Welcome

ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

Dear Colleagues,

On behalf of the International Society for Stem Cell Research (ISSCR), we warmly welcome you to beautiful Amsterdam for the International Symposia, "Stem Cells & Organoids in Development & Disease." A leader in life science and health technologies, Amsterdam was recently ranked as one of the top academic and biomedical communities in Europe. Amsterdam's culture of education and innovation makes it the perfect setting for this timely and important meeting.

Research using organoids is rapidly advancing the field of stem cell science. Recent work has demonstrated that stem cells retain self-organizing properties in culture, recapitulating in vivo processes and forming organ-like tissues. This remarkable ability of stem cells and organ progenitors to form organoids has the potential to revolutionize regenerative medicine. Organoids can help illuminate organ development, degeneration, and cancer progression, and patient-derived stem cells can be used to model specific disease variances and to test drug responses. Additionally, this technology could potentially be used in the future for cell replacement therapy, which would bypass the issue of immune rejection.

We have created a diverse, engaging program that will explore this cutting-edge technology that is pushing the boundaries of stem cell science. During this symposium you will hear from scientists using organoids to model a wide variety of systems, to uncover novel therapeutics, and to repair organs in vivo. The symposium will be bookended by a forum on the policy and ethics of this regenerative medicine and a panel discussion by leaders of the field on the challenges, limitations, and promise of organoids. In addition, the latest tools and technologies will be on display by innovative companies at the Exhibit Hall, a vibrant hub of meeting activities.

This International Symposium presents many opportunities to learn about new areas of research and new approaches to stem cell science, expand your network of colleagues and friends, and build new collaborations that can help your research progress. We hope this meeting will inspire you with new ideas and foster a broader appreciation of the field and its exciting potential. Thank you for your continued support of the ISSCR, and your important work that is increasing our understanding of development and disease and advancing the field of regenerative medicine.

Sincerely,

The Amsterdam Organizing Committee

Paola Arlotta, *Harvard University, USA*

Eduard Batlle, *Institute for Research in Biomedicine, Spain*

Nissim Benvenisty, *The Hebrew University of Jerusalem, Israel*

Meritxell Huch, *University of Cambridge, UK*

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ABOUT THE ISSCR

Mission Statement

The mission of the International Society for Stem Cell Research (ISSCR) is to promote excellence in stem cell science and applications to human health.

History & Philosophy

With about 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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General Information

VENUE

Stem Cells & Organoids in Development & Disease, an ISSCR 2019 International Symposium, takes place at the K.I.T. Royal Tropical Institute, Mauritskade 64, 1092 AD Amsterdam, Netherlands.

Program sessions will take place in the Queen Máxima Hall (Auditorium) of the K.I.T. Royal Tropical Institiute.

Registration and coat check can be found by the entrance of the K.I.T. Royal Tropical Institute. Refreshment breaks, lunches, poster sessions, reception, and the Exhibit Hall are located in the foyer spaces of the venue.

REGISTRATION AND BADGE PICKUP

Pick up your attendee name badge at the ISSCR Registration Desk found by the entrance of the K.I.T. Royal Tropical Institute.

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

Registration Desk and Badge Pickup Hours

Wednesday, 20 February	11:30 – 17:00
Thursday, 21 February	08:30 – 17:00
Friday, 22 February	08:30 – 16:00

INTERNET ACCESS

Complimentary access to the internet is available within the K.I.T. Royal Tropical Institute during the International Symposium.

Network name: Conference & Events
Password: welcometokit

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.

SPECIAL EVENTS

Poster Presentations

Poster presenters will be at their posters for discussion at these times:

Odd numbered posters presented Thursday, 21 February from 13:00 to 14:00

Even numbered posters presented Friday, 22 February from 13:00 to 14:00

Refreshment Breaks

Refreshment breaks will be available in the Marble Hall (Auditorium Foyer) of the venue. Complimentary coffee and tea will be served during the following days and times (times are subject to change):

Wednesday, 20 February	15:30 – 16:00
Thursday, 21 February	08:30 – 09:00 10:30 – 11:00 15:30 – 16:00
Friday, 22 February	08:30 – 09:00 10:30 – 11:00

Lunch Hours

Thursday, 21 February	12:00 – 14:00
Friday, 22 February	12:30 – 14:00

Welcome Reception

Sponsored by Thermo Fisher Scientific

The Welcome Reception will take place at Pompstation, Zeeburgerdijk 52, 1094 AE, Amsterdam, Netherlands.

Wednesday, 20 February	17:30 – 20:00

Networking Reception

A networking reception will take place at the K.I.T. Royal Tropical Institute, in the Marble Hall (Auditorium Foyer).

Thursday, 21 February	17:00 – 18:30

General Information

EXHIBITS INFORMATION

The Exhibition Hall features leading suppliers and vendors. Please support the Exhibitors who help make this Symposium possible.

Exhibit Hours

Wednesday, 20 February	11:30 – 17:00
Thursday, 21 February	08:30 – 18:30
Friday, 22 February	08:30 - 14:00

POSTER SET-UP AND TAKE-DOWN

Poster presenters are responsible for displaying their poster at the appropriate times and removing them at the end of their presentation hour.

Odd numbered posters: displayed Wednesday, 20 February before 16:00 until Thursday, 21 February at 14:00

Even numbered posters: displayed Thursday, 21 February after 15:30 until Friday, 22 February at 14:00

Posters not removed by February 22 at 14:00 will be discarded by the organizer.

RECORDING POLICY

Still photography, video, and/or audio taping of the sessions, presentations, and posters at the International Symposium is strictly prohibited. Communicating or disseminating results or discussion presented at ISSCR events is STRICTLY PROHIBITED until the start of each individual presentation.

Thank you for your cooperation.

ISSCR CODE OF CONDUCT

The ISSCR is committed to providing a safe and productive meeting environment that fosters open dialogue and discussion and the exchange of scientific ideas, while promoting respect and equal treatment for all participants, free of harassment and discrimination. All participants are expected to treat others with respect and consideration, follow venue rules, and alert staff or security of any dangerous situations or anyone in distress. Attendees are expected to uphold standards of scientific integrity and professional ethics. These policies apply to all attendees, speakers, exhibitors, staff, contractors, volunteers, and guests at the meeting and related events.

ISSCR prohibits any form of harassment, sexual or otherwise. Incidents should immediately be reported to security and ISSCR meetings staff at isscr@isscr.org.

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James Wells
Cincinnati Children's Hospital Medical Center, USA

Wednesday, 20 February

08:20 – 11:30 PRE-SYMPOSIUM FORUM

Ethical and policy challenges of organoid research and regenerative medicine applications

08:20 – 08:35 **WELCOME AND OVERVIEW**
Melissa Little, *Murdoch Children's Research Institute, Australia*

08:35 – 09:05 **KEYNOTE ADDRESS**
Wolfgang Burtscher, *European Commission, Belgium*

09:05 – 09:20 **Roger Barker**, *University of Cambridge, UK*

09:20 – 09:35 **Ana Sofia Carvalho**, *Institute of Bioethics, Catholic University of Portugal, Portugal*

09:35 – 09:50 **Arnold Kriegstein**, *University of California San Francisco, USA*

09:50 – 10:15 **REFRESHMENT BREAK**

10:15 – 10:30 **Jeremy Sugarman**, *Johns Hopkins University, USA*

10:30 – 10:45 **Annelien Bredenoord**, *University Medical Center Utrecht, Netherlands*

10:45 – 10:50 **Daniel Klimmeck**, *EMBO Journal, Germany*

10:50 – 11:30 PANEL DISCUSSION

10:50 – 11:30 **Moderator: Hans Clevers**, Hubrecht Institute, Netherlands

EMBO

Co-organized with EMBO

Wednesday, 20 February

13:00 – 14:00 OPENING KEYNOTE SESSION

13:00 – 13:15 **WELCOME REMARKS**

Hans Clevers, *Hubrecht Institute, Netherlands*

Nancy Witty, *ISSCR CEO*

Meritxell Huch, *University of Cambridge, UK*

13:15 – 14:00 **KEYNOTE ADDRESS**

Hans Clevers, *Hubrecht Institute, Netherlands*

STEM CELL-BASED ORGANOIDS AS AVATARS IN HUMAN DISEASE

14:00 – 15:30 RECAPITULATING DEVELOPMENTAL PROCESSES I

Chair: Nissim Benvenisty, *The Hebrew University of Jerusalem, Israel*

14:00 – 14:30 **Anne Grapin-Botton,** *DanStem, University of Copenhagen, Denmark and Max Planck Institute of Molecular Cell Biology and Genetics, Germany*

PANCREAS ORGANOIDS TO DECONSTRUCT DEVELOPMENTAL MECHANISMS

14:30 – 14:45 **Aparna Bhaduri,** *University of California, San Francisco, USA*

EVALUATING THE FIDELITY OF HUMAN CEREBRAL ORGANOIDS TO DEVELOPING HUMAN CORTEX

14:45 – 15:00 **Nicole Repina,** *University of California, Berkeley, USA*

OPTOGENETIC CONTROL OF CANONICAL WNT SIGNALING, DIFFERENTIATION, AND EARLY EMBRYOGENESIS IN HUMAN PLURIPOTENT STEM CELLS

15:00 – 15:30 **James Wells,** *Cincinnati Children's Hospital, USA*

PLURIPOTENT STEM CELL-DERIVED GASTROINTESTINAL ORGANOIDS AS NEW MODELS TO STUDY HUMAN DEVELOPMENT AND DISEASE

15:30 – 16:00 REFRESHMENT BREAK

Wednesday, 20 February

16:00 – 17:00 RECAPITULATING DEVELOPMENTAL PROCESSES II

Chair: Helen Blau, *Stanford University, USA*

16:00 – 16:15 **Nicole Prior,** *University of Cambridge, UK*

LGR5+ STEM/PROGENITOR CELLS RESIDE AT THE APEX OF AN EMBRYONIC HEPATOBLAST POOL

16:15 – 16:30 **Killian Hurley,** *Royal College of Surgeons in Ireland, Ireland*

SINGLE-CELL TRANSCRIPTOMIC PROFILING OF TYPE 2 ALVEOLAR EPITHELIAL CELL DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS: AN EMERGING TOOL TO MODEL INTERSTITIAL LUNG DISEASES

16:30 – 17:00 **Paola Arlotta,** *Harvard University, USA*

UNDERSTANDING BRAIN DEVELOPMENT AND DISEASE: FROM THE EMBRYO TO BRAIN ORGANOIDS

17:30 – 20:00 WELCOME RECEPTION

Sponsored by Thermo Fisher Scientific

Location: Pompstation, Zeeburgerdijk 52, 1094 AE Amsterdam

Thursday, 21 February

09:00 – 10:30 ORGANOIDS AND TISSUE REGENERATION I

Chair: James Wells, *Cincinnati Children's Hospital, USA*

09:00 – 09:30 **Meritxell Huch,** *University of Cambridge, UK*

ADULT LIVER CHOLANGIOCYTES, ORGANOID CULTURES, AND TISSUE REGENERATION

09:30 – 09:45 **Karl Koehler,** *Indiana University School of Medicine, USA*

GENERATION OF HAIR-BEARING SKIN ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

09:45 – 10:00 **Marije Koning,** *Leiden University Medical Centre, Netherlands*

EFFICIENT VASCULARIZATION AND MATURATION OF HUMAN IPSC-DERIVED KIDNEY ORGANOIDS UPON TRANSPLANTATION IN THE COELOMIC CAVITY OF CHICKEN EMBRYOS

10:00 – 10:30 **Botond Roska,** *Institute of Molecular and Clinical Ophthalmology, Switzerland*

TITLE TBA

10:30 – 11:00 REFRESHMENT BREAK

11:00 – 12:00 ORGANOIDS AND TISSUE REGENERATION II

Chair: Calvin Kuo, *Stanford University, USA*

11:00 – 11:15 **Marta Roccio,** *University of Bern, Switzerland*

IN VITRO EXPANSION OF EPCAM+/CD271+ HUMAN FETAL COCHLEAR DUCT CELLS AND DIFFERENTIATION INTO FUNCTIONAL HAIR CELLS IN 3D ORGANOIDS

11:15 – 11:30 **Weng Chuan Peng,** *Stanford University, USA*

TISSUE REGENERATION SIGNALS FOR THE IN VITRO CULTURE OF MOUSE PRIMARY HEPATOCYTES

11:30 – 11:45 **Todd McDevitt,** *Gladstone Institutes, USA*

MEDULLARY HINDBRAIN ORGANOID DEVELOPMENT FROM HUMAN PLURIPOTENT STEM CELLS

11:45 – 12:00 **Andras Nagy,** *Lunenfeld-Tanenbaum Research Institute - Sinai Health System, Canada*

TREATING DISEASE WITH ORGANOIDS REQUIRES SOLUTIONS FOR TWO MAJOR HURDLES

12:00 – 14:00 LUNCH AND POSTER VIEWING

Thursday, 21 February

12:45 – 13:45 MEET THE EDITORS OF *STEM CELL REPORTS*

Location: Meet at the front of the auditorium.

14:00 – 15:30 MODELING DISEASE I

Chair: Eduard Batlle, *Institute for Research in Biomedicine, Spain*

14:00 – 14:30 **Melissa Little,** *Murdoch Children's Research Institute, Australia*
ADVANCING THE APPLICATIONS OF KIDNEY ORGANOIDS FOR DISEASE MODELLING

14:30 – 14:45 **Oded Kopper,** *The Hebrew University of Jerusalem, Israel*
HUMAN DERIVED ORGANOID PLATFORM FOR OVARIAN CANCER CAPTURE INTRA- AND INTER PATIENT HETEROGENEITY

14:45 – 15:00 **Jerome Mertens,** *The Salk Institute for Biological Studies, USA*
AGE-EQUIVALENT AND ADULT-LIKE INDUCED NEURONS REVEAL DE-DIFFERENTIATED NEURONAL STATE IN ALZHEIMER'S DISEASE

15:00 – 15:30 **Toshiro Sato,** *Keio University, Japan*
MODELING OF GASTROINTESTINAL DISEASE USING ORGANOIDS

15:30 – 16:00 REFRESHMENT BREAK

16:00 – 17:00 MODELING DISEASE II

Chair: Paola Arlotta, *Harvard University, USA*

16:00 – 16:15 **Hugo Snippert,** *University Medical Center Utrecht, Netherlands*
SINGLE-CELL DRUG RESPONSE IN PATIENT-DERIVED TUMOR ORGANOIDS

16:15 – 16:30 **Alexander Rialdi,** *Icahn School of Medicine at Mount Sinai, USA*
A PHARMACOGENOMIC APPROACH USING PRECISION MODELS OF HCC TO IDENTIFY NOVEL THERAPEUTICS

16:30 – 17:00 **Arnold Kriegstein,** *University of California, San Francisco, USA*
CEREBRAL ORGANOIDS AS MODELS OF HUMAN-SPECIFIC BRAIN DEVELOPMENT, EVOLUTION, AND DISEASE

17:00 – 18:30 NETWORKING RECEPTION

Location: KIT Royal Tropical Institute, Marble Hall (Auditorium Foyer)

Friday, 22 February

09:00 -10:30 MODELING CELL INTERACTIONS

Chair: Meritxell Huch, *University of Cambridge, UK*

09:00 – 09:30 **Shahin Rafii,** *Weill Cornell Medical, USA*

VASCULARIZATION OF TISSUE-SPECIFIC NORMAL AND MALIGNANT ORGANOIDS WITH ADAPTABLE ENDOTHELIAL CELLS

09:30 – 09:45 **Elisa Giacomelli,** *Leiden University Medical Center, Netherlands*

HUMAN IPSC-DERIVED CARDIAC FIBROBLASTS ENHANCE STRUCTURAL AND FUNCTIONAL CARDIOMYOCYTE MATURATION IN 3D MICROTISSUES

09:45 – 10:00 **Arti Ahluwalia,** *University of Pisa, Italy*

ALLOMETRIC SCALING OF ORGANOIDS

10:00 – 10:30 **Calvin Kuo,** *Stanford University, USA*

MODELING THE TUMOR IMMUNE MICROENVIRONMENT IN ORGANOIDS

10:30 – 11:00 REFRESHMENT BREAK

11:00 -12:30 TISSUE ENGINEERING

Chair: Shahin Rafii, *Weill Cornell Medical, USA*

11:00 – 11:30 **Matthias Lutolf,** *Ecole Polytechnique Federale de Lausanne, Switzerland*

