R1819W mutations were generated. Adult NSCs isolated from mice homozygous for the R1812W mutation display reduced proliferation compared to NSCs isolated from wild-type littermates. Mechanistically, we show that the reduction in NSC proliferation is concomitant with a paradoxical increase in FASN enzyme activity, a dramatic increase in the rate of de novo lipogenesis, an accumulation and redistribution of lipids, and activation of the ER stress response in adult NSCs. Consistent with these findings, both proliferation and survival of adult born NSCs is reduced specifically in the hippocampus of mice homozygous for FASN R1812W in vivo by age 8 months. These data suggest that the FASN point mutation functions as a deleterious gain-of-function allele that enhances FASN enzyme activity, resulting in a lipogenic phenotype and exerting its effects via lipotoxicity in adult NSCs. Our findings support a model of NSC associated lipid metabolism in which the level of de novo lipogenesis must be kept within an optimal range to support normal NSC proliferation. Analyses of hippocampus-dependent behavior in FASN R1812W mice, single cell transcriptomics of NSCs in vivo, and in vitro disease modeling with NSCs derived from FASN R1819W mutant hESCs will clarify the implications of exceeding the cellular capacity to accommodate increased de novo lipogenesis for cognitive impairment in humans with the FASN R1819W mutation

Funding Source

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P011

DEVELOPMENT OF DEFINED CONDITIONS FOR EXPANSION AND DOPAMINERGIC NEURON DIFFERENTIATION OF IPSC-DERIVED NEURAL PROGENITOR CELLS

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Human iPSCs have the capacity to differentiate into all of the somatic cells types and hold great promise for both regenerative medicine and drug discovery. A need for better tools to address neurological disease modeling and neuro-toxicology screening exists. We have developed a scalable process that allows for the generation of large quantities of NPC derived from normal and Parkinson's disease iPSC lines, along with a serum-free defined NPC expansion medium and dopaminergic differentiation medium. To validate the process of NPC derivation, we generated NPCs derived from iPSCs that were reprogrammed with the Sendai virus from the following sources: human foreskin fibroblasts (HFF-1), human CD34+ cells, and human fibroblasts from a patient with Parkinson's disease. Compared to Parkinson's disease patient-derived NPCs, both normal NPC lines demonstrated greater proliferative capacity. Moreover, our CD34+ cell-derived NPCs possessed better tri-lineage differentiation efficiency than that of fibroblast-derived NPCs although all three types of NPCs were capable of differentiating into dopaminergic neurons, astrocytes, and oligodendrocytes. To validate our NPC expansion and dopaminergic differentiation media, we tested 3 types of NPCs derived from iPSCs of various origins as described above as well as three lineage-specific reporter NPC lines (MAP2p-NanoLuc®-HaloTag®, DCXp-GFP, and GFAPp-NanoLuc®-HaloTag®) generated by targeting a NanoLuc®-HaloTag® or GFP construct to the C-terminus of the MAP2, DCX, or GFAP genes via zinc finger nucleases. Regarding morphology, proliferative capacity, and expression of NPC markers, the NPC expansion medium performed well for all six types of NPCs. Importantly, the expanded NPCs maintained the capacity to differentiate into dopaminergic neurons, astrocytes, and oligodendrocytes. Furthermore, the dopaminergic neuron differentiation medium was validated for the efficient differentiation of six types of NPCs into dopaminergic neurons. Through this study we have developed a portfolio of NPCs along with an NPC expansion medium and dopaminergic neuron differentiation medium. Collectively, these provide a complete solution for the expansion and differentiation of NPCs and comprise a powerful tool for neurodegenerative disease modeling and drug screening.

POSTER ABSTRACTS

P013

DRIVING CELLULAR AND PHARMACOLOGICAL DIVERSITY OF INDUCED NEURONS BY DEFINED TRANSCRIPTION FACTOR PAIRS

Lee, Sohyon[1], Tsunemoto, Rachel[1], Szűcs, Attila[2], Sokolova, Irina[1], Chubukov, Pavel[1], Eade, Kevin T.[1], Blanchard, Joel W.[1], Sanna, Pietro P.[1], Baldwin, Kristin K.[1]

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Ion channels and receptors represent a class of genes implicated in virtually all neurological conditions ranging from addiction, mood disorders, and pain. However, due to the lack of primary human neurons and understanding of neuronal subtypes, assessing how genetic differences impact the function of a given receptor has been challenging. Therefore, being able to generate neurons that functionally resemble endogenous neurons would represent a valuable resource for modeling human disease in vitro. In this study, we screened ~600 transcription factor (TF) pairs and identified 76 pairs that produced induced neurons (iNs) from mouse fibroblasts. By comparing the transcriptomes of iNs to endogenous neurons, we were able to link expression of different TF pairs to diversity in neurotransmitter and receptor expression. Expression levels of ion channels and receptors in turn correlated with diverse electrophysiological and pharmacologic properties. TF pairs that induced expression of receptors implicated in diseases such as nicotine addiction, alcoholism, and schizophrenia were also capable of generating functional human iNs. As a proof-of-principle, we examined the cellular impact of the strongest risk haplotype linked to nicotine addiction by generating nicotine responsive human iNs and conducting Ca2+ imaging. We expect the results of this study to be applicable to future pharmacogenomic efforts that aim to reprogram neurons from disease cohorts and diverse genetic backgrounds.

P015

ANTI-INFLAMMATORY EFFECTS OF EXOSOMES DERIVED FROM HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS ON RAT NEUROGLIA

Thomi, Gierin, Joerger-Messerli, Marianne, Oppliger, Byron, Spinelli, Marialuigia, Haesler, Valérie, Surbek, Daniel V., Schoeberlein, Andreina

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Wharton's jelly mesenchymal stem cells (WJ-MSC) have the capacity to reduce inflammation and induce tissue regeneration in perinatal brain damage despite of their low long-term survival in host tissue. The therapeutic function of WJ- MSC is mainly ascribed to their paracrine secretion comprising WJ-MSC-derived exosomes. Therefore, the aim of this study is to evaluate the anti-inflammatory effects of WJ-MSC-derived exosomes on neuroglia in vitro. WJ-MSC derived exosomes were isolated from cell culture supernatants using a protocol consisting of several steps of successive centrifugations and ultra-centrifugations. In vitro models involving oxygen glucose deprivation and reoxygenation (OGD-R) and lipopolysaccharide (LPS) stimulation were used to activate primary astrocytes and immortalized microglia cells. The effects of the exosomes on the expression of inflammation markers in glia cells were assessed in co-culture experiments by real-time PCR, immunocytochemistry and Western blot. WJ-MSC-derived exosomes prevent the upregulation of the astrocyte activation marker glial fibrillary acidic protein (Gfap) in response to 6h of OGD and 48h of reoxygenation on mRNA and protein levels. WJ-MSC-derived exosomes further prevent the upregulation of the microglia activation marker cluster of differentiation molecule 11b (CD11b, integrin alpha M) in response to 24h of LPS stimulation on the mRNA level. Moreover, WJ-MSC-derived exosomes suppress the upregulation of pro-inflammatory cytokines such as interleukin 1 beta (IL-1b) and interleukin 18 (IL-18) on mRNA levels in microglia cells. In conclusion, we demonstrate that WJ-MSC-derived exosomes are potent modulators of neuroglia activation in hypoxia/ischemia and inflammation. Hence not only WJ-MSC, but also WJ-MSC-derived exosomes are able to support tissue regeneration by reducing inflammation. As a result, WJ-MSC-derived exosomes might represent a novel cell-free approach to treat perinatal brain damage.

Funding Source

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POSTER ABSTRACTS

P017

A MICROFLUIDIC FORSKOLIN-INDUCED SWELLING ASSAY ON LGR5+ SMALL INTESTINAL ORGANOIDS

Wilschut, Karlijn J.[1], Kurek, Dorota[1], Trietsch, Sebastiaan[1], van Duinen, Vincent[1], van de Wetering, Marc[2], Vries, Rob[2], Clevers, Hans[2], Lanz, Henriette[1], Joore, Jos[1], Vulto, Paul[1]

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The culture of human organ-specific stem cells into organoids offers novel opportunities for regenerative and personalized medicine. Recently a the forskolin-induced swelling (FIS) assay for primary intestinal organoids was proposed for drug testing in cystic fibrosis . The assay delivers a visual readout of the Cystic Fibrosis Transmembrane Conductance Regulator function (CFTR), a protein mutated in cystic fibrosis patients. We use a high-throughput microtiter plate-based microfluidic platform called OrganoPlate® for the implementation of the assay in an automated setting . The MIMETAS OrganoPlate® technology enables culturing of 3D structures in a high-throughput membrane-free manner with pump-free perfusion. The forskolin assay was implemented on mouse lgr5+ small intestinal organoids after 3-4 days in culture. Since the organoids were grown in a confined microfluidic space, a swollen organoid occupied the complete height of a 120um high channel and expanded further in lateral direction. Automated readout of the swelling assay was realized on a MolDev ImageXpress High Content Imager, followed by image analysis and quantification of the swelling process. The implementation of this assay in a microfluidic platform yields a robust assay that can be processed in high-throughput towards personalized drug response studies in patients.

P019

HIGH-FREQUENCY ENGRAFTMENT OF HUMAN ACUTE MYELOID LEUKEMIA IN IMMUNODEFICIENT MICE

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Repopulation of immunodeficient mice remains the primary method to functionally assess human acute myeloid leukemia (AML). Published data report engraftment of ~40-66% of AML cases, mostly of intermediate or poor risk subtypes. Here we report that extending follow-up beyond the standard analysis end-points of 10 to 16 weeks post-transplantation permitted leukemic engraftment from nearly every xenotransplanted AML (18/19, ~95%). Xenogeneic AML showed conserved immune phenotypes and genetic signatures when compared to corresponding pre-transplant cells, and were furthermore able to induce leukemia in re-transplantation assays. Importantly, bone marrow (BM) biopsies taken at standardized time points failed to detect leukemic cells in 11/18 of AML cases that later on showed robust engraftment (61%, termed long latency engrafters), indicating that leukemic cells can persist over months at undetectable levels without losing disease-initiating properties. Favorable risk AML required longer time to become detectable in NSG mice (27.5±9.4 weeks) than intermediate (21.9±9.4 weeks, $p < 0.01$) or adverse risk subtypes (17±7.6 weeks; $p < 0.0001$), explaining why the engraftment of first was missed with previous protocols. Mechanistically, AML cells engrafting with prolonged latency showed inferior homing to the BM. Finally, we applied our model to favorable risk AML with inv(16); here, we showed that leukemia initiating cells (LIC) were contained in CD34+ (but not CD34-) blasts, which induced robust, long latency engraftment and expressed enhanced levels of stem cell genes. Together, we provide a model that allows in vivo NSG mouse studies with a wide range of molecular AML subtypes previously considered non-engraftable, thus enabling novel insights on leukemogenesis.

POSTER ABSTRACTS

P021

MODELLING MATERNAL IMMUNE ACTIVATION IN HUMAN IPSCS

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The risk of developing autism is markedly increased among individuals whose mothers contracted severe infections during pregnancy – an effect likely to be mediated by cytokines. The purpose of this study is to attempt to mimic these effects during neural differentiation of human induced pluripotent stem cells (IPSCs). Control IPSCs, derived from neurotypical individuals, were neuralised through dual SMAD inhibition. Cells were treated with IFNγ at either, or both, 18 and 30 days after neuralisation. Morphological and transcriptomic changes were assessed by high content screening and RNA sequencing respectively. Treatment with IFNγ at 18 days, the neural progenitor stage, resulted in the disruption of neuro-epithelial rosette formation and long-term changes in neuronal morphology, with treated neurons displaying increased total neurite length. The RNA sequencing analysis also showed that IFNγ treatment at the neural progenitor stage results in changes that endure after differentiation into post-mitotic neurons. The effected genes are enriched for the gene ontology terms "antigen processing" and "cell adhesion", with a notable overrepresentation of MHC class I genes. Furthermore, a subset of "primed" genes show a magnified response to IFNγ in neurons when treated at both early and late time points when compared to cells that were only treated at the later time point. We also see a significant response to treatment in many autism-associated genes. These results demonstrate that exposure to the pro-inflammatory cytokine IFNγ has an acute and lasting impact on iPSC-derived neural progenitors and neurons. The morphological perturbations we observe are similar to those we see in cells derived from sporadic and CNV autism cases, and the transcriptomic analyses highlight potential mechanisms that may mediate these effects.

P023

MODELLING RETINAL DYSTROPHIES USING HUMAN IPSC DERIVED RETINAL ORGANOIDS

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Stem cell derived retinal cells provide an excellent resource to study unique and otherwise inaccessible human cell types. The ability to generate induced pluripotent stem cells (iPSC) presents an opportunity to study the mechanisms of retinal degeneration in individuals harbouring mutations in retinal dystrophy genes. Methods to differentiate human stem cells into retinal cell types have been under development for almost a decade and the use of a three-dimensional 'organoid' approach to differentiation has been a significant advance. IPSC derived from individuals with inherited retinal dystrophies (IRD) were differentiated along side control cell lines using two previously published 3D organoid differentiation protocols. Control and IRD iPSC retinal organoids consisted of a laminated structure containing all the major retinal cells types arranged in their appropriate layers including retinal ganglion cells, bipolar cells, muller glia and photoreceptors. Retinal pigment epithelium (RPE) cells were also produced. Retinal organoids took 5-6 months to develop into mature opsin-expressing photoreceptors and to begin to develop the light sensitive 'outer segment' structure, a highly specialised cilium that is designed to optimise the capture of photons and transduce light into an electrical signal to the underlying bipolar cells. These outer segments were strongly immunoreactive for rhodopsin and components of the visual transduction cascade. Cone cells immunoreactive for blue and red/green opsin were also present in the mature retinal organoids from both patient and control cell lines. These retinal models may serve as useful tools for disease modelling and therapy development.

Funding Source

Fight for Sight

P025

SYNCHRONOUS OSCILLATORY ACTIVITY IN HUMAN IPSC-DERIVED CORTICAL CIRCUITS

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The functional hallmark of the brain is the ability to generate oscillations. Brain oscillations are defined as extracellular recorded field potentials composed of different frequency bands which depends on neuronal and synaptic activity. We addressed the question, if oscillatory activity can be recorded in in vitro generated human cortical circuits obtained from human induced pluripotent stem cells (hiPSC). This type of neuronal network function required full neuronal maturation of neural stem cell-derived neuronal and glia progeny into structured three-dimensional (3D) organized aggregates. Super-confocal-laser microscopy imaging show that neurons are interconnected by mature synapses and are organized as polarized cell-layer embedded into a glial network. We assessed in vitro generated hiPSC-derived 3D-cortical neural assemblies by multi-electrode array technology and revealed the autonomous generation, i. e. in the absence of electrical

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or chemical stimulation, of synchronized activity recorded as population bursting and oscillatory local field potentials (LFPs). By a comparison of oscillatory in vitro generated human cortical circuits LFPs and in in vivo human cortical LFPs it becomes evident that hiPSC-derived neurons show principles of human brain functions.

P027

KAGAMI-OGATA SYNDROME: RARE IMPRINTING DISEASE MODELLING USING PATIENT-DERIVED IPSCS

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Uniparental disomies (UPD) are rare genomic conditions that present the inheritance from only one parent of a pair of homologous autosomal chromosomes or in parts of them (UPD-like syndromes). This condition has been described when a chromosome contains an imprinted locus, a region in which a gene(s) is differentially expressed between maternal- and paternal-inherited chromosomes because of different methylation pattern in regulatory genomic element. Paternal UPD of chromosome 14 has a frequency of < 1/1,000,000 and causes mainly facial dysmorphisms, bell-shaped thorax, abdominal wall defects and polyhydramnios. Also partial UPD due to large deletions of maternal chromosome 14 has been reported and named Kagami-Ogata Syndrome. The imprinted region of chromosome 14 contains two paternally expressed protein-coding genes (DLK1 and RTL1) and several maternally expressed non-protein-coding genes (MEG3, MEG8, RTL1 anti sense, a cluster of SNORDs and a cluster of miRNAs), regulated by two differentially methylated regions which are hypomethylated on the maternal chromosome and hypermethylated on the paternal one. Until now, the pathogenesis of the Kagami-Ogata syndrome and the UPD14 is predicted from the mouse model, but yet to be investigated using a human model in both physiological and pathological basis, resulting in un-fully understanding of variable phenotypes and severities from mild to lethal in these diseases. In this study, we focus on the Kagami-Ogata syndrome modeling using iPS cells technology and create iPSCs derived from an affected family with different severity, which carries around 130kb deletion downstream of the methylated regions. In addition, we modify the deleted chromosome by CRISPR/Cas9 technique to rescue each of deleted genes and to study their roles in this syndrome. Overall, we here characterize human paternal UPD14 in molecular biology basis using first human UPD14-like model.

Funding Source

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P029

THE ROLE OF MICROGLIA IN NEURONAL CEROID LIPOFUSCIONOSIS

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Neuronal ceroid lipofuscionosis (NCL) summarizes a group of human childhood neurodegenerative genetic disorders with the common phenotypic characteristics of dementia and lethal seizures. The incidence rate of NCL ranges between 1:12,500 and 1:100,000 depending on the genetic background of the population. So far, 14 disease-causing genes and their genetic variants are described, however their impact in the fundaments of molecular and cellular dynamics are not known. Although mouse models have provided important insights into the disease pathogen from a neuro-centric view, they lack first, the immune-relevant perspective and second, a direct human correlation in pathophysiology. We are particular addressing microglia because we have previously shown in the retina that microglia express a set of NCL disease genes, which are known to trigger visual loss as a first phenotypic sign. Here, we use genome-editing to induce the most prevalent NCL genetic mutations in human induced pluripotent stem (iPS) cells. We then differentiate those cells into three-dimensional retinal structures as well as microglia to study the direct consequences of microglia in retinal cell type formation. The result of this study will provide important insights into the role of microglia in NCL disease and will widen our knowledge of pathophysiology.

P031

FASCIN MAINTAINS BREAST CANCER STEM CELLS VIA THE REGULATION OF ADHESION MOLECULES

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Recent years have wetness significant advances in breast cancer treatment due to the development of targeted therapy that results in better treatment outcome when use in combination with chemotherapy. Despite this progress, the tumor-related mortality remained high mainly due to relapse and metastasis, which are widely believed to be regulated by a small subpopulation of cancer cells that possess stem cell-like features, and thus called "Cancer Stem cells

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(CSCs)". Fascin is an actin-bundling protein, which we and others have shown that it associates with a worse clinical outcome in breast cancer patients. We have previously reported that fascin is a critical mediator of breast CSCs and chemoresistance, via the activation of Focal Adhesion Kinase (FAK), which directly binds to members of the integrin adhesion molecules. Here we have used fascin loss and gain of function approaches to examine if fascin influences integrin expression to regulate breast CSC function. Our results demonstrated a significant correlation between fascin and increased expression of integrins including: CD49a, CD49C, CD49f, CD29 and CD61. Fascin-mediated regulation of integrins is critical for adhesion, chemoresistance and tumorsphere formation. This study highlights the crucial role of fascin in the maintenance of breast CSCs via the regulation of integrin expression. The outcome of this study will shed light on the possible targeting of fascin as a therapeutic approach for optimal treatment of breast cancer from the root.

Funding Source

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P033

SHORT HAIRPIN SCREEN IDENTIFIES THE E3 UBIQUITIN LIGASE HOMOLOGS ARIH1 AND ARIH2 AS ACHILEES HEELS FOR HUMAN GLIOBLASTOMA STEM CELL GROWTH

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Glioblastoma multiforme (GBM) is an aggressive brain tumor associated with poor prognosis and fatality. The median survival time is approximately 12 months and less that 5% of the patients survive up to 5 years or longer. The standard treatment consists of surgical resection of the tumor followed by a regimen of chemotherapy. Although new drugs have been developed against the disease, none have successfully completed clinical trials. The aim of our study is to identify enzymes, that are critical nodes in pathways driving proliferation of GBM cells, which can serve as novel potential therapeutic targets. To achieve this goal, two new and independently derived proneural cancer stem cell (CSC) lines from glioblastoma patients were screened using lentiviral short hairpin RNA (shRNA) libraries targeting approximately 10000 human genes. Human foreskin fibroblasts were used as a control cells to identify and exclude genes that have adverse growth inhibitory or promoting effects on normal cells thereby selecting for GBM-specific effects. Using this approach we have identified the E3 ubiquitin ligase homologs ARIH1 and ARIH2 as specific vulnerabilities of glioblastoma cancer stem cells. Short hairpin RNA-mediated knockdown of the two E3-ligases prevented growth of GBM CSCs as well as various conventional glioblastoma cell lines. In line with previously published studies we have, in preliminary work, shown that inhibition of ARIH1 and ARIH2 in glioblastoma cells result in DNA damage leading to cell cycle arrest. Furthermore, in ongoing work, we are elucidating the cellular mechanisms, including DNA damage, by which the E3-ligases are required for growth of the glioblastoma CSCs. In order to dissect the molecular mechanisms by which these target genes contribute to the disease, we are at present performing functional genomic studies (mass-spectrometry and RNA-sequencing). In order to investigate the importance of ARIH1 and ARIH2 in disease progression and tumor regression we have established a murine orthotopic xenograft model of glioblastoma. The findings from this study might facilitate the development of novel targeted therapies and thereby improve the overall survival of patients suffering from this lethal disease.

P035

MODELLING MITCHELL-RILEY SYNDROME IN VITRO USING IPS CELLS DERIVED FROM A RFX6 MUTANT PATIENT

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The forkhead transcription factor Regulatory Factor X, 6 (RFX6) is essential for the development of the mammalian pancreas. In humans, RFX6 mutations are responsible for Mitchell-Riley Syndrome (MRS), which is characterized by neonatal diabetes with pancreatic hypoplasia. Similarly, Rfx6 mutant mice fail to develop all islet endocrine cell types and die soon after birth. Although RFX6 mutations yield a dramatic disease phenotype, our knowledge about RFX6 function and regulation is extremely limited. To address this deficit, we have generated human induced pluripotent cell (hiPSC) lines from a Syrian MRS patient with a novel RFX6 null mutation. Based on published, but limited work in the mouse, our expectation was that RFX6 functions specifically at the transition between NGN3+ pancreatic endocrine progenitors to mature hormone-containing islet subtypes. Our data however show that (1) during directed differentiation toward the pancreatic lineage, RFX6 expression precedes the activation of the master regulator gene PDX1, with weak RFX6 expression first detected as early as day 4; (2) MRS iPSC fail to activate the pan-endodermal organ marker SOX9 as well as PDX1; and (3) wild-type H9 human ES cells (hESC)

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routinely produce >85% PDX1+;NKX6-1+ pancreatic progenitors by day 12 of differentiation, whilst MRS iPSC consistently yield < 25%. Taken together, we propose that RFX6 plays a previously unanticipated, earlier role in human pancreatic development and that RFX6 expression is bi-phasic, with increasing levels during primitive gut tube development followed by a decline and then resolving to mature beta cells. RFX6 binds and activates the INSULIN promoter in the human beta cell line EndoC-βH2 and thus, like PDX1, may also function during beta cell homeostasis. Lastly, both humans and mice display developmental defects in the gall bladder and duodenum, suggesting that RFX6 function is not limited to the pancreatic lineage.

P037

ESTABLISHMENT OF A PATIENT-DERIVED MYELOFIBROSIS XENOGRAFT MOUSE MODEL

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A growing number of patient-derived xenograft (PDX) mouse models have been developed over the past few decades that allow engraftment of human hematopoietic stem cell (HSC) malignancies. This is a powerful tool for investigating the evolution of HSC and leukemic stem cells, as well as disease heterogeneity. However, engraftment is often limited due to potential lack of supportive factors in the bone marrow (BM) microenvironment. This limitation facilitated the development of more advanced mouse strains that express human cytokines and growth factors that are needed for efficient human hematopoietic development in vivo. Myelofibrosis (MF) is a HSC disorder characterized by bone marrow fibrosis that has the potential to transform into acute myeloid leukemia depending on the clonal evolution of MF stem cells (MF SCs). However, the engraftment of MF SCs in PDX models is poor. We hypothesized that the constitutive expression of human cytokines and growth factors in a PDX model may promote the development of the human MF clone in vivo. Therefore, we used next-generation mice that express human M-CSF, IL-3, GM-CSF, TPO, and SIRPαTg (MISTRG) in order to develop a pre-clinical MF PDX model. NSG mice (Standard PDX mouse strain) were used as controls. Each mouse strain was transplanted with the same patient sample and sacrificed 5-9 weeks after transplantation. Preliminary results show that NSG and MISTRG mice support unprecedented myeloid engraftment of human MF SCs in 57% of patient samples investigated so far. In order to determine whether specific somatic mutations promote human MF engraftment in PDX models next-generation sequencing will be performed on transplanted patient samples. Moreover, clonal evolution in vivo will be tracked and correlated with disease progression in patients.

P039

DISEASE MODELING OF HYPERTROPHIC CARDIOMYOPATHY USING HUMAN IPSC-DERIVED CARDIOMYOCYTES

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An enhanced understanding of mechanisms involved in complex heart disease has been advanced by the advent of induced pluripotent stem cell (iPSC) technology – which enables the production of human cardiomyocytes (CM) from any individual in quantities sufficient to enable repeatable and scalable research projects in vitro. Hypertrophic cardiomyopathy (HCM), a common genetic heart condition affecting approximately 1 in 500 individuals, is characterized by ventricular wall thickening as a result of enlarged cardiac myocytes, changes in blood pressure due to restricted blood flow, and arrhythmias. One of the most prevalent forms of familial HCM arises from a missense mutation in the gene encoding the beta-myosin heavy chain protein (MYH7), resulting in a change of amino acid 403 from Arg-to-Gln (R403Q). Here we compare the physiological and functional properties of CMs differentiated from a normal healthy donor iPSC line to CMs derived from a patient with the MYH7-R403Q mutation. Hypertrophy can be induced in human iPSC-derived CM with exposure to Endothelin-1 (ET-1). HCM-induced CM exhibit classic hallmarks of cardiac hypertrophy, including up-regulation of fetal genes, cytoskeletal rearrangements, expression of brain natriuretic peptide (BNP), and an increase in cardiomyocyte size. We show that the induced and inherited HCM phenotype in iPSC-derived CM have common features. CM differentiated from MYH7-R403Q iPS cells exhibit typical cardiac morphology and showed autonomous contractile activity similar to the control iPSC-derived CM. Both the patient-derived MYH7-R403Q CM and ET-1 induced HCM in normal CM have similar basal gene expression. ET-1 induction increases BNP expression in both control and MYH7-R403Q cardiomyocytes, but basal BNP levels are higher in MYH7-R403Q cardiomyocytes. These data show the progression of HCM characteristics in MYH7-R403Q cardiomyocytes and underscore the advantages of modeling cardiovascular disease with iPSC technology.

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P041

PHENOTYPING OF HUMAN IPSC-DERIVED DOPAMINERGIC NEURONS CONTAINING THE ENGINEERED A53T ALPHA-SYNUCLEIN MUTATION

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Parkinson's disease (PD) affects ~1% of people over the age of 65 and is the second most common neurodegenerative brain disorder after Alzheimer's. The physiological decline associated with PD is generally thought to be caused by a marked pathological deterioration of dopaminergic neurons located in the substantia nigra. Mutations in several different genes have been clearly linked to PD, including SNCA that encodes the alpha-synuclein (α-syn) protein, which is predominantly expressed in the brain at presynaptic terminals. The mutation in α-syn at A53T renders the protein more susceptible to aggregation and accumulation, which are hallmark indicators of PD pathology. Despite its low occurrence, A53T is one of the most highly penetrant and widely studied mutations. The combination of cutting-edge genome-editing and induced pluripotent stem cell (iPSC) technologies offers the opportunity to study patient-specific risk factors or disease-specific mutations (such as the A53T mutation in α-syn) in a physiologically-relevant cell type (dopaminergic neurons) and compare the function and phenotype in a series of assays to cells derived from healthy control iPSC lines. This approach is revolutionary for disease modeling and drug discovery. In this poster, we show data comparing healthy (WT) and A53T dopaminergic neurons that demonstrate alterations at the synapse, both functionally (electrophysiological MEA readout) and anatomically (neurite outgrowth and branching). The observed differences between healthy and A53T suggest early physiological changes tilted towards producing a more connected and highlyactive neuronal network. In correlation with the known disease pathology, these "aging" cultures show synaptic deterioration and dendritic atrophy. Current studies are underway to further determine if additional hallmarks of PD pathophysiology, including α-syn aggregation or mitochondrial dysfunction, can be measured in these human cell models.

P043

N64Y MUTATION IN NOX3 GENE CAUSES EXCESSIVE PROLIFERATION OF MOUSE CEREBELLAR PROGENITOR AND NEURAL STEM CELL

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Nox3 is a NADPH oxidase that belongs to a family of transmembrane proteins which main function is to reduce molecular oxygen to form reactive oxygen species (ROS). ROS produced by Nox can modulate cell signaling in various physiological processes including proliferation. The cerebellum is the region in the central nervous system responsible for motor coordination and balance. We selected an ENU-mutated mouse lineage that lacks motor coordination and have increased proliferation of cerebellar granule neuron progenitors during early postnatal development as well as disorganized Purkinje cell layer. Genetic mapping by polymorphic microsatellite analysis and Next Generation Sequencing, identified the mutation as an A>T transversion at position 190 of the transcribed sequence of Nox3 gene, located on mouse chromosome 17. The mutation causes the substitution of Asp by Tyr at position 64 of the protein. Furthermore, Nox3N64Y mice show higher expression of Nox1, indicating a possible compensation caused by the expression of mutated Nox3. Because SHH (Sonic Hedgehog), secreted by Purkinje cells, is the main mitogen for neuronal cell precursors in the developing cerebellum, our goal was to study the role of Nox and ROS in the control of proliferation of granule cell progenitors and cerebellar neural stem cells stimulated by SHH signaling pathway. Nox3N64Y mice cerebellar neural stem cell and neuronal progenitors produce higher levels of ROS, and proliferation of cerebellar neural stem after 7 days in vitro is increased when compared to wild type BALB/c. Moreover, Nox3N64Y cerebellum shows upregulation of Gli1, Gli2, Gli3, Cyclin D1, Cyclin B1, Sox2 and Rb1, and downregulation of Cdkn2a and CD133. After inhibition of Nox by apocynin, treated animals showed reduction in the thickness of the external granular layer (EGL). Our hypothesis is that SHH is the main pathway affected by mutated Nox3, and ROS has an important role in the increased proliferation of Nox3N64Y cerebellar neural stem cell and neuronal progenitors.