ENGINEERING ORGANOID DEVELOPMENT

11:30 – 11:45 **Shukry Habib,** *King's College London, UK*

ENGINEERING A HUMAN OSTEOGENIC TISSUE MODEL

11:45 – 12:00 **James Hudson,** *QIMR Berghofer Medical Research Institute, Australia*

HIGH-CONTENT SCREENING IN HUMAN CARDIAC ORGANOIDS IDENTIFIES KEY PROLIFERATIVE PATHWAYS WITHOUT FUNCTIONAL SIDE-EFFECTS

12:00 – 12:30 **Helen Blau,** *Stanford University, USA*

REJUVENATING STEM CELL FUNCTION TO INCREASE MUSCLE STRENGTH

Friday, 22 February

12:30 – 14:00 LUNCH AND POSTER VIEWING

14:00 – 15:00 PANEL DISCUSSION: The Challenges, Limitations, and Promise of Organoids

Moderator: **Hans Clevers,** *Hubrecht Institute, Netherlands*

Panelists: **Arnold Kriegstein,** *University of California, San Francisco, USA*

Anne Grapin-Botton, *DanStem, University of Copenhagen, Denmark and Max Planck Institute of Molecular Cell Biology and Genetics, Germany*

Botond Roska, *Institute of Molecular and Clinical Ophthalmology, Switzerland*

Melissa Little, *Murdoch Children's Research Institute, Australia*

15:00 – 16:00 CLOSING KEYNOTE SESSION

15:00 – 15:45 **Juergen Knoblich,** *IMBA-Institute of Molecular Biotechnology, Austria*

CEREBRAL ORGANOIDS: MODELLING HUMAN BRAIN DEVELOPMENT AND TUMORIGENESIS IN STEM CELL DERIVED 3D CULTURE

15:45 – 16:00 **Eduard Battle,** *Institute for Research in Biomedicine, Spain*

CLOSING REMARKS

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Cell Symposia

Engineering Organoids and Organs

August 25–27, 2019 — Paradise Point, San Diego, USA

Abstract submission deadline:

May 3, 2019

Early registration deadline:

June 14, 2019

The goal of this Cell Symposium is to bring scientists studying organoids and organ engineering together with bioengineers to discuss the exciting opportunities and challenges for engineering complexity in higher-order organ-like systems and to foster collaborations and synergize efforts toward generating cellular platforms that can address a myriad of unmet needs.

Speakers

Hans Clevers, *The Netherlands*
Fred Gage, *USA*
Penney Gilbert, Canada
Tracy Grikscheit, *USA*
Juan Carlos Izpisua Belmonte, *USA*
Rudolf Jaenisch, *USA*
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Madeline Lancaster, *UK*
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www.brainxell.com

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www.chemometec.ocm

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Our primary focus is on cell counting and cell analysis, especially for use in life sciences research, clinical diagnostics and in production and quality control within the pharmaceutical industry.

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Speaker Abstracts

WEDNESDAY, 20 FEBRUARY 2019

OPENING KEYNOTE

STEM CELL-BASED ORGANOIDS AS AVATARS IN HUMAN DISEASE

Clevers, Hans C.

Hubrecht Institute, Utrecht, Netherlands

Stem cells are the foundation of all mammalian life. Stem cells build and maintain our bodies throughout life. Two types of stem cells are discerned. 1) Embryonic stem cells (ES cells) are briefly present in the early human or mouse embryo, a few days after fertilization. These ES cells can be grown indefinitely in the lab and have the potential to build each and every tissue in our body. Because of this 'pluripotency', ES cells hold great promise for therapeutic application in the field of regenerative medicine. However, derivation of ES cells leads to the destruction of the (mouse/human) embryo. This has caused intense debates from ethical, religious and logistical perspectives. A recent development circumvents the destruction of embryos. It is now possible to take skin cells (or other cells) from adults and convert these in the lab into cells with ES properties, so called iPS cells. Many of the hurdles that ES cell technology have faced, do not exist for iPS cells. 2) Adult stem cells. Every organ in our body is believed to harbor its own dedicated stem cells. These adult stem cells replace tissue that is lost due to wear and tear, trauma and disease. Adult stem cells are highly specialized and can only produce the tissue in which they reside; they are 'multipotent'. Examples are bone marrow stem cells that make all blood cells, skin stem cells and gut stem cells. Even the brain is now known to harbor its specialized stem cells. The adult stem cells allow us to live 80-90 years, but this comes at a cost: they are the cells that most easily transform into cancer cells. We have identified a gene (lgr5) that marks a series of known and novel adult stem cells, in organs such as the gut, the liver, the lung and the pancreas. We have learned to grow these stem cells in a dish into mini-versions of the human organs from which they derive. This so called organoid technology opens a range of avenues for the study of development, physiology and disease, and for personalized medicine. In the long run, cultured mini-organs may replace transplant organs from donors and hold promise in gene therapy.

Keywords: Adult/tissue stem cell; Lgr5; Organoid

RECAPITULATING DEVELOPMENTAL PROCESSES I

PANCREAS ORGANOIDS TO DECONSTRUCT DEVELOPMENTAL MECHANISMS

Grapin-Botton, Anne[1,2]

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To understand pancreas development, as a complement to in vivo investigations, we designed simplified in vitro systems that can be monitored and manipulated better than the whole embryo. We established 3D culture conditions that enable the efficient expansion, differentiation and morphogenesis of dissociated mouse embryonic pancreatic progenitors. We will discuss how we used these systems to understand the mechanisms of differentiation of acinar and endocrine cells and the formation of the ductal network that transports digestive enzymes. Focusing on the initial conditions leading to these organoids, we observed that the organoids formed if enough cells were clustered and identified a cooperative community effect. Assembling defined numbers of Notch active and inactive cells shows that their interaction is needed to initiate organoid formation and fuel growth. We will also discuss how the culture medium can be used to control the differentiation trajectory of cells towards acinar or endocrine cells and the associated morphogenetic changes. Developing the model to study human development and model disease, we will present recent data showing the robust expansion, differentiation and morphogenesis of human pancreatic organoids derived from embryonic stem cells and their similarity to human fetal pancreas.

Keywords: pancreas; fetus; morphogenesis

EVALUATING THE FIDELITY OF HUMAN CEREBRAL ORGANOIDS TO DEVELOPING HUMAN CORTEX

Bhaduri, Aparna[1], Andrews, Madeline G.[1], Shin, David[2], Allen, Denise[2], Nowakowski, Tomasz[2]

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Cerebral organoids are an exciting in vitro model that enable otherwise inaccessible long term vital culture and experimental manipulation of human brain development. To evaluate the fidelity of cerebral organoids to primary developing human cortex, we performed single-cell RNA sequencing of 200,000 cortical organoid cells across developmental stages generated with a range of protocols, from undirected signaling to strongly directed forebrain protocols. Using this dataset, as well as published sequencing datasets from cortical organoids, we compared cell type identity and molecular signatures of the organoid model to primary developing human cortex single-cell RNA sequencing data obtained in our laboratory as part of the NIH BRAIN Initiative. Molecular trajectories indicate that while cerebral organoids effectively recapitulate neuronal differentiation programs that characterize the transition from radial glia progenitor cells to neuronal identity, the precise

specification of radial glia and neuronal subtypes observed in normal human development is obscured in the organoid model. Comparisons of molecular maturation states between organoids and primary samples indicate that the cortical organoid models mature substantially faster than primary developing cortical counterparts, and cortical organoid progenitor cells exist in a much smaller physical space compared to the span in primary tissue between the ventricular zone and cortical plate. Interestingly, area-specific neuronal signatures are a hallmark of primary human newborn neurons, and in some cases the organoid newborn neurons recapitulate these identities, but in most cases, they express no previously characterized area-specific neuronal transcriptomes. Across all organoid datasets explored, we find a significantly higher expression of markers of glycolysis and endoplasmic reticulum stress in cortical organoids compared to primary developing cortex that we subsequently validate with immunohistochemistry. Together, these findings highlight that although there are important benefits to in vitro cerebral organoid models, the differences between organoids and primary tissue should be accounted for when designing and interpreting experiments.

Funding Source
NIH awards F32NS103266 to A.B. and U01MH114825 and R35NS097305 and California Institute of Regeneration Medicine GC1R-06673-A to A.R.K.

Keywords: Cortical organoids; Single-cell RNA sequencing; Developmental trajectories

OPTOGENETIC CONTROL OF CANONICAL WNT SIGNALING, DIFFERENTIATION, AND EARLY EMBRYOGENESIS IN HUMAN PLURIPOTENT STEM CELLS

Repina, Nicole A.[1], Bao, Xiaoping[2], Schaffer, David[1]

[1]*Bioengineering, University of California, Berkeley, CA, USA,* [2]*Chemical Engineering, University of California, Berkeley, CA, USA*

Embryonic development is governed by dynamic, spatially and temporally varying signals that guide stem cell differentiation, migration, and tissue morphogenesis to ultimately create the adult organism. However, traditional genetic approaches and current in vitro methods do not allow for precise, dynamic, spatiotemporal control of a signaling pathway and are thus insufficient to readily study how the dynamics of a signal impacts cell and tissue development. Fortunately, unlike soluble proteins, small molecule agonists, or protein-coated beads, optogenetics offers the opportunity to control the location, timing, and intensity of a signal. We present an optogenetic system, named optoWnt, to control the Wnt/B-catenin signaling pathway in human embryonic stem cells (hESCs) and mimic signaling patterns of early embryonic development. Using newly designed illumination devices, we activate the canonical Wnt pathway in hESC lines engineered with a CRISPR knock-in of a Wnt co-receptor fusion with A. thaliana photoreceptor Cryptochrome 2. Wnt pathway activation is induced in a light dose-dependent manner, has a high dynamic range (~1000-fold induction), and displays no detectable dark-state activity or phototoxicity effects. Optogenetic stimulation induces hESC differentiation into mesendoderm, as indicated by fate marker expression such as Brachyury, gain of epithelial-to-mesenchymal transition markers, and loss of pluripotency. To mimic the spatial signaling gradients present in the early mammalian embryo, we use patterning of illumination to spatially localize mesendoderm differentiation and find that differentiating cells adopt a migratory phenotype, migrating beyond the regions of illumination, and assemble into defined structures. Furthermore, mixed co-cultures of wild-type and optoWnt hESCs self-segregate upon illumination, creating sharp boundaries between the two cell populations in a process accompanied by changes in cell adhesion protein expression. Thus, our optogenetic platform and illumination device are a robust system for activating Wnt signaling in hESCs and can be used for studying how spatial and temporal signaling patterns affect gastrulation, primitive streak formation, and tissue self-assembly in early human development.

Keywords: Optogenetics; Gastrulation; Wnt signaling

PLURIPOTENT STEM CELL-DERIVED GASTROINTESTINAL ORGANOIDS AS NEW MODELS TO STUDY HUMAN DEVELOPMENT AND DISEASE

Wells, James M.

Cincinnati Children's Hospital, Cincinnati, OH, USA

Successful efforts to direct the differentiation of human embryonic and induced pluripotent stem cells (PSCs) into specific organ cell types in vitro have largely been guided by studies of embryonic organ development. We have used principles of organogenesis to generate complex, three-dimensional human gastrointestinal organ tissues from PSCs in vitro. We have done this by focusing on the signaling pathways that drive anterior-posterior and dorsal-ventral patterning of the developing endoderm. We can now generate organoids representing all of the organs of the gastrointestinal tract including esophagus, gastric fundus, gastric antrum, small intestine and colon. GI organoids contain complex epithelial structures and diverse cell types that are unique to their representative organ; esophageal organoids develop a stratified squamous epithelium, gastric organoids have a glandular epithelium that secrete digestive enzymes, hormones, and acid, and intestinal organoids additionally absorb nutrients. While the first generation of GI organoids had epithelium and mesenchyme, they were lacking important cell types and functions. We have now engineered additional cellular complexity into organoids, such as small intestinal organoids with a functional enteric nervous and colonic organoids with functional immune cells capable of triggering an inflammatory cascade in response to pathogenic bacteria. Ongoing studies include PSC-derived organoids to identify the underlying mechanisms behind birth defects including Hirschsprung's disease and esophageal atresia, to identify new pathologies in patients with complex GI diseases. Lastly we are using human organoids to investigate the how the GI endocrine system modulates a broad array of metabolic functions including nutrient sensing and absorption.

Funding Source
P01HD093363, R01DK098350, R01DK092456, U01DK103117, U19 AI116491.

Keywords: Human Organoids, pluripotent stem cells, stomach, esophagus, pancreas, intestine, colon, diabetes, digestive disease, embryonic development

Speaker Abstracts

RECAPITULATING DEVELOPMENTAL PROCESSES II

LGR5+ STEM/PROGENITOR CELLS RESIDE AT THE APEX OF AN EMBRYONIC HEPATOBLAST POOL

Prior, Nicole[1], Hindley, Christopher[1], Rost, Fabian[2], Meléndez, Elena[1], Lau, Winnie [3], Göttgens, Bertie[3], Rulands, Steffen[2], Simons, Benjamin[1], Huch, Meritxell[1]

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During mouse embryogenesis, progenitors within the liver bud known as hepatoblasts give rise to the adult liver epithelial hepatocyte and cholangiocyte cells. Contrary to previous reports, we show that the hepatoblast pool is functionally heterogeneous and that a sub-population of E9.5 hepatoblasts exhibit a previously unidentified early commitment to cholangiocyte fate. Importantly, we also identify a sub-population of hepatoblasts which at E9.5 express the adult stem cell marker LGR5 and contribute to liver development by generating both hepatocyte and cholangiocyte progeny that persist for the life-span of the mouse. Using a combination of lineage tracing and single cell RNA sequencing, we show that LGR5 marks bi-potent liver progenitors residing at the apex of a hierarchy of the hepatoblast population. Notably, isolated LGR5+ hepatoblasts can be clonally expanded in vitro into embryonic liver organoids, which can commit to hepatocyte or cholangiocyte fates dependent upon the culture conditions. Our study represents the first functional demonstration of heterogeneity within E9.5 liver progenitors and identifies LGR5 as a marker for a sub-population of truly bi-potent hepatoblasts which contribute to the postnatal liver.

Keywords: Liver stem/progenitor cells; LGR5; Hepatoblast

SINGLE-CELL TRANSCRIPTOMIC PROFILING OF TYPE 2 ALVEOLAR EPITHELIAL CELL DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS: AN EMERGING TOOL TO MODEL INTERSTITIAL LUNG DISEASES

Hurley, Killian J.[1], Jacob, Anjali[2], Ding, Jun[3], Herriges, Michael J.[2], Lin, Chieh[3], Hawkins, Finn[2], Vedaie, Marally[2], Villacorta-Martin, Carlos[2], Bar-Joseph, Ziv[3], Kotton, Darrell[2]

[1]*Department of Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland,* [2]*Center of Regenerative Medicine, Boston University and Boston Medical Center, Boston, MA, USA,* [3]*Computational Biology Department, School of Computer Science, Carnegie Mellon University, Pittsburgh, PA, USA*

Type II alveolar epithelial cells (AEC2) play key roles in the response of lung epithelial cells to injury and the pathogenesis of interstitial lung diseases (ILD). Mutations affecting genes highly expressed in AEC2s, such as surfactant protein (SFTP) C, SFTPB, and ABCA3, result in neonatal respiratory distress or early-onset ILD. Access to human tissue to study AEC2s is difficult, therefore, generating a robust in vitro model of AEC2s from an alternative inexhaustible source could provide insights into disease pathogenesis. We have previously published a directed differentiation protocol to generate putative AEC2 from induced pluripotent stem cell (iPSC), however, we have further shown that the resulting cells are transcriptomically heterogenous. We therefore sought to identify at the single-cell level the developmental pathways involved in the generation of AEC2s. We targeted fluorescent reporters into the endogenous NKX2-1 and SFTPC loci of a human iPSC line. Using a directed differentiation approach, we generated lung progenitors followed by putative AEC2s in 3D 'alveolospheres'. Characterization of the development of putative AEC2 was carried out over a time course by single-cell RNA sequencing (scRNA-seq) and confirmed by bulk qPCR and flow cytometry. Machine learning analysis of the transcriptional profile was carried out using forced-directed layouts of k-nearest-neighbor visualization and continuous lineage trajectory analysis of transcription factors (TF)s. k-nearest neighbor analysis of the time series scRNA-seq reveals early transcriptional heterogeneity with WNT low dependent pathways leading to lung (NKX2-1 high) and WNT high leading to non-lung (NKX2-1 low) cell lineages. Putative AEC2 are found exclusively in the WNT low pathways and were enriched for surfactant processing genes, SFTPB, LPCAT1 and CLDN18. Reconstructing the regulatory networks of TFs reveals that early withdrawal of WNT agonist during differentiation improves the yield of SFTPC expressing cells and suggests important TFs in the differentiation of progenitors to AEC2. Our results provide key transcriptional profiles of engineered AEC2 at single-cell resolution, revealing fundamental mechanisms underpinning alveolar development and provide a guide for improving directed differentiation to AEC2.

Keywords: Induced Pluripotent Stem Cells; Single-cell RNA sequencing; Interstitial lung disease

UNDERSTANDING BRAIN DEVELOPMENT AND DISEASE: FROM THE EMBRYO TO BRAIN ORGANOIDS

Arlotta, Paola[1,2], Velasco, Silvia[1,2], Kedaigle, Amanda J.[1,2,3], Simmons, Sean K.[2,3], Nash, Allison[1,2], Rocha, Marina [1,2], Quadrato, Giorgia[1,4], Nguyen, Lan[3], Adiconis, Xian[2,3], Regev, Aviv[3,5], Levin, Joshua Z.[2,3]

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Much remains to be understood regarding the cellular and molecular principles that govern the development of the mammalian brain, and how these events are affected in

neurodevelopmental disease. I will present some of our work on the generation and long-term development of human brain organoids; their developmental trajectory, cellular diversity and neuronal network features. I will then discuss our most recent progress in developing human brain organoids that are capable of generating the large diversity of cell types found in the cerebral cortex while achieving unprecedented levels of organoid-to-organoid reproducibility in cellular composition and their extended developmental trajectories. Through this work, I will discuss the challenges and opportunities of modeling human brain development within 3D human brain organoids, and the promise that organoids offer to understand complex neurodevelopmental disease.

Keywords: Brain organoids; Neurodevelopment; Cerebral cortex

THURSDAY, 21 FEBRUARY 2019

ORGANOIDS AND TISSUE REGENERATION I

ADULT LIVER CHOLANGIOCYTES, ORGANOID CULTURES AND TISSUE REGENERATION

Huch, Meritxell

The Wellcome/CRUK Gurdon Institute, University of Cambridge, UK

The adult liver exhibits low physiological turnover, however excels for its extensive proliferative capacity and regeneration ability after damage. In conditions where hepatocyte proliferation is impaired, resident ductal cells de-differentiate and acquire a proliferative and progenitor-like state that allows the tissue to regenerate both hepatocytes and ductal cells. In vivo, after tissue damage, proliferating ductal cells increase the expression levels of the stem-cell markers such as Lgr5, FoxL1 and Trop2. Similarly, in vitro, we will show that mouse and human ductal cells recapitulate this process when grown in 3D as liver organoid cultures. Thus, differentiated ductal cells cultured as liver Organoids acquire a bi-potential progenitor state that enables them to self-renew and differentiate into hepatocyte-like cells in vitro and in vivo, upon transplantation. However, the molecular mechanism behind the de-differentiation and acquisition of a proliferative state is largely unknown. Here I will discuss our recent findings on how the regulation of the epigenome is critical for organoid formation and tissue regeneration. We anticipate that epigenetic regulation to be a general mechanism by which adult cells exit dormancy as response to injury/regeneration.

Keywords: liver organoids ; epigenetic regulation; regeneration

GENERATION OF HAIR-BEARING SKIN ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

Koehler, Karl R.

Otolaryngology - Head and Neck Surgery, Indiana University School of Medicine, Indianapolis, IN, USA

Over one hundred million people worldwide suffer from injury or loss of skin due to burns, diseases, or genetic defects. Skin is essential for protecting body by regulating fluid retention and temperature, guarding against external stresses, and mediating touch and pain sensation. Human skin develops from coordinated interactions between multiple cell lineages and is vulnerable and difficult to be reconstructed once damaged. Despite repeated attempts for decades, however, a method of reproducing the full cellular diversity of skin in tissue cultures or in bioengineered skin equivalents has been elusive. Here we report a skin organoid culture system that generates complex skin from human pluripotent stem cells and recapitulate key features of skin development. We found that skin organoids are composed of stratified epidermis, fat-rich dermis, dermal condensate, pigmented hair follicles equipped with sebaceous glands and bulge stem cells, and sensory neuronal cells forming synapses with Merkel cells in organoid hair follicles, mimicking human touch circuitry. Furthermore, the skin organoids are comparable to human fetal facial skin and capable of reconstituting hairy skin in a xenograft mouse model. Together, our results demonstrate that the skin organoids produced in our culture system are the most functional and fully equipped human skin tissue that can be generated in vitro, to date. We anticipate our study provides a foundation for using skin organoids in studying skin development, modeling skin disease processes, or supplying cell source for skin regeneration and transplantation.

Funding Source
Ralph W. and Grace M. Showalter Trust, the Indiana CTSI (core pilot grant UL1 TR001108), and the Indiana Center for Biomedical Innovation (technology enhancement grant).

Keywords: Skin Organoid; Human pluripotent stem cells; Hair follicle

EFFICIENT VASCULARIZATION AND MATURATION OF HUMAN IPSC-DERIVED KIDNEY ORGANOIDS UPON TRANSPLANTATION IN THE COELOMIC CAVITY OF CHICKEN EMBRYOS

Koning, Marije[1], Avramut, Cristina[2], Lievers, Ellen[1], Wiersma, Loes E.[1], Jaffredo, Thierry[3], van den Berg, Bernard M.[1], Howden, Sara E.[4], Little, Melissa H.[4], van den Berg, Cathelijne W.[1], Rabelink, Ton J.[1]

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Human induced pluripotent stem cell (hiPSC) derived kidney organoids closely resemble in vivo kidney tissue, offering unprecedented possibilities for modeling disease and

Speaker Abstracts

development. Despite recent advances in kidney organoid generation, currently available protocols still yield organoids that lack a functional vasculature. As kidneys are highly vascularized organs that depend on the presence of vascular endothelial cells and blood flow for their correct development and functionality, it is not surprising that these organoids remain immature. Recently, it was shown that transplantation of hiPSC-derived kidney organoids under the renal capsule of mice leads to functional vascularization and maturation of the organoids. However, this model is labor-intensive and time consuming, requiring 28 days for optimal maturation. Here, we describe a new model where we transplanted kidney organoids inside the coelomic cavity of early chicken embryos. We observed rapid vascularization by host vasculature as well as reorganization of organoid-derived human endothelial cells. Through intravascular injection of fluorescently-labeled lectins, we show functionality of and anastomosis between the chicken- and human-derived blood vessels. Upon 8 days of transplantation we did not observe misdirected differentiation to stromal tissue. Furthermore, unbiased wide field transmission electron microscopy analysis of these organoids demonstrated glomerular maturation with the formation of podocyte foot processes, slit diaphragms and a glomerular basement membrane. Tubular structures also showed signs of maturation, with the appearance of cell junctions and microvilli. These data demonstrate the suitability and accessibility of this model for studying vascularization and show that intracoelomic transplantation inside chicken embryos is an efficient method to rapidly vascularize and mature kidney organoids.

Keywords: Kidney organoid; Vascularization; Chicken embryo

Roska, Botond

Institute of Molecular and Clinical Ophthalmology, Switzerland

Abstract not available at time of printing.

ORGANOIDS AND TISSUE REGENERATION II

IN VITRO EXPANSION OF EPCAM+/CD271+ HUMAN FETAL COCHLEAR DUCT CELLS AND DIFFERENTIATION INTO FUNCTIONAL HAIR CELLS IN 3D ORGANOIDS

Roccio, Marta[1], Perny, Michael[1,2], Ealy, Megan[3,4], Widmer, Hans Ruedi[5], Heller, Stefan[3], Senn, Pascal[6]

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Sensory hair cells located in the organ of Corti are essential for cochlear mechanosensation. Their loss is irreversible in humans resulting in permanent hearing loss. The development of therapeutic interventions for hearing loss requires fundamental knowledge about similarities and potential differences between animal models and human inner development as well as the establishment of human cell based-assays. We have carried out a systematic analysis of the fetal human inner ear in a temporal window spanning from week 8 to week 12 post conception, when cochlear hair cells become specified. We analyzed gene and protein expression of the developing cochlear duct, the spiral ganglion and vestibular tissue. We have identified surface markers for the cochlear prosensory domain, namely EPCAM and CD271, that allow to purify postmitotic hair cell progenitors. When placed in culture in three-dimensional organoids these regained proliferative potential and at the same time maintained marker expression and features of the native tissue. We could further differentiate these tissue resident progenitors into hair cell-like cells in vitro, displaying expression of hair cell markers such as BRN3C and MYO7A, F-Actin and Espin positive hair bundles and showing active MET-channel transduction. The competence of the generated hair cells to uptake aminoglycosides antibiotics such as gentamycin provides now a platform to test ototoxicity and regeneration. While the expansion and differentiation yields are still not suitable for drug screening purposes, we have started to analyze approaches to optimize both based on “chemical reprogramming” of cochlear progenitors. These results provide a foundation for comparative studies with otic cells generated from human pluripotent stem cells and for establishing novel cell based platforms for drug validation.

Funding Source
EU-FP7 Health, grant number 603029.

Keywords: Inner Ear Organoids; Human Fetal Cochlea Development; Differentiation fetal hair cell progenitors

TISSUE REGENERATION SIGNALS FOR THE IN VITRO CULTURE OF MOUSE PRIMARY HEPATOCYTES

Peng, Weng Chuan[1], Grompe, Markus[3], Li, Bin[3], Nusse, Roel[2]

[1]*HHMI, Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Mountain View, CA, USA,* [2] *Developmental Biology, Stanford University, Stanford, CA, USA,* [3]*Oregon Stem Cell Center, Oregon Health & Science University, Portland, OR, USA*

In the healthy adult liver, most hepatocytes proliferate minimally. However, upon physical or chemical injury to the liver, hepatocytes proliferate extensively in vivo under the direction of multiple extracellular cues, including Wnt and pro-inflammatory signals. Currently, liver organoids can be generated readily in vitro from bile-duct epithelial cells, but not hepatocytes. Here we show that TNFa, an injury-induced

inflammatory cytokine, promotes the expansion of hepatocytes in 3D culture and enables serial passaging and long-term culture for almost a year, at the time of writing. Single-cell RNA sequencing reveals broad expression of hepatocyte markers. Upon the withdrawal of expansion signal Wnt and inflammatory signal TNFa, hepatocytes upregulate the expression of genes related to liver function. Strikingly, in vitro-expanded hepatocytes engrafted, and significantly repopulated, the injured livers of Fah-/- mice. We anticipate tissue repair signals can be harnessed to promote the expansion of otherwise hard-to-culture cell-types, with broad implications.

Funding Source
Howard Hughes Medical Institute (HHMI), California Institute for Regenerative Medicine (CIRM).

Keywords: Liver hepatocyte; Inflammatory Cytokine; Tissue repair/regeneration signals

MEDULLARY HINDBRAIN ORGANOID DEVELOPMENT FROM HUMAN PLURIPOTENT STEM CELLS

McDevitt, Todd[1], Butts, Jessica[1], Mihaly, Eszter[1], Joy, David[1], Lai, Michael[1], Yackle, Kevin[2]

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The hindbrain is the most conserved region of the CNS and controls critical autonomic functions, such as respiration. Specifically, excitatory interneurons (INs) of the pre-Bötzinger complex, a cluster of several thousand neurons in the ventral medulla, generate the pace of breathing. Organoids created from human pluripotent stem cells (PSCs) have been used to mimic various regions of the developing brain, but hindbrain development has lagged significantly behind fore- and midbrain models. We previously generated excitatory caudal INs from PSCs using a combination of neuroinductive morphogenic signals. Subsequent analysis of HOX gene expression along with single cell RNA-Seq revealed that the neuronal population consisted predominantly of 3 neurons normally found in the hindbrain: PHOX2B/PHOX2A+ (chemosensitive), LHX5/PAX2+ (V0 IN), and CHX10/SOX14+ (V2a IN). Based on these findings, 3D aggregates of iPSCs (WTC line; 104 cells/aggregate) were differentiated using a similar protocol in suspension culture for 1 year. Multiple neuroepithelial cystic structures formed within individual aggregates and doubled in size during the first 2 weeks of differentiation. By day 17, coincident emergence of V0, V2a and chemosensing neurons was observed in spatially distinct patterns. The relative proportion of V0:V2a INs was controlled by varying the concentration of purmorphamine (pur), a Shh agonist, analogous to the ventral-to-dorsal SHH gradient in the developing neural tube. Organoids cultured for 100 days exhibited increased neuronal maturation based on NeuN, Tau, VGlut2, and synaptophysin expression. Although glial cells (GFAP+) were not readily detected until day 50, they increased in number thereafter. Starting at ~40 days, organoids from GCaMP6-expressing iPSCs displayed rhythmic Ca++ transients that rapidly dissipated within 2 minutes in normoxic conditions, potentially suggestive of C02 sensing. The patterns of Ca++ waves continued but became less periodic over time (after day 60) as the cellular composition and structure of the organoids changed. These results provide the first description of a human iPSC-derived hindbrain organoid that replicates elements of normal neuronal circuitry and physiologic function, and therefore could be used to model hindbrain development ex vivo.

Keywords: hindbrain; pre-Botzinger; interneurons

TREATING DISEASE WITH ORGANOIDS REQUIRES SOLUTIONS FOR TWO MAJOR HURDLES

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Organoids have proven to be powerful tools for studying organ development and disease processes. Organoids are also instrumental in evaluating disease treatments and they hold promise for the treatment of illness by transplantation into patients. The most obvious examples of such a promise are the pancreatic islet organoids faithfully responding to blood glucose levels with insulin secretion and the liver organoids that rescue liver failure. Here, we report solutions for the two major hurdles faced by transplant recipients, which are the graft safety and the long-term-tolerated allogeneic organoid products. We provide a definition and quantitation of safety by calculating the odds of accidentally generating potentially dangerous therapeutic cells and aim to ameliorate these odds through gene-editing. We introduced an inducible “kill-switch” into the genome thus making the unwanted proliferating cells sensitive to ablation while leaving quiescent cells resistant. The kill-switch was placed into the 3’UTR of a cell division-essential gene in a homozygous manner. This precise genome editing created a bicistronic gene encoding for two proteins; the division essential factor (i.e. Cdk1) and a negatively selectable marker (i.e. HSV-TK) and achieved a high level of protection of the kill-switch (Fail-SafeTM cell system). To obtain allogeneic tolerance, we identified eight immune modulatory cell surface or local acting genes. Transgenic over-expression of this set of genes was sufficient to achieve long-term induced allogeneic cell tolerance (iACT) without the need for systemic immune suppression. By combining the Fail-SafeTM and the iACT systems, we are in the position to provide a single pluripotent cell line to serve as a source for therapeutic cells and organoids for all humankind. *All authors contributed equally.

Funding Source
This work was funded by Canadian Institutes for Health Research, Medicine by Design, and The Foundation Fighting Blindness, Canada.