Funding Source

CNPq and Fapesp

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P045

GENERATION OF MOTOR NEURONS FROM DISTRAL HEREDITARY MOTOR NEUROPATHY PATIENT-DERIVED IPSCS

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Neurons are particularly vulnerable to disorders characterised by imbalances of protein homeostasis (proteostasis); that is, protein synthesis, degradation and traffic coupled to the ability of the cell to deal with stress and correct or clear misfolded proteins. Motor neurons appear to be particularly susceptible to cellular stress and inclusions of aggregated protein are often seen in motor neuropathies such as amyotrophic lateral sclerosis (ALS). Molecular chaperones are a class of proteins closely involved with regulating protein folding and misfolding and as such are important facilitators of proteostasis. HSJ1 (DNAJB2) is a DNAJ protein that functions with HSP70 proteins to shuttle misfolded client proteins to the proteasome for degradation. Here we have generated induced pluripotent stem cells (iPSCs) from two patients with distinct mutations in DNAJB2 that result in distal hereditary motor neuropathy (dHMN) and Charcot Marie Tooth disease type 2 (CMT2) and we have used these to generate motor neurons (MNs), alongside control iPS-MNs, to serve as a model for investigating the cellular consequences of DNAJB2 mutations on motor neuron proteostasis. Therefore, our development of patient cell-based models offer a unique opportunity to further our understanding of the specific proteostasis requirements of motor neurons and define the mechanisms that lead to motor neuron cell death.

P047

EXAMINING ASTROCYTE-MEDIATED SYNAPTIC PLASTICITY AT HUMAN CORTICAL SYNAPSES

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Neurons are the primary signal-carrying cell of the brain, but the functions of neurons are greatly influenced by networks of interacting astrocytes. These supportive glial cells are believed to fulfil multiple active roles, one of the most important being their regulation of synaptic connections. Indeed, the interactions between neurons and astrocytes have been implicated in a number of neurological disorders that involve synaptic dysfunction, such as Alzheimer's Disease (AD). In this project we are taking advantage of induced pluripotent stem cell (iPSC) technology in order to derive human cortical neurons and astrocytes from healthy individuals, or from patients diagnosed with AD. Firstly, we have focused on producing the two cell types of interest from a common progenitor pool and demonstrate that it is possible to generate both cortical neurons and astrocytes with appropriate morphological and functional properties. Subsequently, we have established a co-culture system of human cortical neurons and either rat or human astrocytes, in which we are able to characterise the emergence of synaptic activity within the network using targeted patch clamp recordings. Finally, we have used this co-culture system to examine a form of astrocyte-mediated synaptic plasticity that has been described in rodent models. This involves activity-dependent calcium signalling in astrocytes and the release of gliotransmitters, which can influence the strength of nearby synapses. We are able to reconstitute aspects of this process using optogenetic techniques to stimulate activity in our neuron-astrocyte co-cultures. With long-term optogenetic control of astrocytes, we are able to drive a significant increase in excitatory synaptic network communication between the human cortical neurons. This supports the conclusion that astrocytic activity can regulate human cortical synapses and the tools are now in place to explore neuron-astrocyte interactions at both normal and disease-relevant synapses.

P049

STUDYING SYNAPTIC PHENOTYPES IN IPSC-DERIVED CORTICAL NEURONS FROM ALZHEIMER'S DISEASE PATIENTS

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Alzheimer's disease (AD) is a neurodegenerative disorder that causes a gradual decline in memory and cognition. Synapse loss is the best correlate of cognitive decline and evidence suggests that this is preceded by synaptic dysfunction, manifesting as alterations in synaptic plasticity. In order to explore these events in a human model system, we are utilising induced pluripotent stem cells (iPSCs) from patients with AD and control subjects to generate cortical excitatory neurons in vitro. Using electrophysiology, we have established that with increasing time in culture the neurons become more mature, reflected by a maturation in multiple intrinsic membrane properties, which are significantly improved by co-culture with astrocytes. We have demonstrated that the iPSC-derived human neurons also express functional AMPA and NMDA receptors, which

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are implicated in glutamatergic synaptic plasticity, and we are currently exploring activity-dependent mechanisms that regulate the expression of these receptors. The neurons form synaptic networks, however, the overall levels of glutamatergic synaptic activity are low. Therefore, we are also examining ways to improve the generation of mature, synaptically connected cortical neurons. The approaches we are adopting include forced expression of transcription factors involved in neuronal differentiation, co-culture with rodent neurons and long-term manipulations of neural activity. The impact of these different manipulations is being assessed using targeted whole cell patch clamp recordings and the quantitative assessment of network-generated and stimulus-evoked synaptic transmission.

P051

REVERSAL OF NEUROBEHAVIORAL TERATOGENICITY WITH CELL TRANSPLANTATION TO THE BRAIN: MOUSE AND CHICK MODELS AND THE PROSPECT FOR TRANSLATION

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Our studies focus on the reversal of neurobehavioral teratogenicity with stem cell transplantation. Prenatal (mice) and prehatch (avian) exposure to various neuroteratogens, mainly heroin and chlorpyrifos, evoked deficits in mice hippocampal spatial abilities and in chick imprinting performance, with concomitant alterations in cholinergic pathways, related to deficiencies in cholinergic control of PKCγ and β activation/translocation. Identifying these mechanisms enables the design of various therapies to reverse the impairment, most relevant, neural grafting of cholinergic neurons and more recently, transplantation of embryonic, neural, adult (SVZ), and mesenchymal stem cells (MSC) in mice, and MSC in chicks. Both neural grafting and stem cell transplantation reversed the behavioral deficits and the mechanistically associated neural alterations. Our further studies suggested that one major mechanism by which the transplanted cells exert their therapeutic action is by induction of neurogenesis. Recently, we studied the expression of genes related to the neurobehavioral deficits and their reversal. Mice and chicks were exposed to the neuroteratogens prenatally or prehatch and were transplanted with MSC at adulthood (mice) or prehatch (chicks) a week later. The expression of genes related to neurogenesis and/or various innervation (cholinergic, serotonergic and catecholaminergic) were assessed with real time PCR. Prenatal/prehatch exposure to the teratogens altered the expression of most genes; a tendency mostly associated with a decrease in neurogenesis genes (e.g., BDNF, IGF, DCX) and an increased expression in the neurotransmission genes (e.g., CHT, PKCβ, 5HT1RA). Transplantation with MSC reversed those alterations to normalcy. It is expected that the mechanism of both defects and repair by MSC are attributed to regulating mechanisms of neurogenesis-related gene expression. Indeed, our preliminary studies suggest global epigenetic changes as indicated by changes in DNA methylation. Our studies offer a novel model, never replicated by others yet, with a translational prospect for the reversal of neurobehavioral birth defects with stem cell transplantation.

Funding Source

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P053

HUMAN IPSC-DERIVED FUNCTIONAL BETA-CELLS TO BE USED IN DRUG SCREENING AND DISEASE MODELLING

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Unfortunately, the global prevalence of diabetes has been rising during the last decades. Diabetes mellitus is a condition where the pancreatic beta-cells fail to produce enough insulin acquired to regulate the circulating blood glucose level in the body, thus resulting in hyperglycemia. This can further lead to severe long-term complications such as; renal failure, neuropathy, increased risk of cardiovascular diseases and retinopathy that may cause blindness. Type I diabetes is an autoimmune variant of this disease where the immune system attack and destroy the pancreatic beta-cells. Regularly, Type I diabetes patients are treated by external administration of insulin, but it has lately been shown that it can also be treated by transplanting islets from deceased donors. This treatment is a proof-of-concept as it shows that cell therapy can restore euglycemia. Therefore, many stem cell scientists have been encouraged to develop differentiation protocols generating stem cell-derived beta-cells, in order to produce a reliable and renewable source of beta-cells to be used in regenerative medicine. Besides using these cells for clinical therapies, they can also be embraced in in vitro applications for studying beta-cell functions (e.g. insulin secretion mechanism, calcium influx, pancreatitis) or as a screening tool to identify potential drugs regulating insulin secretion. By employing a four-step differentiation protocol, insulin-producing beta-cells can be generated

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from hiPS cells to be used in in vitro applications. In the last differentiation/maturation step the cells are cryopreserved as single cells and can easily be thawed and seeded in a 2D monolayer, enabling different analyses, such as Glucose-Stimulated-Insulin-Secretion (GSIS), incretin response, response to acetylcholine and GPR. Our hiPS-derived beta-cells express markers characteristics of human primary beta-cells, including MAFA, NKX6.1 and UCN3. Results presented here shows that hiPS-derived beta-cells, an almost inexhaustible source of functional human beta-cells, could replace the use of primary pancreatic islet cells in in vitro studies, thus reducing donor dependence and individual variability.

P055

MODELLING GBA1-ASSOCIATED PARKINSONISM IN PLURIPOTENT STEM CELL-DERIVED NEURONS

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Homozygous mutations in the GBA1 gene encoding the lysosomal hydrolase glucocerebrosidase (GCase) cause the lysosomal storage disorder Gaucher Disease (GD). In addition, GBA1 mutations have been identified as the most common genetic risk factor for Lewy body diseases (LBD) including Parkinson's disease (PD). Many GD and PD associated GBA1 mutations lead to misprocessing and misfolding of GCase. Whether the pathological link between GD and LBDs is due to a gain- or loss-of-function mechanism of GCase still remains elusive. We have created the first human GBA1 loss-of-function pluripotent stem cell-based model to decipher the pathological aspects that can be attributed to the loss of GCase. We show that genome edited GBA -/- hPSC-derived neurons show GD associated pathologies including accumulation of glycosphingolipid substrates accompanied by lysosomal alterations. Furthermore, the complete loss of GCase function in neurons impairs autophagic processes. These findings provide further evidence that the loss of GCase can result in lysosomal/autophagic perturbations. Our data strengthen the hypothesis that lysosomal/autophagic dysfunction could represent a pathological mechanism connecting GBA1 dysfunction and PD. Furthermore, with our isogenic GBA-/- pluripotent stem cell model we add a powerful resource to the GBA-PD modeling tool box.

DRUG DISCOVERY

P057

EPILEPTIFORM ACTIVITIES AND DRUG RESPONSES IN CULTURED HUMAN IPSC-DERIVED NEURONAL NETWORKS USING HIGH-THROUGHPUT MEA SYSTEM

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The functional network of human induced pluripotent stem cell (hiPSC)-derived neurons is a potentially powerful in vitro model for evaluating disease mechanisms and drug discovery. To evaluate the drug effect in cultured hiPSC-derived neurons, we used the high-throughput 384 multi-electrode array (MEA) system, where we simultaneously record extracellular potentials for 16 channels per well across 24-well plates. We firstly confirmed the modulation of activity by typical glutamatergic and GABAergic receptor antagonists/agonists in spontaneous firings and evoked responses. Next, to evaluate the utility of these human iPS-derived neural networks for modeling human disease states and drug screening, we examined chemically evoked epileptiform activity. Electrophysiological seizes were induced by pentylentetrazole (PTZ) and 4-Aminopyridine (4-AP), the most widely used chemical convulsant in animal models to screen for new anti-epilepsy drugs. We also examined the anti-convulsant effects of two common clinical anti-epilepsy drugs (AEDs), phenytoin and sodium valproate (VPA). PTZ and 4-AP induced a rapid increase in synchronized burst firings (SBFs) in a concentration-dependent manner. Phenytoin and VPA also suppressed PTZ-induced epileptiform activity. From these results, we suggest that the electrophysiological assay in cultured human iPSC-derived neuron using high-throughput MEA system is a useful to investigate the neuronal toxicity in drug screening and pharmacological effects of human neurological disease.

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P059

MICROVESICLES FROM BRAIN-EXTRACTM—TREATED MESENCHYMAL STEM CELLS IMPROVE NEUROLOGICAL FUNCTIONS IN A RAT MODEL OF ISCHEMIC STROKE

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Transplantation of mesenchymal stem cells (MSCs) was reported to improve functional outcomes in a rat model of ischemic stroke, and subsequent studies suggest that MSC-derived microvesicles (MVs) can replace the beneficial effects of MSCs. Here, we evaluated three different MSC-derived MVs, including MVs from untreated MSCs (MSC-MVs), MVs from MSCs treated with normal rat brain extract (NBE-MSC-MVs), and MVs from MSCs treated with stroke-injured rat brain extract (SBE-MSC-MVs), and tested their effects on ischemic brain injury induced by permanent middle cerebral artery occlusion (pMCAO) in rats. NBE-MSC-MVs and SBE-MSC-MVs had significantly greater efficacy than MSC-MVs for ameliorating ischemic brain injury with improved functional recovery. We found similar profiles of key signalling proteins in NBE-MSC-MVs and SBE-MSC-MVs, which account for their similar therapeutic efficacies. Immunohistochemical analyses suggest that brain-extract—treated MSC-MVs reduce inflammation, enhance angiogenesis, and increase endogenous neurogenesis in the rat brain. We performed mass spectrometry proteomic analyses and found that the total proteomes of brain-extract—treated MSCMVs are highly enriched for known vesicular proteins. Notably, MSC-MV proteins upregulated by brain extracts tend to be modular for tissue repair pathways. We suggest that MSC-MV proteins stimulated by the brain microenvironment are paracrine effectors that enhance MSC therapy for stroke injury.

Funding Source

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P061

RAPID AND EFFICIENT GENERATION OF HUMAN OLIGODENDROCYTES FROM INDUCED PLURIPOTENT STEM CELLS FOR DISEASE MODELLING AND DRUG DISCOVERY

Ehrlich, Marc[1], Mozafari, Sabah[2], Glatza, Michael[3], Starost, Laura[3], Velychko, Sergiy[1], Hallmann, Anna-Lena[1], Cui, Qiao-Ling[4], Bachelin, Corinne[2], Marteyn, Antoine[2], Antel, Jack[4], Sterneckert, Jared[5], Zaehres, Holm[6], Schöler, Hans Robert[1], Evercooren, Anne Baron-van[2], Kuhlmann, Tanja[4]

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The ability to generate human oligodendrocytes (OL) from patient-specific induced pluripotent stem cells (iPSC) offers a promising approach to understand the biology of myelin diseases and to develop treatments for such disorders. However, current OL differentiation protocols are very time-consuming and inefficient limiting the applicability of this technique. In this study, we demonstrate that the induction of key transcription factors in iPSC-derived neural progenitor cells is sufficient to rapidly generate O4+ OL with an efficiency of up to 70 % in 28 days. These iPSC-derived OL present a gene expression profile highly comparable to adult human OL and myelinate the CNS of Mbp shi/shi Rag-/- mice during development and after demyelination. Moreover, we show that these cells are suitable for in vitro myelination assays, disease modelling and screening of pharmacological compounds potentially promoting oligodendroglial differentiation. Thus, our strategy for the generation of iPSC-derived OL provides rapid access to large numbers of human iPSC-derived OL which may facilitate the investigation of human myelin diseases and the development of high-throughput screening platforms for drug discovery.

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P063

INDOLACTAM V AND DERIVATIVES : POTENTIAL TOOLS FOR CONTACT-INDEPENDANT IPSC CULTURE

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Induced pluripotent stem cells (iPSCs) provide an excellent source of cells to study human diseases mechanisms, develop regenerative therapies and screen for lead molecules for drug discovery. A fundamental element needed to exploit the potential of stem cells throughout these applications is the ability to produce a large number of cells of a consistent quality and most importantly in a cost-effective manner. Traditional culture methods currently used require cell-matrix interactions to maintain both cells' pluripotency and viability. However this approach is disadvantaged by its inability to produce iPSCs on a sufficient scale. One highly desirable strategy of overcoming this difficulty is to develop a 3D suspension culture method which would allow a reliable, cost effective and industrial scale production of iPSCs. During the past years, our group has been focusing on establishing a possible method to develop a single-cell suspension culture of iPSCs that could be obtained through the disruption of one of the key players in cell-cell adhesion, the transmembrane E-Cadherin protein. Therefore, we have been working on the development of chemical compounds that would allow the cells to grow in a contact-independent manner while maintaining viability and pluripotency in both 2D and 3D suspension culture. A screening of compounds has enabled the identification of the natural product (-)-indolactam V (ILV), which appears to disrupt cell-cell contact in a PKC-dependent manner. This discovery provided a valuable starting point for the project as our research now focuses on the effect that subtype-selective PKC activation would have on iPSCs. Based on modifications of the ILV skeleton, several small molecules with reported isozyme selectivity were synthesized and tested, the most promising of which reduces iPSCs cell-cell contact in both 2D and 3D suspension culture over multiple passages while maintaining pluripotency and high viability.

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P065

ENHANCED WOUND HEALING EFFECT OF CONDITIONED MEDIA FROM AMNIOTIC FLUID-DERIVED MESENCHYMALL STEM CELL BY SCRATCH STIMULATION

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Wound healing is a complex process of cell proliferation, migration, and interaction between epidermal and dermal region. Many paracrine factors which are secreted from cells in wound region can accelerate the wound healing process. Human mesenchymal stem cells(MSCs) have multipotency and take spotlight in the regenerative medicine field. Moreover, conditioned media from MSCs, which contains paracrine factors of MSCs, is proved to have an effect on wound healing process. Our previous studies showed that the paracrine factors from human amniotic fluid-derived mesenchymal stem cells(AF-MSCs) enhanced the proliferation and migration of dermal fibroblast so that accelerated the wound healing process. Also, other studies showed that mechanical, chemical, and environmental stimuli trigger celluar responses such as secretion of cytokines, increased cell migration as well as enhanced cell proliferation rate. In this study, we hypothesize that scratch stimulation, which mimic wound of tissues, would enhance secretion of paracrine factors in AF-MSCs and accelerate the wound healing process consequentially. The results showed that the proliferation and cytokine release of AF-MSCs were increased by scratch stimulation compared to non-stimulation. In addition, conditioned media from AF-MSCs(CM-AF-MSCs) by scratch stimulation had the increased proliferation and migration of human dermal fibroblasts. Also, treatment of CM-AF-MSCs stimulated with scratches to mouse wound model showed the effective and expeditious wound healing process compared to normal conditioned media. In scratch stimulated CM-AF-MSCs, expression of a variety of wound healing related cytokines(TGF-β1, EGF, TNF-α1, and IL-4/6) were increased compared to normal CM-AF-MSC and consequently, cellular signalling pathways(AKT, ERK, SMAD2/3) also were activated by treatment of scratch induced CM-AF-MSCs in fibroblasts. Overall, our research suggests that scratch stimulation to AF-MSCs, which is similar with in vitro wound model, can enhance the wound healing process in-vivo and in-vivo and it can be useful for medical application.

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P067

MODELLING ALZHEIMER'S DISEASE EMPLOYING HUMAN PATIENT-DERIVED NEURONS BASED ON INDUCED PLURIPOTENT STEM CELL (IPSC) TECHNOLOGY

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In this study we developed and optimized two neuronal differentiation protocols to generate specific neuronal subtypes (i.e. cortical and midbrain) to setup a phenotypic in vitro models for Alzheimer´s disease (AD). These protocols were optimized for application with an automated cell culture system to increase reproducibility and to allow for a medium- to high-throughput scale. Molecular characterization studies, carried out during several stages of the differentiation process employing RT-PCR and immunofluorescence staining, demonstrated an increase in expression of neuronal markers (e.g. vGLUT1/2, GAD65, Tuj1 and Map2), as well as the presence of synapses (e.g. synaptophysin and PSD95). The hiPSC-derived neurons regional identity was confirmed by using a customized RT-PCR-based gene expression array. Neurite outgrowth was investigated in the presence or absence of different growth-promoting or -inhibiting compounds employing live cell and high content imaging technologies.To establish disease-relevant phenotypic assays neuronally differentiated hiPSCs, derived from age-matched healthy controls and familial AD patients, were compared regarding the production of Aß40 and Aß42 in a time course experiment. Dysregulations in Calcium signaling were revealed employing calcium imaging by investigating spontaneous Calcium oscillations in healthy controls and familiar AD neurons. Taken together, our studies demonstrate the utility of patient-derived iPSC-based neurons for the implementation of human disease-relevant model systems for drug discovery, which will support the development of new effective therapies.

Funding Source

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P069

QR-110 TREATMENT FOR LEBER'S CONGENITAL AMAUROSIS TYPE 10: RESTORATION OF CEP290 LEVELS IN 3D IPSC-DERIVED OPTIC CUPS

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Leber's congenital amaurosis (LCA) Type 10, caused by mutations in CEP290, is the most frequent form of LCA (~15%) and generally results in a severe congenital blindness. In this study, we assessed the effect of the antisense oligonucleotide QR-110 as a potential therapy for the CEP290-LCA mutation c.2991+1655A>G (p.Cys998X). This mutation creates a cryptic splice donor site in intron 26 of the pre-mRNA, which results in the inclusion of an aberrant exon of 128 base pairs in to the CEP290 mRNA. This cryptic exon introduces a premature stop codon leading to truncated CEP290 protein. QR-110 is an antisense, single stranded, fully phosphorothioate and 2'-O-methyl modified RNA oligonucleotide. QR-110 targets the splicing mutation of the CEP290 through a mechanism of RNA modulation, by which it skips the inclusion of the cryptic exon and thus restoring the open reading frame of CEP290. Human three-dimensional optic cup organoids with photoreceptors were generated from homozygous c.2991+1655A>G CEP290-LCA patient derived induced pluripotent stem cells (iPSC). A range of QR-110 doses were tested and aberrant splicing was studied using RT-PCR and ddPCR. Ciliogenesis defects were assessed by immunofluorescence. The data show that QR-110 can block the cryptic exon, leading to rescue of wild-type CEP290 mRNA and improved ciliogenesis. These data support the use of QR-110 as a potential treatment for LCA10.

Funding Source

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POSTER ABSTRACTS

P071

NON-HUMAN PRIMATE STEM CELL-DERIVED MODEL TO STUDY ENDOTHELIAL RESPONSES ACROSS SPECIES

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In biomedical research, non-human primates (NHPs) play a unique role in translational science by bridging the gap between basic and clinical investigations due to their close resemblance to humans. In drug development NHPs are primarily used for safety assessment of new medicines, but also for evaluation of potency and mechanism of action of novel drug candidates. In recent years, the number of animal studies has constantly increased, primarily driven by the increase in the development of novel therapeutics that by nature possess a higher species-specificity, such as therapeutic monoclonal antibodies or oligonucleotides (e.g. ASOs), for which NHPs are deemed to be the only relevant animal model. This trend implies an increasing demand for NHP in vitro models to analyze in vivo findings and potentially reduce or refine animal experiments. Here, we present a NHP in vitro endothelial cell system using induced pluripotent stem cells (iPSCs) from Cynomolgus monkey (Macaca fascicularis). Forming the inner layer of blood vessels, endothelial cells are involved in multiple important physiological processes, such as barrier function, angiogenesis, and inflammation. Therefore, endothelial cells play a crucial role in drug uptake and distribution, and their function is associated with adverse drug reactions, such as drug-induced inflammatory responses. iPSC-based in vitro model systems per se represent valuable tools for studying cellular and developmental processes. The NHP endothelial cells generated in this study exhibit typical endothelial marker expression and functions and are highly similar to human primary and human iPSC-derived endothelial cells regarding gene expression patterns and key functional features. Thus, NHP IPSC-derived endothelial cells represent a predictive model to compare drug responses across species. Such a model could be extremely useful for the development of novel drugs by comparing head-to-head human and NHP endothelial cells to evaluate drug safety and efficacy. Moreover, the assessment of drug efficacy in such in vitro assay models will help to select the best drug candidates prior to in vivo studies. Such strategies will not only improve drug development processes but also potentially help to reduce the number of animal experiments.

P073

HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES: FILLING THE GAP FOR TRANSLATION OF IN VITRO ASSESSMENTS TO CLINICAL OBSERVATIONS OF DRUG-INDUCED CARDIAC ARRHYTHMIAS AND LONG-TERM TOXICITY

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Human induced pluripotent stem cell (iPSC)-derived cardiomyocytes represent a promising model for in vitro prediction of cardiac arrhythmias. Currently, two commercially available hiPSC-derived cardioymocyte products - including Axiogenesis Cor.4U human cardiomyocytes - are validated in an international multi-coresite study inside the Comprehensive in vitro Pro-arrhythmia (CiPA) consortium. This aims to change the paradigm of safety pharmacological assessment from the assessment of drug interaction with the hERG channel to a potentially clinically more relevant assays system using human iPSC-derived cardiomyocytes in MEA-, voltage- and calcium-sensitive dye recordings. Besides CiPA, a variety of oncological drugs including tyrosine kinase and HDAC inhibitors have been reported to induce long-term cardiac toxicity in the clinic. Recent findings clearly show that Cor.4U cardioymocytes can be applied to a long-term impedance assay and the results from known drug perfectly translate to the clinical observations. The results shown here imply that human iPSC-derived Cor.4U human cardioymocytes are a translational in vitro cell model for the prediction of clinically relevant drug-induced cardiac arrhythmias and long-term toxicity

P075

APPLICATION OF HUMAN-INDUCED PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES IN HIGH-THROUGHPUT SCREENING ASSAYS FOR DRUG SAFETY AND EFFICACY TESTING

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In drug development it is important to assess potential efficacy as well as toxicity of drug candidates as early in the drug development pipeline as possible. hiPSC-derived cardiomyocytes hold great potential as a novel in vitro model for cardiac disease modelling and cardiac safety screening. We have generated fully functional hiPSC-derived ventricular cardiomyocytes (Pluricyte®

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Cardiomyocytes) without the use of genetic modification or purification/selection procedures. This provides the opportunity to manufacture cardiomyocytes at a high yield with very low batch-to-batch variation, which is necessary to test compound safety and efficacy in high-throughput settings. Currently, Pluricyte® Cardiomyocytes are used extensively for (medium-throughput) safety pharmacology applications using electrophysiology- and contractility- based assays. However, there is a clear need for a better human cardiac model which can also be used within drug discovery and lead optimization phases, in order to improve predictivity of assays used early in the drug development pipeline. To assess the potential of Pluricyte® Cardiomyocytes for application in high-throughput assays, we analyzed the pharmacological responses of Pluricyte® Cardiomyocytes to a set of cardioactive compounds (e.g. Nilotinib, E4031, BayK-8644, diliazem and isoproterenol) using a 384-well plate based Ca2+-signalling assay on a Hamamatsu Photonics FDSS/µCell system. The results were compared to data obtained with a medium-throughput MEA/impedance assay. Both assays succeeded in capturing the relevant cardioactive effects of the tested compounds. In addition, impedance measurements showed profiles in line with the Ca2+-transient data of the cells. We conclude that Pluricyte® Cardiomyocytes combined with HTS-compatible assays will form highly useful tools to study pharmacological and toxicological responses of large numbers of drug candidates. Implementation of such tools in drug discovery processes could further improve the predictivity of early phase drug screenings.

Funding Source

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EMERGING TECHNOLOGIES

P077

PRONGF ACTS AS CELL-TYPE SPECIFIC MITOGEN, ALLOWING STABLY EXPANSION OF ADULT AND INDUCED NEURAL STEM CELLS

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The role of ProNGF, the immature form of Nerve Growth Factor (NGF), on the biology of adult neural stem cells is still object of controversy. We wanted to test the effect of an unclivable form of proNGF (ProNGFKR) on the proliferation of adult hippocampal progenitors derived from the dentate gyrus (DG) of 5 month-old mice. We found that ProNGFKR significantly increases the proliferation of hippocampal progenitors by the time (2 weeks) when added to the mitogens present in the medium culture (EGF and bFGF) at low concentration (0.4 nM), while it exerts opposite effect at higher concentration (4 nM). Due to the heterogeneity of these progenitors culture in terms of cell-type and stage of maturation, this result might suggest a dual role of proNGF: i) to induce the survival and the expansion of the putative stem cells with truly self-renewal capacity and ii) to negatively affect proliferation and survival of progenitors at later stage of development. To better address this issue, we enriched the hippocampal population in GFAP+/Nestin+ cells, representing the putative slowly cycling neural stem cells (Ming and Song, 2011), by LIF (leukemia inhibitory factor) selection in vitro (Pitman, 2004). The selected GFAP+/Nestin+ cells represent less than 1% of the total population and express p75NTR at high level compared to the late progenitors. Interestingly, this subpopulation is present also in vivo, as demonstrated by immunofluorescence analyses performed ex vivo on cells isolated from freshly-dissociated DG. By a clonogenic assay performed in the absence of mitogens we showed that these neural stem cells respond to ProNGF by reactivating their cell-cycle and thus leading to neurospheres formation. We also investigated the effect of ProNGF on induced Neural Stem Cells (iNSCs) derived from mouse embryonic fibroblasts reprogrammed with Sox2 (Ring et al., 2012). This protocol produces mainly late Nestin+/DCX+ progenitors, that have limited propagation potential. Chronic exposure of iNSCs to ProNGFKR led to the expansion of few clones that reach greater dimension than the untreated cells, while the percentage of new forming neurosphere remained unchanged. Altogether our results show for the first time that ProNGF acts as mitogen on neural and induced stem cells.

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P079

IMPROVING MATURATION OF CARDIOMYOCYTES DERIVED FROM HUMAN PLURIPOTENT STEM CELLS: AN "-OMICS" DRIVEN APPROACH

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The production of human pluripotent stem cell derived cardiomyocytes (hPSC-CM) holds great promise for cell therapy and drug testing. However, the complex nets of signaling pathways involved in cardiomyogenesis compromises the effectiveness of the existing differentiation protocols to reproducibly produce highly pure and mature hPSC-CM. In this study we aim to overcome this hurdle by devising an integrated strategy for production and maturation of functional hPSC-CM. hPSC (hiPSC and hESC lines) were differentiated into CM, using a directed differentiation protocol adapted from the ones described in literature. With this protocol we were able to robustly originate pure 2D monolayers and 3D aggregates of hiPSC-CM. 13C-Metabolic Flux Analysis revealed that these hPSC-CM are metabolic immature, relying almost exclusively on glycolysis as major energy source. Thus, we assessed whether alteration of hPSC-CM culture medium composition to mimic in vivo substrate usage during cardiac development would promote a glycolytic-to-oxidative metabolic shift and ultimately induce hPSC-CM maturation in vitro. Combining a set of "-omics" tools (metabolomics, fluxomics and transcriptomics) with structural and functional analyses we demonstrated that hiPSC-CM cultured in glucose depleted medium supplemented with fatty acids and galactose display features that resemble more mature CM, than hiPSC-CM cultured in standard glucose rich medium, namely: energetically efficient oxidative metabolism, transcriptional signatures closer to ventricular CM; more elongated morphologies; organized sarcomeric structures; higher myofibril alignment; improved calcium handling, contractility and action potential kinetics. In sum, this work shows that glycolytic-to-oxidative metabolic shift is a cause, rather than a consequence, of hPSC-CM maturation in vitro. These findings may have important implications in the stem cell field contributing to improve the scalable production of more mature and functional hPSC-CMs.