Keywords: Safe Cells; Allogeneic Cell Tolerance; Cell and Organoid Therapy

Speaker Abstracts

MODELING DISEASE I

ADVANCING THE APPLICATIONS OF KIDNEY ORGANOIDS FOR DISEASE MODELLING

Little, Melissa H.[1], Hale, Lorn[1,2], Forbes, Thomas[1], Howden, Sara[1], Er, Pei[1], Kynan, Lawlor[1], Khan, Shahnaz[1], Ghobrial, Irene[1], Combes, Alexander[1], Phipson, Belinda[1], Zappia, Luke[2], Oshlack, Alicia[2]

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The development of protocols for the differentiation of human pluripotent cells to complex multicellular organoids provides novel opportunities for stem cell medicine. We have developed a protocol for the generation of multicellular human kidney organoids from human pluripotent stem cells. The application of kidney organoids for disease modelling will require robustness, transferability between line, developmental accuracy, appropriate controls and an appropriate and tractable readout. Single cell RNAseq has facilitated the direct comparison between human fetal kidney and kidney organoids, revealing strong conservation of cell type. Comprehensive evaluation of transcriptional variation between differentiation experiments has identified technical variation as the major source of differences between organoids. CRISPR-Cas9 gene editing was then used to generate isogenic patient lines representative of heritable monogenic kidney disease. Using these lines to create kidney organoids, we are able to replicate tubular and glomerular disease phenotypes and demonstrate causative mutations. Together, this is moving us closer to the application of patient-derived kidney organoids for disease modelling.

Funding Source
National Institute of Health USA, National Health and Medical Research Council

Keywords: kidney organoid; disease modelling; pluripotent stem cell

HUMAN DERIVED ORGANOID PLATFORM FOR OVARIAN CANCER CAPTURE INTRA- AND INTER PATIENT HETEROGENEITY

Kopper, Oded[1], De Witte, Chris J.[2], Lõhmussaar, Kadi[3], Espejo Valle-Inclan, Jose[2], Hami, Nizar[2], Kester, Lennart[3], Balgobind, Anjali[3], Korving, Jeroen[3], Proost, Natalie[4], Begthel, Harry[3], van Wijk, Lise M.5, Van De Ven, Marieke[4], Vrieling, Harry[5], Vreeswijk, Maaike P.G[5], Jonges, Trudy[6], Zweemer, Ronald P.[7], Snippert, Hugo[8], Kloosterman, Wigard P.[2]

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Ovarian cancer (OC) is a heterogeneous disease usually diagnosed at a late stage. Experimental in vitro models that faithfully capture the hallmarks and tumor heterogeneity of OC are limited and hard to establish. We present a novel protocol that enables efficient derivation and long-term expansion of OC organoids. Utilizing this protocol, we have established 56 organoid lines from 32 patients, representing the spectrum of ovarian neoplasms, including non-malignant borderline tumors, as well as mucinous, clear-cell, endometrioid, low- and high-grade serous carcinomas. OC organoids recapitulate histological and genomic features of the pertinent lesion from which they were derived, illustrating intra- and inter-patient heterogeneity, and can be genetically modified. We show that OC organoids can be used for drug screening assays and capture different tumor subtype responses to the gold standard platinum-based chemotherapy, including acquisition of chemoresistance in recurrent disease. Finally, OC organoids can be xenografted, recapitulating in vitro drug sensitivity. Taken together, this demonstrates their potential application for research and personalized medicine.

Keywords: Ovarian cancer; Organoids; Disease modeling

AGE-EQUIVALENT AND ADULT-LIKE INDUCED NEURONS REVEAL DE-DIFFERENTIATED NEURONAL STATE IN ALZHEIMER'S DISEASE

Mertens, Jerome[1], Herdy, Joseph[1], Böhnke, Lena[2], Traxler, Larissa[2], Schäfer, Simon[1], Galasko, Douglas[3]

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Sporadic forms of Alzheimer's Disease (AD) exclusively affect people at old age and represent the overwhelming majority of all AD cases, as genetically defined familial cases are the rare exception. Still, most research on AD has been performed on genetic causes and their directly related pathways, also because we were in lack of models that can reflect complex human genetics, physiology, and age in an appropriate human neuronal context. While patient-specific iPSC-based models represent an attractive solution, iPSC reprogramming results in cellular rejuvenation and thus yields phenotypically young neurons. By contrast, direct conversion of old patient fibroblasts into induced neurons (iNs) preserves endogenous signatures of aging. To control for the involvement of aging in human neuronal models for AD, we took advantage of combining both technologies and generated age-equivalent fibroblast-derived iNs, as well as rejuvenated iPSC-derived neurons from a large cohort of AD patients and controls. In addition to their rejuvenated state,

we found that iPSC neurons transcriptionally resemble prenatal developmental stages, while iNs reflect adult-like neuronal stages and show little correlation with the prenatal brain. Thus not surprisingly, only age-equivalent adult-like iNs, but not rejuvenated prenatal-like iPSC neurons, revealed a strong AD patient-specific transcriptome signature, which shows high concordance with previous human post-mortem AD studies, and highlights functional gene categories known to be involved in neurodegeneration. Based on AD patient-specific transcriptional, functional, and epigenetic changes, we found that AD iNs display a more de-differentiated neuronal state than control iNs, which might underlie many of the here and previously observed changes in AD. These data show that iNs represent a unique tool for studying age-related neurodegeneration, and support a view where a partially de-differentiated state of old cells might permit the loss of the specialized neuronal fitness in AD.

Funding Source
National Institute on Aging R01-AG056306-01 and K99-AG056679-01;H2020-MSCA-IF-2017 797205; Paul G. Allen Family Foundation; Shiley-Marcos Alzheimer's Disease Research Center at the University of California San Diego.

Keywords: Induced neurons (iNs) ; Alzheimer's Disease; Aging

MODELING OF GASTROINTESTINAL DISEASE USING ORGANOIDS

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The biological understanding of gastrointestinal cancer requires faithful disease modeling that recapitulates the disease heterogeneity and pathobiological traits of original cancers. We optimized stem cell niche factor medium for gastrointestinal tumor organoids and established over 100 patient-derived organoid lines from various tissue origins and histological subtypes including previously uncultured rare tumors. Tumor organoids reproduced the histopathological grade and differentiation capacity of parental tumors in vitro and upon xenografting. Integrated molecular and biological analyses of tumor organoids revealed variable degree of niche independency along with accumulating genetic mutations. In general, cancer organoids acquire niche independent growth capacity through corresponding signal mutations, however, we noted that some cancers do not follow this rule. For instance, pancreas cancers often gained Wnt-niche independency through epigenetic reprogramming such that they no longer required Wnt signal activation for their long-term self-renewal. In this session, we would like to show our recent progress of disease modeling using patient-derived cancer organoids and share our biological understanding how cancers acquire their niche independency.

Keywords: Intestinal stem cells; Colorectal cancer; Gastric cancer

MODELING DISEASE II

SINGLE-CELL DRUG RESPONSE IN PATIENT-DERIVED TUMOR ORGANOIDS

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Patient-derived tumor organoids recapitulate the histopathological features of native tumors, including patient specific responses towards therapies. Moreover, organoids develop cellular heterogeneities in respect to cell fate and signaling activities, providing a platform to study single cell drug responses towards targeted inhibitors in the context of population dynamics and therapy resistance. To understand functional heterogeneities of RAS signaling activity in colorectal cancers (CRC), we established quantitative single-cell activity measurements of the downstream effector ERK in patient-derived CRC organoids. Using improved sensors and calibration strategies, we can scale recorded profiles of all individual cells within an CRC organoid to the full physiological ERK activity range. We use patient-derived CRC organoids of variable genetic backgrounds to monitor heterogeneity between ERK activity profiles. Moreover, we CRISPR-engineered isogenic tumor organoids where we introduced common mutations of the RAS pathway to study mutation specific signaling characteristics. Intriguingly, we reveal markedly different profiles and drug-responses among individual cells within clonal organoids. Of note, we identify ERK re-activation, a signaling rewiring phenomenon that is generally hold responsible for therapy resistance, to occur only in a subset of tumor cells. Together, single-cell measurements of real-time RAS signaling activity in CRC organoids shows great potential to improve our understanding of cellular drug responses and the nature of resistant cells that give rise to cancer recurrence.

Funding Source
KWF; SU2C; ERC starting grant; HFSP young investigator grant.

Keywords: Organoids; Colorectal cancer; Real-time imaging

A PHARMACOGENOMIC APPROACH USING PRECISION MODELS OF HCC TO IDENTIFY NOVEL THERAPEUTICS

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Hepatocellular carcinoma (HCC) presents unique challenges for the identification of drug targets and therapeutics. The most prevalent mutations, amplifications, or transcriptional de-regulations are within tumor suppressors or yet-to-be drugged oncogenes. Only small number of kinase inhibitors (KIs) are approved for advanced HCC, which only extend life

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by ~3 months and cause a variety of side effects. Most importantly, there have been no attempts to stratify HCC patients for personalized medicine approaches. The aforementioned data highlight the urgent need for: (I) the identification of novel clinical compounds, and (II) the understanding of genetic and epigenetic drivers that impact drug efficacy on tumor cells. Herein, we describe a multidimensional approach to target validation and drug discovery that focuses on kinase networks within the context of sophisticated HCC murine models and tumor organoids. We have used chemical biology to generate libraries of KIs, which are built off known HCC-approved drugs thereby retaining drug-like properties and allowing us to optimize activity in multiple genetic systems. To do so, we have adopted a precision system to quickly induce autochthonous and mosaic liver tumors that are driven by genetic alterations observed in the human population. Tumor organoids are derived from each model to be used for drug screening and for the transcriptomic and epigenomic understanding of drug response mechanisms. This pharmacogenomic approach has been scaled to five common genetic alterations in HCC: CTNNB1 activation, TP53 loss, TERT amplification, PTEN loss, and KMT2C loss. Our data suggests that individual driver alterations establish unique chromatin landscapes, which in turn, drive transcriptomic modulation of targetable kinomes. Our KI library screening approach has revealed a number of chemicals that are active across all models, but more importantly, chemicals that act in a genotype-specific manner. To the best of our knowledge, we are the first to demonstrate a scalable organoid platform for chemical genetic screening of HCC therapeutics. We believe that informed modifications to KIs will lead to stratified and more effective therapeutics for HCC.

Keywords: Hepatocellular carcinoma; Pharmacogenomics; Drug discovery

CEREBRAL ORGANOIDS AS MODELS OF HUMAN-SPECIFIC BRAIN DEVELOPMENT, EVOLUTION, AND DISEASE

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The human cerebral cortex has the largest number of neurons among living mammals. The features of cortical development that underlie the evolutionary expansion of the human cortex as well as susceptibility to disease are largely unexplored. We have identified outer radial glia (oRG) as a neural stem cell population that appear to generate a significant number of human cortical neurons, and have sequenced mRNA from single human progenitor cells and young neurons for unbiased classification of cell identity and for detection of activated signaling pathways. oRG cells are enriched in genes related to extracellular matrix production, epithelial-mesenchymal transition, and stem cell maintenance, suggesting mechanisms by which human oRG cells actively maintain their neural stem cell niche. Additionally, our genomic data has informed a novel model of primate corticogenesis, suggested a relationship between oRG cells and brain tumors and provided insights into the specific cell types affected by genetic forms of lissencephaly. Our census of cell types and lineages along with their molecular and physiological properties also supports comparisons of the same cell subtypes generated from pluripotent stem cells in in vitro models of development. To identify human-specific features of cortical development and disease, we leveraged recent innovations that permit generating pluripotent stem cell-derived cerebral organoids from human and non-human primates. We systematically evaluated the fidelity of organoid models to primary human and macaque cortex, finding organoid models preserve gene regulatory networks related to cell types and developmental processes but exhibit increased metabolic stress. Our molecular insights also show that human oRG cells are dependent upon mTOR signaling, and are thus likely to be targeted by disease causing mutations of this signaling pathway, as for example, in autism.

Funding Source
Supported by grants from the NINDS, the NIMH, and the California Institute of Regenerative Medicine.

Keywords: organoid; cortex; mTOR

FRIDAY, 22 FEBRUARY 2019

MODELING CELL INTERACTIONS

VASCULARIZATION OF TISSUE-SPECIFIC NORMAL AND MALIGNANT ORGANOIDS WITH ADAPTABLE ENDOTHELIAL CELLS

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Normal organoids and tumoroids are ideal biological replica of the organ-specific tissues. Nonetheless, most of the organoid cultures are devoid of interactive niches, which has diminished the physiological and translational relevance of this technology. Tissue-specific vascular endothelial cells (VECs) represent essential niche cells, that by supplying key paracrine angiocrine signals regulate the metabolic, morphogenic and patterning during organ regeneration and tumorigenesis. However, current sources of adult VECs fail to arborize, adapt and interact with the various organoids in three-dimensional (3D) co-cultures. Here, we show that transient expression of the embryonic-restricted ETS variant 2-transcription factor (ETV2) along with defined extracellular matrix, "Reset" adult human mature endothelial cells into adaptable VECs (R-VECs) capable of remodeling into long-lasting compliant tissue-specific vessels. In defined Laminin, Entactin, CollagenIV (L.E.C) extracellular matrix, without the constraints of artificial scaffolds or enforced perfusion, R-VECs acquire the molecular and cellular attributes

of interactive and malleable VECs. Upon seeding into defined L.E.C matrix, R-VECs undergo harmonized tubulogenesis and self-assemble into lumenized vascular network. In 3D co-cultures, R-VECs avidly arborize and co-opt tissue-specific organoids and tumoroids supporting *in vivo* and *in vitro* organogenesis and tumorigenesis. R-VECs serve as an adaptive vascular niche cells that by deploying key angiocrine factors accelerate intestinal, islet, and glomerular epithelial organoid expansion and patterning. By contrast, R-VECs maladapt to various tumor organoids forming abnormal vessels supporting aberrant tumor cell growth and invasive behaviour. Deciphering the cross-talk among R-VECs and co-opted organoid cells could facilitate identification of factors that determine tissue/tumor-specific EC heterogeneity, enable physiological chemical screens and set the stage for functional organ regeneration and tumor targeting.

Keywords: Adaptable endothelium, Organoid vascularization, Tumuroid vascularization

HUMAN IPSC-DERIVED CARDIAC FIBROBLASTS ENHANCE STRUCTURAL AND FUNCTIONAL CARDIOMYOCYTE MATURATION IN 3D MICRO-TISSUES

Giacomelli, Elisa[1], Meraviglia, Viviana[1], Campostrini, Giulia[1], Garcia, Ana Krotenberg[1], Slieker, Roderick[2], van Helden, Ruben WJ[1], Jost, Carolina[2], Mulder, Aat[2], Mei, Hailiang[3], Koster, Abraham AJ[2], Tertoolen, Leon GJ[1], Orlova, Valeria[1], Bellin, Milena[1]

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) in vitro are structurally and functionally immature, unless incorporated into engineered tissues or forced to undergo cyclic contraction, thus limiting their use in many potential applications. Previously, we described that scaffold-free, miniaturized three-dimensional (3D) cardiac microtissues (MT-CMECs), containing hiPSC-CMs and hiPSC-derived cardiac endothelial cells, exhibited some features of maturation. Here, we increased microtissue complexity through inclusion of cardiac fibroblasts derived from hiPSC-epicardium (MT-CMECFs) and we showed an enhancement of structural, electrical, mechanical and metabolic maturation in hiPSC-CMs compared to MT-CMECs. MT-CMECFs were larger and showed more homogeneous endothelial cell distribution compared to MT-CMECs. Direct coupling occurred between hiPSC-CMs and cardiac fibroblasts and intercellular paracrine signals appeared to contribute to hiPSC-CM maturation. hiPSC-CMs in MT-CMECFs showed enhanced sarcomere alignment, T-tubule formation, electrophysiology, contractility, and canonical responses to pharmacological regulators of inotropy compared to MT-CMEC. Primary adult cardiac- but not dermal fibroblasts could replace hiPSC-cardiac fibroblasts. Our results indicate that cardiac fibroblasts play crucial roles in enhancing CM maturation. Using just 5000 cells per microtissue we were thus able to induce structural, electrical, mechanical and metabolic maturation of hiPSC-CMs. In conclusion, this system represents a scalable and affordable platform to study cardiovascular development, disease and cardiotoxicity in vitro.

Funding Source
European Research Council; European Community's Seventh Framework Programme; European Union's Horizon 2020 research and innovation Programme; Netherlands Organ-on-Chip Initiative (NWO Gravitation project).