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P081

DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TOWARD MESENCHYMAL STEM CELLS IN SUSPENSION CULTURE

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Mesenchymal stem cells (MSCs) can be derived from various adult and fetal tissues. However, the availability of tissues for the isolation of adult or fetal MSCs remains limited, requiring often invasive procedures and their quality expansion remains problematic. Traditionally, two-dimensional (2D) adherent culture conditions have been used as standard techniques for in vitro expansion of MSCs. Nevertheless, these processes are cumbersome, highly artificial and offer less physiological environment, as some in vivo characteristics and traits are lost or compromised. In contrast, non-adherent, 3D cell culture is regarded as more physiological enabling these traits to be better preserved. In addition, tissue engineering and cell-based therapy require large-scale expansion and production of pure population of lineage restricted stem cells that can be easily induced to differentiate into a specific cell type. Therefore, human pluripotent stem cells (hPSCs), either -embryonic or induced, can potentially provide alternative, unlimited and reproducible source of MSCs. Recently we have developed carrier-free suspension culture systems for PSCs, which enable culture of PSCs at high densities of 10 million cells per ml and above. The systems allow directed differentiation of PSC towered ectodermal, endodermal and mesodermal progenitors in suspension. Here we present, novel, specific and efficient directed differentiation of hPSCs towards MSCs systems in non-adherent suspension culture. hPSCs were cultured in suspension in spinner flask, and following few days in differentiation medium, our protocols generated high density MSCs which stably expressed CD105, CD146, CD90, and CD44 and lack the expression of CD45 and CD31. These MSCs exhibited high-rate differentiation toward adipocytes, chondrocytes and osteoblasts in-vitro. Therefore, our novel media and protocol provide means to generate mass production of hPSC derived-MSCs with differentiation potential into several mesodermal lineages useful for the field of cell-based therapy.

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P083

COMPARISON OF DEVELOPMENTAL DYNAMICS IN HUMAN FETAL RETINA AND HUMAN PLURIPOTENT STEM CELL DERIVED RETINAL TISSUE

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Retinal degenerative diseases such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP) are incurable blinding conditions, severely impacting quality of life and affecting millions of people. Finding efficient treatment for these devastating diseases is among the greatest unmet clinical needs. Retinal replacement strategy can bring a piece of healthy mutation-free human retina into a patient's eye to replace degenerated retinal tissue. The only donor tissue, which was demonstrated to work in animals with retinal degeneration as well as in RP patients is human fetal retinal tissue. However, fetal retina has limited availability and ethical constraints. The 3D retinal tissue (retinal organoids) derived from human pluripotent stem cells (hPSCs) shares many similarities with human fetal retina and may be an excellent replacement of fetal retinal tissue in retinal transplantation experiments. The aim of this study is to find similarities and differences in distribution and expression of molecular markers in human fetal retina and in hPSC-derived 3D retinal tissue. To assess the similarities between fetal retina and hPSC derived 3D retinal tissue we used immunohistochemistry and RNA-seq methods. Preliminary results showed high correlation in gene expression profiles between human fetal retina and hPSC derived 3D retinal tissue. Immunohistochemical profiling of developing human fetal retinal tissue 8 -16 weeks showed strong expression of retinal pigment epithelium (RPE) markers (EZRIN, Beta-catenin), retinal progenitor markers (OTX2, CRX, PAX6), photoreceptor marker (RCVRN), amacrine marker (CALB2) and ganglion marker (BRN3B). Our study will streamline the development of hPSC-3D retinal tissue technologies aimed at repairing and replacing human retina affected by degeneration and causing irreversible blindness.

P085

AFFINITY-SELECTION OF BIOACTIVE HEPARAN SULFATE SUGARS: A PROMISING STRATEGY TO AUGMENT STEM CELL THERAPY

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Bone marrow-derived mesenchymal stem cells (hMSCs) are a valuable resource for cell-based therapy. However, their abundance is low, necessitating ex vivo expansion to reach useful numbers; such expansion compromises their "stemness". Fibroblast growth factor-2 (FGF2), now in widespread use, increases hMSC proliferation, but triggers premature differentiation. Certain heparan sulfate (HS) glycosaminoglycan variants, abundant in stem cell extracellular matrix (ECM), are known to regulate the activity of many growth factors, including FGF2. Heparin has been used extensively to support human stem cell expansion as an analogue of the more physiologically relevant HS, although it adversely affects hMSCs, resulting in senescence. To obviate the need for heparin, we have isolated HS variants better targeted to the growth factor FGF2. An affinity chromatographic approach was utilized to extract an HS variant (HS8), using peptide sequences derived from the heparin-binding domains of FGF2 as bait. ELISA assays demonstrated that HS8 binds to FGF2 with much higher affinity than to other FGFs, as well as to other heparin-binding factors such as PDGF or VEGF. The melting temperature of FGF2 was markedly increased by HS8, indicating it acts to stabilize FGF2, so prolonging its activity. Both FGF2-stimulated ERK signaling and proliferation were amplified by HS8 in hMSCs. Crucially, hMSC cultures expanded with HS8 supplementation yielded a subpopulation of cells enriched for the early marker Stro-1+ as well as displaying greater CFU-F capacity. When applied into critical-sized calvarial defects in rats, HS8 significantly accelerated bone healing. Our work demonstrates that affinity-selection of HS is able to enrich for HS variants that can trigger faster hMSC ex vivo expansion without adversely changing their biological properties or potential. Such HS preparations are components for the scale-up technologies required to meet the expanding clinical need for adult stem cells.

Funding Source

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P087

IMPACT OF AGGREGATE CULTURE ON CARDIOMYOCYTE DIFFERENTIATION AND HYPOTHERMIC STORAGE

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Three dimensional (3D) cultures of human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) hold great promise for drug discovery and regenerative medicine applications. However, the transition of CM differentiation protocols from two dimensional (2D) to 3D cultures has been challenging typically resulting in lower CM purities and reduced reproducibility. Moreover, the applicability of hPSC-CMs in the clinic/industry is highly dependent on the development of efficient methods for worldwide shipment of these cells. In order to address these issues we established a differentiation protocol capable to produce highly pure 3D aggregate cultures of hPSC-CMs that relies on the aggregation of hPSC-derived cardiac progenitors. Also, we evaluated the feasibility to cold store 2D monolayers and 3D aggregates of functional hPSC-CMs using a fully defined clinical-compatible preservation formulation and investigated the time frame that hPSC-CMs could be subjected to hypothermic storage. We showed that a 3D aggregate culture of hPSC-derived cardiac progenitors: improves CM enrichment and commitment. Additionally, we demonstrated that hPSC-CMs are more resistant to prolonged hypothermic storage-induced cell injury in 3D aggregates than in 2D monolayers, showing high cell recoveries (>70%) after 7 days of storage. Importantly, hPSC-CMs maintained their typical (ultra)structure, gene and protein expression profile, electrophysiological profiles, and drug responsiveness after hypothermic storage. Overall, this study provides novel insights on the impact of 3D culture on CM differentiation and hypothermic storage that could be valuable in improving global commercial distribution of hPSC-CMs.

Funding Source

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P089

REGENERATIVE MEDICINE: GENE AND STEM CELL THERAPY FOR LUNG INJURY

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Lung is a vital organ with highly complex architectural structure and contains a variety of cell populations. Lung diseases, such as acute respiratory distress syndrome (ARDS) or chronic obstructive pulmonary disease (COPD), are both major public health problems but currently without any effective pharmacologic approach for the treatment. Stem cell therapy based on transplantation of in vitro propagated stem/progenitor cells has been proposed as a potential solution to restore lung functions. However, due to the complexity of cell source and lung microenvironment, whether transplanted cells have differentiated for reconstitution of airway/alveolar epithelium were questioned. Furthermore, safety issues have raised concerning the use of stem cells in vivo. Gene therapy has long been considered as a promising approach for the treatment of a variety of diseases. Our lab has focused on in vivo gene delivery of stemness genes in somatic lung epithelial cells using PEI nanoparticles for lung injury treatment. In mouse model of elastase-induced emphysema, we found that the transient gene delivery of BMI-1 in alveolar epithelial cells post-injury induced efficient regeneration of alveolar epithelium and improved pulmonary function. The regenerated regions showed normal alveolar epithelial phenotype and extracellular matrix components, without the symptoms of neoplasia. Furthermore, the treatment enriched the population of slow-cycling cells that appeared after injury. The BMI-1 target cells were further sorted and cultured in vitro, which formed colonies within a week. Whether these cells may undergo an in vivo reprogramming process and transiently acquire the stemness property for tissue regeneration remains to be further clarified by gene expression and epigenetic profile analyses. In summary, our study suggests that in vivo delivery of stemness genes in somatic cells in pathologic loci is a feasible approach for tissue regeneration. The target cells could proliferate and differentiate more efficiently due to the native identity and microenvironment. Such approach also avoids the complexities of in vitro propagating stem/progenitor cells resulting from differences in cell source and culture conditions. In vivo gene delivery may hold promise for the future treatment of lung diseases such as ARDS or COPD

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P091

ALTERNATIVE PLURIPOTENT STATE ENHANCES SUSPENSION MANUFACTURABILITY OF HUMAN PLURIPOTENT STEM CELL DERIVED TISSUES FOR CELL THERAPY AND DRUG SCREENING

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As stem cell technologies are translated out of the laboratory, human pluripotent stem cells (PSC) have emerged as a robust, reliable source of cells for generating inputs for a wide range of cell therapy and drug screening applications. However, the scalable bioreactor technologies required to manufacture sufficient quantities of cells for these applications typically yield only 10% of the cells that laboratory-scale adherent systems generate. Cell engineering to convert traditional PSC cultures to an alternative pluripotent state with enhanced manufacturability can overcome this bottleneck. Small molecule-directed conversion to a naïve-like(nl) state by inhibition of GSK3b, ERK, and other pathways generates nlPSC with a sustained pluripotent phenotype of >85% Oct4/Sox2+, distinct colony morphology, trilineage germ layer differentiation capacity, and a distinct gene expression fingerprint. In suspension, nlPSC formed clonal aggregates at efficiencies of 6.5±0.9%, 5x higher than PSC efficiencies. In a small scale suspension bioreactor system, nlPSC formed a larger number of smaller-sized aggregates and had reduced levels of apoptosis. nlPSC reached densities of 5.3±0.9*10^6 cells/mL in 6 days, representing a 26 fold expansion, whereas PSC expanded 3.5 fold, to 7.7±0.25*10^5 cells/mL. High yield nlPSC maintained a pluripotent phenotype for 5 passages in suspension. Suspension nlPSC had reduced specific uptake of glucose and secretion of lactate and ammonium, suggesting altered metabolic utilization between the states. Differentiation to pancreatic tissue for future cell therapies or drug screening can be achieved directly from the nlPSC state; however, reconverting cultures to the PSC state prior to differentiation generates higher purity outputs with 80±15% definitive endoderm (c-kit/cxcr4+) and 47±4% pancreatic progenitors (nkx6.1/pdx1+) at a yield of 0.7 pancreatic progenitors per input PSC. Improving efficiency of differentiation to more differentiated cell types is being explored together with application of these cells in tissue engineering and drug screening contexts. Through cell state engineering, this nlPSC culture system can enable highly efficient, scalable manufacturing of the cells required for translation of stem cell therapies and drug discovery strategies.

Funding Source

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P093

TRANSCRIPTION FACTOR-WIDE ENGINEERING OF HUMAN CELL TYPES

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The ability to produce any human cell type in a robust and facile manner would advance the development of realistic microtissues and organoids. To expand the breadth and access of in vitro cell types, we conducted large-scale cell fate engineering approach to generate cell types from human induced pluripotent stem cells (hiPSCs). First, we created a comprehensive human transcription factor (TF) expression library (the "human TFome") to systematically screen TFs that differentiate human stem cells. Second, we mapped transcription factor-cell type relationships by pooled TFome over-expression and RNA sequencing. From these approaches, we identified 78 single TFs that are potent to program stem cells into differentiated cell types of multiple tissues. We achieved potent (>80%) cellular programming without the addition of growth factors, mechanical processes or purifications by maximizing TF expression and selecting potent TF isoforms. Engineered cell types include electrically active neurons, lumen-forming endothelial cells and myoblasts. Large-scale engineering of human cell identity could pave the way towards the production of many human cell types in vitro.

P095

PATHOGEN REDUCTION THROUGH ADDITIVE-FREE SHORT-WAVE UV LIGHT IRRADIATION RETAINS THE OPTIMAL EFFICACY OF HUMAN PLATELET LYSATE FOR THE EXPANSION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS

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We recently developed and characterized a standardized and clinical grade human Platelet Lysate (hPL) that constitutes an advantageous substitute for fetal bovine serum (FBS) for human mesenchymal stem cell (hMSC) expansion required in cell therapy procedures, avoiding xenogenic risks (virological and immunological) and ethical issue. Because of the progressive use of pathogen reduced (PR) labile blood components, we evaluated the impact of the novel procedure

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THERAFLEX UV-Platelets for pathogen reduction on hPL quality (growth factors content) and efficacy (as a medium supplement for hMSC expansion). This technology is based on short-wave ultraviolet light (UV-C) and has the main advantage not to need the addition of any photosensitizing additive compounds (that might secondary interfere with hMSCs). We applied THERAFLEX UV-Platelets procedure on fresh platelet concentrates (PCs) suspended in platelet additive solution and prepared hPL from these treated PCs. We compared the quality and efficacy of PR-hPL with the corresponding non-PR ones. We showed no impact on the content in 5 cytokines tested (EGF, bFGF, PDGF-AB, VEGF and IGF) and a significant decrease in TGF-beta1 (-21%, n=16, $p < 0.01$). We performed large scale culture of hMSCs during 3 passages and showed that hPL or PR-hPL at 8% triggered comparable hMSC proliferation than FBS at 10% plus bFGF (n=3). Moreover, after proliferation of hMSCs in hPL or PR-hPL containing medium, their profile of membrane marker expression, their clonogenic potential and immunosuppressive properties (inhibition of T-cell proliferation) were maintained, in comparison with hMSCs cultured in FBS conditions. We quantitatively compared the potential to differentiate in adipogenic and osteogenic lineages of hMSCs cultured in parallel in the 3 conditions and showed that they remained also identical. In conclusion, we demonstrated the feasibility to use UV-C treatment to subsequently obtain pathogen reduced hPL, while preserving its optimal quality and efficacy for hMSC expansion for cell therapy applications.

Funding Source

Judith LORANT received financial support from French government (National Research Agency), Nantes Metropole and the Region Pays de la Loire.

MODELING TISSUE DEVELOPMENT

P097

HUMAN ADIPOSE-DERIVED STEM CELLS ON THE MICRO-PATTERNED HYDROGEL ENHANCE NERVE REGENERATION IN A RAT MODEL OF CRUSHING SCIATIC NERVE INJURY

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Neuropathic pain is the results of disorder or damage of the nervous system. Presenting symptoms include dysesthesia, hyperalgesia and allodynia. The fundamental treatment of neuropathic pain is not yet developed. However, we demonstrated that adipose-derived stem cells (ASCs) improve neuropathic pain and enhance nerve regeneration in a rat model of crushing sciatic nerve injury in our previous study. In advance, we evaluated the transplantation effect of ASCs on the micro-patterned hydrogel. Male Sprague-Dawley rats (n=29, 200g) were randomized into three groups: Group 1=PBS epidural soaking, Group 2=ASC epidural soaking, Group 3=ASCs/hydrogel epidural soaking. Transplantation was conducted 1 week later after crushing injury and subjects were observed for 5 weeks. To evaluate pain degree, each group was examined with paw withdraw latency, cold allodynia, and somatosensory evoked potentials (SSEP), and motor evoked potentials (MEP). Also to estimate nerve regeneration, the sciatic function index (SFI) was estimated and histology of sciatic nerve tissue was also performed. Group 3 showed significantly decreased paw withdraw latency and enhanced SFI value more than Group 1 and Group 2 ($p < 0.05$). Also, both SSEP and MEP improved more significantly in Group 3 compared with Group 1 and Group 2 ($p < 0.05$). Histological analysis of the sciatic nerve tissue revealed that ASCs enhanced nerve regeneration in the injured sciatic nerve. ASCs improve neuropathic pain and enhance nerve regeneration in a rat model of crushing sciatic nerve injury. Moreover, hydrogel enhances the regeneration effect of ASCs. ASCs/hydrogel epidural soaking may be used for treating neuropathic pain and neural injury in the future.

Funding Source

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P099

OPTICAL CONTROL OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CONE PHOTORECEPTORS

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Human pluripotent stem cells are an unlimited and unique source for cell replacement, tissue engineering and in vitro disease modeling. Given their differentiation capacity they can be used to generate photoreceptors among other retinal phenotypes. Despite the advances in the recent years of numerous groups to obtain mature and functional photoreceptors for cell replacement strategies, up to date no functional outer segments have been well described. Optogenetics offers the possibility to modify cells by expressing light sensitive ion channels or pumps. Here, we

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have developed a protocol of differentiation to obtain cone photoreceptors, which have been genetically engineered to carry a hyperpolarizing chloride pump that renders them responsive to light even in the absence of outer segments. In accordance with recent publications, demonstrating that development can be recapitulated better in 3D conditions, we have generated a 3D retinal organoid system. Our retinal organoids efficiently differentiated towards photoreceptor cells, expressing the major specific markers for this population such as: RCVN, CAR, OTX2 and CRX. Furthermore they have been modified to express a light-sensitive protein at their membrane, a pump hyperpolarizing the cells upon light stimulation. The colocalization studies demonstrated this was specific of cones. Using a reporter system we corroborated the functionality of our approach by targeting the engineered cones, and by using whole cell patch-clamp recording we observed that they responded to light in a fast and robust manner.

P101

MOLECULAR CHARACTERIZATION AND PROSPECTIVE ISOLATION OF HUMAN FETAL INNER EAR PRO-SENSORY DOMAIN PROGENITOR CELLS

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Mechanosensitive hair cells (HC) located in the cochlea are the sensory cells essential for the detection of sound. Their loss is irreversible in humans and a major cause of permanent hearing loss. Unraveling the mechanisms of human inner ear development and hair cell specification might enable establishment of novel therapeutic directions such as the development of human cell-based assays for screening of otoprotective and otoregenerative compounds. Hair cells develop in inner ear sensory epithelia that in turn are derived from so-called pro-sensory domains (PSD). Our current knowledge of the developmental mechanisms and timing leading to hair cell formation relies on molecular studies performed in mice. Here we have carried out a systematic analysis of the fetal human cochlea, in a temporal window spanning from week 8 to week 12 postconception when hair cells become specified, and analyzed gene and protein expression of the developing PSD. We have identified a surface marker combination, based on the co-expression of the epithelial marker EpCAM and the neurotrophin receptor CD271, that can be used for prospective isolation by facs sorting of PSD resident somatic progenitors. To validate our sorting strategy, we have been making use of organoid culture methods to derive human HCs from the sorted putative progenitors. Here we present data showing that organoids conserve protein expression and localization typical of their in vivo counterparts, namely epithelial marker expression (CD49f, EpCAM, E-Cadherin, β-Catenin), apical basal polarity (Zo-1), cochlear duct markers (sox9) and PSD markers (sox2; CD271). Moreover, cells within the human inner ear organoids can be induced to differentiate in vitro to express the hair cell markers MYO7A and POU4F3. Morphological and functional maturation, however, has not yet been demonstrated. A Fluidigm-based assay of 192 genes has been used for comparative gene expression analyses of the sorted populations, the derived organoids, as well as to follow spatiotemporal expression of known developmental inner ear markers during cochlea development. Our data provides beyond state-of-the art insight into the development of the human cochlea with particular emphasis on new methods for selection and expansion of inner ear /hair cell progenitors in vitro.

Funding Source

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P103

HUMAN PLURIPOTENT AND NEURAL STEM CELLS DIFFERENTIATE INTO CELLS BELONGING TO THE NEURAL LINEAGE IN A THREE-DIMENSIONAL RAT BRAIN CELL ENVIRONMENT

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In vitro studies of the human nervous system are mainly carried out in monolayer and employing single cell types. Two-dimensional monocultures, however, do not truly recapitulate the complex cell-cell interactions existing in the nervous system, especially those involving different cell types. Three-dimensional (3D) cultures derived from the human pluripotent or neural stem cells are being developed, but contain mainly neurons and astrocytes. None of these models contains oligodendrocytes and microglia, which play a central role in the correct development and maintenance of the brain functions and in pathological mechanisms. Rat 3D aggregating brain cell cultures are produced from brains of rat embryos and are composed of post-mitotic neurons, astrocyte progenitors, oligodendrocyte progenitors and

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microglia. Over time, these cells proliferate, differentiate and mature, giving rise to a functional tissue-like structure, where neurons form synapses, becoming electrically active and oligodendrocytes wrap their myelin sheath around axons. At the same time, astrocytes and microglial cells reach a highly differentiated phenotype. The present study describes a protocol for the incorporation of human pluripotent and neural stem cells into the rat brain aggregates. The rat neural environment induces the human cells to differentiate into neurons, astrocytes and oligodendrocytes in a time frame shorter than the currently available cultures composed of only human cells. The rat/human aggregates are a valuable tool for the study of physiology and disease in the brain, by enabling the manipulation of the rat environment and/or the human cells independently, making at the same time possible to discriminate the behaviour of the sole human cells by exploiting human-specific assays. This method could furthermore be translated from rat to mouse to take advantage of the numerous already available genetically modified mice models.

Funding Source

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P105

A SYSTEMATIC APPROACH TO UNRAVEL MOLECULAR DRIVERS OF CELL FATE SPECIFICATION IN HUMAN EMBRYONIC STEM CELLS

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The core transcriptional factor (TF) complex consisting of OCT4, SOX2 and NANOG maintains the hESC state by occupying regulatory regions and activating genes responsible for pluripotency, while suppressing genes that initiate lineage specification. This delicate balance is maintained by extracellular signalling clues of the Fibroblast Growth Factor (FGF) and TGF-β families. They create a spatiotemporal pattern of activity that is important to specify different regions of the primitive streak and subsequent definitive endoderm (DE) and mesoderm (ME) derivation by controlling the activity of downstream TF complexes to specific regulatory elements - enhancers. Such enhancers are called "poised" due to the fact that during pluripotency they are bookmarked with a unique chromatin and TF signature repressing their activity. However, it is not clear 1) how these levels of regulation intersect and interact to drive pluripotency and cell fate specification and, 2) which are the signalling mediators binding to "poised" enhancers that drive early DE and ME induction. To gain deeper mechanistic insights, we take advantage of mass spectrometry (MS)-coupled to DNA pull down, an assay we have recently established. For this, enhancer regions of genes important for pluripotency or ME and DE formation were selected based on their co-occupancy by SMAD2, OCT4 and NANOG and their compliance with the classical epigenetic signature. These fragments were labelled by PCR amplification using biotinylated oligonucleotides and the resulting biotinylated fragments were incubated with cell lysates from pluripotent or differentiated hESCs followed by DNA pull down. The differentially bound proteins between the two states were identified by MS and classified according to the number of corresponding peptides identified under the different conditions. In a further step the candidates will be validated and their role in early DE and ME differentiation will be assessed using CRISPR/Cas9 and CRISPR interference (CRISPRi) technology in human ESCs and induced PSCs. In summary, by pursuing these aims, we will shed light on new hESCs regulatory networks, which will help to understand early embryonic development and to refine culture protocols for efficient directed differentiation of clinically relevant tissues for therapeutic purposes.

P107

THE EFFECT OF MIRNA-31 AND BMP-2 ON OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STROMAL CELLS DERIVED FROM PLACENTA

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Osteogenic differentiation of human mesenchymal stromal cells (MSCs) has been widely studied both in vitro and in vivo as a potential tool for regenerative medicine and tissue engineering. While most of the studies focus on bone marrow derived MSCs (BM-MSCs), however, the use of BM-MSCs has several limitations including the invasive procedure for harvesting bone marrow and limited amount of MSCs in bone marrow especially in aging population. In addition to BM-MSCs, MSCs derived from placenta (PL-MSCs) seem to be a good alternative source of MSCs. They can be differentiated into osteoblast with a phenotypic

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similarity to that of BM-MSCs. However, the differentiation capacity is not consistent. In addition, osteogenic differentiation of PL-MSCs took a longer period of time than BM-MSCs. To date, there is an increasing information addressing the involvement of miRNAs and BMP-2 on osteogenic differentiation of BM-MSCs. However, the miRNAs and BMP-2 induced osteogenic differentiation of PL-MSCs is not fully examined. The aim of this study was to examine the effect of miRNA-31 and BMP-2 on osteogenic differentiation of PL-MSCs in comparison with BM-MSCs. Isolated MSCs were characterized morphologically and the expression of MSC markers was characterized using flow cytometer. The degree of osteogenic differentiation after BMP-2 treatment was confirmed by cell staining and alkaline phosphatase (ALP) activity assay. The expression of osteogenic genes and specific microRNAs involved in osteogenic differentiation of MSCs were also evaluated using quantitative RT-PCR. The results demonstrated that PL-MSCs could be isolated and easily expanded in culture. BMP-2 could enhance osteogenic differentiation of both PL-MSCs and BM-MSCs by up-regulating the expression of osteogenic genes including RUNX-2, OSX, and OCN. The ALP activity was increased during osteogenic differentiation of both PL-MSCs and BM-MSCs. In contrast to expression of osteogenic genes, the expression of miR-31 was decreased during osteogenic differentiation especially in BMP-2 treatment. The results obtained might be lead to the progress of bone regeneration using PL-MSCs and provide new insights for the potential of using PL-MSCs as alternative source for bone engineering or cell therapy in regenerative medicine.

Funding Source

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P109

TOWARDS RETINAL CELL REPLACEMENT THERAPY: ISOLATION OF HUMAN STEM-CELL-DERIVED PHOTORECEPTORS USING CELL SURFACE BIOMARKERS

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Loss of photoreceptor cells due to retinal degeneration is one of the main causes of blindness in the developed world. Although there is currently no effective treatment available, cell replacement therapy, using stem-cell-derived photoreceptor precursor cells, may be a feasible future treatment option. In order to ensure safety and efficacy of this approach, robust cell isolation and purification protocols must be developed. To this end, we previously developed a biomarker panel for the isolation of mouse photoreceptor precursors from the developing mouse retina and mouse embryonic stem cell cultures. In the current study we extended this approach to the human pluripotent stem cell (hPSC) system, and identified biomarkers, which alone or in combination can be leveraged for the isolation of human rod and cone photoreceptors. Human retinal samples and hPSC-derived retinal organoid cultures were screened against 242 human monoclonal antibodies using a high through-put flow cytometry approach. We identified 31 biomarkers with significant expression levels in the human retina and hPSC differentiation cultures, 16 of which showed expression in discrete cell populations. Human retinal cell samples, either from fetal tissue or derived from embryonic and induced pluripotent stem cell cultures, were FAC-sorted using select candidate biomarkers and subjected to immunocytochemical analysis with photoreceptor-specific antibodies and Ki-67, a marker of mitotically active cells, which need to be excluded from donor cell preparations. Quantitative real-time PCR analysis was conducted to assess the enrichment of photoreceptor-specific transcripts in the sorted populations. We established a biomarker combination, which enables the robust isolation of human photoreceptors from both human retinae and hPSC-derived organoid cultures. Lastly, we demonstrate that human photoreceptors isolated via this novel biomarker panel can survive within wild-type and rd1 mutant retinae following sub-retinal transplantation.

P111

MACROPHAGES INDUCE AKT-DEPENDENT LGR5+ STEM CELL HAIR FOLLICLE REGENERATION THROUGH TNF

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Skin stem cells can regenerate epidermal appendages; however, hair follicles (HF) lost as a result of injury are barely regenerated. Here we show that macrophages in wounds activate HF stem cells, leading to telogen-anagen transition (TAT) around the wound and de novo HF regeneration, mostly through TNF signalling. Depletion of Ly6C+ inflammatory macrophages but not CX3CR1+ tissue-resident macrophages attenuates wounding induced TAT and HF neogenesis. TNF is upregulated in the injured tissue around the wound in the early stage of wounding healing and subsequently in the newly formed tissue in the wound center. Both TNF knockout and over-expression attenuate HF neogenesis in wounds, suggesting dose dependent induction of HF neogenesis by TNF, which is consistent with TNF-induced AKT signalling in epidermal stem cells in vitro. TNF-induced β-catenin accumulation is dependent on AKT but not Wnt signalling.

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Inhibition of PI3K/AKT blocks depilation-induced HF TAT. Notably, Pten loss in Lgr-5+ HF stem cells results in HF TAT independent of injury and promotes HF neogenesis after wounding. Moreover, lineage tracing analysis indicates that the progeny of Lgr5+ cells (mT-/mG+) were detected in over 40% of neogenic HFs in the wound, and the number of Lgr5+ cell progeny in individual neogenic HFs differed greatly and ranged from less than 3% to over 90% of total HF cells. Thus, our results suggest that macrophage-TNF-induced AKT/β-catenin signalling in Lgr5+ HF stem cells has a crucial role in promoting HF regrowth and neogenesis after wounding.

P113

DEFINED AND SCALABLE DIFFERENTIATION OF HUMAN OLIGODENDROCYTE PRECURSORS FROM PLURIPOTENT STEM CELLS IN A 3D CULTURE SYSTEM

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Cell therapy has emerged as a potential approach for treating demyelinating diseases and conditions, including pediatric leukodystrophies, spinal cord injuries, and diffuse demyelination after radiation therapy for cancer. The use of primary oligodendrocyte precursor cells (OPCs) for remyelination therapy has been challenging as they are difficult to access and are not highly expandable; however, human pluripotent stem cells (hPSCs) are a promising source for OPCs. Here, we demonstrate that OPCs can be reproducibly differentiated from human pluripotent stem cells (hPSCs) in a three-dimensional (3D), scalable, and fully-defined thermoresponsive biomaterial system. We used CRISPR-Cas9-mediated genome engineering to establish a NKX2.2-EGFP reporter cell line and investigate early OPC differentiation in the 3D hydrogel. Harnessing this line to test multiple differentiation conditions in a simple and real-time manner, we identified a method that markedly increased the number of progenitor cells that expressed both OLIG2 (~70%) and NKX2.2 (~80%), by day 18 of the differentiation. Based on the early appearance of O4- and MBP-positive OPCs and oligodendrocytes, respectively, we also show that this 3D synthetic biomaterial culture systems is particularly well suited for OPC maturation. Furthermore, straightforward transplantation of the resulting, non-sorted OPCs into NOD/SCID mice revealed that OPCs differentiated for 50 days could engraft, migrate and mature into myelinating oligodendrocytes. In conclusion, we developed a rapid, simple, and efficient approach for generating OPCs by combining the benefits afforded by scalable 3D culture with hESC reporter line guided optimization.