Keywords: Three-dimensional culture model; Cardiac microtissue; Cell-cell interactions

ALLOMETRIC SCALING OF ORGANOIDS

Ahluwalia, Arti, Magliaro, Chiara

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Despite their remarkable resemblance to downscaled mammalian organs, organoids are often reported to possess non-viable cores due to oxygen diffusion limits. To assess their potential as physiologically relevant in vitro models, we determined a working window in which quarter power allometric scaling as well as a minimum threshold oxygen concentration is maintained during organoid growth. Oxygen consumption and diffusion in organoids at different stages of growth were computed using three-dimensional finite element models. The results show that it is impossible to establish mature organoids free of necrotic cores using current protocols. However, micro-fluidic oxygen delivery methods can be designed to ensure that oxygen levels are above a minimum viable threshold throughout the constructs and that they follow physiological metabolic scaling laws. The results provide new insights into the significance of allometric scaling in systems not equipped with a resource- supplying network. They may also be used to guide the
experimental set up and design of more predictive and physiologically-relevant in vitro models, providing an
effective alternative to animals in research.

Keywords: Computational modelling; Allometric scaling; Oxygen

MODELING THE TUMOR IMMUNE MICROENVIRONMENT IN ORGANOIDS

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The tumor microenvironment contains diverse populations including fibroblasts, vasculature and immune cells, all of which critically regulate tumor progression. A major historical impediment to basic and translational cancer studies has been the lack of in vitro methods that allow tumor epithelium to be robustly cultured together with stromal components. This talk will describe advances in 3D tumor organoid culture that allow tumor cells to be grown in a manner that preserves their native interaction with diverse tumor-infiltrating immune cells and

Speaker Abstracts

recapitulates immune checkpoint inhibition. Such organoid modeling of the tumor immune microenvironment within human clinical cancer biopsies will facilitate basic and translational immunooncology studies, allow a more holistic in vitro modeling of cancer biology and potentially facilitate determination of personalized treatment responses.

Keywords: Organoids, Immunotherapy, checkpoint inhibition, PD-1

TISSUE ENGINEERING

ENGINEERING ORGANOID DEVELOPMENT

Lutolf, Matthias P.

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Organoids form through poorly understood morphogenetic processes in which initially homogeneous ensembles of stem cells spontaneously self-organize in suspension or within permissive three-dimensional extracellular matrices. Yet, the absence of virtually any predefined patterning influences such as morphogen gradients or mechanical cues results in an extensive heterogeneity. Moreover, the current mismatch in shape, size and lifespan between native organs and their in vitro counterparts hinders their even wider applicability. In this talk I will discuss some of our ongoing efforts in developing programmable organoids that are assembled by guiding cell-intrinsic self-patterning through tissue engineering.

Keywords: Organoid; Self-organization; Tissue engineering

ENGINEERING A HUMAN OSTEOGENIC TISSUE MODEL

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Tissue engineering focuses on the development of tissue and organ substitutes by generating a controlled 3D biological and biophysical environment. Stem cell-based therapies that integrate tissue-engineering technologies and biomaterials science are
a fundamental pillar of regenerative medicine. Wnt signalling regulates, with high spatial control, the function of skeletal stem cells (SSCs) and is essential for bone remodelling. We have recently pioneered a novel engineered system that recapitulates a human bone niche. Our system consists of an immobilised Wnt-platform that maintains primary human SSC population and directs the production of multilayers of increasingly differentiated cells with an osteogenic phenotype in an organised fashion within one week. We have characterised the mechanism of the formation of the Wnt induced osteogenic tissue model (WIOTM). Our findings indicate that the immobilised Wnt polarises the Wnt/beta-catenin machinery, controls the spindle orientation and induces asymmetric cell division of the SSCs in 3D. This implicates the role of spatially confined Wnt signals in the maintenance of stem cells and directed cell differentiation. Therefore, spatial presentation of Wnt signals to cells in a 3D context can be used for tissue engineering purposes. Our ultimate goal is to mimic basic cellular, signalling, and mechanical elements of the bone environment by generating a controlled microsystem of stem cells and a directed differentiation into oestrogenic cells in 3D culture.

Funding Source
Sir Henry Dale Fellowship and the UK Regenerative Medicine Platform (Medical Research council).

Keywords: Bone regeneration and tissue engineering; Asymmetric stem cell division, Wnt-bandage; Biomaterials

HIGH-CONTENT SCREENING IN HUMAN CARDIAC ORGANOIDS IDENTIFIES KEY PROLIFERATIVE PATHWAYS WITHOUT FUNCTIONAL SIDE-EFFECTS

Hudson, James

Cell and Molecular Biology, QIMR Berghofer Medical Research Institute, Brisbane, Australia

Human pluripotent stem cell-derived cardiomyocytes are emerging as a powerful platform for cardiovascular drug discovery and toxicology. However, standard 2D cultures are typically immature, which limits their capacity to predict human biology and disease mechanisms. To address this problem, we have recently developed a high-throughput bioengineered human cardiac organoid (hCO) platform, which provides functional contractile tissue with biological properties similar to native heart tissue including mature, cell cycle-arrested cardiomyocytes. Here, we take advantage of the screening capabilities of our mature hCO system to perform functional screening of 105 small molecules with pro-regenerative potential. Our findings reveal a surprising discordance between the number of pro-proliferative compounds identified in our mature hCO system compared with traditional 2D assays. In addition, functional analyses uncovered detrimental effects of many hit compounds on cardiac contractility and rhythm. By eliminating compounds that had detrimental effects on cardiac function, we identified two small molecules that were capable of inducing cardiomyocyte proliferation without any detrimental impacts on function. High-throughput proteomics on single cardiac organoids revealed the underlying mechanism driving proliferation, which involved synergistic activation of the mevalonate pathway and a cell cycle network. In vivo validation studies confirmed that the mevalonate pathway was shut down during postnatal
heart maturation in mice and statin-mediated inhibition of the pathway inhibited proliferation and heart growth during the neonatal window. This study highlights the utility of human cardiac organoids for pro-regenerative drug development including identification of underlying biological mechanisms and minimization of adverse side-effects.

Funding Source
National Health and Medical Research Council and The National Heart Foundation of Australia

Keywords: Cardiac Tissue Engineering; Regeneration; Drug Screening

REJUVENATING STEM CELL FUNCTION TO INCREASE MUSCLE STRENGTH

Blau, Helen

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Regenerative medicine holds great promise for local enhancement of skeletal muscle repair to treat muscular dystrophies and aging-associated muscle wasting. Muscle stem cells (MuSCs) are a potent population that resides within muscle tissues, poised to repair muscle damage throughout life. However, the therapeutic utility of MuSCs is currently limited by their rarity and their inefficient survival, self-renewal, and differentiation after injection into muscle tissue. We have devised bioengineering strategies and discovered novel molecular regulators to surmount these hurdles. By defining the myogenic stem cell progression by single cell mass cytometry (CyTOF), we can target metabolic functions that dictate cell fate transitions. By fabricating biomimetic hydrogels with differing elasticity matching muscle tissue,
we can overcome the loss of stem cells on traditional plastic cultureware. Fibrosis, which causes dysfunction and ultimate failure of numerous tissues with aging, is characterized by increased tissue stiffness. We are developing a dynamic material platform to enable mechanistic studies of cellular dysfunction as fibrosis progresses in real time. Cell autonomous defects in MuSC function accompany aging. By targeting these molecular pathways, we can rejuvenate stem cell function. As an alternative to cell therapy, we are seeking to stimulate the function of endogenous quiescent satellite stem cells within muscle tissues. Through an in silico screen, we identified a potent regulator that robustly augments stem cell function and may serve as a novel therapeutic agent to induce muscle regeneration and counter debilitating muscle wasting in the elderly.

Keywords: rejuvenation ; muscle stem cell; hydrogel microenvironment

CLOSING KEYNOTE

CEREBRAL ORGANOIDS: MODELLING HUMAN BRAIN DEVELOPMENT AND TUMORIGENESIS IN STEM CELL DERIVED 3D CULTURE

Knoblich, Juergen A.

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The human brain is unique in size and complexity, but also the source of some of the most devastating human diseases. While many of these disorders have been successfully studied in model organisms, recent experiments have emphasized unique features that cannot easily be modeled in animals. We have therefore developed a 3D organoid culture system derived from human pluripotent stem cells that recapitulates many aspects of human brain development. These cerebral organoids are capable of generating several brain regions including a well-organized cerebral cortex. Furthermore, human cerebral organoids display stem cell properties and progenitor zone organization that show characteristics specific to humans. We have used patient specific iPS cells to model microcephaly, a human neurodevelopmental disorder that has been difficult to recapitulate in mice. This approach reveals premature neuronal differentiation with loss of the microcephaly protein CDK5RAP2, a defect that could explain the disease phenotype. More recently, we have been able to generate organoid based models for human brain cancer and demonstrated their feasibility for drug testing. Our data demonstrate an in vitro approach that recapitulates development of even this most complex organ, which can be used to gain insights into disease mechanisms.

Keywords: Organoids; tumorigenesis; stem cells

Poster Abstracts

ODD NUMBERED POSTERS PRESENTED THURSDAY, 21 FEBRUARY FROM 13:00 TO 14:00.

EVEN NUMBERED POSTERS PRESENTED FRIDAY, 22 FEBRUARY FROM 13:00 TO 14:00.

ABSTRACTS ARE INCLUDED ON PAGES 31–106.

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GENERATION AND CHARACTERIZATION OF TASMANIAN DEVIL (SARCOPHILUS HARRISSI) INDUCED PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS

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Marsupials have long attracted scientific interest because of their unique biological features and their position in mammalian evolution. Mesenchymal stem cells (MSCs) have attracted considerable research interest in translational medicine due to their immunomodulatory, anti-inflammatory, regenerative, migratory and homing properties. MSCs have been harvested from various tissues in numerous eutherian species; however, there are no descriptions of MSCs derived from a marsupial. In this study, we have generated Tasmanian devil (Sarcophilus harrisii) MSCs from devil induced pluripotent stem cells (iPSCs), thus providing an unlimited source of devil MSCs circumventing the need to harvest tissues from live animals. Devil iPSCs were differentiated into MSCs (iMSCs) through both embryoid body formation assays (EB-iMSCs) and via inhibition of the transforming growth factor beta (TGF-β)/activin signalling pathway (SB-iMSCs). Both EB-iMSCs and SB-iMSCs are highly proliferative, express the specific MSC surface markers CD73, CD90 and CD105, in addition to the pluripotency factors OCT4, SOX2 and NANOG. Moreover, devil iMSCs readily differentiate along the adipogenic, osteogenic and chondrogenic pathways in vitro, confirming their trilineage differentiation potential. Importantly, while in vitro teratoma assays confirmed the pluripotency of devil iPSCs, iMSCs only form derivatives of the mesodermal germ layer. Therefore, devil iMSCs will be an indispensable tool for further studies on marsupial biology but, perhaps most significant, is their potential in the development of an effective MSCs based treatment strategy against Devil Facial Tumour Disease (DFTD) which is threatening Tasmanian devils with extinction.

Funding Source
This study was funded by a Morris Animal Foundation (USA) grant offered to Deanne J. Whitworth (Grant Number: D14ZO-080) and an Australian Government Research Training Program Scholarship offered to Prasanna Weeratunga.

Keywords: Mesenchymal stem cells; marsupial; Devil Facial Tumour Disease

003

INVESTIGATING THE HUMAN TEASHIRT GENE TSHZ3 IN PANCREATIC AND ENDOCRINE LINEAGE COMMITMENT USING GENOME MODIFIED HUMAN EMBRYONIC STEM CELLS (HESC)

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PDX1 (Pancreatic and Duodenal Homeobox 1) encodes an evolutionarily-conserved homeobox transcription factor expressed during the earliest stages of human pancreatic development. PDX1 transcripts label early pancreatic progenitor (ePP) cells that are multipotent, giving rise to all components of the adult organ. Strikingly, loss of PDX1 results in complete pancreatic agenesis in man and mice, a drastic phenotype that emphasizes PDX1's critical role in coordinating the morphogenesis of this indispensable organ. In the Dunn laboratory, a human embryonic stem cell (hESC) differentiation protocol was developed 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. This finding mirrors the human congenital disorder of pancreatic agenesis that results from the loss of the PDX1 gene. Unpublished microarray studies comparing wild-type and PDX1 knock-out hESC lines that are available in the laboratory reveal a list of differentially expressed genes, which are hypothesized to play key roles in orchestrating pancreatic development downstream of PDX1. Corresponding microarray studies of PDX1 null mutant hESC with PDX1 Chromatin Immunoprecipitation-Sequencing data revealed a novel list of candidate PDX1 transcriptional targets including TSHZ3, the human homolog of the Drosophila homeotic gene teashirt, which is involved in gut and limb development. The gene's functions are analyzed in detail for its effect on pancreatic differentiation through loss-of-function cell lines generated using CRISPR/Cas9 genome-engineering. Functionalizing the candidate gene will greatly expand our knowledge of human pancreatic development and impact our understanding of pancreatic homeostasis and disease. Our results provide insights into the importance of TSHZ3 in endocrine commitment and pancreatic differentiation, and interestingly, the gene's influence on neural development.

Keywords: Pancreatic differentiation; TEASHIRT; PDX1

004

BART-SEQ: COST-EFFECTIVE MASSIVELY PARALLEL TARGETED SEQUENCING FOR SINGLE CELL TRANSCRIPTOMICS

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The introduction of techniques for single cell transcriptomics by next-generation sequencing (NGS) has remarkably accelerated our understanding of stem cell regulation. Majority of these, however, suffer from shallow coverage because they are global (unbiased), thus require numerous reads to cover the gene repertoire. Targeted approaches can remove this bottleneck by lowering the number of analyzed genes (thus, increase the coverage), but current methods are not compatible with gene expression measurements of single cells. We developed the first highly sensitive and quantitative barcoding technique for enriching selected transcripts from thousands of samples/single cells cost-effectively, using common laboratory equipment and reagents, and multiplexing them for conventional NGS. The novel workflow, named Barcode Assembly foR Targeted Sequencing (BART-Seq), is based on a simple method for synthesizing differentially barcoded forward and reverse primers, which are assembled as matrices to generate sample-specific combinatorial amplicon labels. Moreover, we developed tools for designing the primers and barcodes, and decoding the barcoded NGS reads. We demonstrated the technique by analyzing the expression of selected pluripotency markers in human embryonic stem cells (hESCs) grown on different media (mTeSR, E8, and bFGF), which might account for the variations in multi-lineage differentiation capacities of the cells maintained with them. Furthermore, we have observed distinguishable subpopulations emerging from hESCs upon 72 hours of Wnt/b-catenin pathway activation using CHIR, ectopic expression of constitutively active b-catenin, or recombinant Wnt3a protein. Taken together, BART-Seq will complement low-sensitivity global single cell transcriptomics approaches for discovering the mechanisms of stem cell regulation, by cost-effective analysis of thousands of single cells across the dynamic range of gene expression.

Keywords: Targeted single cell RNA sequencing; Human pluripotent/embryoic stem cells; Barcoding

005

HDSPC-DERIVED CONDITIONED MEDIUM PLAYS A PROTECTIVE ROLE AGAINST UVA IRRADIATION IN NORMAL HUMAN EPIDERMAL KERATINOCYTES

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This study was performed to define the epidermal moisturizing effects of human dermal stem/progenitor cell-derived conditioned medium (hDSPC-CM) on normal human epidermal keratinocytes (NHEKs). To investigate epidermal moisturizing effect of hDSPC-CM on NHEKs, I measured superoxide scavenging activity, cell viability assay, gene expressions, and hyaluronic acid (HA)-ELISA assay. In this study, I could elucidate the effects of hDSPC-CM on AQP3, HAS2, KRT1, and KRT10 mRNA expressions and hyaluronic acid production. Quantitative Real-time RT-PCR presented that hDSPC-CM increased gene expression levels of HAS2, AQP3, KRT1, and KRT10 and HA-ELISA also revealed that hDSPC-CM increased HA production in NHEKs. I identified the epidermal moisturizing effects of hDSPC-CM, and this outpot showed that the hDSPC-CM can be a potent moisturizing cosmetic ingredient for epdiermal moisturizing. Based on this study, I anticipated further studies about the mechanisms of hDSPC-CM on epidermal keratinocytes to develop not only cosmetics but for healthcare medicines.

Funding Source
This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP; Ministry of Science, ICT & Future Planning) (No. 2017R1C1B5076217).

Keywords: hDSPC-CM, Moisturizing; HAS2; AQP3

006

OVOL1 INFLUENCES THE DETERMINATION AND EXPANSION OF IPSC REPROGRAMMING INTERMEDIATES

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During somatic cell reprogramming to induced pluripotent stem cells (iPSCs), fibroblasts undergo dynamic cellular and molecular changes. These changes include a mesenchymal-to-epithelial transition (MET) and gradual gain of gene expression regulating pluripotency; processes which are highly influenced by Yamanaka factor (Oct3/4, Sox2, Klf4, and c-Myc) stoichiometry. For example, in early reprogramming, high Klf4 levels are correlated with the induction of MET genes which sustain expression in iPSCs, as well as MET genes with transient expression, whose functions in reprogramming remain undefined. Here, we identified the cell surface protein TROP2 as a marker for cells with transient MET gene expression in the high-Klf4 condition. We observed the emergence of cells expressing the pluripotency marker SSEA-1+ mainly from within the TROP2+ fraction. Upon isolation by cell sorting, double-positive cells which subsequently lost TROP2 displayed a high reprogramming potential. Using TROP2 as a marker in CRISPR/Cas9-mediated candidate screening of MET genes, we identified the transcription factor Ovol1 as a potential regulator of an alternative epithelial cell fate characterized by the expression of non-iPSC MET genes and low cell proliferation. Our study sheds light on how reprogramming factor stoichiometry alters the spectrum of intermediate cell fates, ultimately influencing reprogramming outcomes.

Keywords: Mouse somatic cell reprogramming; Mesenchymal-to-epithelial transition; CRISPR/Cas9-mediated screening

Poster Abstracts

007

CHARACTERISING EMBRYONIC STEM CELL DERIVED TENOCYTES AND DETERMINING THE CHANGING ROLE OF SCLERAXIS DURING TENDON DEVELOPMENT

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Tendon injuries are common in human and equine athletes. Adult tendon injuries heal via the formation of biomechanically inferior scar tissue resulting in high re-injury rates. However, fetal tendon injuries undergo total scar-less regeneration. Novel cell therapies should therefore try to recapitulate this scar-less fetal tendon regeneration. This study aims to build upon research into the use of equine embryonic stem cells (ESCs) to aid tendon regeneration by determining if tenocytes derived from ESCs are more representative of the fetal or adult stage of tendon development. Using genome wide transcriptional analysis, we compared fetal, adult and ESC-derived tenocytes cultured and differentiated in a 3D collagen matrix. 2,260 genes were differentially expressed between adult and ESC-derived tenocytes, whereas 1,254 genes were differentially expressed between fetal and ESC-derived tenocytes (log fold change ±2; q-value <0.05). Genes which were significantly upregulated in adult tenocytes tended to be expressed at similar levels in fetal and ESC-tenocytes and were highly associated with immune system processes and the recognition of foreign external stimulus. Genes which were significantly upregulated in fetal tenocytes tended to be even further upregulated in ESC-derived tenocytes and were highly associated with cellular adhesions. To further study how adult and fetal tenocytes differ, scleraxis (SCX), an important transcription factor involved in tendon formation was knocked down in four lines of fetal and adult tenocytes. Quantitative PCR determined that SCX knock-down had differential effects in adult and fetal cells on the expression of several genes including matrix metalloproteinases and collagens. RNA-sequencing is currently being performed to provide more global information on the effects of SCX knock-down in fetal and adult tenocytes. In summary, our initial work towards global gene expression profiling in fetal and adult tenocytes has yielded useful information into key over-represented pathways and novel genes that are likely to play a role in regulating tendon development and healing. Future work will now delve into the developmental differences between ESC-derived, fetal and adult tenocytes.

Funding Source
Petplan Charitable Trust; BBSRC

Keywords: Equine; ESC-derived Tenocytes; Tendon-injury

008

AIPL1-/- END-STAGE RETINAL DEGENERATION MICE AS A MODEL FOR CELL TRANSPLANTATION

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Cell transplantation is a promising therapeutic approach to treat degenerative retinal diseases, the leading cause for blindness in the western world. Although hESC-derived photoreceptors have been used extensively for transplantation studies in various mouse models of retinal degeneration, further optimization is still required to achieve substantial improvement of visual function. We have previously shown that hESC-derived cone photoreceptors can survive following transplantation into Aipl1-/- mice. Here we sought to characterize in detail the degeneration and remodelling of the Aipl1-/- retina to optimize the transplantation of hESC-derived cone photoreceptors into this model. Advanced photoreceptor degeneration was already evident by post-natal stage 18 (P18) with a single row of rod and cone photoreceptors in the ONL. In the INL rod bipolar cells were displaced and their axon processes appeared retracted. Both horizontal and amacrine cells are reduced in number. Post-synaptic markers are absent in interneurons at 3 months of age. No difference in the number of ganglion cells was observed. Following transplantation, no significant improvement in visual function was detected by the methods used, but cell maturation and pre-synaptic proteins were identified in the transplanted hESC-derived cone photoreceptors. This data shows that Aipl1-/- retina suffers severe remodelling as it degenerates. hESC-derived cone photoreceptors survive and mature following transplantation. Our data suggests that by 3 months of age, the Aipl1-/- retina might have undergone extensive remodelling that prevents the establishment of functional connections with the transplanted cone photoreceptors. Further studies are required to establish the optimal conditions for achieving functional rescue.

Funding Source
MRC; erc; NIHR; Moorfields eye Charity; Fighting for Blindness

Keywords: Cell Transplantation; Human Stem cells; End-Degeneration retina

009

THE ENGINEERED ACTIVIN A PROVIDES PROLONGED AND SUSTAINABLE SIGNALLING ACTIVITY IN HUMAN STEM CELL CULTURING

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Activin A, a member of TGF-beta superfamily, is widely used in human stem cell culturing to maintain the cell pluripotency and induce differentiation. Its signalling activity also induces the cells to produce follistatin, a secreted antagonist of activin A, which binds to activin A and inhibits its signalling in a negative-feedback loop. This leads to gradual reduction of activin A bioactivity during the stem cell culturing. With the aim to develop an engineered form of activin A with more sustainable activities, we have designed a number of activin A mutants that preserve the wild-type signalling activity, but resist inhibition by follistatin. We have used activin A-responsive luciferase assay to confirm that these mutants have more sustained signalling activities during the culturing of human stem cells. These engineered forms of activin A could provide a more stable and sustainable signalling environment for stem cell culturing. Moreover, there are potentials to reduce the amount of the engineered activin A added into the culture due to their longer half-life times.

Funding Source
This work is supported by the Biotechnology and Biological Sciences Research Council (BBSRC) Follow-on funding.

Keywords: Human stem cell culturing; TGF-beta superfamily; Sustained activity

010

FUNDAMENTAL ROLE FOR HIF-1A IN INTESTINAL HOMEOSTASIS AND AUTOIMMUNE DISEASE

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HIF transcription factors (HIF-1a and HIF-2a) are central mediators of cellular adaptation to hypoxia. Because the resting partial pressure of oxygen is low in the intestinal lumen, epithelial cells are believed to be mildly hypoxic. Having recently established a link between HIF and mucosal inflammation and dysbiosis, we hypothesized that HIFs stabilized in the hypoxic intestinal epithelium, may also play critical roles in regulating intestinal homeostasis. To explore this idea, we generated small intestine organoids and cultivated them under normoxia (21% O2), or Hypoxia (1% O2) conditions. As expected, hypoxia strongly influenced the viability of organoids. Organoids maintained for 8 hours under hypoxia conditions displayed high levels of HIF1a and elevated numbers of apoptotic cells. Next we generated conditional knockout mice that lacked Hif1a specifically in the intestinal epithelium (HIF-1αΔIEC mice). Using in vitro HIF-1αΔIEC organoid culture, we showed that HIF-1α is essential for organoid cell survive after hypoxia exposure. Moreover, intestinal inflammation and dysbiosis have been linked to autoimmune diseases such as rheumatoid arthritis (RA), however, the underlying mechanisms remain incompletely understood. Using collagen induced arthritis in HIF-1αΔIEC mice, we demonstrated that deficient mice have severer than littermate controls, which was correlated with an increase of intestinal IgA+ B cells and splenic Th1 cells in HIF-1αΔIEC mice.Taking together, these finding indicated that HIF-1a play an important role in small intestine homeostasis and autoimmune diseases by controlling epithelial cell survival.

Funding Source
DFG

Keywords: HIF1a(hypoxia induced factor), organoid; inflammation; epithelial

011

SCALABLE MULTIWELL MICROELECTRODE ARRAY (MEA) TECHNOLOGY FOR THE EVALUATION OF CARDIAC AND NEURAL THREE-DIMENSIONAL CELL CULTURES

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Three-dimensional induced pluripotent stem cell (iPSC)-derived in vitro models, commonly referred to as spheroids, organoids, or “mini-brains”, more accurately recreate the multicellular organization and structure of in vivo tissues when compared to traditional monolayer stem cell cultures. However, to effectively characterize 3D iPSC cell cultures, or to extract meaningful and predictive information from these models, new technology is required for evaluating functional cellular and network responses. For electro-active cells, like cardiomyocytes or neurons, measurements of electrophysiological activity across a networked population of cells provide a comprehensive view of function. Microelectrode array (MEA) technology offers such a platform by directly connecting key biological variables, such as gene expression or ion channel distributions, to measures of cellular and network function. Furthermore, the advent of multiwell MEA platforms has enabled scalable throughput capacity for 3D cell culture applications including toxicological and safety screening, disease modeling, and developmental biology. Raw neural or cardiac electrical activity can be captured simultaneously from each electrode on an MEA multiwell plate and in relation to the position of the spheroid on the array. Here, we present data supporting the use of multiwell MEA technology as an efficient non-invasive approach to capture electrophysiological activity from individual iPSC-derived spheroids. Human iPSC-derived cardiomyocytes and neural spheroids were cultured on 6-well MEA plates and monitored throughout maturation of their network connections. Cardiac electrophysiological activity, including spike amplitude and field potential duration, were recorded in response to compounds to provide information on the depolarization and repolarization of the cardiomyocyte action potential. For neural cultures, functional endpoints, such as network bursting and synchrony, were measured to define cellular activity across neural spheroids within a network. These results support the continued development and use of human iPSC-derived cardiomyocyte and neural spheroid assays on multiwell MEA technology for high throughput drug toxicity and safety assessment, evaluation of phenotypic disease-in-a dish models, and cell development.

Keywords: microelectrode array (MEA); spheroids; electrophysiology

012

PRIMARY HUMAN HEPATOCTYE 3D SPHEROIDS FOR STUDYING HEPATIC FUNCTION AND DRUG TOXICITY

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Primary Human Hepatocyte (PHH) culture provides the closest in vitro model to human liver that can produce a metabolic profile of a given drug very similar to that found in vivo. Hence, PHH culture is the gold standard for studying the in vitro hepatic biology, liver function, and drug induced hepatotoxicity. The conventional 2-dimensional (2D) PHH culture is limited by de-differentiation and rapidly loss of hepatic specific functions. Therefore, there is a need for more robust in vitro models that reflects in vivo liver biology with better culture longevity. Recently, 3-dimensional (3D) in vitro models for hepatocytes have gained a lot of attention for their ability to recapitulate the hepatic function and greater longevity. Recently we have developed an easy-to-assemble in vitro PHH 3D-spheroid model. Our initial work shows that PHH can assemble into spheroids using low attachment 96-well U-bottom plates within 5 days of seeding. Interestingly, we have also found that not every lot of PHH can assem-

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ble into 3D-spheroids. We have shown that seeding 1,500 PHH/well resulted in spheroid formation with homogenous morphology and consistent size (~200 µm diameter). These PHH spheroids can live up to 28 days in culture and can retain hepatocyte-specific functions. To assess whether hepatocyte-specific functions were maintained in the PHH spheroids during prolonged culture, albumin secretion, CYP3A4 activity and levels of ATP synthesized were analyzed. These parameters were found to remain stable during prolonged culture period. Also, gene expression profiles at 5, 7, 14 and 21 days showed a relatively higher expression of hepatocyte specific genes, such as albumin and CYP3A4, compared to that of the 2D-culture. Finally, we have performed cytotoxicity assay using compounds causing drug induced liver injury (DILI), such as Chlorpromazine and Diclofenac, and found comparable IC50 values between the 2D and 3D cultures using PHH. These results indicate that the PHH 3D-spheroid system developed by us constitutes a promising in vitro tool to evaluate hepatic function. As part of our future work, we are investigating the possibility of introducing nonparenchymal liver cells like Kupffer and Stellate cells to the spheroid system to assess feasibility of creating various liver disease models.

Keywords: Organoid; Disease modeling; Hepatic spheroid

013

IN VITRO DRUG EFFICACY EVALUATION IN CULTURED HUMAN IPSC-DERIVED NEURONS USING MEA SYSTEM

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Micro-electrode array (MEA) assay using human iPSC-derived neurons are expected to one of in vitro assays to predict the toxicity and predict the mechanism of action of drugs. Human iPSC-derived cortical neurons and astrocytes were cultured on 24-wells MEA plate for extracellular recording using MED64 Presto. Twelve compounds (pentylenetetrazole, picrotoxin, 4-aminopyrdine, linopyridine, amoxapine, strychnine, pilocarpine, amoxicillin, chlorpromazine, enoxacin, phenytoin, and acetaminophen) and dimethyl sulfoxide were tested at 5 concentrations for each compound (n>6). In this study, we aimed to develop an analytical method enabling the detection of toxicity of convulsants and the prediction of mechanism of action (MoA) using deep learning. We firstly had artificial intelligence (AI) learned the data of convulsants and the data of non-convulsants. Next this AI predicted the Toxicity of the data that not used for learning. The toxicity probability of unlearned sample data was 90% or more, and the toxicity probability of the unlearned convulsants was also 80% or more. In addition, the negative probability of non-convulsants and the prediction probability of MoA was more than 80%. These results indicated that this AI analysis method is useful for predicting the convulsion toxicity and the MoA of new drugs using hiPSC-derived neurons.

Keywords: MEA; iPSC-derived neurons; drug

014

PRECISE LEVELS OF SNF5 ARE REQUIRED FOR HUMAN PLURIPOTENT STEM CELLS DEVELOPMENTAL FATE REGULATION

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SNF5 is one of the core subunits of the SWI/SNF chromatin-remodeling complex. Therefore, loss of function (LOF) or gain of function (GOF) of this protein might have significant effects on the epigenetic state of the cells and on their phenotype. Indeed, SNF5 LOF is the solely genetic lesion in Rhabdoid tumor (RT) of the kidney and in AT/RT tumor in the brain. SNF5 is also required for early embryonic development as it was shown that SNF5-/- mouse blastocysts lose their hatching capacity and that SNF5 regulates the expression of Nanog during the differentiation of mouse embryonic stem cells. Here we aim to study the effect of SNF5 LOF and SNF5 GOF on human pluripotent stem cells (hPSCs). For this purpose, we targeted the endogenous SNF5 gene and introduced a conditional SNF5 over-expressing cassette into the AAVS1 locus. Using this system, we show that both SNF5 GOF and SNF5 LOF lead to rapid changes in cell fate, however in a different manner. SNF5 GOF results in down-regulation of pluripotency markers such as Oct4 and in cell differentiation. SNF5 LOF by contrast, doesn't show a direct effect on the expression of pluripotency markers but rather seems to affect the morphology of the cell colonies which start to grow as 3D structures instead of flat monolayer colonies. Global gene expression analysis reveals significant down-regulation in pathways related to the extra cellular matrix (ECM) and integrins upon SNF5 LOF. Notably, earlier attempts to downregulate SNF5 in embryonal carcinoma cells (NCCIT) led to similar morphological changes while down-regulation of the other SWI/SNF core subunits in hPSCs not. Taken together, these results reveal that SNF5, but not other SWI/SNF subunits, regulates the interactions between hPSCs and the ECM. This observation is further supported by the fact that re-expression of SNF5 in mouse RT cells leads to upregulation of cellular pathways related to cell adhesion, extracellular space, integrin signaling etc. To conclude, our results reveal that SNF5 levels have to be tightly regulated in hPSCs. Furthermore, the opposite effect of SNF re-expression in RT cell line and SNF5 LOF in hPSCs demonstrates the capacity of our system to be used as a novel tool to study RT formation.