Funding Source

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P115

DEVELOPMENT OF AN ACELLULAR URETHRAL SCAFFOLD FROM SEX REASSIGMENT PATIENTS: CRYOPRESERVATION EFFECTS AND BIOCOMPATIBILITY EVALUATION

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Different tissues have been used for urethral repair. These substitutes have limitations compared to urethral tissue (UT). Acellular scaffolds from human urethra may be a suitable alternative. The objectives were to develop a decellularization method for UT, evaluate cryopreservation effects and biocompatibility of acellular scaffold. 7 urethral samples from male patients were used. 2 decellularization protocols in 2 periods (3 or 7 days) were analyzed: sodium deoxycholate 1% (PR1) and Triton X-100 1% (PR2). 2 freezing media with DMSO 0.7M (PRA) and 1.5M (PRB) were evaluated. Decellularization and structural integrity were assessed by histological analysis, actin WB, DNA levels and scanning electron microscopy (SEM). Extracellular matrix (EM) proteins (collagen I and IV, laminin, fibronectin and elastin) and VEGF were studied by IHC and dot blot. To evaluate biocompatibility, scaffolds were implanted into the subcutaneous space of 8 week old male mice (2 BALB/c and 2 athymic nude). Mice were sacrificed after 3 weeks. The implants were fixed in formalin to histological analysis and IF of cytokeratin and vimentin. PR1 and PR2 applied for 3 days, maintained high cellular components, while PR2 applied for 7 days showed total decellularization, undetectable DNA and actin levels with high structural integrity evaluated by SEM. EM proteins and VEGF were higher in PR2 than PR1. Comparing the 2 freezing protocols, PRA presented better integrity and protein levels

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than PRB. PRA/PR2 showed the highest levels of EM proteins and VEGF, even better than PR2 without freezing cycle. Mice implants showed no pathological inflammatory responses with both decellularization protocols. Histological analysis showed high fibroblastic infiltrating cells with the presence of some macrophages. Angiogenic activity associated with the external implant surface was observed mainly in PR2 protocol. Vimentin showed strong expression in infiltrating cells. These results show that PR2 applied for 7 days is the best decellularization protocol and suggest that a freezing cycle, previously to decellularization promotes the UT integrity. Furthermore, our results suggest that both PR1 and PR2 have high degree of biocompatibility. Urethral scaffold from sex reassignment patients represents a feasible tissue for urethral repair.

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P117

HYPOXIA-SPECIFIC VEGF-EXPRESSING SYSTEM USING ERYTHROPOIETIC ENHANCER LEAD TO RECOVERY OF NEUROPATHIC PAIN

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Vascular endothelial growth factor (VEGF) is an angiogenic cytokine that stimulates the differentiation and function of vascular endothelial cells. VEGF has been implicated in improving nervous system function after injury. However, uncontrolled overexpression of VEGF increases the risk of tumor formation at the site of gene delivery. For this reason, VEGF expression needs to be strictly controlled. The goal of the present study was to understand the effects of hypoxia-induced gene expression system to control VEGF gene expression in neural stem cells (NSCs) on the regeneration of neural tissue after sciatic nerve injury. In this study, we used the erythropoietin (Epo) enhancer-SV40 promoter system (EpoSV-VEGF-NSCs) for hypoxia-specific VEGF expression. We used three types of NSCs: DsRed-NSCs as controls, SV-VEGF-NSCs as uncontrolled VEGF overexpressing NSCs, and EpoSV-VEGF-NSCs. For comparison of VEGF expression at normoxia and hypoxia, we measured the amount of VEGF secreted. VEGF expression decreased at normoxia and increased at hypoxia for EpoSV-VEGF-NSCs; thus, EpoSV-VEGF-NSCs controlled VEGF expression, dependent upon oxygenation condition. To demonstrate the therapeutic effect of EpoSV-VEGF-NSCs, we transplanted each cell line in a neuropathic pain sciatic nerve injury rat model. The transplanted EpoSV-VEGF-NSCs improved sciatic nerve functional index (SFI), mechanical allodynia, and re-myelination similar to the SV-VEGF-NSCs. Additionally, the number of blood vessels increased to a level similar to that of the SV-VEGF-NSCs. However, we did not observe tumor generation in the EpoSV-VEGF-NSC animals that were unlikely to have tumor formation in the SV-VEGF-NSCs. From our results, we determined that EpoSV-VEGF-NSCs safely regulate VEGF gene expression which is dependent upon oxygenation status. In addition, we found that they are therapeutically appropriate for treating sciatic nerve injury.

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P119

DIFFERENTIATION AND TRANSPLANTATION OF EMBRYONIC STEM CELL-DERIVED CONE PHOTORECEPTORS INTO A MOUSE MODEL OF END-STAGE RETINAL DEGENERATION

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The loss of cone photoreceptors that mediate daylight vision represents a leading cause of blindness, for which cell replacement by transplantation offers a promising treatment strategy. Here, we characterise cone differentiation in retinae derived from mouse embryonic stem cells (mESCs). Similar to in vivo development, a temporal pattern of progenitor marker expression is followed by the differentiation of early thyroid hormone receptor β2-positive precursors and, subsequently, photoreceptors exhibiting cone-specific phototransduction-related proteins. We establish that stage-specific inhibition of the Notch pathway increases commitment to the cone lineage, whilst retinoic acid signalling regulates cone maturation, comparable to their actions in vivo. MESC-derived cones can be isolated in large numbers and transplanted into adult mouse eyes, showing capacity to survive and mature in the subretinal space of Aipl1-/- mice, a model of end-stage retinal degeneration. Together, this work identifies a robust, renewable cell source for cone

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replacement by purified cell suspension transplantation.

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P121

NEURAL TUBE MORPHOGENESIS IN SYNTHETIC 3D MICROENVIRONMENTS

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The earliest steps of development are characterized by cellular reorganization and differentiation within a three-dimensional (3D) microenvironment. This 3D context allows for a complex spatial interplay between biochemical and physical signals, and governs important cellular rearrangements leading to morphogenesis. In-vitro approaches have attempted to recapitulate key features of these processes, and it has now become possible to generate an increasing variety of self-organizing multicellular tissue constructs termed organoids. While important aspects of the 3D in-vivo organization have been recreated in these organoid systems[1-3], such studies have been exclusively performed in MatrigelTM, a poorly defined proteinaceous mixture whose properties cannot be readily modulated. As such, the uncharacterized interactions between cells and this extracellular matrix (ECM) have proven to be a major challenge to understanding the underlying regulatory mechanisms governing morphogenesis. In this work, we employ tunable synthetic ECM hydrogels in a high throughput screening approach in order to disentangle the contributions of biochemical and physical components of the microenvironment and to elucidate the mechanisms controlling early neuroepithelial commitment of single mouse embryonic stem cells[4]. We show the synergistic roles of matrix elasticity, degradability and ECM protein composition in specifying neural fate and initiating apico-basal polarity. We explore how matrix characteristics relate to dynamic symmetry-breaking events in such multicellular constructs, and show how apico-basal polarity is required for initiating subsequent dorso-ventral patterning. We demonstrate that these morphogenetic processes are tightly coupled to the physical characteristics of the matrix, and demonstrate for the first time that a patterned neural tube-like organoid can be generated within an optimized, fully synthetic matrix.

OTHER

P125

ENGINEERED BONE ORGANS TO STUDY AND ENVIRONMENTALLY REGULATE THE ENGRAFTMENT OF HUMAN HEMATOPOIETIC STEM CELLS WITHIN HUMAN ORIGIN NICHES.

Pigeot, Sébastien[1], Bourgine, Paul E.[2], Fritsch, Kristin[3], Takizawa, Hitoshi[4], Kunz, Leo[2], Kokkaliaris, Konstantinos[2], Coutu, Daniel[2], Manz, Markus G.[3], Martin, Ivan[1], Schroeder, Timm[2]

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Hematopoietic stem cells (HSC) reside and are regulated in a specialized microenvironment in the bone marrow (BM), called "HSC niche". So far, little is known about the cellular and molecular components of the human BM niche, also due to the limited availability and customization potential of humanized models. We previously demonstrated that human bone marrow mesenchymal stromal cells (BM-MSCs), induced in vitro to differentiate into hypertrophic cartilage (HyC) tissues and subsequently implanted subcutaneously in nude mice, can robustly generate endochondral bones (ossicles). Here, we report the successful engineering of humanized BM niches as a platform to engraft, maintain and study human hematopoiesis. BM-MSCs transduced with a VENUS reporter or a VENUS reporter fused to the SDF1α protein were seeded onto collagen sponges (6mm diameter, 2 mm thick), chondrogenically differentiated and implanted in Rag2-/- mice mutants expressing human thrombopoietin (hTPO) and human signal regulatory protein alpha (hSIRPa). After 4 weeks, mice were sub-lethally irradiated and transplanted with CD34+ cells derived from third party-donor cord blood (CB). After 2 months post transplantation, ossicles were explanted and the retrieved cells analysed cytofluorimetrically to quantify human HSCs and progenitors. The VENUS reporter allowed histological analysis of the human stroma environment in association to the human HSCs by deep confocal microscopy analysis. VENUS ossicles showed similar engraftment as the mouse bone whereas VENUS-SDF1α ossicles generated a 2 to 2.5 fold increase in HSCs and progenitors engraftment. Finally, the VENUS reporter combined with multidimensional deep imaging allowed mapping of the BM-MSCs fate into osteoblasts, osteocytes and also in association with vasculature structures. The present study demonstrates the feasibility to engineer and customize human MSC-based marrow organoids to investigate and regulate interactions with human HSC.

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MICROGLIA IN THE RAT SUBVENTRICULAR ZONE REMAIN ACTIVATED AFTER NEONATAL HYPOXIA-ISCHEMIA AND SUPPORT NEUROSPHERE GENERATION IN VITRO

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Recent studies in rodents indicate that microglia in the subventricular zone (SVZ) support neurogenesis in healthy newborn and after adult stroke. We characterized temporal changes in SVZ microglia after hypoxia-ischemia (HI) in rat neonates, and analyzed the effect of microglia depletion on neurosphere generation in vitro. Postnatal day (P) 7 rats were subjected to right-hemispheric (ipsilateral) HI or sham surgery and were sacrificed at P10, P20 or P40. Microglia were immunohistochemically analyzed in the anterior SVZ, the adjacent M2 cortex (CX) and median corpus callosum (CC). For a neurosphere assay, periventricular tissue including the ipsilateral SVZ from P10 sham or HI animals was dissociated and cultured. Microglia were selectively depleted in vitro by saporin-conjugated antibodies. Our results show that in sham ipsilateral SVZ, microglial density (Iba1+ cells per SVZ area) was constant between P10 and P40. The number of phagocytic microglia (ball-and-chain Iba1+ cells) and the ratio of activated microglia (CD68+Iba1+/Iba1+ cells) declined significantly after P10. However, in HI animals, microglial density and number of phagocytic microglia remained significantly increased until P40, and microglial activation until P20 respectively, when compared to age-matched sham SVZ. These findings were observed in the SVZ only and did not occur in the adjacent CX or CC. In periventricular cell cultures from both P10 sham and HI animals, neurosphere numbers were significantly reduced if microglia were depleted. To conclude, microglia in the postnatal SVZ undergo unique developmental phenotypic changes. While microglial activation rapidly decreases in sham SVZ, neonatal HI significantly alters microglial development with cell accumulation, prolonged phagocytosis and activation. Reduction of neurosphere numbers after microglia depletion in vitro suggests a supportive role of microglia for neurogenesis in early postnatal development and HI.

Funding Source

Swiss National Science Foundation (SNF) 146632; Departement of Neurosurgery, University Hospital Basel, University of Basel

P129

SELF-CONSTRUSTION OF AN AFFORDABLE CLEAN AIR FACILITY FOR (STEM) CELL THERAPY

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We have worked extensively on the differentiation potential of mouse embryonic stem cells to blood islands containing embryoid bodies (EBs) in culture. Such EBs contain hematopoietic stem- precursor cells (HSCs) committed to lymphoid, myeloid lineages and cells possessing Natural Killer (NK) cell marker. Further differentiation of such EBs to mature B- lymphoid cells can be demonstrated in a second stage of tissue culture, by co-culturing disrupted cells with a mitogen, Lipopolysaccharide (LPS). The demonstration of differentiation of such cells to mature T- and B-lymphoid cells was performed in vivo, i.e., by implanting subcutaneously EBs in nude mice. A scale up process of differentiation of ESCs to EBs became a necessity in order to obtain enough HSCs containing EBs for a second stage of differentiation, in vitro and in vivo. To select HSCs containing EBs must be performed manually under microscope outside the tissue culture incubator. Unlike the research project, it is recommended to be free of antibiotics in the medium for clinical grade product, however, the conventional laboratory environment is not sufficient to keep the culture sterile. A GMP (Good Manufacture Production) facility with clean air became necessary. The commercial clean air facility used in the operation room and/or by the industry was not affordable by us. We have developed an affordable GMP facility for clinical research within our budget. It worked and the price for such a GMP facility is 10% of the industrial construction. This work will devote to describe and to share our experiences of constructing such affordable GMP facilities from scratch.

Funding Source

ISPI e.V. internal funding

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HUMAN WHARTON'S JELLY MESENCHYMAL STEM CELLS POSSESS THE POTENTIAL TO DIFFERENTIATE INTO OOCYTE-LIKE CELLS AFTER INDUCTION BY FOLLICULAR FLUID

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Wharton's jelly-derived mesenchymal stem (WJ-MSCs) have combination of prenatal and postnatal MSCs properties. Also WJ-MSCs have a same differentiation origin with primordial germ cells. Follicular fluid (FF) is a rich source of essential components for oocyte development. Here we examined the FF ability for differentiation of oocyte like cells from WJ-MSCs. WJ-MSCs were isolated by explant method. Then, we used flow cytometry in order to analyze the expression of mesenchymal-specific markers in cultured WJ-MSCs at passage three. Next, WJ-MSCs were induced to female germ line cells in Î±-MEM containing 10% human follicular fluid for 21 days. Expression of oocyte and germ cells specific markers including ZP1, ZP2, ZP3, GDF-9, VASA, C-KIT, and SYCP3 were evaluated by Real-Time RT PCR on days 0, 7, 14 and 21 and expression of ZP3, GDF9, VASA, and SYCP3 were investigated by immunofleurescent staining at protein level on day 21 of differentiation. Flow cytometry analysis showed that the WJ-MSCs do not express hematopoietic cell marker (CD34-45) but express mesenchymal specific markers (CD73, CD90 and CD105). During the differentiation, a subpopulation of round-large cells (~50 Âµm) appeared in culture that were similar to oocytes morphologically. Hereafter, we called these cells as oocyte like cells (OLCs). Real time RT-PCR analysis and immunofleurescent staining demonstrated that FF induced differentiation in WJ-MSCs. Differentiated WJ-MSCs expressed oocyte and germ cells specific markers and by significantly increased on day 7 of induction compared to undifferentiated WJ-MSCs. Conclusion: The present study demonstrates that FF induced OLCs from WJ-MSCs differentiated.

P133

DEVELOPMENT OF A COMPUTATIONAL PREDICTION MODEL FOR MIGRATION OF EXOGENOUS L-MYC EXPRESSING NEURAL STEM CELL IN THE BRAIN

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A major obstacle to successful pharmacological treatment of brain tumors is the blood-brain barrier, which prevents most anti-cancer agents from entering the brain parenchyma in amounts sufficient to eradicate tumors. Neural stem cells (NSCs)—immature cells that can regenerate any type of cell in the nervous system—offer a potential solution to treating neurodegenerative diseases. NSCs have been investigated as a delivery vehicle for prodrug activating enzymes, nanoparticles, and other anti-cancer agents in the context of intracranial (IC), intravenous (IV), and intranasal (IN) delivery. However, in order for NSC therapy to be effective, a sufficient number of viable cells must reach the diseased or damaged area(s) in the brain, but the paths that the NSCs take to the tumors, as well as the location of tumors within the brain may affect the ultimate number of viable cells that reach the tumor. Recently, we have developed a new human NSC line expressing L-myc gene, LM-NSC008, with favorable physiological, multi-lineage differentiation, and migratory properties (Li Z. et al, 2016). In mouse models of brain tumors and traumatic brain injury, LM-NSC008 cells migrate specifically towards sites of brain injury and tumors. In our preclinical studies using 3D reconstructions of serial tissue sections, we have also observed that LM-NSC008 were found migrating along white matter tracts. In this study, we used computational analyses of tissue anisotropy to identify white matter tracts amenable to NSC migration, which provides the ability to analyze and predict routes of migration and biodistribution of LM-NSC008 cells within the brain. A successful outcome of developing a computational predictive model of NSC migration in the brain would provide a quantitative method for non-invasive, repeat treatments via IN, IC, or IV, delivery, which could change the NSC treatment administration paradigm for patients with brain tumors or injuries. The

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proposed computational method will greatly enhance the translational potential of delivery of NSCs to treat brain tumors and other brain pathologies by allowing us to identify the most efficient targets for NSC therapy based on the most likely route of migration to the target site.

Funding Source

Pediatric Cancer Research Foundation Alex Lemonade Stand Foundation

P135

NEUROGENIC STEM CELLS IN A DORMANT NICHE ARE ACTIVATED BY ANTIDEPRESSANT FLUOXETINE AND SUPPRESSD BY NOTCH2 SIGNALING

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The brains of adult mammals contain neural stem cells (NSCs) with the potential to produce neurons throughout life. However, neurogenesis progressively diminishes with age and this has been linked to stem cell exhaustion and mitotic quiescence, but the actual cause is not known. Notch signaling is a key regulator of NSC maintenance, proliferation and differentiation. The individual contribution of different Notch receptors to these processes has not been depicted in detail. We have analyzed the role of Notch signaling in adult forebrain neurogenesis by the cross analysis of different Notch signaling component knockout (Notch1, Notch2, RBPJk) animals from adult NSCs in the murine subventricular zone. We found that distinct Notch receptors play unique roles in adult neurogenesis. Notch2, but not Notch1, reversibly represses dormant NSCs in the SVZ. We identified a dormant stem cell niche in the dorsal medial wall (dMW) of the SVZ. This dormant niche displays primary characteristics of a germinal zone, containing Notch2-dependent, inactive NSCs, which are responsive to antidepressant treatment (Fluoxetine). The ablation of Notch2 from quiescent NSCs in the adult dMW induces ectopic production of new neurons in the septum. Our results indicate that NSCs with latent neurogenic potential remain within dormant niches in the adult mouse brain and can be reanimated to produce neurons.

P137

MANAGEMENT OF OSTEOPOROSIS USING STEM CELL THERAPY: IS IT POSSIBLE?

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Osteoporosis and its complications is one of the major healthcare issue in the world today. Drugs are available for the management of osteoporosis and has its own complications. The objective of this study to assess infusion of osteoblasts for the treatment of osteoporosis in rats. Osteoporosis was induced in 20 female Sprague-Dawley rats by performing ovairectomy in 4 weeks old. After obtaining ethical approval from the

University of Dammam, 20 Sprague-Dawley female rats were procured and kept for a three days before the study was started. All animals were housed and handled in accordance with the guidelines. At 1 week bilateral ovariectomy (OVX) was carried out. At 3 months a biopsy of the iliac crest was made to assess the bone quality and the same site bone marrow was harvested. From the bone marrow aspirate, MSCs were separated as described by Piao et al. (2005)1. Once Osteoblasts were separated and were characterized using Alizarin red staining. At 6 months tetracycline doubled labelled biospy carried out. Osteoblasts were injected in the tail vein of 10 rats. 2 weeks later post injection of osteoblasts, a second biopsy was done. Animals were euthanized after 8 weeks of osteoblasts infusion by overdose of ketamine mixed with xylazine. The whole femurs and lumbar spine were dissected out and the specimens were stored in 2% formalin. The specimens were analysed using HRpQCT (High resolution peripheral quantitative computerized tomography (Î¼CT 100, SCANCO Medical AG, BrÃ¼ttisellen, Switzerland). Statistical Analysis was performed using SPSS Inc Version 19. The statistical level of significance was < 0.05 was considered significant. There were no complications of infection or deaths in either the group. In all the 10 animals whom the bone aspirate was done, osteoblasts were cultured and were transplanted. Analysis showed that there was significant bone formation at bone sites of distal femur and lumbar

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spine (P < 0.001), with increased number of trabeculae and thickness (P < 0.001). Further analysis revealed that there was robust bone formation in the animals who had osteoblasts injection. This preliminary study indicates that osteoblasts infusion can lead to new bone formation in osteoporosis induced by ovariectomy in rats. Our results warrants further studies.

Funding Source

University of Dammam

P139

THE HEARTPATCH, A VERSATILE CELL-ALGINATE BASED SCAFFOLD FOR CARDIAC TISSUE ENGINEERING

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Nearly half of all deaths in Europe are caused by cardiovascular diseases. Full recovery of affected hearts through current therapies is not possible and many patients still progress towards end-stage heart failure. Regenerative approaches aiming to cure these conditions face substantial problems with incorporation and survival of injected cell suspensions. We designed a 3D printed, hydrogel-based heartpatch that sustains cardiac muscle identity as a functional unit over a long time, thus making it a promising candidate for regenerative applications. Highly biocompatible and xeno-free ultra-high viscosity (UHV) alginates obtained from a special blend of algae (Alginatec, Germany) are 3D printed using the BioScaffolder 2.1 (GeSiM, Germany). In concert with a novel viscosity-independent printing method, it allows both randomized patterned surface and tunable scaffold stiffness. Additionally, adjustable surface modifications allow for specific ECM protein compositions of the cardiac niche through a carbodiimide-based linker chemistry. Human induced pluripotent stem cell-derived cardiomyocytes (abbr. hiPSC-CMs) are cultured on the alginate scaffolds for over 3 months, while exhibiting self-sustained macro contractions, visible with the bare eye. Gene expression profiling indicates increased cardiomyocyte maturation and immunocytochemistry analysis shows enhanced cell-cell interactions with a high degree of electrophysiological coupling and cytoskeletal maturation. As a result, and supported by an optical-based analysis of the self-sustained depolarizations, force transduction resembles the in vivo situation more closely. In conclusion, our viscoelastic hydrogel provides an additional degree of freedom to hiPSC-CMs, leading to an enhanced cardiac muscle model with possible applications in regenerative medicine.

P141

DIFFERENTIATION AND SURVIVAL OF NEUROBLASTOMA DEPENDS ON UROKINASE RECEPTOR (UPAR) ACTIVITY

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Neuroblastoma is an undifferentiated malignant tumor of the sympathetic nervous system. It is common in children and is treated by retinoic acid to induce differentiation. Expression of receptor tyrosine kinase EGFR is up-regulated in malignant neuroblastoma, while inhibition of EGFR-dependent signaling blocks neuroblastoma differentiation. It has been shown in vitro that phosphorylation of EGFR and activation of EGFR-dependent MAPK-kinases (Erk1/2 and Akt) are drastically increased in N2a cells upon serum withdrawal. Urokinase (uPA), present in the serum, and its specific receptor (uPAR) facilitates tumor progression. uPA and uPAR mediate the activation of extracellular proteolysis and intracellular signaling via receptors that interact laterally with uPAR. We studied the effects of uPAR expression on N2a differentiation and intracellular signaling. The time of serum deprivation correlated positively with the increase in uPAR expression. After 72 hours uPAR was increased by 50% compared to control. uPAR co-localized with phosphor-EGFR on the plasma membrane and uPAR overexpression increased this co-localization. uPAR overexpression didn't affect the total amount of EGFR or NeuN (neuronal specific nuclear protein), while uPAR blocking by antibody resulted in a rapid (5 min) reduction in phospho-EGFR (Y1068) in N2a cells. uPAR blocking resulted in the activation of EGFR and its downstream kinases (MAPK-p38, Akt, and c-Src) and in the decrease of Erk1/2 phosphorylation. Such signaling is known to decrease cell viability and promote apoptosis. uPAR blocking led to a 4-fold reduction in N2a proliferation, DNA fragmentation and generation of apoptotic PARP-1 fragments. This study demonstrates for the first time uPAR's role in the regulation of differentiation and survival pathways in neuroblastoma, suggesting that uPAR can be considered a therapeutic target in the treatment of neuroblastoma. Source of funding: Grant of the Russian Science Foundation (№14-24-00086).

Funding Source

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UNDERSTANDING THE QUIESCENT-ACTIVATED SWITCH: ROLE OF ID4 IN NEURAL STEM CELLS REGULATION

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Neural stem cells (NSCs) are normally in a quiescent or dormant state in the adult brain. Quiescence is essential for NSC pool maintenance and avoids stem cell exhaustion. Some of the genes implicated in the maintenance of the quiescence have been recently identified. However, the transcription factors and the molecular mechanisms regulating the NSC quiescent-activated switch remain largely unknown. Here, we have identified the transcription factor Id4 (Inhibitor of DNA-binding protein) – a downstream effector of BMP signalling pathway – as one of the factors implicated in maintaining quiescence in NSCs from the adult subventricular zone (SVZ). Id4 is the last described member of the Id family and it has been shown to be involved in the development of the central nervous system, participating in several cellular processes such as proliferation, differentiation and apoptosis. Nevertheless, the implication of Id4 protein in a non-proliferative role such as quiescence is a pathway that has not been undertaken. We have reported high levels of Id4 protein expression in NSCs, astrocytes and ependymal cells, which are quiescent cells in the adult brain. In order to understand Id4 function in this context, we used the Id4 conditional mouse line under a Glast reporter to specifically delete Id4 in SVZ stem cells at a given time point. In vitro and in vivo analyses showed that deletion of Id4 in stem cell population increases proliferation supporting the hypothesis of Id4 as quiescent regulator. In this sense, our study identifies for the first time Id4 as an important regulator of stem cell quiescence in the adult SVZ.

Funding Source

Marie Skłodowska-Curie Individual fellowship

P145

FRACTIONATED HUMAN ADIPOSE AS A NATIVE CONSTRUCT FOR THE GENERATION OF A BONE ORGAN BY ENDOCHONDRAL OSSIFICATION.

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Department of Biomedecin and Tissue Engineering, University of Basel, Switzerland

Adipose stem cells (ASC) generate bone organs through endochondral ossification if suitably primed in vitro (Osinga et al., 2016). However, many steps are required to generate such osteogenic grafts, from cell isolation to in vitro monolayer expansion of ASC, who has been shown to strongly decrease their differentiation potential. We hypothesize that human adipose tissue is not only a source of regenerative cells but could also serve as an ideal scaffolding material, thanks to its native extracellular matrix and microenvironment. The goal of this study was thus i) to develop protocols allowing cell expansion, new matrix formation and chondrogenic commitment of ASC directly inside the native adipose tissue, and ii) to evaluate the ectopic bone formation capacity of such constructs in vivo by endochondral ossification. Human liposuctions were fractionated and cultured to allow cell expansion. After 3 weeks, 4-mm cylinders were punched out of the resulting constructs (named Adiscaff) and cultured for 4 weeks with chondrogenic induction medium and 2 more weeks with hypertrophic induction medium. Minimally expanded ASC from the same donors, seeded in 4-mm cylinders of collagen (UltrafoamTM, Osinga et al., 2016) and cultured with the same differentiation media were used as control. Both constructs types were then implanted subcutaneously in nude mice for 8 weeks. Cells in Adiscaff proliferated in vitro and generated a stromal matrix contained ASC as well as endothelial cells. After the chondrogenic phase, Adiscaff produced cartilage, containing high levels glycosaminoglycans and type II collagen. Upon hypertrophic culture, gene and protein expression analyses showed upregulation of markers of terminal chondrogenic differentiation. In vivo, Adiscaff constructs generated bone tissue, both of cortical and trabecular types, vascularized and included a bone marrow compartment. In situ hybridization for human-specific sequences revealed a direct contribution of human implanted cells to bone formation in Adiscaff. These data demonstrate that native human adipose tissue can be used as a construct for the generation of bone by endochondral ossification. This novel paradigm bypasses the steps of cell isolation and monolayer expansion, and has translational relevance in the field of bone regeneration therapies.

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P147

DIRECT INDUCTION OF ENDOCHONDRAL OSSIFICATION OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS IN A FUNCTIONALIZED HYDROGEL SYSTEM

Stüdle, Chiara[1], Barbero, Andrea[1], Centola, Matteo[2], Haumer, Alexander[1], Metzger, Stéphanie[3], Ehrbar, Martin[3], Martin, Ivan[1]

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Endochondral ossification encompasses the process during long bone development that starts with the condensation of embryonic mesenchymal cells forming the cartilage anlagen which then is remodelled into bone. Earlier, we demonstrated that adult human bone marrow-derived mesenchymal stromal cells (BMSCs) can form bone upon ectopic implantation through the recapitulation of the endochondral process. However, for efficient production of ossicles, a several weeks long in vitro culture step to generate a mature hypertrophic tissue is required prior to implantation. Moreover, the process suffers from high donor to donor variability. Here, the goal was to develop a system where the in vitro priming step can be omitted and the BMSCs are instructed to undergo chondrogenesis directly in vivo. For this, an enzymatically cross-linkable and cell degradable poly(ethylene glycol) based hydrogel was used to be functionalized with TGFβ3 by employing an affinity binding strategy. BMSCs underwent chondrogenic differentiation in functionalized hydrogels within two weeks upon immediate subcutaneous implantation. These cartilaginous constructs were subsequently remodelled leading finally after 12 weeks to ossicles consisting of a rim of cortical bone surrounding a host-derived hematopoietic compartment. In contrast, in non-functionalized gels the cells remained undifferentiated. These findings suggest that the BMSCs are induced to take the endochondral route by the presentation of TGFβ3 in the gel resulting in very reproducible ossicle formation. Through the functionalization with TGFβ3 not only the otherwise needed in vitro culture step is bypassed, but also the inter donor variability is reduced. Aiming at generating a properly integrated cartilage-bone interface in the context of osteochondral repair, in an ongoing study the TGFβ3 functionalized gel is extended by a second layer containing human chondrocytes.

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A STANDARDIZED AND CHARACTERIZED CLINICAL GRADE HUMAN PLATELET LYSATE FOR EFFICIENT EXPANSION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS

Viau, Sabrina[1], EAP, Sandy[1], Chabrand, Lucie[1], Lorant, Judith[2], Bertholet, Pauline[1], Bouckenooghe, Thomas[1], Lagrange, Anaïs[1], Goudaliez, Francis[3], Rouger, Karl[2], Delorme, Bruno[1]

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Human platelet lysate (hPL) is rich in growth factors (GF) and nutritive elements and represents an advantageous xeno-free alternative to fetal bovine serum (FBS) for mesenchymal stem cell (hMSC) proliferation. However, there is a great variability in hPL sources and production protocols, resulting in discrepancies in product quality, low management of product safety and poor batch-to-batch standardization. We describe here the development and the characterization of a standardized hPL prepared from outdated transfusional grade screened normal human donor platelet concentrates (PCs), manufactured on an industrial scale (batch sizes of 10 L; 250 donors) and following a highly qualified process (clean room, trained operators, validated filtration). PCs were frozen at -80°C and thawed at +4°C to lyse platelets. Cell debris were removed by centrifugation and the supernatant (hPL) was recovered. Clinical grade 10L batches of aseptic filtered hPL were characterized. First, we showed that hPL prepared from a limited number of donors displayed a variability in terms of GF contents. On the contrary, we observed a robust standardization between 10L-industrial batches of hPL in terms of GF contents (bFGF, EGF, VEGF, PDGF-AB, TGF-beta1 and IGF-1), biochemical analyses (total proteins, albumin, fibrinogen and iron) and efficacy on bone marrow (BM)-hMSC proliferation. Secondly, we compared expansion and functional characteristics of BM-hMSCs grown in clinical grade hPL versus MSC-screened FBS batch. We showed a reproducible increase in cell growth kinetics using hPL, a maintenance of BM-hMSC clonogenic potential and membrane marker expression (with however a strong overexpression of CD90). We observed a similar adipogenic and osteogenic differentiation potential and finally that immunosuppressive properties of BM-hMSCs (inhibition of T-cell proliferation) cultivated in parallel in both conditions remained also identical. Finally, we documented the stability over time of hPL stored at -80°C and -20°C. In conclusion, we demonstrated the feasibility to use a standardized, efficient and clinical grade hPL for research and cell therapy applications.

Funding Source

Judith Lorant received financial support from French government (National Research Agency), Nantes Metropole and the Region Pays de la Loire.

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EVEN NUMBERED POSTERS WILL BE PRESENTED WEDNESDAY, 1 MARCH 12:30 TO 14:00

POSTER SESSION II

Note: Abstracts for Poster Session I (odd-numbered posters) can be found on page 35

CELLULAR DISEASE MODELS

P002

GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELL LINE FROM CHARCOT-MARIE-TOOTH DISEASE PATIENT WITH GAP JUNCTION BETA1 MUTATION

Son, Daryeon, Kang, Phil Jun, Kim, Dae-Sung, You, Seungkwon
Korea University, Seoul, Korea

Charcot-Marie-Tooth (CMT) disease is inherited neurological disorder that affects peripheral nerve system. Patients who have CMT disease have muscle weakness in legs, feet and also have highly arched feet. One of the common type of CMT disease is the X-linked form (CMT1X) which is caused by mutation in gap junction beta 1 gene (GJB1). Currently, it may be possible to relieve some symptoms by physical therapy, occupational therapy and orthopedic devices but there is still no cure for CMT disease. Induced pluripotent stem cells (iPSCs) have great potential as personalized medicine and disease modeling because they represent personal genetic information. Therefore, iPSCs are suitable for CMT disease modeling that are useful for finding disease mechanism and therapeutic method. Here, we generated iPSCs from CMT1X disease patient fibroblasts by electroporation with episomal vectors encoding OCT4, SOX2, KLF4, L-MYC, LIN28 and shRNA-p53. Established iPSCs expressed various pluripotency markers, had differentiation potential of three germ layers in vitro and in vivo with normal karyotype. In addition, these cells also retained one allele transition (G>A) of GJB1 gene which substitutes Methionine for Valine at codon 139. This iPSC line could be useful for CMT1X disease research.

P004

CELLULAR MODELING OF FABRY DISEASE USING HUMAN INDUCED PLURIPOTENT STEM CELLS

Do, Hyo-Sang[1], Park, Sang-Wook[2], Lee, Beom-Hee [3], Yoo, Han-Wook[3], Han, Yong-Mahn[1]
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Fabry disease(FD) is a recessive X-linked inherited lysosomal storage disorder which is caused by α-galactosidase(GLA) deficiency. Reduced of GLA activity results in immoderate globotriaosylceramide(Gb3) accumulation in the major cell types, thereby causing to progressive complications. In particular, accumulation of Gb3 in vascular cells causes life-threatening complications such as ischemic stroke, hypertrophic cardiomyopathy, and renal failure at the terminal stage of FD. But, the pathophysiological mechanism of this vasculopathy in FD is unclear. To investigate these mechanisms, FD induced pluripotent stem cells(FD-iPSCs) were generated from patient dermal fibroblasts. FD-iPSCs showed low alpha galactosidase activity and excessive Gb3 accumulation like the Fabry patient's fibroblast in undifferentiated state. Also, FD-iPSCs could differentiate vascular cells such as endothelial cells(ECs) and smooth muscle cells(SMCs) with functionality of tubule-like structure formation, although FD-ECs and SMCs represented Gb3 accumulation. Accumulated Gb3 was cleared by treatment with alpha-galactosidase recombinant protein(Fabrazyme®) during endothelial differentiation of FD-iPSCs. FD-ECs showed endothelial dysfunction and decreased eNOS expression. These results demonstrate that endothelial dysfunction may be caused by low activity of eNOS in Fabry disease.

P006

IPSC CELL LINE WITH ENHANCED MODULAR INTEGRATION CAPACITY

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Sigma, St. Louis, U.S.

The use of CRISPR-Cas9 systems as tools for functional genomics has become prevalent in recent years. Libraries of guide RNAs for gene knockout and gene activation/repression to conduct genetic screens have been or are currently being developed by a number of different sources. With an abundance of such tools available to the researcher, a need for standardization among cellular models is apparent, yet unrealized. With this goal in mind, we have created a system that will allow for rapid integration of Cas9 and Cas9-based effector molecules, coupled with the ability to switch from knockout to non-knockout modes of genetic regulation. This system relies on the use of donor

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molecules containing an appropriate arrangement of trans-recombining elements that permit rapid screening for the integration of a "landing pad" within an inert locus within the human genome. Further, this landing pad can be used as a target site for effector molecule exchange, promoter swapping, or removal of the Cas9 system entirely. This system can not only be used in any human cell type to standardize screening platforms, but can also allow for creation of modified cell lines in a more rapid and reproducible manner.

P008

ENDOTHELIAL DYSFUNCTION IN NEURODEGENERATIVE DISEASES

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Neurons rely on vascular function for oxygen and nutrient supply as well as for removal of carbon dioxide and toxic metabolites. Endothelial cells at the inner surface of blood vessels form the interface between circulating blood and tissues. They are exposed to various physiological and pathological stimuli and their main function is to form a selectively permeable barrier, but they also serve several other specialized functions including production of nitric oxide, taking part in inflammatory response and in controlling blood flow and clotting. Many neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinsons disease (PD) are associated with microvascular dysfunction and/or defects in the blood-brain barrier (BBB) and angiopathy of small vessels is present in MELAS syndrome (mitochondrial encephalomyopathy with lactic acidosis and stroke like episodes). The key pathways of vascular dysfunction linked to neurodegenerative disease include BBB breakdown and metabolic dysfunction of endothelial cells, but whether vascular dysfunction actually leads to neuronal dysfunction, the order of the events and the mechanisms remain unknown. We have generated endothelial cells from iPS cells derived from patients with different neurodegenerative disorders, including AD, PD and MELAS, and are now studying metabolism and mitochondrial function of these cells. Further, we have also derived astrocytes from these same iPS cells, so we can co-culture them with the endothelial cells and model BBB in vitro in microfluidic-based organ-on-a-chip system.

P010

IMPAIRED PHAGOCYTOSIS IN IPSC-DERIVED MACROPHAGE MODELS OF NEURODEGENERATIVE DISEASE

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The innate immune system has a crucial influence on the progression of neurodegenerative diseases. Professional phagocytes in the brain, primarily microglia, have an important neuroprotective role in removing dying cells, incompetent synapses, and aggregates of amyloidogenic proteins. Conversely, failure of microglial phagocytosis and the activation of microglia by pro-inflammatory stimuli can exacerbate neurodegeneration. Both Alzheimer's disease (AD) and Parkinson's disease (PD) have been linked to genetic defects that alter microglial function. Furthermore, AD and PD are associated with the pathological accumulation of amyloid-beta and alpha-synuclein proteins respectively, which activate microglia and promote neuroinflammation. To study changes to phagocytosis in neurodegenerative disease, we have developed phagocytosis assays in induced pluripotent stem cell (iPSC)-derived macrophages. iPSC-derived macrophages are very similar to microglia in characteristics and ontogeny, being MYB-independent, and can be differentiated further towards microglia by co-culture with neurons. We initially studied the effect of alpha-synuclein on phagocytosis using iPSC-derived macrophage lines from PD patients with the SNCA gene triplication. SNCA triplication macrophages exhibited decreased phagocytosis relative to healthy controls. This phagocytic defect was phenocopied by addition of excess monomeric alpha-synuclein to non-PD iPSC-derived macrophages. Currently we are evaluating whether similar changes to microglial phagocytosis occur in iPSC models of AD. Impaired microglial phagocytosis has previously been demonstrated in APPPS1 mice, so we are developing phagocytosis assays in iPSC-derived macrophages exposed to amyloid-beta monomers and aggregates, and iPSC-derived macrophages expressing AD-associated gene mutations and variants. Using iPSC models in this way enables dissection of the mechanisms by which specific AD-associated mutations impair microglial function, and can be scaled for drug-screening.

Funding Source

Alzheimer's Research UK

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P012

INTRANASAL GSK3B INHIBITOR DELIVERY PROMOTES SPONTANEOUS CORTICAL REPAIR FOLLOWING PREMATURE BRAIN INJURIES

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Perinatal hypoxia leads to degeneration, atrophy and delayed maturation of oligodendrocytes (OLs) and cortical glutamatergic neurons. Previous studies demonstrated a spontaneous but partial regeneration of those cell types following hypoxia. In this study we investigated the contribution of dorsal subventricular zone (dSVZ) stem cells, which we have shown to continue producing OLs and glutamatergic progenitors (OPCs and Glu progenitors) after birth, to this regenerative attempt. We next assessed the appropriate specification of newborn cells as well as the amenability of this regenerative attempt to pharmacological manipulation. Our results reveal that OPCs and Glu progenitor populations are differentially affected by the period of hypoxia, as illustrated by a reduction in the number of Glu progenitors while OPC number was increased. Both populations however displayed increased proliferation at the end of the hypoxic period, suggesting their effective recruitment during the recovery period. Permanent labelling of dSVZ neural stem cells revealed an increased migration of OLs as well as the induction of de novo glutamatergic neurogenesis into the cortex of hypoxic mice. Expression of cortical layer specific markers by new-born glutamatergic neurons supports their appropriate specification. Pharmacological activation of Wnt/β-catenin signalling pathway by intranasal GSK3β inhibitor administration immediately after hypoxia, promoted both cortical oligodendrogenesis and neurogenesis. Our results highlight a dynamic and lineage-specific response of dorsal neural stem cells to hypoxia and identify the early postnatal dSVZ as a malleable source of stem cells for forebrain repair following trauma that occur early in life.

P014

HUMAN PLURIPOTENT STEM CELL DERIVED MICROGLIA/NEURON CO-CULTURES REPRESENT AN AUTHENTIC MICROGLIA MODEL

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Microglia are increasingly implicated in brain pathology, particularly neurodegenerative disease, with many Alzheimer's and Parkinson's Disease-implicated genes expressed in microglia. There is, therefore, a need for authentic in vitro models to study human microglial pathological mechanisms. Microglia originate from the yolk sac as MYB-independent macrophages, migrating into the developing brain to complete differentiation. We recapitulate microglial ontogeny by co-culturing iPSC-derived macrophages, which we have demonstrated to be MYB-independent, with iPSC-derived cortical neurons. These co-culture microglia express key microglia-specific markers C1QA, GAS6, GPR34, PROS1, MERTK, P2RY12 and TMEM119 and many neurodegenerative-disease-relevant genes. Importantly, the Alzheimer's Disease-relevant genes TREM2, APOE and FERMT2 are highly expressed in iPSC-derived microglia and human primary microglia but are not expressed in blood monocytes, demonstrating the superiority of iPSC-microglia for modelling neuroinflammation in Alzheimer's Disease versus using patient blood monocytes. The presence of iPSC-microglia is compatible with the development of deep layer and upper layer iPSC-cortical neurons, and electrical activity is maintained in these co-cultures. Co-culture microglia develop highly dynamic ramifications and are phagocytic. Upon activation they become more amoeboid, cluster and release multiple microglia-relevant cytokines. Importantly, co-culture microglia downregulate pathogen-response pathways, upregulate homeostatic function pathways and promote a more anti-inflammatory and pro-remodelling cytokine response than corresponding mono-cultures, demonstrating that co-cultures are preferable for modelling authentic microglial physiology.

Funding Source

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P016

DEVELOPMENT OF RETINAL ENDOTHELIAL CELLS IN VITRO MODEL WITH HIGH-RESISTANCE BARRIER USING ENDOTHELIAL CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

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Disruption and dysfunction of retinal endothelial cell barrier are associated with ocular pathologies (wet-AMD and diabetic retinopathy). Our team has recently established a method to generate functional endothelial cells (ECs) from human pluripotent stem cells (hPSCs) in large quantities in vitro (Patsch et al. Nat Cell Biol. 2015). Using hPSC-ECs we plan to establish an in vitro model of retinal endothelial cells which form a high-resistance barrier. In vivo, endothelial cells that form high-resistance barriers (retinal-blood barrier, brain-blood barrier) express high levels of claudin 5 (CLDN5). Therefore, we have generated a CLDN5 transcriptional reporter in hPSCs to serve as a surrogate marker for high-resistance endothelial barrier. The reporter cell line was generated by inserting a GFP at the 3' end of the CLDN5 gene by genome editing. We have differentiated hPSC cells with an integrated CLDN5 reporter to ECs and observed a 10 % GFP+ population. We have FACS sorted the GFP+ population and validated high expression of CLDN5 and GFP. We are currently investigating by RNA-seq the gene expression network that is necessary to induce CLDN5 expression and form a high-resistance barrier. Based on gene expression signature of CLDN5high hESC-EC population and gene expression signature of in vivo/ex vivo isolated retinal endothelial cells we plan to identify critical transcription factors that lead to formation of retinal endothelial barrier. Further, using an evidence based chemical-probe library, designed to span a large number of molecular targets, we are screening for chemical-probes that induce CLDN5 expression. Together, these studies will allow us to develop a reliable and reproducible model of retinal endothelial cells that would enable the discovery of novel therapeutics of ocular pathologies.

Funding Source

Claas Meyer and Chad Cowan have contributed equally to the research

P018

3D PARKINSON'S DISEASE MODEL OF HUMAN IPSC-DERIVED DOPAMINERGIC NEURONS FOR NOVEL DRUG CANDIDATES SCREENING

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Parkinson's disease (PD) is a neurodegenerative disease which is characterized by motor dysfunction and progressive loss of dopaminergic neurons in the substantia nigra. Important advances have been made in the understanding of the pathogenic pathways that underlies PD, though this has not yet been translated into any neuroprotective treatment that slows the progression of this neurodegenerative disease. The high heterogeneity of the disease and the lack of preclinical models that recapitulate the features of PD prohibiting successful development of neuroprotective therapies. By using induced pluripotent stem cell (iPSCs) technology we have a powerful tool in hand to develop in vitro PD disease models applicable in the field of personalized medicine. At Mimetas, we have developed a high-throughput culture platform, the OrganoPlate®, that is an easy to use microfluidic device supporting the creation of more physiologically relevant 3D tissue cultures. The tissue models may consist of several cell types structured layered in a membrane-free manner due to the PhaseGuide™ technology allowing passive liquid handling with a minimal requirement of cell material. In this study, patients iPSCs were differentiated toward an in vitro phenotype resembling that of substantia nigra dopaminergic neurons in vivo. The intermediate human neuroepithelial stem cells were seeded pre-mixed with an extracellular matrix gel in the OrganoPlate® and differentiated for 6 weeks towards functional dopaminergic neurons in a 3D environment. Immunostaining confirmed the presence of dopaminergic neurons and formation of nerve terminals. Furthermore, imaging of calcium fluctuations showed the spontaneously electro physiologically activity of the neurons, which is a characteristic feature of dopaminergic neurons in vivo. We aim to develop an industrial quality microfluidic PD model for high-throughput screening advancing personalized medicine in the discovering of new candidate neuroprotective agents tailored to these patients.

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P020

NOVEL NANO GRADIENT TECHNOLOGY CREATES CONDITIONS ESSENTIAL TO MIMIC LIMB DEVELOPMENT IN AN OSTEOARTHRITIS-IPSC-MODEL

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Osteoarthritis (OA) disease can be very painful and has a severe impact on the quality of life due to inflammation and erosion of articular cartilage covering the joint surfaces normally responsible for smooth joint movement. Treatment options are limited to joint replacements. The only protein significantly linked to OA is Growth Differentiation Factor 5 (GDF5) and mutations of the GDF5 gene cause a lack of limb joints (brachypodia). Limbs are formed during development due to orchestration of growth factors that functions as morphogenes, GDF5 belongs to this family. Such proteins specify more than one cell type by forming a concentration gradient in vivo. To generate a disease-model, an induced pluripotent stem cell (iPSC) line from a patient, with a genotype protective against OA, was genetically edited to knock down the GDF5 gene. A novel patented nano gradient technology was used to screen for cell behavior and gene expression depending on GDF5 concentration. At a specific interval of high GDF5 density a budding zone and an apoptotic zone were observed for both the wild type and the knock down line, though the effected genes differed between the two cell lines. Both budding and apoptosis are important for joint development. We demonstrated that this specific molecular density interval was essential to induce cellular clusters. The presentation will show how the gradient technology was used as an in vitro molecular model of OA and limb bud formation from iPSCs.

P022

PROBING THE FUNCTION OF ARL3 IN CRISPR MODIFIED CELL MODELS

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ARL3 is closely related to the Arf family of regulatory GTPases. Recently described missense variants in ARL3 have been associated with dominantly inherited retinal dystrophies, but their significance is unclear. Constitutive Arl3 knock-out in mice is lethal, making the function of Arl3 difficult to examine. ARL3 has been suggested to be important in cilia associated lipidated protein traffic and the ARL3 effectors, UNC119 and PrBP, and GTPase activating protein RP2 are important for retinal function. Therefore, we wanted to examine the function of ARL3 in the retina. Initially, CRISPR-Cas9 was used to disrupt ARL3 production in hTERT-RPE cells, where the introduction of a homozygous 1 bp insertion led to a frame shift resulting in a premature stop codon and thereby knocked out ARL3 synthesis. Cells lacking ARL3 had defects in cellular proliferation and, most notably, cilia incidence and length were significantly reduced. To further probe the function of ARL3 in human retinal cells, CRISPR-Cas9 gene editing was applied while simultaneously reprogramming control human dermal fibroblasts. The resultant iPSC colonies were analysed and two lines were selected for further investigation. The first had a homozygous 1 bp deletion leading to a complete knock out of ARL3 protein in the cells. The second line had a 2 bp deletion in one allele leading to a premature stop codon, while the other allele was unaffected. The heterozygous cells expressed approximately half the amount of ARL3 protein compared to control cells. These iPSC will be differentiated into 3D retinal organoids to delineate the role the ARL3 plays within the cells of the retina and test if complete loss or haploinsufficieny of ARL3 could lead to retinal dystrophies. This highlights the importance of gene edited stem cell models as tools for studying human retinal dystrophies and understanding the function of the retina.

P024

AN IPSC-DERIVED VASCULAR SMOOTH MUSCLE CELL DISEASE MODEL OF MARFAN SYNDROME IDENTIFIES KEY ABERRANT MOLECULAR PATHWAYS

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Marfan Syndrome (MFS) is a connective tissue disorder that affects 1 in 4,000 newborns and is caused by mutations in the FBN1 gene encoding for the extracellular matrix glycoprotein fibrillin-1. Major pathologic manifestations affect the skeletal, ocular and cardiovascular systems. Progressive aneurysm within the ascending thoracic aorta is the most notable clinical characteristic of MFS that when left untreated may progress to dissection and rupture, the leading cause of morbidity and mortality in MFS patients. This phenotype has been associated with vascular smooth muscle cells (VSMCs). To establish a disease model that allows for characterization of potential molecular abnormalities in VSMCs in MFS and to identify novel therapeutic targets, we have generated induced pluripotent stem cells (iPSCs) from skin fibroblasts of MFS patients. We have subsequently differentiated MFS iPSCs into VSMCs through a neural crest intermediate that represents the

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developmental origin of the ascending thoracic aorta. VSMCs had spindle shaped or rounded morphology typical of contractile or synthetic VSMCs, expressed the VSMC markers alpha-actin-2 and transgelin, and exhibited contractility. We then performed whole transcriptome sequencing of mRNA from VSMCs-derived from iPSCs from two MFS patients, a control subject and H9 human embryonic stem cells. Differential gene expression analysis confirmed transgelin, to be upregulated, which was found to be overexpressed in aortas of MFS patients.. Network and gene set enrichment analysis identified muscle contractility processes among the top down-regulated biological processes. We are expanding our analysis to additional patient-specific iPSC-derived VSMCs, to identify drugs that target differentially expressed genes or their transcriptional regulators and evaluate the effects of modulating them in overexpression/inhibition assays for characterization of the changes in the functional signatures. Furthermore, we are characterizing contractile properties and calcium-signaling dynamics of these patient-specific VSMCs and engineered vascular tissues. In summary, our iPSC-based MFS model provides a patient-specific in vitro platform to dissect molecular and biomechanical mechanisms of MFS and may help identify novel drug targets and therapeutics for MFS.

P026

VISUALIZATION OF LGR5+ STEM CELLS AND IMMUNE ENVIRONMENT IN MOUSE INFLAMMATORY COLON TISSUE

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The adult intestinal epithelium is a rapidly self-renewing tissue, maintained by stem cells that reside within the crypts. These stem cells express Lgr5 (leucine-rich-repeat-containing G protein-coupled receptor 5) and require Wnt/β-catenin signaling, which is augmented by R-Spondin. With its rapid turnover and well-defined structure in cellular orientation and differentiation, the intestine provides a great model to understand the mechanisms behind adult stem cell functions and insights towards regenerative medicine. It is also of great interest to understand the relationship between stem and immune cells in the inflammatory environment of gut mucosa during disease progression and therapeutic treatments. However, investigation of the specific functions and mechanisms of these cell types at single cell resolution in intact tissue architecture has been challenged by the lack of reliable and specific antibodies to detect key target receptors, such as Lgr5 and Fzd, and to identify key functional secreted molecules, such as Wnt, R-Spondin, and cytokines. In this study, we used the RNAscope technology, a highly sensitive and specific in-situ hybridization method, to evaluate the expression and function of Lgr5+ stem cells and immune cells in the TNBS-induced mouse model for inflammatory bowel disease (IBD). We examined the impact of inflammation on the Lgr5+ stem cells in FFPE colon tissue sections from healthy and TNBS-treated mice. The expression of Wnt3a and R-Spondin 1 in the crypt epithelium was evaluated in relation to their receptors by duplex analysis. Using serial sections, we evaluated the function of regulatory T cells and Th17 cells by the expression of key transcription factors and cytokines, such as FoxP3/Il10 and RorgT/Il17. Specific mRNAs were visualized as distinct punctate dots in different cell types within the morphological context. Further relationships between stem cell receptor-ligand and immune markers are shown and quantified in the inflammatory environment, especially with TNFα expression. The presented method may help understand mechanisms behind the pathogenesis of IBD and other inflammatory diseases and also evaluate the development of potential therapies.

P028

EFFICIENT DIFFERENTIATION OF MICROGLIAL PRECURSORS FROM HUMAN EMBRYONIC STEM CELLS AND THEIR CHARACTERIZATION BY STAGE SPECIFIC MARKERS FOR THERAPEUTIC IMPLICATIONS

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Microglia are the resident brain macrophages those continuously patrol neuronal synapse, prune neuronal synapses for functional maturation and engulf diseased or dead cells following brain injury or neuropathology. It is noteworthy that under abnormal circumstances, these microglial cells either under-prune or over-trim dendritic spines leading to damaged functional synapses. However, there is a paucity in the understanding of exact roles of microglial cells involved in neurogenesis and onset of neurological disease progression. To generate a cellular model of human microglia for translational application relevant to neurological diseases, we have optimized a fast and efficient protocol to derive microglia precursors from human embryonic stem cells (hESCs) using conditioned microenvironments. This process employs four stages for microglial conversion, including; i) formation of embryonic body (EB), ii) induction of microglial cells, iii) lineage commitment towards microglial precursors, iv) maturation of microglial precursors. To authenticate stage specific progression of hESC-derived microglial

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precursors, we have evaluated expression of several crucial genes involved in microglial lineage commitment and maturation, using flow cytometry and confocal imaging based applications. To name a few, at 21 days in vitro (DIV), Iba1+/ CD68+ microglial cell populations could be detected, those represented 60% and 80% of the total cultured cells. From 31 DIV onwards predominant expression of Pentraxin-3 (PTX3), an acute-phase protein could be detected in Iba1+ microglial precursors. Interestingly, Phospholipase D4 (PLD4), a gene specific to postnatal cerebral white matter was found to be specially enriched in Iba1+ microglial precursors. In summary, our study has proposed a fast and efficient strategy to generate mature human microglia precursors, which can be used as a cellular model in future for unraveling microglial roles in brain disease and development.

Funding Source

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P030

HUMAN IPSC-BASED DJ1-PARKINSONISM DISEASE MODELING

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There is an expanding list of genetic variants of Parkinson's disease (PD) and parkinsonism, where mutations in the PARK7 locus have been described to have a relatively benign phenotype. PARK7 encodes DJ1, a protein involved in transcriptional regulation, kinase activity regulation, protein ubiquitination and oxidative stress. The number of patients with DJ1 mutations remains small in the literature with only 24 patients from 8 families previously reported. Although the pathogenic contributions of DJ1 mutations to PD remain elusive, strong evidence obtained from animal and in vitro studies suggests that these mutations result in mitochondrial dysfunction. We have characterized a patient with a novel homozygous DJ1 mutation as the cause of a severe, early onset sporadic atypical complex dystonia-parkinsonism syndrome. We have generated and characterized fibroblast-derived iPSCs from the DJ1 mutation patient and his unaffected sibling. These iPSC lines were then differentiated towards dopaminergic fate using the Gibco PSC Dopaminergic Neuron Differentiation Kit. This culture protocol generated floorplate progenitors (FPPs) and mature (TH-positive) dopaminergic neurons. Gene expression analysis in iPSCs, FPPs, and mature neurons revealed a near absence of DJ1 transcript(s) and absence of DJ1 protein was confirmed by immunocytochemistry and Western Blotting. To avoid potential confounding genetic background effects on phenotypic read-out in the DJ1 cell lines, we generated isogenic patient- and control iPSC lines using CRISPR/Cas9 to repair and introduce the DJ1 mutation, respectively. Editing was done using Gibco StemFlex media, specifically formulated to better support PSCs throughout the gene editing process. We are currently characterizing the cohort of gene edited, isogenic iPSC lines, and will differentiate these cells to dopaminergic neuronal cells. Upon dopaminergic (DA) neuron differentiation of this cohort of isogenic DJ1 mutation iPSC lines, we will: 1) screen for general cell health, neuronal morphology and DA neuronal markers; 2) perform RNA-seq and transcriptional profiling; and 3) assay mitochondrial function. The generation and use of this human DJ1-PD disease model in-a-dish will prove invaluable for elucidating the contribution of DJ1 mutations to PD pathogenesis.

Funding Source

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P032

ASTROCYTES GENERATED FROM INDUCED PLURIPOTENT HUMAN STEM CELLS DERIVED FROM PSYCHIATRIC PATIENTS

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Astrocytes play an important role in maintaining synaptic function but their contribution to the development of psychiatric diseases is still underexplored. Therefore, psychiatric patient iPSC derived astrocytes are potential tools for developing disease relevant cellular models for research in academia as well as pharmaceutical industry. In this study we differentiated astrocytes from iPS cells over a period of 200 days. After neuronal induction and neuronal rosette selection, cells were cultivated as neurospheres

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in 3D culture up to 90 days. Then, cells were dissociated and cultivated in the presence of fibroblast growth factor, epidermal growth factor and fetal bovine serum for up to 200 days. Immunocytochemistry was performed at different time points of differentiation, using astrocyte markers such as GFAP, S100, ALDH1L1, EAAT1, glutamine synthase and Itgb5. For comparison, primary human astrocytes and two different commercially available iPSC derived astrocyte lines where analyzed. Cultures were treated with proinflammatory cytokine tumor necrosis factor alpha (TNF alpha) and lipopolysaccharide (LPS). RNA samples were taken to study differences between the four astrocyte lines at the transcriptome level. All four cell lines expressed the typical astrocyte markers at different expression levels. However morphological appearance of the four cell lines differed. Albeit the morphology of our own differentiated astrocytes was comparable to primary astrocytes, they lost GFAP expression over the cultivation period. Treatment with TNF alpha and LPS could not stimulate GFAP expression. Transcriptome analysis will help to compare the activation state and potential brain region or subtype specificity of the iPSC derived astrocytes. Co-culture of patient iPSC-derived neurons and astrocytes will be used to create a stable cell culture model for addressing the mutual influence of neurons and astrocytes in psychiatric diseases.

P036

HUMAN UMBILICAL CORD DRIVEN MESENCHYMAL STEM CELLS AS WELL AS THEIR CONDITIONED MEDIUM, SUPPRESS THE PROLIFERATION OF INFLAMMATORY CELLS FROM SYNOVIUM OF RHEUMATOID ARTHRITIS PATIENTS, IN-VITRO

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Rheumatoid arthritis (RA) is a T-cell-mediated systematic inflammatory disease and is usually accompanied by bone and cartilage damage. Current remedies include highly toxic chemotherapy accompanied with anti-inflammatory agents. Recently cellular therapy has been proposed to have some potential. Mesenchymal stem cells (MSCs) are being explored for having the dual advantage of being immunosuppressive in nature as well as have the ability to differentiate into osteocytes and chondrocytes, making them a potential therapeutic agent for RA. In the present study, we planned to evaluate the anti-inflammatory effect of umbilical cord-derived mesenchymal stem cells (hUCMSCs) ex-vivo and check its regenerative potential in the rat model of RA. Mesenchymal Stem Cells (MSCs) were isolated and cultured by explant method from Umbilical cord tissue in alpha-minimum essential medium. Human umbilical cord derived MSCs (hUCMSC) were phenotypically characterized for cell surface markers (CD73+,CD90+,CD105+,CD34-,CD45-) by flowcytometry and the grown cells showed 95% positivity for the above cell surface markers. These cells differentiated nicely into Adipocytes, Osteocytes and Chondrocytes in vitro. Besides cells, their conditioned medium when co-cultured with mononuclear cells isolated from the synovial fluid and peripheral blood of the RA patients, indicated immunosuppressive effects on the lymphocyte proliferation (CFSE Assay) and activation (CD25,CD38 and HLA-DR). Collagen Induced Arthritis Rat Model has been successfully generated to check the regenerative potential of MSCs in vivo. Thus, the preliminary findings of our study provide hope of addressing the complications caused by RA, by suppressing the inflammation and regenerating the damaged tissue.