Keywords: SWI/SNF complex; Rhabdoid tumor formation; Cell-ECM interactions

015

EVIDENCE FOR NEURONAL PLASTICITY IN BIOENGINEERED NEURONAL ORGANOIDS

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To study human neuronal network function, we developed a defined, Matrigel-free 3D cell culture system termed human bioengineered neuronal organoids (BENOs). Neural differentiation of pluripotent stem cells (iPSCs) embedded in a collagen matrix was directed under defined serum-free conditions. Calcium imaging revealed spontaneous tetrodotoxin (1 µM)-sensitive signals by d25. To test spontaneous neuronal network activity, BENOs (day 30-60) were subjected to GABAergic (picrotoxin, 58 µM; saclofen, 330 µM) inhibition. Spontaneous Ca2+ signals of synchronized neurons became asynchronous upon GABAR inhibition (2 independent experiments). Antagonist washout restored synchronicity suggesting the presence of functional GABAergic networks. Stimulation (injected current: 20-100 µA)-evoked Ca2+ influx in remote regions (distance from electrode 0.5 to 1.5 mm) suggested a neuronal network that extends throughout the organoid. Multi-pulse stimulation demonstrated a Ca2+ influx pattern similar to paired pulse depression (PPD). The PPD-like Ca2+ signal pattern was alleviated by a GABA-A inhibition (picrotoxin 58 µM) and was restored upon washout (2 independent experiments). Field potential measurements revealed high frequency stimulation-induced long-term potentiation (n=3) suggesting neuronal plasticity. In conclusion, BENOs from human pluripotent stem cells contain electrically active neuronal networks that exhibit typical forms of plasticity observed in the human brain.

Keywords: Neuronal netwotk function; Neuronal Plasticity; Bioengineered neuronal organoids

016

WNT AGONIST ENHANCES THE FUNCTION OF PROGRANULIN FOR HUMAN DOPAMINERGIC NEURONAL DIFFERENTIATION

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The method to enrich dopaminergic neurons in vitro is essential for Parkinson's cell therapy. Our hypothesis is that maintenance of progranulin (PGRN) expression in neural precursors may be essential to ensure neuronal differentiation. The reported results from our previous studies indicate that the dopaminergic neuronal differentiation (DND) occurs only among the nestin+/CD133+ human fetal brain cells (HFBC) rather than the cells with same markers isolated from adult human brain tissue. We also notice that the level of PGRN expression in fetal neural precursor cultures can be elevated after the cells treated with Wnt signal enhancer. The increased PGRN expression is benefit to the DND. To explore the molecular mechanism of PGRN to regulate DND, both PGRN antisense deoxynucleotides (PGRN-ADON) and human recombinant PGRN protein were used for this study. Prior incubation of the nestin+/CD133+ HFBC in dopaminergic differentiation medium, cells were cultured with the PGRN-ADON medium. After PGRN antisense treatment, the level of PGRN expression in the nestin+/CD133+ HFBCs was reduced. The decreased PGRN expression was confirmed using RT-PCR, Northern blotting and protein assays. No elevated apoptotic signal was detected after the cultures treated with PGRN-ADON. Interestingly, inhibition of PGRN expression declines the potential of dopaminergic differentiation among nestin+/CD133+ HFBCs. Meanwhile, it is likely reducing of PGRN expression increases GFAP expression and astrocytic differentiation. In contrast, using exogenous recombinated-PGRN protein to pretreat the nestin+/CD133+ HFBCs is able to supress the PGRN-ADON induced glio-differentiation. The results from this study indicate that high level of PGRN promotes neuronal differentiation. Although the mechanism of DND may be not limited to the intracellular PGRN expression level, effect of PGRN on DND deserves further investigation.

Funding Source
University internal fundings; University hospital internal fundings.

Keywords: Wnt agonist, active Wnt signals; Progranulin, differentiation regulator; Dopaminergic neurons, produce dopamine in CNS

017

ELECTROPHYSIOLOGICAL PHENOTYPE CHARACTERIZATION OF HUMAN IPSC-DERIVED DOPAMINERGIC NEURONAL LINES BY MEANS OF HIGH-RESOLUTION MICROELECTRODE ARRAYS

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High-resolution-microelectrode-array (HD-MEA) technology enables to study neuronal dynamics at different scales, ranging from axonal physiology to network connectivity. We have used this HD-MEA technology to characterize and compare the electrical phenotypes of commercially available human dopaminergic neurons. Furthermore, we have studied the effect of human astrocytes on neural-culture development. Astrocyte/neuron co-cultures showed higher signal amplitudes and higher firing rates than neural cultures without astrocytes. Adding astrocytes to neural cultures changed the whole culture morphology by promoting cell clustering. Interestingly, astrocyte/neuron co-cultures showed a lower sample-to-sample variability across multiple preparations and HD-MEA recordings compared to neural cultures without astrocytes. We compared action potential propagation velocities along axons between dopaminergic A53T α-synu-

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clein neurons and the wild-type isogenic control cell line. We found that in both, wild-type and disease-model neurons, axonal action potential propagation velocities were lower than those in rat primary cortical neurons. Furthermore, we found different axonal-action-potential-velocity-development profiles of A53T α-synuclein dopaminergic neurons and the wild-type counterpart. Finally, we were able to precisely evoke action potentials in individual single human neurons by subcellular-resolution electrical stimulation. HD-MEA systems were found to enable access to novel electrophysiological parameters of iPSC-derived neurons, which can be potentially used as biomarkers for phenotype screening and drug testing.

Funding Source
EU, ERC Advanced Grant "neuroXscales", contract number 694829; EU, ERC-PoC "MwHresEP", contract number 755383; CH, Project CTI-No. 25933.2 PFLS-LS "Multi-well electrophysiology platform for high-throughput cell-based assays"

Keywords: iPSC; microelectrode, neurodegeneration

018

SALIVARY GLAND STEM CELL DESMOSOME DYSREGULATION AND LYMPHOEPITHELIAL LESIONS IN PRIMARY SJÖGREN'S SYNDROME

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We have previously demonstrated that hyposalivation in the autoimmune disease Sjogren's syndrome (pSS) is likely due in part to replication-induced senescence of salivary gland stem cells (SGSCs), resident in the ducts of salivary glands (SGs). In order to establish if SGSCs are capable themselves of producing cytokines driving their own proliferation, we stimulated SGSC-derived organoid toll-like receptors using TLR stimulants Poly(I:C) and Imiquimod (specific for TLR3 and TLR7, respectively). Organoid exposure to IMQ induced initial cell death (passages 1-3) followed by partial rescue of proliferation (passage 4). TLR3 stimulation induced similar but less significant effects. Surviving organoids at p4 of IMQ treatment upregulated expression of proinflammatory cytokines (TNFα, IFNα, IFNβ, IL6) and became disorganized in morphology. In p4 cultures containing these disorganized structures, desmosomes components were downregulated at transcript level, and appeared to be scarce when examined by electron microscopy. Lymphoepithelial lesions (LELs) are poorly understood facets of pSS SG pathology, and presumed to be the precursor to MALT lymphomas in pSS. They develop from the striated ducts (SDs), and involve proliferation and loss of organization of the epithelial cells, beginning from the cells in the SD basal layer (BSD). The BSD layer is also one of the stem cell niches in the human SG. When immunostained for the BSD marker K14, we observed proliferation, lack of organization, and aberrant differentiation of BSD cells into acinar cells, in LELs. Reasoning that TLR-induced desmosome dysregulation in BSD stem cells may lead to this phenotype ad be important in LELs formation (organoid cultures used contained a majority of SD cells), we examined LELs from pSS patient tissue, using a previously established LEL severity grading system. Desmosomes in all LEL stages (1-3) were difficult to identify in BSD cells. We hypothesize that TLR-induced desmosome dysregulation in BSD resident SGSCs, in combination with their ability to attract B cells through cytokine/chemokine expression, represents the first stages of lymphoepithelial lesion formation. and therefore also potentially of MALT lymphomas in pSS patients.

Funding Source
This research was funded by the Dutch Arthritis Foundation Translational Research Grant (T015-052) and Cancer Research UK (grant number: C17199/A18246), and unrestricted research grant from Bristol-Myers Squibb.

Keywords: primary Sjogren's syndrome; Salivary gland stem cells/organoids; Lymphoepithelial lesion

019

GENERATING DISEASE MODELS USING CRISPR-CAS9 GENE EDITING OF HUMAN PLURIPOTENT STEM CELLS AND CEREBRAL ORGANOIDS

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Human pluripotent stem cells (hPSCs), in combination with CRISPR/Cas9 gene editing technologies, are emerging as important tools to study mechanisms of pathogenesis and modelling human diseases. Here we used CRISPR-Cas9 to target the CDK5 regulatory subunit-associated protein 2 (CDK5RAP2), a gene implicated in the development of primary microcephaly. Briefly, a blood-derived hPSC line was dissociated into a single cell suspension, electroporated with the ArciTect™ CRISPR-Cas9 ribonucleoprotein complex and allowed to recover in mTeSR1™ supplemented with CloneR™. After the initial round of clone selection, heterozygous clones were subjected to a second round of gene editing to generate a compound heterozygous clone harbouring frameshift mutations in both alleles of CDK5RAP2. Clones were expanded in mTeSR1™ and screened for cell quality attributes including karyotype, pluripotency and hPSC marker expression. Expression levels of CDK5RAP2 were confirmed by RT-qPCR and immunostaining, with only the compound heterozygote exhibiting decreased CDK5RAP2 expression. Compound heterozygote lines were then used to generate cerebral organoids using the STEMdiff™ Cerebral Organoid Kit to investigate effects of CDK5RAP2 on organoid formation, morphology and size. Cerebral organoids generated from the compound heterozygote line were approximately 10% - 40% smaller in diameter compared to the heterozygote and control at the EB formation (Day 5), neural induction (Day 7), expansion (Day 10), and maturation stages (Day 18); n=4 per clonal line, 12 - 16 EBs per measurement. Expression of neuronal

markers (DCX and TuJ-1) were increased while markers for neural progenitors (SOX2 and PAX6) were slightly decreased in the compound heterozygote compared to the heterozygote and control lines. Morphology of the cortical region in the compound heterozygote also exhibited decreased organization of the ventricular zone and cortical plate with an increase in neuronal marker expression. In summary, we have optimized a gene editing protocol using the ArciTect™ CRISPR-Cas9 system combined with mTeSR1™ supplemented with CloneR™ to derive high quality clonal edited hPSC lines, which can be used to generate and study brain development in a cerebral organoid culture system.

Keywords: Human Cerebral Organoid; Gene editing; Human pluripotent stem cell

020

DEVELOPMENT OF CORTICAL ORGANOIDS TO STUDY CACH/VWM SYNDROME DEVELOPMENTAL ALTERATIONS

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EIF2B-related disorders are autosomal recessive leukodystrophies due to mutations in Eukaryotic Initiation Factor 2B genes (EIF2B). EIF2B genes are involved in the translation initiation and protein synthesis regulation and are expressed ubiquitously in the tissues. But Childhood Ataxia with Central Hypomyelination/Vanishing White Matter Syndrome (CACH/VWM) is a neurodegenerative disease and the archetype form of those eIF2B-related disorders. It affects mainly the development and full maturation of the brain and is characterized by either a quick or a progressive evolution depending on the identified point mutation in the various EIF2B genes. In the present work, we developed an in vitro model allowing us to mimic the developmental evolution and alteration resulting from the CACH/VWM Syndrome. We generated cortical organoids from human induced pluripotent stem cells (hiPSc) derived either from CACH patients (EIF2B5 R113H/R113H – a moderate phenotype) or from sex and aged matched control patients using a 3D specific protocol that starts with cell aggregates. The results of this approach allowed us i) to validate a robust protocol for generation of long term cultured cortical organoids, ii) to start to decipher the molecular mechanisms presiding the defects observed in this unique developmental genetic disease and iii) to identify morphological alterations in the neuroepithelial structures observed during the organoïds formation. Development of these 3D cortical organoïds helps us to mimic the physiopathological disorders specific to this neurodegenerative disease.

Keywords: cortical organoïds; neurodegenerative disease; leukodystrophy

021

VISUALIZATION OF STEM CELL NICHE METABOLISM AND PROLIFERATION IN THE INTESTINAL ORGANOID MODEL

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Studies of 3D tissue models reveal dual role of cell metabolism for multicellular organization and development: it plays both bioenergetic and cell signaling functions. Recent studies demonstrate that cell metabolism depends on the cell type and it can directly play an instructive role in regulation of the stem cell niche, balancing proliferation and differentiation. Growing interest in this area correlates with the need of new live imaging methods for detailed analysis of cell composition, proliferation and cellular metabolism in toto with organoid and related 3D tissue models. Here we report the application of phosphorescence lifetime imaging microscopy (PLIM) with the cell-penetrating O2-sensitive probe for analysis of oxygenation, an important marker of aerobic metabolism, in mouse intestinal organoid culture. O2-PLIM method allows for high-resolution quantitative imaging of O2 in individual organoids in real-time. Our preliminary experiments with primary adult stem cell-derived organoids revealed strong inter-cultural heterogeneity of oxygenation (27-92 μM O2 for individual organoids); in addition, the oxygenation also varied within the single organoids and resulted in transmembrane gradients. However, even with such variability, organoids displayed high activity of the mitochondria and responded to stimulation with uncoupling agent (FCCP) and inhibitor of respiration metformin. We also studied differences in oxygenation, depending on the glucose content in the growth medium. We further demonstrated that O2-PLIM method can be multiplexed with fluorescence lifetime imaging microscopy (FLIM) of DNA-binding probe Hoechst 33342 for detection of BrdU-accumulating cells in S phase, conventional fluorescence microscopy of Lgr5-GFP-positive cells and some other biomarkers. We found that the same live organoid can be analyzed for presence of stem cells (Lgr5), proliferating cells (Hoechst FLIM) and oxygenation. This allows investigation of organoid metabolism and cell fate, by localizing and identifying new regions with respect to their oxidative phosphorylation and proliferation status.

Funding Source
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Keywords: Hypoxia imaging; Intestinal organoid; Cell proliferation

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022

KIDNEY ORGANOIDS FROM NEPHROTIC DISEASE DERIVED IPSCS EXHIBIT IMMATURE NEPHRIN LOCALIZATION AND SLIT DIAPHRAGM FORMATION IN PODOCYTES

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Mutations in the NPHS1 gene, which encodes NEPHRIN, cause congenital nephrotic syndrome, characterized by massive proteinurea after birth due to impaired kidney slit diaphragm (SD). However, methods for SD reconstitution have been unavailable, thereby limiting studies in the field. In the present study, we established human induced pluripotent stem cells (iPSCs) from a patient with an NPHS1 missense mutation and reproduced the SD formation process using iPSC-derived kidney organoids. The mutant NEPHRIN failed to become localized on the cell surface for pre-SD domain formation in the induced podocytes. Upon transplantation, the mutant podocytes developed foot processes, but exhibited impaired SD formation. Overexpression of mutated protein in Human embryonic kidney (HEK293) cell line indicated that impaired translocation might be due to impaired glycosylation, resulting in retention of mutated protein in the cytoplasm. Treatment with a combination of chemicals revealed improved translocation of the mutated protein in HEK293 cells. However, similar treatment to patient iPSC exhibited no effect. In contrast, genetic correction of the single amino acid mutation restored NEPHRIN localization and phosphorylation, colocalization of other SD-associated proteins, and SD formation. Thus, these kidney organoids from patient-derived iPSCs identified SD abnormalities in the podocytes at the initial phase of congenital nephrotic disease.

Funding Source
The study was supported by: Japan Society for the Promotion of Science, Japan Agency for Medical Research and Development (AMED).