P038

NOVEL IPSC-DERIVED CELLULAR SYSTEMS FOR IN VITRO DISEASE MODELING

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A major challenge in drug discovery research is modeling human biology in an in vitro system that is both physiologically relevant and predictive of the disease state. Human induced pluripotent stem cell (iPSC) technology allows for the generation of unlimited quantities of virtually any cell type in the human body from numerous donors. This technology also enables access to human disease models which have been shown to recapitulate the native phenotype in vitro. The functional relevance of human iPSC-derived cell types in research and drug discovery programs is being demonstrated by a rapidly growing body of literature. Here, we present case study examples of induced, engineered, and innate disease models generated by the production of iPSC-derived cell types environmentally stimulated to elicit a disease phenotype, genetically modified to introduce a disease mutation, or from patient-derived material, respectively. In particular, we describe the application of iPSC-derived hepatocytes in hepatitis C virus (HCV) infectivity. We present an induced model of Alzheimer's disease (AD) where beta-amyloid-dependent toxicity is induced in iPSC-derived cortical neurons. This assay was developed for a pilot screen to

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identify compounds protective against AD. We also provide data from iPSC lines genetically modified to carry point mutations in the amyloid precursor protein yielding another neuron-based Alzheimer's model. Finally, we showcase an iPSC-derived diabetic cardiomyopathy model, in which culture conditions were optimized to induce the disease state in apparently normal iPSC-derived cardiomyocytes. This model was used in a phenotypic screen for rescue from the pathological phenotype during diabetic stress and identified candidate molecules that were subsequently shown to be protective in cardiomyocytes derived from diabetic patient-specific iPS cell lines. Overall, these illustrate how iPSC technology offers reliable and predictive model systems not otherwise attainable using currently available primary and immortalized cells, thus creating new tools and opportunities in drug discovery.

P040

TOWARDS DEVELOPING PATIENT-SPECIFIC LIMB-GIRDLE MUSCULAR DYSTROPHY INDUCED PLURIPOTENT STEM CELL BASED ASSAYS FOR DISEASE MODELING AND DRUG DISCOVERY

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Limb-girdle muscular dystrophies (LGMDs) are a group of clinically and genetically heterogeneous genetic disorders that currently have no known cure. Although these disorders are classified as rare, the autosomal recessive forms are overrepresented in Middle Eastern societies due to the high rate of consanguinity and represent an unmet medical need that will greatly benefit from the development of patient-specific induced pluripotent stem cell (iPSC)-based disease models to screen for drugs. Towards this aim, eleven genetically undiagnosed families in Kuwait that presented with weakness in the shoulder and hip region were screened against a panel of 63 OMIM genes associated with LGMDs and other neuromuscular disorders. The panel identified the disease mutation in 64% of families (7/11: 4 LGMD2A, 1 LGMD2B, & 2 LGMD2I) and 2 of the remaining 4 undiagnosed patients were shown to carry unreported genetic variants in known LGMD genes (suspected LGMD2D and LGMD2I) that require further investigation to confirm diagnosis. iPSC lines were successfully generated from the fibroblasts of consenting patients by repeated transfection with mRNA encoding the four reprogramming factors OCT4, SOX2, cMYC and KLF4 and pluripotency was characterized at the protein level by the expression of pluripotency markers (Oct-4 and Tra1-60). These preliminary results demonstrate the advantage of using neurological gene panels in the diagnosis of heterogeneous diseases such as LGMDs and the generation of LGMD iPSC lines provides a platform to model and study the pathology of the disease and screen for therapies in the future.

P042

INVESTIGATION OF THE UNDERLYING CAUSE OF RETINAL DYSTROPHIES CAUSED BY IFT140 MUTATIONS

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Primary cilia are hair-like protrusions on most cells, which function as environmental sensors that are built and maintained by intraflagellar transport (IFT), but the precise function of IFT proteins is not well understood. The photoreceptor outer segment is a highly specialised light sensing primary cilium. Disruption of IFT as a result of IFT gene mutations cause a range of disease manifestations from isolated retinal degeneration to pleiotropic, systemic ciliopathies. Importantly, different mutations in the same IFT gene can cause different ciliopathy phenotypes. For example, mutations in IFT140 cause Mainzer-Saldino syndrome (MSS) and Jeune Asphyxiating Thoracic Dystrophy (JATD), but also isolated non-syndromic retinal degeneration. The underlying mechanism for this phenotypic variability is unknown and it is unclear why retinal degeneration is such a highly penetrant phenotype of ciliopathies. Here, we have analysed ciliary protein expression in fibroblasts and iPS cells from patients harbouring either a homozygous missense change or a compound heterozygous missense and frame shift/stop change in the IFT140 gene, causing MSS. We show that ciliary proteins, such as components of the IFT machinery are mislocalised in patient fibroblast cells compared to controls. In addition, cilia length is reduced in IFT140 E664K fibroblasts, compared to Y311C and control cells, suggesting that this IFT140 missense mutation affects ciliary tubulin traffic. Future differentiation into retinal pigmented epithelial (RPE) cells and 3D optic cups will be used to investigate the effect on photoreceptor biology.

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P044

LEPTIN STIMULATES ADULT SVZ NEUROGENESIS IN A TRANSGENIC MICE OVEREXPRESSING AMYLOID PRECURSOR PROTEIN

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It is widely acknowledged that neural stem cells generate new neurons through the process of neurogenesis in the adult brain. In mammals, adult neurogenesis occurs in two areas of the CNS: the subventricular zone and the subgranular zone of the dentate gyrus of the hippocampus. Alzheimer's disease (AD) is the most common cause of dementiaand is an age-related, progressive and irreversible neurodegenerative disease. Although two of the main pathological features of AD (amyloid plaques and neurofibrillary tangles) were already recognized, the pathogenesis of the disease remains unsettled.Recently, it was shown that leptin, acentrally-acting hormone controlling metabolic pathways, can reduce βamyloid levels both in vitro and in vivo and have neurogenic potential. Herein, we sought to determine if there are differences in the proliferation and in expression of genes related to neural and glial differentiation by SVZ-derived neural stem cells from double transgenic animal models of AD from 3 and 12 months of age, treated with leptin, in vitro and in vivo. After 7 days of leptin administration, we quantified the number of neurospheres. Interestingly, the quantitative analysis demonstrated a higher formation of neurospheres in cultures of cells extracted from older AD animals when compared to younger AD and WT mice of both ages. In addition, the neurospheres from AD mice are significantly larger. On the other hand, we noticed a decrease in cellular proliferation in the AD mice at 12 months of age. However, the number of BrdU-positive cells increased after treatment with leptin in both lineages and ages analyzed. This result is compatible with that observed by qPCR results, which showed that animals treated with leptin had an increase in the expression of genes related to neural and glial differentiation such as DCX, a neuroblast marker and GFAP, a glial marker.Our results indicate that acute administration of leptinincrease proliferation and differentiation of neural progenitor cells in SVZ.Therefore, it seems that the neurogenesis-stimulating leptin could be proposed as a potential therapy for AD.

Funding Source

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P046

A HUMAN 3D NEURAL CULTURE MODEL UTILISING IPSCS FROM AN ALZHEIMER'S PATIENT TO STUDY NEURODEGENERATIVE DISEASES

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Alzheimer's disease (AD) is the leading cause of dementia and with a population of increasing longevity is set to become a major burden on health services. Currently human tissue from AD cases is only available for research from post mortem brain but we can now utilise iPSC technology to obtain neural stem cells from AD patients and produce mature neurons with the same genetic signature of AD patients. Using IPSCs from an early onset AD patient containing the Presenilin 1 mutation (L286V) we have created a 3D human neural culture system for the study of early onset Alzheimer's. Using immunofluorescence, western blotting and electrophysiology we have characterised neuronal morphology, disease-protein profiles and synaptic function. Neural progenitors derived from AD IPSCs were plated in matrigel and self-organised into 3D structures by week 3. These cultures contained a heterogeneous cell population expressing differentiation markers for neurons and astrocytes and importantly AD-associated proteins; amyloid precursor protein/Abeta and tau. These 3D cultures were maintained in vitro for 18+ weeks where they expressed ABeta and the mature 4R tau isoform in the AD cultures by week 7 and exhibited repetitive firing by week 12. Together these results suggest a maturation of the neurons within these cultures. In conclusion we have created 3D neural cultures derived from the fibroblasts of an AD patient to study the disease pathology in a fully human model. This is a viable new in vitro 3D neural model for the study of neurodegeneration that addresses some of the limitations of current 2D models and which we believe can used as a powerful new tool in the fight against Alzheimer's disease.

Funding Source

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POSTER ABSTRACTS

P048

HUMAN-INDUCED PLURIPOTENT STEM CELL-DERIVED MODELS OF DEVELOPMENTAL AND PARKINSON'S DISEASE ASSOCIATED NEUROTOXICITY

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Despite significant differences between human and rodent brains and the relatively low sensitivity, low throughput and high cost of rodent–based neurotoxicological models they have been the primary approach for detecting potential human neurotoxicants. Increased concerns regarding species-specific neurotoxicant sensitivities drive the development of alternative model systems that reflect more closely human neurotoxicant sensitivities. Here we describe the use of human neurons of varied lineages differentiated from human-induced pluripotent stem cells (hiPSC) to assess mechanism underlying the toxicity of developmental neurotoxicants, as well as environmental stressors that have been linked to Parkinson's disease. Methylmercury (MeHg) is an abundant environmental neurodevelopmental toxicant with a high affinity for sulfhydryl groups and hence for proteins containing cysteines. The E1 enzyme is the initiating enzyme of the ubiquitin signaling cascade and binding of an ubiquitin to a cysteine sulfhydryl residue in the catalytic site activates the E1 enzyme. Here we show that MeHg decreases ubiquitinylation and thus activation of the E1-enzyme in hiPSC-derived human cortical glutamatergic neurons. Ziram, a member of a family of dithiocarbamate pesticides which has been associated with increased risk for Parkinson's disease and has a high affinity for SH-groups causes a loss of ubiquitinated E1-enzyme in cortical glutamatergic and mesencephalic dopaminergic neurons. We are presently evaluating the effects of Ziram and MeHg on the ubiquitinylation of targets downstream of E1. The pesticide rotenone and the metal manganese are both environmental stressors associated with Parkinson's disease, and both are known inhibitors of mitochondrial oxidative phosphorylation and thus inducers of oxidative stress. Interestingly, in spite of their presumed shared mechanism of action, we found the oxidative stress signatures of rotenone and manganese to be significantly different in human mesencephalic dopamine neurons. We demonstrate here that hiPSC-derived human neurotoxicological models are applicable to assess the mechanism of action of a wide variety of environmental neurotoxicants.

Funding Source

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P050

A NOVEL DIFFERENTIATION SYSTEM TO GENERATE HPS CELL-DERIVED HEPATOCYTES IN A ROBUST MANNER WITH POTENTIAL APPLICATION TO DRUG DISCOVERY, DRUG METABOLISM RESEARCH, AND HEPATOTOXICITY STUDIES

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Human induced pluripotent stem (hiPS) cell-derived hepatocytes have the potential to serve as predictive human in vitro model systems for drug discovery, drug metabolism research, and hepatotoxicity studies provided that they possess relevant hepatic function. Until recently, however, the functionality of hiPS cell-derived hepatocytes has been insufficient for applications that demand high expression of multiple drug metabolizing enzymes. We have recently developed a novel, robust differentiation system to generate hepatocytes from human induced pluripotent stem cells. These resulting hiPS cell-derived hepatocytes have substantial CYP1A, 2C9, 2C19, 2D6, and 3A4 enzyme activities and important adult hepatic features, such as low expression of fetal genes (e.g., CYP3A7 and alpha-fetoprotein) and high expression of adult genes (e.g., CYP2C9, 2C19, and 3A4).

In this study, we illustrate the system's differentiation protocol that begins with culturing any hiPS cell line, continues with directed differentiation into definitive endoderm (DE) cells, and ends with further differentiation into hepatocytes, thereby mimicking normal embryonic development. Morphological images and immunostaining data demonstrate the robustness and reproducibility of the system to generate hepatocytes from several different cell lines. Of 28 different hPSCs tested, all 28 lines were efficiently differentiated into hepatocytes that exhibit substantial CYP1A, 2C9, 2C19, 2D6, and 3A4 enzyme activity and important adult hepatic features, such as low expression of fetal genes and high expression of adult genes. More importantly, these hepatocytes generated from multiple hiPSC lines show diverse CYP activity profiles, indicative of the inter-individual variation present in the human population. hiPS cell-derived hepatocytes are potentially suitable for toxicity assays: they correctly identify known hepatotoxins and respond similarly to human primary hepatocytes when exposed to test compounds. Now we can reliably generate a virtually inexhaustible source of human hepatocytes from different genetic backgrounds for use in in vitro drug discovery, drug metabolism research, toxicology-related studies, and disease modelling.

POSTER ABSTRACTS

P052

AMYLOID-INDUCED MOLECULAR MECHANISMS IN NEURAL STEM CELLS ENABLE ENHANCED PLASTICITY AND NEUROGENESIS RESPONSE IN VERTEBRATE BRAINS

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Tissue regeneration is a fascinating phenomenon, and it manifests quite differently in animals. We humans are very poor regenerators, but other vertebrates, for instance the zebrafish, is stunningly regenerative. Our main goal in our lab is to find out the molecular basis of how we can use stem cells for regenerative therapies, especially for neurodegenerative diseases in humans by investigating the zebrafish brain. In Alzheimer's disease (AD), where patients progressively lose neurons yet cannot form new ones, the proper differentiation/survival response of stem cell-derived neurons ceases., To explore the potential of zebrafish in circumventing AD, we generated an Aβ42-dependent neurotoxic model in where phenotypes are highly reminiscent of amyloid pathophysiology in humans. Upon Aβ42, zebrafish induced neural stem cell proliferation and enhanced its neurogenesis, which is not the case in our brains. We found that an Interleukin-4 is activated primarily in neurons and microglia/macrophages in response to Aβ42 and is sufficient to increase stem cell proliferation and neurogenesis in fish brain. Our detailed work shows a direct crosstalk of neurons and glia via immune-related molecules, which imposes plasticity to endogenous stem cells in vertebrate brains, and this role is novel and distinct to the known effects of interleukins on immune cells. Furthermore, in order to compare our findings to human brains, we developed a novel and highly tunable and defined glycosaminoglycan (GAG)-based 3D matrix system to culture primary human cortical cells, which form extensive mature cortical networks in a tissue mimetic manner. We also recapitulated the cellular symptoms of Alzheimer's disease by modeling amyloid toxicity in this 3D system. By using our system we identified a molecule that reverts the Amyloid toxicity and restores the NSPC plasticity and network forming ability. Our new 3D culture platform provides a novel and improved model to study the plasticity of human neural stem cells and disease states in real time. This system can be used to address questions regarding stem cell proliferation, neurodevelopment and neurodegenerative diseases in a preclinical setting. In my presentation, I propose to discuss our findings in zebrafish and 3D cultures, and our future prospects.

P054

CHARACTERIZATION OF DE NOVO MUTATIONS IDENTIFIED IN A SCHIZOPHRENIC PATIENT BY PARALLEL SOMATIC CELL REPROGRAMMING OF BLOOD CELLS FROM A FAMILY TRIO

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De novo mutations (DNMs) have been implicated in the etiology of schizophrenia (SCZ), a chronic debilitating psychiatric. The large scale identification of DNMs has become feasible with the advent of next generation sequencing. While several DNMs have been uncovered by examining SCZ cases and their unaffected parents, in most cases the biological significance of these mutations remains inconclusive. To overcome this limitation we have developed an approach of using somatic cell reprogramming to generate induced pluripotent stem cell (IPSC) lines from each member of a case-control trio, in order to investigate the effects of DNMs in cellular progenies of interest, particularly in hippocampal dentate gyrus granule cells. Here we describe a patient with SCZ characterized by early onset of both positive and negative symptoms, who is a carrier of 3 non-synonymous DNMs in genes LRRC7 (leucine rich repeat containing 7), KHSRP (KH-Type Splicing Regulatory Protein), and KIR2DL1 (Killer Cell Immunoglobulin-Like Receptor, Two Domains, Long Cytoplasmic Tail, 1) identified by exome sequencing. LRRC7 encodes densin-180, a postsynaptic density protein in glutamatergic synapses, while KHSRP is an RNA-binding protein implicated in miRNA regulation, axonal growth and dendritic spine development. IPSC lines were generated from the patient's and his parents' peripheral blood mononuclear cells using Sendai virus based reprogramming. Then IPSCs were characterized using alkaline phosphatase staining, qPCR, spontaneous differentiation assay and immunofluorescence staining. The DNMs were validated in the IPSC lines by Sanger sequencing. We have used an established protocol to generate neuronal progenitor cells and mature neurons from all three cell lines. Currently we are investigating the molecular effects of the DNMs in the target proteins and various neuronal phenotypes in the derivative cells including neuronal morphology, neurite outgrowth, synaptic connectivity and electrophysiological activity. The

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presented approach of reprogramming blood cells from family trios enables more accurate investigation of disease causing mutations by comparing cell lines with reduced variation in genetic background.

Funding Source

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DRUG DISCOVERY

P058

PROANTHOCYANIDIN IN THE PREVENTION AND TREATMENT OF LPS, NICOTINE OR HYPOXIA INDUCED PERIODONTITIS

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Proanthocyanidin, an oligomeric component extracted from grape seed has shown to be an effective anti-oxidant agent. Periodontitis is a chronic inflammatory disease in the periodontal tissues and results in the tissue destruction during advanced status. The aim of this study is to evaluate the use of proanthocyanidin in preventing or reversing the inflammation in periodontal ligament stem cells caused by LPS or Nicotine under hypoxia. Proanthocyanidin toxicity for human periodontal ligament stem cells were assessed by MTT assay and the optimal dosage of PA for in vitro studies was determined. Human periodontal ligament stem cells were incubated with LPS derived from P. gingivalis 10µg/ml and 1mmol of Nicotine for 24 hours under hypoxic conditions. Cells were treated with 20mg/ml PA for 10 minutes as pretreatment for one group before the pathological stimuli and as post-treatment for another group after the same stimuli. After the inflammation cells were cultured for another 24 hours in normoxic condition the cells were collected for quantitative polymerase chain reaction (RT-PCR), western blotting and Amplex red assay for H2O2. The culture media were also collected and the concentration of NO2 and PGE2 in the media were determined.The optimal PA concentration was determined as 20mg/ml Proanthocyanidin treatment for 10 minutes. The PA pretreatment significantly down regulated the NOX4, TNF α and MMP-2 expression ($P < 0.05$). Furthermore the NOX4 protein expression was also reduced significantly in the PA pretreated groups ($P < 0.05$). The use of PA as a pre-treatment agent has reduced the production of H2O2, NO2 and PGE2 ($P < 0.05$). Proanthocyanidin can prevent the inflammation in periodontal ligament stem cells induced by LPS or nicotine under hypoxia. Therefore it might has a therapeutic potential in preventing periodontitis.

P060

GENE NETWORK ANALYSIS OF INTERSTITIAL MACROPHAGES AFTER TREATMENT WITH INDUCED PLURIPOTENT STEM CELLS SECRETOME (IPSC-CM) IN THE BLEOMYCIN INJURED RAT LUNG

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Idiopathic pulmonary fibrosis (IPF) is a complex disease involving various cell types. Macrophages are essential in maintenance of physiological homeostasis, wound repair and fibrosis in the lung. Macrophages play a crucial role in repair and remodeling by altering their phenotype and secretory pattern in response to injury. The secretome of induced pluripotent stem cells (iPSC-cm) attenuates injury and fibrosis in bleomycin injured rat lungs. In the current study, we evaluate the effect of iPSC-cm on interstitial macrophage gene expression and phenotype in bleomycin injured rat lungs in vivo. iPSC-cm was intratracheally instilled 7 days after bleomycin induced lung injury and assessed histologically and biochemically 7 days later. Single cell isolation of the lungs was performed. Macrophages were FACS sorted and microarray analysis was performed. Using transcriptional profiling, we characterized changes in the rat lung macrophages. iPSC-cm reduced the total collagen content of the lung and reduced different macrophage populations. Microarray analysis revealed 831 downregulated and 384 upregulated genes. Gene set enrichment analysis revealed involvement of three essential pathways (a) immune modulation, (b) branching morphogenesis and (c) canonical Wnt signaling. This study demonstrates that iPSC-cm reduces fibrosis in bleomycin injured rat lung by partially altering the macrophages and regulating their gene expression.

Funding Source

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POSTER ABSTRACTS

P062

MODULATION OF UPR RESPONSE IN IPSC-DERIVED MOTOR NEURONS FROM ALS-PATIENTS

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One pathway strongly activated in ALS is the unfolded protein response (UPR), a signalling network that orchestrates the recovery of homoeostasis or triggers apoptosis depending on the level of protein folding demand in the lumen of the endoplasmic reticulum (ER). Familiar and sporadic ALS patients show accumulation of misfolded proteins and elevated levels of UPR markers. In ALS model mice, studies indicate upregulation of UPR markers early in disease and provide evidence that ER stress correlates with selective vulnerability of specific motor neuron populations. This has generated widespread interest in targeting the UPR response as a therapeutic strategy in ALS. First small molecules targeting different nodes of the UPR are under investigation but highlight the need for molecules with more efficacy, lower risk of adverse events, and a mechanism of action that prevents apoptosis but enhances the buffering capacity of the proteostasis network. We have used motor neurons derived from iPSCs from familial ALS patients and their isogenic controls as a basic model of disease. Standardization and upscaling of motor neuron differentiation in 384-well plates allows us to phenotypically screen thousands of small molecule compounds from focused sets ranging from bioannotated known drugs and tools compounds to high chemical diversity and natural product derivatives. Here we present on our phenotypic drug discovery strategy including validation of hits in secondary in vitro assays of the UPR pathway, progression towards identification of the underlying molecular targets of selected hits, assessment of selected hits on ALS-specific phenotypes of patient motor neurons, and the testing of selected hits on motor neurons of multiple familiar ALS genotypes. As a result selected novel compounds may progress to a lead optimization program towards development of novel drug candidates for the treatment of ALS and further neurodegenerative diseases.

P064

ANTI-ADIPOGENIC EFFECT OF CELASTROL ON THE HUMAN ADIPOSE DERIVED STEM CELLS.

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Obesity become a serious health problem in worldwide, however, existing anti-adipogenic drugs does not fulfill a requisition of obesity patient. For this reason, it is important to discover new drug to restrict the adipogenic property of mesenchymal stem cell. Celastrol, chemical compound that extract from thunder god vein, was reported that it impairs adipocyte differentiation by regulating pparg2 & C/EBPa signaling on mouse cell line. But there are few reports which applicate celastrol on human system. Here, we report that celastrol has anti-adipogenic effect on the various age groups of human adipose derived stem cells(hADSCs) in vitro. First, we tested the toxicity of each celastrol concentration and there was no toxicity up to 500nM. Second, hADSC were treated with celastrol with adipocyte differentiation medium and then optimal concentration and duration of celastrol treatment was established. We found that 3 days of celastrol treatment was sufficient to repress adipogenesis of hADSC as tested by quantifying Oil Red O staining dye and expression of adipocyte marker genes (PPARg, C/EBPa, LPL…) by qPCR. We also conducted lipolysis assay to evaluate lipolytic effect of celastrol on hADSCs, in contrast to the lipolytic effect on 3T3L1 cell, but there was no lipolytic effect on hADSCs. Finally, we treated celastrol on the various donor of hADSC and verified that celastrol has anti-adipogenic effect on hADSCs regardless of ages and sexes. In conclusion, we identified optimal concentration and minimum duration time of celastrol treatment and confirmed the anti-adipogenic effect of celastrol on various age groups of hADSCs. Our research showed that celastrol has anti-adipogenic effect on human primary cell and indicated that it can be a promising anti-adipogenic drug.

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P066

THE USE OF IPSC-DERIVED RENAL PROXIMAL TUBULAR CELLS AND PODOCYTES IN NEPHROTOXICITY SCREENING

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Renal injury is a frequent side effect of pharmaceutical exposure. Due to the high blood perfusion rate and the expression of a wide variety of xenobiotic transporters and metabolizing enzymes, the glomerulus and the proximal tubule are exposed to high concentrations of compounds, making them the most susceptible regions of the nephron. Human-derived cell culture models offer specific advantages over animal-based models, including species-relevant expression of transporters and metabolizing enzymes, lower cost and increased applicability to molecular investigations. The utilization of donor specific iPSC derived target tissues could potentially add additional important information, including population dynamics, genetic disease backgrounds and gender differences. The aim of this project was to develop methods to differentiate human iPSC into relatively pure proximal tubular cells (PTs) and podocytes that can be applied to molecular pharmacological and safety testing approaches. iPSC that have been generated in the StemBANCC consortium were differentiated into either podocyte-like cells or PT-like cells with a combination of small molecules and growth factors. Characterization of these cells show expression of specific podocyte marker, including nephrin, synaptopodin and podocin, as well as specific PT markers, including megalin and claudin 2. Further, PT-like cells show a typical polarized phenotype. The cells have been challenged with several compounds including Doxorubicin, a gold standard compound for glomerular toxicity, as well as with Bardoxolone methyl (aka CDDO) an activator of the Nrf2 oxidative stress response pathway. Deeper molecular investigations are underway with a wider set of compounds. In conclusion, we have developed optimized protocols to create podocyte-like and PT like cells. Efforts are now underway to better characterize the cells with respect to tissue and donor specific responses.

Funding Source

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P068

HIGH THROUGHPUT SCREENING USING HUMAN BROWN ADIPOCYTES REVEALS NEW METABOLIC STIMULANTS DERIVED FROM EDIBLE PARTS OF PLANTS

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Changed dietary habits, sedentary lifestyle, and/or defects in metabolism have led to a worldwide increase in the incidence of obesity and associated disorders, such as type II diabetes, cardiovascular disease, hypertension, non-alcoholic fatty liver disease, stroke, some cancers, and Alzheimer's disease. Chronic obesity affects 1.4 billion adults globally and is the second most common preventable cause of death in Western society. With the continuing growth of the aged population, obesity has become one of the biggest underlying drivers of preventable chronic diseases and healthcare costs. Current strategies to combat obesity via life style changes and pharmaceuticals are inefficient and/or unsafe. Novel approaches are therefore urgently needed to prevent and manage this chronic high risk condition. A promising anti-obesity therapeutic target is represented by brown adipose tissue, a tissue specialised in the dissipation of chemical energy in the form of heat. Identifying new compounds that induce the endogenous brown adipocytes could have important therapeutic implications. We have developed a robust and efficient protocol to differentiate human adult adipose-derived stem cells (ADSCs) into brown adipocyte-like cells using CombiCult® technology. The CombiCult® derived differentiation protocol works in monolayer and in 3D culture facilitated by specialised matrices. The cells generated show all the properties of brown adipocytes, including high expression levels of UCP1, expression of typical brown fat transcription factors, and higher rates of oxidation than white adipocytes and parental ADSCs. The CombiCult® derived brown adipocyte-like cells were used to screen a library of natural products for UCP1 inducers. A number of hits were validated in dose response and tested in a functional assay designed to measure oxygen consumption. Some extracts derived from the edible parts of plants were observed to increase the metabolic activity of human brown adipocytes but not white adipocytes. These findings may help to provide a safe method of controlling obesity and associated disorders.

POSTER ABSTRACTS

P070

DEVELOPMENT OF A THERAPEUTIC DRUG-SCREEN ASSAY FOR MARFAN SYNDROME USING AN IN VITRO HUMAN IPSC-DERIVED SMOOTH MUSCLE CELL DISEASE MODEL

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Marfan syndrome (MFS) is a connective tissue disorder with pleiotropic manifestations. Severe cardiovascular complications, such as aortic aneurysms are caused by mutations in the extracellular matrix structural component, fibrillin-1. As a result, MFS patients display impairment in fibrillin-1 deposition, increased extracellular matrix degradation, increased cell death and excessive signalling by the transforming growth factor beta (TGF-β) pathway. Currently, MFS treatments focus on minimising aortic wall stress by controlling blood pressure. Although blockade of non-canonical TGF-β signalling using losartan has successfully prevented aortic dilatation in a MFS mouse model (Habashi et al. Science 2006), similar attempts have been largely unsuccessful in clinical trials. To further dissect the molecular components of MFS pathogenicity, we established a human induced pluripotent stem cell (hiPSC)-derived in vitro model system for MFS. HiPSC lines have been generated from several MFS patients harbouring different mutations in FBN1, which have subsequently been differentiated via neural crest into smooth muscle cells (SMC) that display most of the pathological features of MFS (Granata et al. Nature Genetics 2016). Our latest results showed that neither broad upstream inhibition of TGF-β signalling nor treatment with losartan were capable of fully reversing the MFS phenotype. However, targeting the p38 MAP kinase pathway was able to normalise pathogenic characteristics, such as abnormal cell death and proteolysis. Our aim is shed light on alternative molecular pathways involved in MFS disease development and identify novel therapeutic targets. Here, we compare two fluorometric strategies aimed at developing a reliable assay for high throughput screening purposes. Our first tactic quantifies matrix metalloproteinase activity as a read-out for matrix degradation. Our second tactic uses cell viability as a read-out. We show that MFS SMC cultured in 96-wells and treated with certain small molecule inhibitors display reduced proteolysis and reduced apoptosis. These preliminary results indicate that both strategies have potential for screening purposes. Further studies will quantify their reliability and suitability for screening before embarking on a high throughput strategy.

Funding Source

BHF Oxbridge CRM: In 2013, the British Heart Foundation (BHF) established Research Centres of Regenerative Medicine. The Centre Director is Prof Paul Riley (Oxford) with leadership by Prof Martin Bennett for the Cambridge part.

P072

A SIMPLE METHOD FOR RAPID IDENTIFICATION OF BACTERIAL CONTAMINATION IN CELLULAR THERAPY PRODUCTS

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Post-transplantation infection due to the bacterial contamination of cellular therapy products is a potential risk in regenerative medicine, therefore the sterility tests are crucial within the quality control. These tests are usually performed by automated blood culture systems, which yield accurate results, but take several days, and cannot provide the sterility data before transplantation. It's possible to detect bacteria in cellular therapy products using polymerase chain reaction (PCR) with universal primers, amplifying the 16S rDNA gene of all eubacteria species. This method is fast and cost-effective, but often gives false-positive results due to high PCR sensitivity and reagents contamination with traces of bacterial DNA. There are various ways to deal with false-positive results, but all of them require additional time and cannot prevent failure due to occasional contamination during PCR setup. We have developed a simple method, which allows to eliminate false-positive results and adjust the sensitivity of PCR-based sterility test. For PCR we used 0,4 μM of each primer for 16S rDNA gene (5'-TCCTACGGGAGGCAGCAGT-3'; 5'-GGACTACCAGGGTATCTAATCCTGTT-3') and 0,4 μM of each primer for human beta-actin gene (5'-GCGCCGTTCCGAAAGTT-3'; 5'-CGGCGGATCGGCAAA-3'). The reaction conditions were 95°C for 5 min, 50 cycles of 95°C for 10 s and 54°C or 55°C for 40 s. Results were detected by agarose gel electrophoresis. The sensitivity was determined by six 10-fold serial dilutions of the E.coli suspension (105-1 CFU) added to 1ml aliquots of the medium containing 105 of human adipose-derived mesenchymal stromal cells. DNA was isolated from these

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mixtures and PCR was performed as described. When PCR was performed with an annealing temperature of 54°C sensitivity of the method was 1000 CFU/ml and with an annealing temperature of 55°C sensitivity was 10-100 CFU/ml, which considered to be sufficient to prevent adverse reactions. No positive results were found in negative controls containing human cells only, but false-positive results were detected in no template controls. Thus, our method has the internal control, which allows to avoid false-positive results due to the competition between the reactions. The method is also configurable to adjust the sensitivity, which can be useful for various applications.

P074

DYNAMIC 3D BIOREACTOR TECHNOLOGY FOR HEPATIC DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSC)

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The generation of hepatocytes from human induced pluripotent stem cells (hiPSC) holds the potential to provide patient-specific cells for preclinical studies on hepatic drug metabolism and toxicity. To support hepatic differentiation of hiPSC, our approach is based on a dynamic four-compartment culture technology that approximates the natural environment of the cells in the organ. Cells are cultivated within a three-dimensional network of hollow-fiber capillaries that serve for nutrient supply, metabolite removal and decentralized oxygenation with low mass gradients. Hepatic differentiation of hiPSC was investigated in the bioreactor system or in standard 2D cultures using a three-step differentiation protocol for endodermal differentiation and hepatic maturation. The analysis of hepatic differentiation markers showed that alpha-fetoprotein (AFP), a marker for premature hepatocytes, was released in larger amounts in 2D cultures than in 3D cultures, while the secretion of albumin, a marker for mature hepatocytes, was higher in 3D cultures. Functional analysis of different cytochrome P450 isoenzymes (CYP) showed activity of CYP1A2, CYP2B6 and CYP3A4, with a significantly ($p < 0.05$) higher level of CYP2B6 activity in 3D bioreactors as compared with 2D cultures. Results were confirmed by gene expression analysis of hepatic proteins. Immunohistochemical analysis revealed the expression of albumin, cytokeratin (CK) 18 and CK 19 in 2D and 3D cultures, while formation of bile-duct-like structures and expression of tight-junction protein-1 (TJP1) were only observed in 3D bioreactors. Further improvement of hepatic maturation of hiPSC with respect to mRNA expression and secretion of AFP and albumin was achieved by using an optimized culture medium enriched with endothelial cell growth supplements. The results indicate a better promotion of hepatic maturation of hiPSC in the 3D bioreactor system as compared to 2D cultures. Thus, the bioreactor system opens the perspective for conduction of in vitro toxicity studies using hiPSC-derived hepatic cells in a complex 3D environment.

Funding Source

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EMERGING TECHNOLOGIES

P078

QUANTITATIVE ANALYSIS OF MOUSE ESC PHENOTYPES USING SINGLE CELL LIVE IMAGING

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Embryonic stem cells (ESCs) have the unique abilities to self-renew and give rise to derivatives of all three primary germ layers. Even though ESCs consist of a genetically homogeneous cell population, a number of studies have reported heterogeneities in ESCs cultures as a result of the fluctuation of the expression of key regulators responsible for maintaining the embryonic stem cell identity. In addition, even under defined self-renewing conditions, ESCs display a range of different phenotypes that are rarely observed or reported in literature, mainly due to technical challenges. Here we combine time-resolved, single-cell live imaging of high spatiotemporal resolution with automated computational approaches to enable the quantitative analysis of the phenotypic characteristics of mouse ESCs in culture. Using our method, we quantify features related to morphology, behavior and gene expression. Finally, we use the extracted features to delineate phenotypically distinct ESCs subpopulations and to analyze the molecular basis of ESCs fate control.

Funding Source

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P080

CELL CULTURE CONTAMINATION RISK REDUCTION FOR HUMAN MSC, PROGENITOR CELLS, AND CAR-T PRODUCTION BY FULL-TIME SEPARATION OF THE ROOM AIR AND CELL PROCESSING ENVIRONMENTS

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For clinical translation of novel cellular therapeutics, the risk of microbial contamination of the cell product must be addressed. Patient cell cultures are most exposed to risks during open cell handling steps of cell isolation, expansion, gene modification, differentiation, selection, or harvest. Even when performing manipulations in a clean room, personnel present the largest contaminant source. A barrier isolator physically separates the cell environment from personnel-borne bioburden. We set out to determine if performing routine cell production in a Cytocentric barrier isolator could control the risk of microbial contamination. We performed three different routine cell production protocols using a permissive color-changing microbial culture broth as a cell culture surrogate. The first protocol was the thawing step of any cell type. The second was a multi-step production of an adherent cell type like mesenchymal stromal/stem cells or progenitor cells. The third protocol was passaging of a non-adherent cell type like CAR-T cell cultures. Samples of final preparations were sealed into sterile vials for long-term incubation. Each protocol was performed at least three times. Contact plates were used to assess the sterility of cell processing surfaces inside the isolator after the process was completed. Positive controls were exposed to the room outside of the isolator. After incubation for at least 5 days, test broth samples and contact test plates showed no evidence of contamination (0 CFU) while positive control broth samples and contact plates showed vigorous microbial growth. Even after 30 days of incubation, test broth samples were negative for microbial growth. Full-time particle sensors on the isolator modules also showed extremely low particle levels during open handling steps. We concluded that separation of the cell processing of the cell product surrogate from the room air environment was able to effectively prevent microbial contamination.

P082

CRYOVIZ CRYO-IMAGING FOR DEVELOPING MSC THERAPY IN LIVER DISEASE

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'MERLIN' (Mesenchymal stem cells to reduce liver inflammation) is an EU FP7-funded project in which BioInVision, Inc. (Cleveland, OH, USA), in partnership with EU establishments, is developing MSC cellular therapy for primary sclerosing cholangitis (PSC), a type of liver disease. In MERLIN project, BioInVision's CryoViz cryo-imaging system is being used to study homing, global and local biodistribution, mechanism of action, and immunogenicity of MSC in mouse models of PSC. CryoViz consists of a fully automated system for sectioning and tiled microscopic imaging of tissues, providing anatomical brightfield and molecular fluorescence with single cell sensitivity. In one set of pre-clinical experiments, CryoViz was employed to study the mechanism of action of MSC. Cryo-imaging uniquely revealed that cells stayed localized in the vicinity of the inoculation site, and as compared to wild type MSC, sorted MSC dispersed further from the inoculation site. In another experiment, investigators employed CryoViz to study MSC homing and biodistribution for different longitudinal time points in chronic liver injury and acute liver injury models. In this study, CryoViz cryo-imaging has revealed unique sites of homing within liver and spleen tissue, and a different 3D biodistribution pattern of MSC in injured animals versus non-injured controls, all not possible with other imaging modalities. Third, in an MSC immunogenicity study, MSC survival was studied under various pre-treatments in a CCL4 liver injury whole mouse model. In this study, CryoViz cryo-imaging revealed that MSC could be optimized to become less immunogenic and more immunomodulatory, which is an important step towards further development of MSC for cellular therapy.

Funding Source

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P084

TOWARDS AUTOLOGOUS CELL THERAPIES ON CHIP: AN AUTOMATED AND INSTRUMENTED MICROFLUIDIC DEVICE FOR CELL REPROGRAMMING AND CULTURE

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To fulfil the promise of personalised cell therapies it is first necessary to generate clinically viable induced pluripotent stem cells from a patient-specific tissue sample. Current derivation methods are constrained by the challenges of primary cell culture, high cost of transfecting agents and low efficiency. Exquisite control over the cellular microenvironment, the dramatic reduction of reagent volumes and the facility for parallelisation and automation make microfluidics highly suitable to determine optimal conditions for reprogramming. To succeed in this venture the microfluidic device must be capable of efficient transfection and homogeneous culture; i.e. to uniformly deliver reprogramming factors and maintain pluripotency of the derived cells. We present a microfluidic culture device (MFCD) which has been validated for both cell transfection and expansion. In particular, our device offers automated transfection of cells and the non-invasive determination of cell culture characteristics, such as cell density and (specific) oxygen uptake. To transfect on-chip we automate micro-volume injections into our MFCD using an injection valve and demonstrate its application with mouse embryonic stem cells (mESCs) using an eGFP episomal vector. Transfection compared with 24-well plate controls showed a higher efficacy in the MFCD, which may be due to the restricted device geometries (lower surface area to volume ratio) combined with an increased concentration of transfection reagent. The expansion of mESCs under fully-defined, homogeneous perfusion conditions was also successfully demonstrated. Time-course profiles for culture confluency and cell density are acquired using novel image processing algorithms and phase contrast microscopy images of the entire culture chamber, without disruption to the cells. Similarly, cellular oxygen kinetics are determined in real time using optical sensors. By combining both analytical approaches we can quantify the specific oxygen uptake rate over the culture time-course. A multiplexed version of the MFCD allows the comparison of different medium conditions in parallel. This design facilitates the current development of single-use culture cassettes with optimal material properties that can be clamped into a fluidic docking station.

Funding Source

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P086

ADVANCING DRUG DISCOVERY AND DISEASE MODELING: AUTOMATION AND OPTOGENETICS FOR PATCH-CLAMP AND MEA TECHNOLOGY

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In the constantly evolving field of drug discovery and safety, novel technologies and application have become a priority. Here, we describe diverse approaches and present data dissecting electrophysiological characteristics and activity patterns of different iPSCs. We present novel additions, enabling researchers to elevate their assays to a higher level, using automation and optogenetics as powerful tools to increase throughput and obtain more freedom in experimental design. The need for simple, reliable and predictive pre-clinical assays for cardiac safety has motivated initiatives worldwide including the Comprehensive in vitro Proarrhythmia Assay (CiPA). Automated high throughput planar patch clamp systems complement this need. Specialized protocols for reduced cell usage, increased throughput and integration into robotic environments improve cost efficiency, precision and are speeding up the HTS process of drug development and safety screening. Additionally, pioneering the fully automated MEA system, integrating MEA plate preparation, maintenance and full MEA assay execution, complement automated patch-clamp measurements. This system seamlessly integrates a sterile compact workstation, which includes a robotic liquid handler, 44-plate capacity incubator, environmental controller and

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HEPA filtration system. Here, we present the data obtained during Phase II of the CiPA initiative study, performed using these two automation systems and diverse iPSC derived cardiomyocytes. Both approaches demonstrated high throughput, sensitive and reproducible performance on various sites. Furthermore, we demonstrate novel solutions for specific cell stimulation (pacing) or silencing using multiwell light delivery add-ons for optogenetics. Multiwell optogenetic stimulation further excels impedance and MEA-based disease modeling and drug discovery. Through even illumination of the wells and lack of induced artifact, optogenetic stimulation exhibits improved reliability across wells, as compared to electrical stimulation. In summary, we present novel experimental possibilities by incorporating automation and optogenetics approaches into already available and widely used technologies, yielding higher throughput, sensitivity and precision.

P088

CARTILAGE-MIMICS BY 3D BIOPRINTING OF CARTILAGE-DERIVED IPSCS IN A NANOCELLULOSE COMPOSITE BIOINK

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Cartilage lesions can progress into osteoarthritis and causes degenerations of cartilage and severe pain in numerous patients. As a prospective treatment of such lesions is cartilage-mimics that was here generated by 3D bioprinting induced pluripotent stem cells (iPSCs) with nanofibrillated cellulose (NFC) composite bioink. Two bioinks were investigated; NFC with alginate (NFC/A) or hyaluronic acid (NFC/HA). While low proliferation and phenotypic changes away from pluripotency was seen in NFC/HA, pluripotency was initially maintained and after five weeks of chondrogenic differentiation, hyaline-like cartilaginous tissue expressing collagen type II, glucosaminoglycan (GAG) and loss of tumorigenic-Oct4 were seen in 3D bioprinted NFC/A constructs. Visualization of the 3D arrangement of extracellular matrix (ECM) in living cells was achieved by using nonlinear microscopy to simultaneously acquire second harmonic generation (SHG) images of collagen and two-photon excited auto-fluorescence (TPEF) images of unlabeled, printed constructs. Inclusion of GDF5 in the chondrogenic differentiation protocol increased the SHG signal and an ordered structure similar to native cartilage was seen. Moreover, increased cell number were detected by 2-photon-fluorescence-microscopy within the cartilaginous tissue, indicating the importance of high cell densities and quest for good survival after printing. We conclude that NFC/A bioink is suitable for bioprinting iPSCs to support cartilage engineering.

P090

HUMAN IPSC-DERIVED NEURONS FOR FUNCTIONAL ASSESSMENT OF IN VITRO NEUROTOXICITY AND SEIZURE LIABILITY

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In vitro pharmacology profiling of new chemical entities during early phases of drug discovery has recently become an essential tool to predict and avoid clinical adverse effects. While for cardiac safety testing, several validated platforms are available, specific in vitro neurotoxic panels are not, and only in vivo models are used. However, correlations between animal and human data are often weak; in addition, animal studies are expensive, ethically questionable and require large amounts of chemical compounds. Here, we present two assay systems that address different in vitro neurotoxicity endpoints in a human system using induced pluripotent stem cell (iPSC)-derived neurons and multiwell microelectrode array (MEA)-technology. Peri.4U are iPSC-derived peripheral neurons that reveal clear burst-like activity after 3-4 day culture on MEA chips, indicating the presence and establishment of a functional neuronal network. Reference compounds with a known neurotoxic potential, such as neuroleptica, antidepressiva, neurotransmitter blockers, pesticides or plant toxins, were analyzed for their effect on neuronal network behavior. Peri.4U showed at least similar or even higher sensitivity to reference compounds compared to other neuronal in vitro models. This demonstrates the potency of Peri.4U for reliable detection and quantification of neurotoxic compound actions. CNS.4U represent a highly physiological iPSC-based co-culture model of neurons (glutamatergic, GABAergic and dopaminergic) and astrocytes. CNS.4U rapidly form neuronal networks in culture and show synchronous network activity assessed by MEA technology over c. 5 weeks in culture. Here, we provide proof-of-concept results that demonstrate the suitability of CNS.4U for seizure liability assays based on dose-dependent reactivity to compounds that are known to affect seizure.

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P092

FOOTPRINT-FREE GENE EDITING USING CRISPR/CAS9 AND SINGLE-CELL CLONING OF EDITED HUMAN IPS CELLS

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The combination of two powerful technologies allows for a new level of sophistication in cell biology research and disease model development. The ability to create hiPS cell lines from different donors and to determine the effects of specific mutations created via gene editing within the donor-specific genetic background will enable discoveries with a new level of granularity. However, while the introduction of CRISPR/Cas9 technology has made gene editing easier to achieve, obtaining single-cell clones of edited hiPS cells has been a bottleneck. Traditionally hiPS cells are grown and passaged as colonies. To obtain single cells for cloning purposes, the colonies must first be dissociated into a single-cells, which often results in cell death or premature differentiation. We have instead used a culture system that allows culturing of hiPS cells in a monolayer and permits single-cell isolation. The specific compositions of the culture system allow for a high rate of single-cell survival and clone expansion. We applied this culture system to develop a complete workflow, starting with CRISPR/Cas9-mediated editing, using Cas9/sgRNA ribonucleoprotein complexes delivered into hiPS cells via either electroporation or cell-derived nanoparticles called gesicles, followed by successful single-cell cloning of edited hiPS cells. We chose non- DNA-based delivery methods to guarantee footprint-free editing of the hiPS cells. We achieved endogenous gene knockout efficiencies of up to 85% for the membrane protein CD81 in a hiPS cell population. A homogeneous population of edited cells were obtained by FACS and individual, edited hiPS cells were single cell seeded into a 96-well plate, either by limiting dilution or repeated FACS. The use of limiting dilution resulted in a high recovery rate of single hiPS cells which went on to form clonal colonies. We also demonstrated that all edited hiPS clones obtained with the described workflow were still pluripotent after gene editing, single cell seeding and expansion. The data show that the described workflow of footprint-free editing via efficient delivery of Cas9/sgRNA RNP complexes and single-cell cloning of hiPS cells using a pampering culture system results in a high number of edited and expandable hiPS clones that maintain the hallmarks of pluripotency.

P094

QUANTITATIVE MULTIDIMENSIONAL IMAGING OF BONE MARROW IN HEALTH AND DISEASE

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The bone marrow, while harboring hematopoietic and skeletal stem cells and their progeny, is also an important site for hematopoietic malignancies and solid tumor metastases. While qualitative and lately also quantitative imaging-based characterization of bone marrow cells in the healthy state has yielded important information in the field, literature on quantitative imaging based exploration of the bone marrow in disease models is scarce. For the characterization of diseases in the bone marrow, imaging of complex immunphenotypes and single cell events in large tissue volumes – important e.g. for early stage disease detection – is needed, but reproducible quantitative information is key in faithfully assessing disease burden and monitoring disease kinetics. Using a novel 3D multicolor (up to 8 colors in the same sample) imaging approach, combined with an analysis pipeline for quantitative analysis, consisting of a combination of commercially available and self-developed software (X-dimensional image quantification Toolbox, XiT). This combination allows easy hypothesis generation and fast and comprehensive data analysis of large imaging data sets – from full bones to single cells. Initial cell/structure visualization and segmentation is done in Imaris®, whereas the rest of the data analysis is performed in XiT. Imaging data can be curated and flow cytometry-like analyses as well as imaging specific plots/tasks (Spatial gating, heatmaps, random dot generation etc.) can be carried out in a user-friendly graphical interface. Also, fully automated and unbiased subpopulation analyses can be performed. Using this approach we plan to investigate the progression of bone marrow affecting diseases with an emphasis on solid cancer metastases in their early stages and put our findings in perspective to the healthy state.

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MODELING TISSUE DEVELOPMENT

P096

EBIO DOES NOT INDUCE CARDIOMYOGENESIS IN HUMAN PLURIPOTENT STEM CELLS BUT MODULATES CARDIAC SUBTYPE ENRICHMENT BY LINEAGE-SELECTIVE SURVIVAL

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Subtype-specific human cardiomyocytes (CMs) are valuable for basic and applied research. In mouse pluripotent stem cells (mPSCs) induction of cardiomyogenesis and nodal-like CMs enrichment by 1-ethyl-2-benzimidazolinone (EBIO), a chemical modulator of small/intermediate conductance Ca2+-activated potassium channels (SKs 1-4), was described. Investigating EBIO in human PSCs we have applied three independent differentiation protocols of low-to-high cardiomyogenic efficiency. Equivalent to mPSCs, timed EBIO supplementation during hPSC differentiation resulted in dose-dependent enrichment of up to 80% CMs, including an increase in nodal- and atrial-like phenotypes. However, our study revealed extensive EBIO-triggered cell loss favoring cardiac progenitor preservation and subsequently CMs with shortened action potentials. Proliferative cells were generally more sensitive to EBIO presumably via SK-independent mechanism. Together, EBIO did not promote cardiogenic differentiation of PSCs opposing previous findings but triggered lineage-selective survival at a cardiac progenitor stage, which we propose as a pharmacological strategy to modulate CMs subtype composition.

Funding Source

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P098

THE ROLE OF BMP SIGNALING IN EARLY HUMAN GONADAL DEVELOPMENT

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Gonads arise at gestational week 5 as a pair of epithelial thickenings on the surface of intermediate mesoderm-derived mesonephros. They are first bipotential and capable of developing into male or female gonads, until SRY initiates sex determination and induces the downstream signaling events to generate testes or ovaries. The regulation of gonadal development prior to sex determination is poorly understood. The primary objective of this study is to generate an in vitro protocol for studying the development of human gonads. We directed human embryonic stem cells (hESCs) with high Activin A and CHIR-99021 (Wnt agonist) into primitive streak-like cells expressing BRACHYURY at day 1-2 of differentiation as analyzed by quantitative PCR (qPCR). Mesendoderm induction was followed by subsequent expression of intermediate mesoderm markers PAX2, LHX1 and OSR1 by day 4 of differentiation. Importantly, we introduced to the culture sequential short-term activation and inhibition of BMP signaling pathway with BMP7 and dorsomorphin, respectively. By day 8 of differentiation, our protocol produced cells expressing bipotential gonadal genes EMX2, LHX9, GATA4 and WT1. By contrast, absence of BMP signaling inhibition on the first days of differentiation decreased the expression of bipotential gonadal genes and instead, induced expression of trophectoderm and extraembryonic markers. Hence, by carefully adjusting the delicate balance between BMP signaling activation and inhibition, we have generated a protocol that produces cells of bipotential gonad-like identity. This protocol will be valuable in studying the early development of human gonads.

Funding Source

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P100

CLINICALLY COMPLIANT HUMAN PSC CULTURE CONDITIONS SUPPORT EFFICIENT CLONAL SURVIVAL AND RAPID SCALE-UP

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The lack of defined, xeno-free, easy and robust methods for efficient expansion of human pluripotent stem cells (PSCs) has hindered both the advancement of basic research and human cell therapy, much due to high experimental variation and poor quality cells with phenotypic and genetic changes. Laminin-521 (LN-521) is a protein naturally expressed by human PSCs and is a critical factor of the pluripotent stem cell niche. Laminins influence adhesion, differentiation, migration, phenotypic stability, anoikis resistance and functionality of all cells associated to it. LN-521 is a human and recombinant protein and can easily be used as a cell culture substrate. When used for human PSCs they can be cultured for over 80 single cell passages without any abnormal genetic aberrations and with maintained expression of pluripotency markers. Human PSCs on LN-521 expand twice as fast compared to other matrices and can be split 1:20 or up to 1:30 as single cells without the addition of ROCK inhibitor. Furthermore, LN-521 can be used as microcarrier coating for generating clinically relevant quantities of human PSCs thus offering a scalable and GMP-compatible bioprocessing platform. Moreover, true clonal culture, important for cell fate tracking, gene function analyses and editing, is possible by using LN-521 in combination with E-cadherin. Human embryonic stem cell (hESC) lines can even be derived from a single blastomere under chemically defined and xeno-free condition on LN-521, thereby circumventing the ethical issues associated with hESCs. The simplicity and reliability of the culture procedure, the rapid cell amplification and the genetic stability of the cells make LN-521 a suitable as reagent in clinical trials for human PSC-based therapy. In conclusion, we show that LN-521 is an optimal matrix for human PSC culture due to its biological relevance that allows derivation, clonal cultivation, stable long-term pluripotent cell growth and scalability. The robust method allows minimum culture maintenance and standardized protocols, which can easily be adapted to automation platforms, making LN-521 a suitable reagent choice for human cell therapy trials.

P102

HUMAN EMBRYONIC AND APICAL PAPILLA STEM CELL INTERACTION: AN IN VITRO MODEL TO DEVELOP RETINAL PIGMENT EPITHELIUM

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We have identified human exfoliated deciduous teeth (SHED) showed a stromal cell-derived inducing activity (SDIA) that induced efficient neural crest cell (NCC) differentiation of co-cultured human embryonic stem cells (hESCs). To understand the range of competence of SDIA of SHED as apical papilla stem cells, we have examined in vitro differentiation of hESCs into retinal pigment epithelium (RPE). In comparison with Matrigel cultured hESCs, SDIA; accumulated on SHED cells induces cocultured hESCs significantly to differentiate into rostral neural cells as demonstrated by upregulation of related markers, OTX2 and PAX6 and dow-regulation of EN1, HOXB4 and HOXC8 under serum-free conditions without the use of either exogenous factors or embryoid bodies. Furthermore, the differentiated cells significantly expressed eye-field markers, RX, PAX6, LHX2 and SIX3. The pigmented colonies were 4 times more than Matrigel cultured hESCs. The expanded hESC-RPE were hexagonal and contained significant amounts of pigment. The RPE expressed typical RPE markers: ZO-1, RPE65, bestrophin, CRALBP and MITF. They were able to phagocytose pHRodo particles. Our report is the first on the expression of specific markers of RPE in SCAP-induced hESCs. Our results suggest that this system can be used for studying molecular and cellular events in the effect of stromal cells on RPE, as well as for producing normal human RPE for developing therapies for diseases such as Age-Related Macular Degeneration.

Funding Source

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P104

TISSUE SPECIFIC LAMININS GENERATE AUTHENTIC AND CLINICALLY COMPLIANT CELLS

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The lack of defined, xeno-free, robust methods for expansion and specialization of human pluripotent stem cells (hPSC) towards different cell types has hindered both the advancement of basic research and the translation into clinical settings. The expression and composition of the basement membrane proteins are essential for embryonic morphogenesis and adult tissue functions. Laminins are the only basement membrane proteins that are tissue specific and with the use of the specific combination of xeno-free and defined human recombinant laminins, the natural environment for each specific cell type can be created, generating high quality cell with homogenous phenotypes. Laminin-521 (LN-521) is the laminin isoform naturally expressed by hPSCs and is also one of the most commonly expressed laminins after birth. LN-521 maintains high degree of hPSC homogeneity, pluripotency and genetic stability when used in vitro. By using different laminins, differentiation of hPSCs can be made robust, reliable and with increased efficiency. Differentiation of LN-521 cultured hPSCs can be made to: 1) dopaminergic progenitors with LN-111, resulting in an increased yield of >40 times compared to standard EB-based protocols. The GMP-compatible LN-111 based protocol gives a very homogenous population of cells predicted to have a good graft outcome. Due to the high yield the cost of reagent per transplant is very low. 2) RPE cells, exhibiting native characteristics including morphology, pigmentation, marker expression, polarization and phagocytic activity. Transplanted cells exhibit long-term integration and photoreceptor rescue capacity. 3) Hepatocytes that in vitro are highly organized, similar to primary tissue, and with significantly increase in metabolic functions. 4) Endothelial cells through a defined and xeno-free protocol rendering 95% functional cells facilitating production of stable endothelial cells for vascular disease modeling and treatment. Laminin coating substrates, compatible with GMP requirements, are being developed which allow the transition of these pre-clinical research protocols into clinical settings.

P106

IL-7 OVER-EXPRESSION ENHANCES REGENERATION POTENTIAL OF MESENCHYMAL STEM CELLS IN A RAT DIABETIC WOUND MODEL

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Delayed wound healing is a prevalent consequence of diabetes that often leads to limb amputations. Stem cells hold significant potential as an alternative therapeutic approach for chronic wounds in diabetes. Degeneration of vessels and failure of angiogenesis in diabetic wound bed plays an important role in the delay of healing process. The present study was aimed to genetically modify rat mesenchymal stem cells (MSCs) through interleukin-7 (IL-7) gene over-expression in order to enhance their healing potential against chronic diabetic wounds. IL-7 gene was over-expressed in MSCs by retroviral transfection and confirmed through reverse transcriptase (RT) PCR. Diabetic wound models were established and the IL-7 transfected MSCs were transplanted to assess their wound healing potential in vivo. Wound area was measurement for 15 days and histological analysis was performed. Gene analysis showed a significant increase not only in the IL-7 gene ($p < 0.001$) but also angiogenic genes, angiopoietin-1, vascular endothelial growth factor (VEGF) ($p < 0.01$), and hepatocyte growth factor (HGF) ($p < 0.05$). In vitro analysis of IL-7 transfected MSCs exhibited intense cell-cell connections and tube formation as compared to the normal MSCs. Rate of wound closure was significantly increased ($p < 0.001$) in diabetic group transplanted with IL-7 transfected MSCs. Histological examination revealed enhanced vascular supply in the tissues of diabetic animals transplanted with IL-7 transfected cells as compared to normal MSCs. We can conclude from our study that IL-7 enhances the regeneration potential of rat bone marrow MSCs aiding in the closure of wounds in diabetic animals through increased expression of angiogenic genes.

Funding Source

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POSTER ABSTRACTS

P108

ADULT-LIKE DENDRITIC CELL GENERATION FROM PLURIPOTENT STEM CELLS BY FORCED EXPRESSION OF RUNX3

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Cell therapeutic application of dendritic cells (DCs) provides a promising approach to immunotherapy. However only a limited number of DCs can be manufactured from adult precursors. In contrast, pluripotent embryonic stem (ES) cells would represent an inexhaustible source for DC production, although, it remains a major challenge to steer directional differentiation because ES-derived cells are typically immature with impaired functional capacity. Consistent with this notion, we found that mouse ES cell-derived DCs (ES-DCs) represented less mature cells than bone marrow-derived DCs (BM-DCs). This finding prompted us to compare the gene expression profile of the ES cell- and adult progenitor-derived, GM-CSF instructed, non-conventional DC subsets. We quantified the mRNA level of 17 DC specific transcription factors and observed that three transcriptional regulators (Irf4, Spi-B and Runx3) showed lower expression in ES-DCs than BM-DCs. In the light of this altered gene expression, we probed the effects of these transcription factors in developing mouse ES-DCs with an isogenic expression screen. Our analysis revealed that forced expression of Irf4 repressed the ES-DC development, in contrast, Runx3 and Spi-B positively regulated the development of the myeloid precursors of ES-DCs. More importantly, enhanced DC maturation with improved chemotactic activity was detected on Runx3-instructed ES cell-derived DCs suggesting that reintroduction of a missing transcription factor can greatly enhance the immunogenicity of the pluripotent stem cell-derived antigen presenting cells.

Funding Source

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P110

NOTCH SIGNALING PROMOTES ENDOTHELIAL-TO-HEMATOPOIETIC TRANSITION AND DEFINITIVE HEMATOPOIESIS FROM ARTERIAL-TYPE HEMOGENIC ENDOTHELIUM DURING HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION

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Generating autologous hematopoietic stem cells (HSCs) from induced pluripotent stem cells (iPSCs) represents a promising approach for novel patient-specific gene therapies. While multiple studies generated hematopoietic stem/progenitor cells (HSPCs) with a definitive-HSC phenotype from mouse and human pluripotent stem cells (hPSCs), these HSPCs failed to produce long-term multilineage engraftment. Our molecular profile analysis of hPSC-derived HSPCs against their in vivo counterparts revealed that in vitro-derived HSPCs have aberrant NOTCH signaling. Thus, understanding the role of NOTCH signaling during hemogenic endothelial (HE) development, endothelial-to-hematopoietic transition (EHT), and definitive HSC specification during hPSC differentiation is essential to produce HSCs de novo. Using a chemically defined and embryoid body-free hPSC differentiation system, combined with the use of DLL1-Fc and the small molecule DAPT to manipulate the level and timing of NOTCH activation on specific populations, we demonstrated that NOTCH signaling facilitates the transition of CD144+CD43-CD73- hemogenic endothelial progenitors (HEP) to definitive-type HSPCs and promotes post-transition expansion. Furthermore, we have identified an arterial-type CD144+CD43-CD73-DLL4+RUNX1+23-GFP+ HE population (AHEs) with NOTCH-dependent capacity to form hematopoietic progenitors with broad lympho-myeloid and definitive erythroid potentials. In contrast, NOTCH modulation has limited effect on EHT from the CD144+CD43-CD73-DLL4- HE population, indicating that NOTCH-dependent definitive hematopoietic activity segregates to the AHE. Together, our studies revealed that activation of NOTCH is essential for the specification of AHE, which appears to be is the proper precursor for HSC formation in the embryo.

Funding Source

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POSTER ABSTRACTS

P112

BULK CELL DENSITY AND WNT/TGFBETA SIGNALING REGULATE MESENDODERMAL PATTERNING OF HUMAN PLURIPOTENT STEM CELLS

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In vitro differentiation of human pluripotent stem cells (hPSCs) recapitulates early aspects of human embryogenesis, but the underlying processes are poorly understood and controlled. Here we show that modulating the bulk cell density (BCD: cell number per culture volume) deterministically alters anteroposterior patterning of primitive streak (PS)-like priming. The BCD in conjunction with the chemical WNT pathway activator CHIR99021 results in distinct paracrine microenvironments codifying hPSCs towards definitive endoderm, precardiac- or presomitic mesoderm within the first 24h of differentiation, respectively. Global gene expression and secretome analysis reveals that TGFß superfamily members, antagonist of Nodal signaling LEFTY1 and CER1 are paracrine determinants restricting PS-progression. These data result in a tangible model disclosing how hPSC-released factors deflect CHIR99021-induced lineage commitment over time. By demonstrating BCDs' decisive role, we show its utility as a method to control lineage-specific differentiation. Vice-versa, these findings have profound consequences for inter-experimental comparability, reproducibility, bioprocess optimization and scale-up.

Funding Source

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P114

INVESTIGATING THE ROLE OF DNA DEMETHYLATING AGENT 5-AZA-2-DEOXYCYTIDINE AND HISTONE DEACETYLASE INHIBITOR SUBERANILOHYDROXAMIC ACID IN STEM CELL DIFFERENTIATION INTO BETA CELLS

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Diabetes Mellitus is a serious disease that affects millions of patients worldwide and is associated with many complications. While insulin injection is the ultimate treatment option, patient compliance and adaptation of the dose to the dietary sugar intake is still a major challenge. Mesenchymal stem cells have been studied for their ability to differentiate into beta cells with controversial results and failure of maturation and secretion in many instances. Epigenetic modifiers have been shown to enhance the stem cell differentiation by rendering the genome more responsive to the culturing condition. In this study, we investigated the effect of the DNA methylation inhibitor 5-Aza-deoxycytidine (Aza) and the histone deacetylse inhibitor Suberoylanilide hydroxamic acid (SAHA) as an additive to a classical differentiation protocol for beta cells, in MG63 cell line which has the characteristics of mesenchymal stem cells. The cells were pretreated with one of the two agents for three consequent days and then cultured in in the specialized media, according to our previously published protocol. While the cells pretreated with SAHA showed increase in the insulin production within the cells (showed by immunofluorescence), those pretreated with Aza showed further increase in the production as well as the secretion of insulin into the media (as detected by ELISA) , especially in response to high glucose challenge. These data as well as the underlying molecular mechanism will be discussed.

Funding Source

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POSTER ABSTRACTS

P116

A MICROFLUIDIC BASED HUMAN GUT-ON-A-CHIP FOR 3D ORGAN AND DISEASE MODELLING

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Intestinal barrier disruption as a result of pathological states or drug-induced toxicity can lead to life-threatening conditions and cease drug discovery. Current in vitro 2D intestinal models lack the features of the in vivo settings like tubular structure or perfusion, and fail in clinical translation. MIMETAS OrganoPlate® technology enables culturing of 3D tube-like structures in a high-throughput manner with a membrane-free extracellular membrane boundary with a pump-free perfusion system1. We show the development of a human gut-on-a-chip consisting of gut epithelial cells grown into a barrier tissue supported by the microfluidic channel of the OrganoPlate®. This epithelial barrier model of the intestinal tract showing rapid cell polarization, tight junction formation and proper expression of intestinal markers. These intestinal tubular structures are suitable for high-throughput toxicity screening, real-time imaging of transport and barrier leakage. Additionally, the OrganoPlate® technology allows the development of complex models by mimicking an in vivo metabolic and immunological response following their co-culture with immune cells and/or bacteria. By this, gut-on-a-chip models allow us to study inflammatory bowel diseases (IBD) like Crohn´s disease and Ulcerative colitis, and support screening for potential drug targets for these diseases. We applied the OrganoPlate® platform to develop cytokine-induced IBD CACO2 model suitable for target discovery and validation. Stimulation of gut tubules with pro-inflammatory cytokines resulted in disruption of epithelial barrier that could be prevented by treatment with known IBD therapeutics. Culture of these IBD gut tubules in the OrganoPlate® were applied to automated readout of the barrier integrity followed by image analysis and quantification. MIMETAS OrganoPlate® technology offers novel ways for studying organ development in a more physiological way and thus allowing us to better understanding disease mechanisms.

P118

MODULATION OF ADIPOGENESIS BY THE EPIGENETIC MODIFIERS 5-AZA-DEOXYCYTIDINE AND SUBEROYLANILIDE HYDROXAMIC ACID

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Burn victims and mastectomy patients have high psychological burden and low quality of life, especially that the current management plans are not physiological and associated with complications and co-morbidities. Mesenchymal stem cells have been studied for their ability to differentiate into adipocytes with controversial results. Epigenetic modifiers have been shown to enhance the stem cell differentiation by rendering the genome more responsive to the culturing condition. In this study, we investigated the effect of the DNA methylation inhibitor 5-Aza-dC and the histone deacetylse inhibitor Suberoylanilide hydroxamic acid (SAHA) as an additive to the classical adipogenic differentiation protocol in MG63 cell line, which has the characteristics of mesenchymal stem cells. Fat accumulation, as evident by oil red staining, was enhanced in SAHA pretreated cells while decreased with 5-Aza-dC in comparison to the vehicle or control groups. The same pattern has been shown with gene expression including the peroxisome proliferator-activated receptor gamma, adiponectin receptor type I and II and glucose transporter type 4. The secretion of monocyte chemotactic protein-1, an adipogenic inducer, reached up to 165% in the culture supernatant with SAHA and less than 22% with 5-Aza-dC in comparison to the control. Next generation sequencing showed different panels of gene expression. This study does not only highlight the potential role for SAHA in fat regeneration but also open the door to investigate the potential role of 5-Aza-dC as anti-obesity agent.

POSTER ABSTRACTS

P120

BONE MARROW NICHE-MIMETICS REMODEL HSPC FUNCTION VIA INTEGRIN SIGNALING

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The bone marrow (BM) microenvironment presents beside chemokines and cytokines preliminary physical cues and accommodates hematopoietic stem cells (HSPCs) via cell-matrix interaction. These stimuli are essential for maintaining HSPCs and significantly contribute to fate decision, however, less is known about matrix communication and signal transduction. In contrast, due to a lack of suitable culture methods regarding particularly the extracellular matrix (ECM) contact, stem cell expansion is mainly facilitated in suspension cultures using cytokine cocktails or co-cultures with mesenchymal stromal cells (MSC). Here, we used BM-mimetic decellularized ECM scaffolds derived from SCP-1 mesenchymal cells to target exclusively the interaction of HSPCs and ECM compartment. We found increased expansion of HSPCs ex vivo and active recognition of matrix incorporated stromal derived factor one (SDF-1). However, a minority of peripheral blood (PB) HSPCs mobilized using G-CSF were capable to adhere. Probing cell mechanics, these cells were found to be stiffer than non-adherent supernatant (SN) cells indicating physical recognition and adaptation via focal contacts. RGD-binding integrin expression, namely integrin αII2 (CD41), αV (CD51) and β3 (CD61) were found to be induced by ECM scaffold contact. Our data identifed focal contacts via integrin (ITG) β3 as mediator of cell adhesion and migration to BM stroma and highlight ECM interaction as modulator of HSPC function.

Funding Source

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P122

GENERATION AND EXPLOITATION OF NAÏVE HUMAN PLURIPOTENT STEM CELLS

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The recently described 'naïve' (or 'ground state') human pluripotent stem cells (hPSCs) resemble an earlier time-point in embryogenesis compared to conventional, 'primed' hPSCs. We have directly compared protocols for generation of naïve hPSCs and have revealed large differences in the expression of key 'naïve' genes in the resulting cells. We have also characterised the naïve-to-primed transition, a process resembling in vivo embryogenesis. Disturbing miRNA biogenesis prevents naïve-primed transition in mouse embryos and prevents differentiation in mouse (naïve) embryonic stem cells in vitro. I am using miRNA-seq to uncover miRNAs upregulated during the exit from naïve pluripotency, and investigating whether any of these govern the transition process. Inhibition of these miRNAs could reversibly lock cells in the naïve state, thereby improving homogeneity, facilitating manipulation and preventing the common problem of premature differentiation.

Funding Source

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POSTER ABSTRACTS

OTHER

P124

EXPANSION AND DIFFERENTIATION CAPACITY OF HUMAN PLURIPOTENT STEM CELLS CULTURED IN A XENO-FREE AND DEFINED CULTURE MEDIUM, STEMFIT®

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We developed a defined and xeno-free culture medium, StemFit®, for feeder-free culture of human pluripotent stem cells (hPSCs). Using StemFit®, in combination with commercially available extracellular matrices (ECM) such as recombinant human laminin-511 E8 fragment (LN511E8), hPSCs can be easily and stably passaged as dissociated single cells for long periods without any karyotype abnormalities. StemFit® showed higher growth efficiencies for dispersed human induced pluripotent stem cells (hiPSCs) than the conventional defined medium. StemFit® efficiently supported growth and colony formation of hiPSCs even when the seeding density was much lower than that for the ordinary maintaining culture, suggesting that it was a preferable medium for scalable expansion and cloning of hPSCs. hiPSCs cultured in StemFit® maintained propensity for differentiation onto the three germ layers (Neuron, Cardiomyocyte and Definitive Endoderm). We further assessed the differentiation capacity of them with the comparison of hiPSCs cultured on feeder cells. Though the differences of cell lines and markers were determined, the differentiation propensity was not changed between on feeder culture and feeder free culture with StemFit®. These results suggest that StemFit® is not only a reliable platform for the clinical applications of hPSCs but also a versatile tool for the stem cell basic research.

P126

INVESTIGATING THE HUMAN TEASHIRT GENES TSHZ2 AND TSHZ3 IN PANCREATIC AND ENDOCRINE LINEAGE COMMITMENT

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The homeobox transcription factor gene PDX1 (Pancreatic and Duodenal Homeobox 1) is expressed at the earliest stages of human pancreatic development. PDX1 transcripts label early pancreatic progenitor (ePP) cells that are multipotent, giving rise to the exocrine, endocrine and ductal components of the adult organ. Strikingly, loss of PDX1 results in complete pancreatic agenesis in man and mice, indicating that PDX1 is a master regulator for pancreatic development. Our laboratory developed a human embryonic stem cell (hESC) differentiation protocol that tightly adheres to developmental logic and yields abundant PDX1+ ePP cells after roughly 17 days of in vitro culture. These ePP cells display a molecular signature that significantly overlaps with the developing pancreatic primordium in vivo. PDX1 knock-out hESC lines were also generated using TALEN gene editing technology, and it was found that PDX1 null cells expectedly fail to activate the pancreatic transcriptional program and divert to alternate fates in vitro. We performed microarray studies comparing wild-type and PDX1-deficient hESC lines at selected days of differentiation to identify differentially expressed genes, which we hypothesize play key roles in orchestrating pancreatic development downstream of PDX1. Combining these microarray studies with PDX1 Chromatin Immunoprecipitation-Sequencing data revealed a novel list of candidate PDX1 transcriptional targets, including TSHZ2 and TSHZ3, the human homologs of the Drosophila homeotic gene teashirt. TSHZ2 and TSHZ3 encode transcription factors containing five widely spaced C2-H2 zinc fingers as well as a DNA-binding homeodomain. In the mouse, Teashirt genes play diverse roles in the development of mesodermal (ureter) and ectodermal (neural cortex) lineages, but to date their function in derivatives of the endoderm, including the pancreas, has not been established. We therefore engineered mutations in TSHZ2 and TSHZ3 using the CRISPR-Cas9 system in H9 cells in order to investigate the roles of TSHZ2 and TSHZ3 as candidate PDX1 target genes during directed differentiation into the pancreatic lineage. Our preliminary results will be presented.

POSTER ABSTRACTS

P128

AUTOLOGOUS ADIPOSE TISSUE DERIVED STROMAL CELLS TO FOR THE MANAGEMENT OF MUSCLES FIBROSIS IN CUTANEOUS RADIATION SYNDROME: A NEW THERAPEUTIC APPROACH IN A MINIPIG MODEL

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The cutaneous radiation syndrome is the delayed consequence of a local high dose irradiation, mainly characterized by extensive inflammation, necrosis or fibrosis of cutaneous and muscular tissues. Adipose tissue derived stromal cells sub-cutaneous injections have previously shown benefit on skin wound healing in a minipig model of acute local irradiation (50 Gy; gamma ray) but a persistent muscle fibrosis remained, due to radio-induced muscles inflammation. Thus, the association of subcutaneous and intra-muscular injections of adipose tissue derived stromal cells has been evaluated on tissue recovery.Six female minipigs were locally irradiated (50 Gy using a 60Co source (0.6 Gy.min−1)) and randomly divided into 2 groups. The vehicle (phosphate-buffer-saline solution) was injected in three animals and three animals received 3 sub-cutaneous injections of 50x106 adipose tissue derived stromal/stem cells and three intra muscular injections of 25.106 cells on days 25, 46 and 66 post-irradiation. The muscle regeneration pathways, the polarization of the inflammatory response of irradiated muscle as well as myofibroblasts recruitment (tissue remodeling marker) were assessed by western-blot, and immunohistochemistry 76 days after irradiation. A homogeneous skin evolution was observed for all minipigs. Macroscopic observation of irradiated muscles showed prominent fibrosis and necrosis areas in PBS group opposed to adipose tissue derived stromal/stem cells injected animals. Moreover, a recruitment of myofibroblasts, a macrophage 2 (M2) polarization of the inflammatory response and muscle regeneration pathway activation after intramuscular injections of adipose tissue derived stromal/stem cells were observed on muscle biopsies. Globally, these preliminary data are consistent with muscle regeneration improvement in the cutaneous radiation syndrome in minipig model after intramuscular injections of adipose tissue derived stromal cells. Work is required to evaluate this therapeutic strategy on a larger cohort with a longer clinical follow-up.

P132

ENDOTHELIAL DIFFERENTIATION OF DENTAL STEM CELLS IN A THREE-DIMENSIONAL MICROTISSUE CULTURE SYSTEM

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Dental stem cells have been demonstrated to have potential to differentiate into several lineages, including endothelial cells. However, the efficiency of in-vitro endothelial differentiation of dental pulp stem cells (DPSCs) and stem cells from exfoliated human deciduous teeth (SHED) is questionable. The present study aimed to investigate the endothelial differentiation potential of DPSCs and SHED in a three-dimensional microtissue spheroid system. Three-dimensional (3D) microtissue-spheroids of DPSCs and SHED were fabricated using 12-series micro-molds (MicroTissues Inc.). DPSCs and SHED cultured on two-dimensional (2D) six-well culture plates were used as the control groups. Twenty-four hours after DPSCs and SHED were seeded in plates (2D) or micro-molds (3D), the culture medium was changed to fully supplemented endothelial growth medium -2 (Lonza Biologics Inc.) After 3-, 6-, and 9- days of induction, 3D microtissues were transferred onto the adherent culture surfaces and allowed to dissociate into cells, while 2D cultures were trypsinized and sub-cultured. Then, the cells were analyzed for expression of endothelial markers – eNOS, vWF, CD31, VE-cadherin, VEGFR-1 and 2 via immunofluorescence and qPCR. Matrigel assay was performed to assess the cells' ability to form capillary-like tube structures in-vitro. Immunofluorescence and qPCR results indicated an enhanced endothelial differentiation of 3D microtissue derived cells compared with 2D induced cells in relation to both DPSCs and SHED. SHED dissociated from induced microtissues were positive for endothelial markers and were able to form endothelial-like tube structures on matrigel. In contrast, 2D induced SHED failed to form such structures on Matrigel. However, DPSCs induced in either 3D or 2D could not form endothelial-like tube structures on matrigel. Three-dimensional microtissue is a promising culture system for promoting endothelial differentiation efficiency of dental stem cells in-vitro. SHED hold a higher potential for endothelial differentiation compared to that of DPSCs.

Funding Source

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POSTER ABSTRACTS

P134

NEONATAL HYPOXIA-ISCHEMIA IN RAT DISRUPTS THE DEVELOPMENTAL TIME COURSE OF DOUBLECORTIN RELEASE IN THE CEREBROSPINAL FLUID

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Doublecortin (DCX) is commonly used as a marker of neurogenesis in immunohistochemical (IHC) stainings of adult rodent brains. Using a recently developed immunoassay that enables objective quantification of DCX in tissues and body fluids, we examined whether DCX in the cerebrospinal fluid (CSF) may be a valid indicator of endogenous neurogenesis. This question was addressed in the rat model of neonatal hypoxia-ischemia (HI), a brain injury documented to stimulate neurogenesis. HI was elicited at postnatal day (P) 7 in Sprague-Dawley rats via ligation of the right common carotid artery and 40 minutes exposure to 8% O2. Control animals received a sham surgery without HI. CSF was collected serially from the cisterna magna at P5 and P10, or at P10 and P15. Bromodeoxyuridine (BrdU) was administered intraperitoneally from P7 to P9 to label dividing cells, and P10 brains were processed for IHC analyses. Neural progenitor cells (NPCs) isolated from rat embryos were differentiated in vitro to evaluate DCX release. In sham-exposed neonates, a sharp, significant drop in the mean concentration of DCX in the CSF (CSF-DCX) occurred between P5 and P15. In HI-exposed neonates, CSF-DCX increased significantly between P5-P10, but declined between P10-P15; yet, at P15, CSF-DCX remained significantly higher in HI than in control neonates. In the P10 HI group, CSF-DCX correlated positively with stroke severity. DCX immunointensity and the number of BrdU-positive cells were significantly increased in the ipsilateral neurogenic niches from P10 HI neonates in comparison to that from age-matched sham neonates. DCX expressing cells were negative for cleaved caspase-3, a cellular death marker, in both groups. In vitro, DCX could be quantified in the cell culture medium of differentiating rat NPCs, and emanated from healthy living cells. To conclude, neonatal HI brain injury remarkably disrupts the developmental time course of DCX release in the CSF. The data indicate that the increase in CSF-DCX after neonatal HI reflects both injury-associated cell death and injury-associated neurogenic response.

Funding Source

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P136

POTENTIAL OF HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS FOR THE TREATMENT OF SALIVARY GLAND DYSFUNCTION

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Salivary gland atrophy occurs after irradiation for the treatment of head and neck cancer, resulting in irreversible xerostomia. Medications such as -agonists have been used for those patients but the regeneration of atrophic glandular tissue is not feasible. Recently, the potential of cell therapy has attracted much attention for the treatment of various chronic conditions. In this study, we focused on umbilical cord-derived mesenchymal stem cells (UC-MSCs) and examined the therapeutic effect on radiation-damaged salivary glands. All animal experiments were approved by a local committee and the experiments using human cells were approved by the ethical committee of IMSUT, the University of Tokyo. We established an in vitro salivary gland model that maintains terminally differentiated salivary gland epithelial cells. The cultured cells express both acinar and ductal cell markers that decrease after irradiation. UC-MSCs were obtained from healthy donors after informed consent. The effect of co-culturing epithelial cells with UC-MSCs was investigated. Co-culture of irradiated salivary gland epithelial cells with US-MSCs showed upregulation of an acinar cell marker (AQP-5) and the difference was significant compared with that of the control, which was cultured without US-MSCs. Interestingly, this effect was achieved without cell-to-cell contact. We also examined the effect of cell transplantation using an in vivo model. After irradiation to the head and neck region of mice, UC-MSCs were administered via tail vein immediately after irradiation and one week later. Saliva flow rate was evaluated up to 8 weeks after cell transplantation. The in vivo experiment showed that the amount of saliva increased significantly in the group treated with UC-MSCs. The effect was identical to the animals that were administered bone marrow-derived mesenchymal stem cells (stromal cells) instead of UC-MSCs. In conclusion, the results of this study showed the efficacy of UC-MSCs on the recovery of radiation-damaged salivary glands, enhancing the function of acinar cells. UC-MSCs are readily available and might be a useful cell source for the treatment of radiation-damaged salivary glands.

POSTER ABSTRACTS

P138

EFFECT OF CELL PROCESSING PROTOCOL ON THE CLINICAL RESULT OF BONE TISSUE ENGINEERING

Kagami, Hideaki[1], Inoue, Minoru[2], Asahina, Izumi[3], Nagamura, Tokiko[4], Tojo, Arinobu[4], Yamashita, Naohide[4]

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Bone tissue engineering is a promising field of regenerative medicine in which cultured cells, scaffolds, and osteogenic inductive signals are used to regenerate bone. Human bone marrow stromal cells (BMSCs) have the ability to form bone when transplanted and BMSCs are the most commonly used cell source for bone tissue engineering. Although it is known that cell culture and induction protocols significantly affect the in vivo bone forming ability of BMSCs, the responsible factors are poorly understood. The results from recent studies using human BMSCs have shown that factors such as passage number and length of osteogenic induction significantly affect ectopic bone formation although such differences hardly affected alkaline phosphatase activity or gene expression of osteogenic markers. Application of basic fibroblast growth factor helped to maintain the in vivo osteogenic ability of BMSCs. Importantly, in vivo osteogenic ability and its change during culture showed significant individual variation. The results from these studies strongly suggest that there are several required conditions for human BMSCs to efficiently demonstrate their bone-forming capabilities in vivo. In this paper, we focus on bone marrow stromal cells. The characteristics of these cells, the suitable culture conditions for bone tissue engineering, and the effect of cell processing protocol on the clinical results will be shown with discussion of possible caveats.

Funding Source

Health Labour Sciences Research Grants" from the Ministry of Health, Labour and Welfare of Japan, "Grant-in-Aid for scientific research (B) (25293416, 16H05546), and "Translational Research Network Program" from MEXT of Japan.

P140

DOWNREGULATION OF POLO-LIKE KINASE 2 ENHANCES LINEAGE COMMITMENT AND FACILITATES ENDOTHELIAL DIFFERENTAITION IN DIFFERENT TYPES OF CARDIAC PROGENITOR CELLS

Mochizuki, Michika[1], Lorenz, Vera[2], Ivanek, Robert [2], Della Verde, Giacomo[2], Gaudiello, Emanuele[2], Marsano, Anna[2], Pfister, Otmar [2], Kuster, Gabriela M.[1]

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Although recent studies support regenerative potential based on adult cardiac progenitor cells (CPCs), little is known about the regulators of CPC fate. Cell fate decisions are influenced by the microenvironment and involve cues from the extracellular matrix (ECM). In this study we use c-kit+ and Sca1+/CD31- side population CPCs in a simplified model of ECM protein-dependent CPC regulation to specify key molecular determinants of CPC fate. We show that fibronectin-based adhesion supports the CPC proliferative phenotype, whereas laminin-based adhesion enhances endothelial and – more moderately – cardiomyogenic lineage commitment. Under endothelial differentiation conditions, facilitated differentiation and maturation into endothelial-like cells is observed on laminin. Comparing the early transcriptomic shift (< 2 hours) between fibronectin- and laminin-based adhesion using RNA sequencing, we identify Polo-like kinase 2 (Plk2) as a novel cell fate cue. Consistent with previous reports that the ECM regulates Yes-associated protein (YAP), we find rapid cytosolic sequestration and degradation of YAP on laminin as opposed to its nuclear enrichment on fibronectin. Furthermore, Plk2 expression depends on YAP stability, is rapidly downregulated on laminin, and its upregulation is sufficient to inhibit and downregulation to promote lineage gene expression and facilitate endothelial differentiation, respectively. We conclude that Plk2 acts as a mediator of ECM-instructed CPC fate downstream of YAP coordinating cell proliferation and lineage specification and may represent a target to promote therapeutic neovascularization.

Funding Source

Swiss National Science Foundation Foundation for Cardio-vascular Research, Basel University of Basel

POSTER ABSTRACTS

P142

EXOSOMES FROM HUMAN STEM CELLS OF THE UMBILICAL CORD TISSUE PRIME RAT NEURAL PROGENITOR CELLS TOWARDS AN OLIGODENDROGLIAL CELL FATE

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Perinatal brain damage is mainly characterized by oligodendrocyte progenitor cell loss. The neuroregenerative effects of mesenchymal stem cells (MSC) as a graft in animal models of perinatal brain damage are presumed to rely on secreted factors including MSC-derived exosomes. Thus, the aim of this study is to evaluate the capacity of exosomes from human Wharton's jelly-derived MSC (WJ-MSC) to drive the cell fate specification of neural progenitor cells (NPC) towards an oligodendroglial identity. WJ-MSC-derived exosomes were isolated from culture supernatants by serial centrifugation, characterized by the expression of endosomal markers (membrane-based antibody array) and their size (electron microscopy). The exosomal microRNA (miRNA) content was assessed by real time PCR. The exosomes were stained with the red fluorescent celltracker dye CM-DIL to analyze the potential interaction with NPC. Exosomal RNA was fluorescently labeled to investigate its potential release into NPC. After the culture with WJ-MSC-derived exosomes, NPC were evaluated for the expression of markers involved in oligodendroglial cell fate specification and differentiation by real-time PCR. WJ-MSC-derived exosomes were positive for endosomal markers, including TSG101 and ALIX, and had a mean diameter of 19 nm. The exosomes contained miRNAs that are involved in oligodendroglial cell fate determination and differentiation (miR-338, miR-9, miR-19b, miR-138). Fluorescently labeled exosomal RNA was detected in NPC after co-culture. The expression of miR-338-3p and miR-219-5p, known to regulate oligodendrocyte specification and differentiation, was increased in NPC post co-culture with exosomes. Furthermore, the gene expression of the transcription factor HES5 and the transcription factor inhibitor of DNA binding protein (ID) 2, which block oligodendrogenesis, were reduced in NPC after co-culture with exosomes. In conclusion, we successfully isolated WJ-MSC-derived exosomes. We further show that the exosomes release their RNA content into NPC and trigger them towards oligodendrocyte specification, ascribing a potential neuroregenerative role to WJ-MSC-derived exosomes.

Funding Source

Financial support by CryoSave Switzerland.

P144

DISTINCT BRD2-BRD4 FUNCTIONALITIES COORDINATE PLURIPOTENT EXIT WITH SMAD2-DEPENDENT LINEAGE SPECIFICATION

Fernandez-Alonso, Rosalia, Davidson, Lindsay , Hukelmann, Jens, Williams, Charles A.C., Zengerle, Michael, Prescott, Alan R , Lamond, Angus, Ciulli, Alessio , Sapkota, Gopal P , Findlay, Greg M.

University of Dundee, United Kingdom

Pluripotent Stem Cells (PSCs) hold great clinical potential, as they possess the capacity to differentiate into fully specialised tissues such as pancreas, liver, neurons and cardiac muscle. However, the molecular mechanisms that coordinate pluripotent exit with lineage specification remain poorly understood. To address this question, we perform a small molecule screen to systematically identify novel regulators of the Smad2 signalling network, a key determinant of PSC fate. We reveal an essential function for BET family bromodomain proteins in Smad2 activation, distinct from the role of Brd4 in pluripotency maintenance. Mechanistically, BET proteins specifically engage Nodal gene regulatory elements (NREs) to promote Nodal signalling and Smad2 developmental responses. In pluripotent cells, Brd2-Brd4 occupy NREs, but only Brd4 is required for pluripotency gene expression. Brd4 downregulation facilitates pluripotent exit and drives enhanced Brd2 NRE occupancy, thereby unveiling a specific function for Brd2 in differentiative Nodal-Smad2 signalling. Therefore, distinct BET functionalities and Brd4-Brd2 isoform switching at NREs coordinates pluripotent exit with lineage specification.

Funding Source

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POSTER ABSTRACTS

P146

PHOSPHODIESTERASE INHIBITORS ENHANCE POSITIVE INOTROPIC EFFECTS OF CATECHOLAMINES IN HUMAN IPSC-DERIVED CARDIOMYOCYTES

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Phosphodiesterases (PDE) break down cAMP and thereby restrict beta-adrenergic stimulation. Accordingly, PDE inhibitors increase the sensitivity to beta adrenergic stimulation, with inhibitors of the PDE3 isoform being most effective in the adult human heart. Our study aimed at determining the relevance of different PDE isoforms in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) in an engineered heart tissue model (EHT). Three-dimensional, force-generating fibrin-based EHT were prepared from hiPSC-CM and contractile force was analyzed by video-optical recording. PDE4 inhibitors resulted in a greater increase in sensitivity of EHTs to isoprenaline than PDE3 inhibitors. The EC50 of isoprenaline shifted from 12.4 nM (isoprenaline alone, n=45) to 0.93 nM or 1.38 nM in the presence of the PDE4 inhibitors rolipram (10 µM, n=11) or roflumilast (10 nM, n=22, $P < 0.05$), respectively, to 2.9 nM in the presence of the mixed PDE3/4 inhibitor milrinone (10 µM, n=13) and to 6.0 nM in the presence of the selective PDE3 inhibitor cilostamide (300 nM, n=16). None of the PDE inhibitors produced a significant increase in contraction force when applied alone. RT-PCR indicated PDE4 mRNA levels to be similar and PDE3 lower in hiPSC-EHTs than in non-failing human heart. The data show that hiPSC-EHT respond qualitatively normal to beta-adrenergic stimulation, but that control of this signalling pathway by PDE4 is more pronounced than that by PDE3. Predominance of PDE4 differs from native human heart.

P148

INTRACOCHLEAR TRANSPLANTATION OF NEURONAL PROGENITORS TO REPLENISH SPIRAL GANGLION NEURONS LOST AFTER EXPERIMENTAL BACTERIAL MENINGITIS

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Bacterial meningitis (BM) is the most common cause of acquired profound bilateral sensorineural hearing loss (SNHL) in childhood occurring in up to 30% of patients with Streptococcus pneumoniae infection. Hearing loss is irreversible because spiral ganglion neurons (SGNs) and hair cells have a limited regenerative capacity. Surviving SGNs are a prerequisite for the correct functioning of cochlear implants, which represents the only treatment option for severe forms of SNHL. The aim of the project is to develop a stem cell-based therapy to replenish lost SGNs after experimental BM in infant rats. In order to analyze the fate and functional effect of transplanted stem cells in the inner ear, we characterized SNHL in a rat model of BM. We show in great detail how SGNs and hair cells are lost upon BM with the biggest damage occurring in the basal part of the cochlea. The initial bacterial inoculum accurately determines the severity of cochlear cell pathologies and thereby allows us study the fate of transplanted cells in different cochlear microenvironments. Three different sources of SGN progenitors are being examined for transplantation purposes. (1) Somatic progenitor cells from the spiral ganglion were cultured as neurospheres and characterized based on the expression of SGN developmental genes and their capacity to differentiate in vitro to bipolar neurons. (2) Mouse embryonic stem cells are used as an alternative to derive otic sensory neurons. We have established a stepwise differentiation protocol, based on 3D organoid cultures. SGNs obtained by these means are currently being characterized and will be used for transplantations. (3) Human embryonic stem cell-derived otic neural progenitors (hONPs) will be transplanted and compared with the rodent cell sources. In conclusion, our initial data demonstrates proof of concept for effective surgical cell transplantation into the cochlear nerve trunk with minimal damage to residual hearing. Identification of the optimal cell source, optimization of the surgical access to minimize traumatic damage to inner ear structures and evaluation of the functional role of grafted cells on hearing function is currently ongoing. This paves the way for potential regeneration of the SGNs to improve the efficacy of cochlear implants in children with hearing deficits.

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