Keywords: iPS cells; Nephrotic syndrome; Kidney, Podocyte

023

TUBEROUS SCLEROSIS COMPLEX MUTATIONS AFFECT HUMAN IPSC DERIVED ASTROCYTES

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Tuberous Sclerosis Complex (TSC) is a genetic multisystem disorder caused by mutations in the TSC1 or TSC2 gene. Patients show benign tumor formation in multiple organs including the brain. Additional neurological problems like epilepsy, autism and intellectual disability are common and the major cause of disability in TSC patients. Astrocytes, one of the major cell types in the brain, are responsible for keeping the homeostasis of the brain and are involved in most, if not all, brain processes like neuronal signaling, myelination and maintenance of the blood-brain barrier. Astrocytes have been implicated in morphological abnormalities (tubers and supependymal giant astrocytoma's) that are observed in post-mortem brain tissue from TSC patients. Glutamate and potassium buffering is affected in TSC-mutated astrocytes, which can promote neuronal excitability and seizures. Most studies looking at TSC brain pathology used transgenic mice or post-mortem brain tissue. We have previously made human induced pluripotent stem cells (hiPSC) lines from TSC patients and controls and used these to model neuronal and oligodendrocytic abnormalities. In the current study we used hiPSC lines to look at astrocytic pathology. We differentiated hiPSC towards astrocytes using previously established protocols and looked at proliferation rates, marker expression and cell morphology. Preliminary data suggest an increased cell proliferation and increased expression of GFAP, a marker for astrocyte reactivity. In a first pilot study no morphological changes in astrocytes were observed. We are now planning to analyze differences between TSC and control hiPSC derived astrocytes with RNA sequencing and to study the effects of TSC astrocytes on neuronal functioning and OPC maturation. The first results of this study show that human astrocytes are affected by TSC mutations and may be important targets in therapy development.

Keywords: Tuberous Sclerosis Complex; Astrocytes; human iPSCs

024

CTIP2-DEPENDENT PKA ACTIVATION REGULATES CA2+ SIGNALING AND DARPP32 PHOSPHORYLATION IN HUMAN MEDIUM SPINY NEURONS

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The mechanisms underlying the selective degeneration of medium spiny neurons (MSNs) in Huntington's disease (HD) remain largely unknown. CTIP2, a transcription factor

expressed by all MSNs, is implicated in HD pathogenesis due to its interactions with mutant huntingtin. We demonstrate that human ESC-derived MSNs deficient in CTIP2 display impaired mitochondrial health, increased vulnerability to oxidative stress and abnormal responses to physiological stimuli. We uncovered a key role for CTIP2 in Ca2+ transport and protein phosphorylation via governing protein kinase A (PKA) signaling. This is demonstrated by a PKA-dependent rescue of Ca2+ signaling deficits and significant reduction in phosphorylation of DARPP32 and GLUR1, two PKA targets in CTIP2-deficient MSNs. Moreover, we show that CTIP2-dependent dysregulation of protein phosphorylation is shared by HD hPSC-derived MSNs and striatal tissues of two HD mouse models. Our study therefore establishes an essential role for CTIP2 in human MSN homeostasis and provides mechanistic and potential therapeutic insight into striatal neurodegeneration.

Funding Source
This work has been funded by MRC UK and European Commission within its FP7 Programme.

Keywords: CTIP2-deficient medium spiny neuron; Neural differentiation; Huntington's disease

026

THE LOSS OF FRAGILE X MENTAL RETARDATION PROTEIN ALTERS THE DEVELOPMENT OF HUMAN FOREBRAIN ORGANOIDS

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Fragile X syndrome (FXS) is the most common inherited form of intellectual disability and a leading genetic cause of autism. FXS is caused by the loss of functional fragile X mental retardation protein (FMRP). FMRP is an RNA-binding protein forming a messenger ribonucleoprotein complex with polyribosomes in the regulation of protein synthesis. Despite major progress to characterize underlying disease mechanisms in animal models that has led to several clinical trials, improvements of behavioral and cognitive outcomes in patients have unfortunately been unsuccessful, a strong need for human-specific models of FXS to understand the unique factors that underlie human disease and to test the efficacy of candidate compounds. Three-dimensional (3D) organoid culture of human-induced pluripotent stem cells (iPSCs) has evolved from embryoid body cultures, quite faithfully following human organogenesis, and provides a new platform to investigate human brain development in a dish, otherwise inaccessible to experimentation. To determine whether the loss of FMRP could alter the development of human brain organoids, we have generated forebrain organoids from three FXS male patients and three healthy male controls. We observed dysregulated proliferation of neural progenitor cells and neural differentiation as well as perturbed cell migration in FXS forebrain organoids. Interestingly these deficits were not observed with FXS mouse model. To compare the differential gene expression caused by the loss of FMRP between human and mouse, we then performed RNA-seq to identify the differentially expressed genes using both mouse embryonic brain cortex and human forebrain organoids at the comparable developmental stages. We detected very few genes differentially expressed in the absence of Fmrp in mouse. However, we identified 200 genes downregulated and 126 genes up-regulated in human FXS organoids, indicating human-specific impact caused by the loss of FMRP. These results together suggest that the loss of FMRP could cause neurodevelopmental deficits specifically in human, and fragile X organoids could provide a unique platform to study the molecular pathogenesis of FXS and identify human-specific druggable targets for FXS and autism in general.

Funding Source
National Institutes of Health, FRAXA Research Foundation, Emory Woodruff Health Sciences Center.

Keywords: Fragile X syndrome; FMRP; Forebrain organoids

027

MODELLING THE NEURODEVELOPMENTAL DISORDER FRAGILE X SYNDROME BY DIRECT CELLULAR REPROGRAMMING OF ADULT HUMAN FIBROBLASTS

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Fragile X Syndrome (FXS) is the leading monogenic cause of intellectual impairment and autism spectrum disorder. FXS results from epigenetic silencing of the FMR1 gene due to expansion of the CGG repeat in the 5′UTR of FMR1. Study of the molecular and cellular mechanisms that link FMR1 inactivation to impaired neuronal development and function is limited by the inability to access live FXS affected human neurons. We have established a direct cellular reprogramming strategy using a chemically modified mRNA gene delivery system to enhance the conversion of adult human dermal fibroblasts (HDFs) to induced neural precursor cells (hiNPs) from which cortical forebrain neurons can be generated. We propose that by generating hiNPs directly from HDFs derived from FXS patients, we will improve our understanding of the molecular, cellular and pathological basis of FXS, with potential to identify new therapeutic compounds. This study shows the ability to directly reprogram FXS patient-derived HDFs to hiNPs by transient ectopic expression of the pro-neural transcription factors, SOX2 and PAX6 using chemical modified mRNA (cmRNA) transfection. A mixed population of hiNP-derived neurons and astrocytes were derived after differentiation in defined media, exhibiting expression of the astrocyte marker S100B as well as glutamatergic genes TBR2 and VGLUT1 and GABAergic genes MEIS2 and GAD67. FMR1 was not detected in independent FXS lines (n = 3) following differentiation. We observed an increase in neurite length as well an alteration in the ratio of neurons to astrocytes in FXS hiNP-derived neurons compared to unaffected controls (n = 3/condition).

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These observations were correlated with the CGG-repeat dynamics and epigenetic profile of independent FXS lines using genotyping and DNA methylation analyses. Overall, these findings provide proof-of-concept for the application of direct-to-hiNP reprogramming to undertake further research analyzing the molecular and cellular basis of FXS.

Funding Source
The Neurological Foundation of New Zealand.

Keywords: Cell reprogramming; Fragile X Syndrome; Neural precursor cells

028

AUTISM SPECTRUM DISORDERS: FROM DYNAMIC DEVELOPMENTAL DISEASE TRAJECTORIES TO GENE NETWORK HETEROCHRONICITY

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Autism is a neurodevelopmental disorder with a complex genetic etiology. Recent advances in systems biology approaches have caused a paradigm shift in the field from a single gene causation model to pathway perturbation models and the cellular and molecular events that lead to autism likely occur very early during human fetal cortical development. However, one of the current challenges in understanding autism pathophysiology is to determine critical neurodevelopmental periods and cellular states that might provide the ground for disease propensity. This task requires an in-depth exploration of the temporal dimension of shifting biological processes by treating the disorder as an evolving, dynamic system. Using a time series approach to monitor patient-derived induced pluripotent stem cells (iPSCs) throughout early stages of neurodevelopment enabled us to reconstruct the dynamics of transcriptional gene network programs and led to the identification of an intrinsic network-specific heterochronicity. Our data show that these ASD-related changes are likely to be primed during an earlier stage of development and we propose that studying molecular disease trajectories could maximize our chance of capturing relevant mechanistic disease states, as well as the processes by which these states unfold.

Funding Source
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Keywords: Autism; Development; Gene networks

029

DECIPHERING THE ETIOLOGY OF MICROCEPHALY USING CEREBRAL ORGANOIDS GENERATED FROM NIJMEGEN BREAKAGE SYNDROME PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

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Nijmegen Breakage Syndrome (NBS) is a rare autosomal recessive genetic disorder caused by a mutation within the NBN gene and thus resulting in genomic instability, premature aging, microcephaly, growth retardation, immune deficiency, impaired puberty and infertility in females. The consequence of these manifestations is a decrease in average life span, caused by cancer or infection of the respiratory and urinary tract. We previous reported that fibroblasts from NBS patients can be reprogrammed into induced pluripotent stem cells (iPSCS) and thus by-passing premature senescence. Global transcriptome analysis comparing NBS fibroblasts to healthy cells and NBS-iPSCs to hESCs unveiled de-regulated cancer related pathways such as p53, cell cycle and glycolysis. Furthermore, molecular analysis of NBS-iPSCs derived neural progenitor cells (NPCs) revealed de-regulated expression of neural developmental genes in-part due to NBS-NPCs inability to maintain normal levels of P53. Recent advances in in vitro culture of 3D cerebral organoids derived from iPSCs have illuminated the early mechanisms of mammalian neuro-development. To decipher the etiology of microcephaly in NBS patients, we first generated another iPSC line from a NBS patient bearing the homozygous 657del5 within NBN. Next, we used the generated NBS-iPSC line together with the previously reported NBS-iPSC line bearing the heterozygous 657del5 and a healthy control iPSC line to comparatively generate cerebral organoids. Here, we show that NBS-derived organoids are significantly smaller during the early differentiation stage, showed decrease proliferation of the neural progenitor cells (NPCs) and key pathways related to neurogenesis, DNA damage response and cell cycle are differentially regulated compared to the healthy control iPSC line. In conclusion, our observations are (i) successful generation of a 3D cerebral organoid model of NBS, (ii) delayed neurogenesis of the NBS organoids probably due to less proliferation of the NPCs and (iii) de-regulated expression of neurogenesis-associated genes and P53, which might account for the development of microcephaly in NBS patients.

Funding Source
Medical faculty of Heinrich Heine University Düsseldorf.

Keywords: Microcephaly; Nijmegen Breakage Syndrome; Cerebral organoids

Poster Abstracts

030

GENERATION AND CULTURE OF MURINE PANCREATIC EXOCRINE ORGANOIDS USING PANCREACULT™ SERUM-FREE MEDIUM

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Despite the prevalence and urgency of pancreatic cancer research throughout the previous decades, a method for the long-term in vitro maintenance of pancreatic exocrine tissue as 3-dimensional (3D) organoids has been described only recently. Pancreatic exocrine organoids are composed of a polarised monolayer epithelium that retains many of the features of in vivo exocrine pancreatic tissue, and thus can serve as a physiological model system to address diverse research questions related to pancreatic development, stem cell biology, secretory function, and disease modeling. We have developed PancreaCult™ Organoid Growth Medium (Mouse), a defined cell culture medium for the initiation and long-term maintenance of pancreatic exocrine organoids. To establish these cultures, resected mouse pancreatic tissue was enzymatically dissociated to liberate ductal fragments that contain the putative stem cell niche. When embedded into Corning® Matrigel® domes and cultured in PancreaCult™, these ductal fragments formed spherical organoids within 1 week (n = 59 mice). Established organoids were passaged weekly at an average split ratio of 1:25 and maintained in continuous culture for > 1 year. Expanded organoids were also cryopreserved using CryoStor® CS10 for long-term storage. Pancreatic exocrine organoids cultured in PancreaCult™ are composed of cells expressing genes specific for pancreatic stem cells (Lgr5), progenitor cells (Pdx1, Sox9), and ductal cells (Car2, Muc1, Krt19, Cftr). Additionally, we observed that primary and metastatic tumor cells isolated from Kras+/LSL-G12D; Trp53+/LSL-R172H; Pdx-Cre mice and cultured in PancreaCult™ generate tumor organoids that recapitulate the features of the original tumor, thus providing a model system to study ductal pancreatic carcinoma. Due to the robust growth of pancreatic exocrine organoids in PancreaCult™ and their close resemblance to the in vivo pancreatic epithelium, this organoid technology can complement or replace other experimental methodologies for studying the exocrine pancreas and could reduce or even eliminate the need for animal experimentation.

Keywords: Pancreas; Organoids; Cancer

031

EXPLOITING IPSC-DERIVED NEUROSPHEROIDS TO RECAPITULATE HUMAN BRAIN MICROENVIRONMENT IN DISEASE

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Brain microenvironment plays important roles in neurodevelopment and pathology. Neural cell culture typically relies on the use of heterologous matrices that poorly resemble brain ECM or reflect its pathological features. We have shown that perfusion bioreactor-based 3D differentiation of iPSC-derived human neural stem cells (iPSC-NSC) sustains the concomitant differentiation of the three neural cell lineages. We hypothesized that if the neurospheroid strategy would also allow deposition of native neural ECM, it would be possible to (i) mimic cellular and microenvironment remodeling occurring during neural differentiation, without the confounding effects of exogenous matrices and (ii) recapitulate pathological phenotypic features of diseases in which alterations in homotypic/heterotypic cell-cell interactions and ECM are relevant. Quantitative transcriptome (NGS) and proteome (SWATH-MS) analysis showed that neurogenic developmental pathways were recapitulated in our system, with significant changes in cell membrane and ECM composition. We observed a significant enrichment in structural proteoglycans typical of brain ECM, a downregulation of basement membrane constituents and higher expression of synaptic and ion transport machinery. Neurospheroids were generated using iPSC-NSC derived from Mucopolysaccharidosis type VII (MPS VII) patient. MPS VII is a neuronopathic lysosomal storage disease caused by deficient β-glucuronidase (β-gluc) activity, leading to glycosaminoglycan (GAGs) accumulation in the brain. The main MPS VII molecular hallmarks were recapitulated, e.g., accumulation of GAGs. MPS VII neurospheroids showed reduced neuronal activity and disturbance in network functionality, with alterations in connectivity and synchronization. The proteome of MPS VII neurospheroids is being analysed to further elucidate molecular alterations related with the neuronal dysfunction observed in the disease. These data provide insight into the interplay between reduced β-gluc activity, GAG accumulation, alterations in the neural network, and its impact on MPS VII-associated cognitive defects. Further, our model provides a platform to unveil the cellular alterations responsible for brain dysfunction in neurological diseases and to test and optimize new therapies.

Funding Source
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Keywords: Neurospheroids; Mucopolysaccharidosis type VII; Brain microenvironment

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DEVELOPMENT AND CHARACTERIZATION OF BRAIN REGION-SPECIFIC ORGANOIDS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS FOR MODELLING OF RETT SYNDROME

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Organoid biology and process engineering strategies provide a novel platform to model brain development and neurological related disorders, by providing a more reliable recapitulation of the neurodevelopment process when compared to 2D culture systems. Among these diseases, Rett Syndrome (RTT), a rare neurodevelopmental disorder that affects multiple neuronal subtypes originated from forebrain sub-regions, can greatly benefit from the merging of these technologies. The main objective of this work is the development and characterization of standardized ventral and dorsal forebrain organoids from both RTT patient-specific and wild-type human induced pluripotent stem cells (hiPSC). We demonstrated that these organoids successfully recapitulate ventral and dorsal forebrain progenitor zone organization, as well as the process of neurogenesis. Importantly, both regions contain the two major populations of cortical neurons, glutamatergic pyramidal neurons and GABAergic interneurons, as determined by qRT-PCR, immunocytochemistry and spontaneous currents recorded by whole-cell path clamp. However, while ventral organoids from wild-type hiPSC displayed a predominant neuronal-like profile, revealed by an increase in Ca2+ levels in response to KCl but not to histamine stimulation, ventral organoids obtained from RTT hiPSC lines exhibited a more immature-like profile. Moreover, it was found that along the maturation process of ventral organoids, DCX+ immature neurons are present mostly in RTT cell lines, while MAP2+ neurons are enhanced in organoids differentiated from wild-type cell lines. These neurons are currently being characterized in terms of their electrophysiology profile. Preliminary results from these studies suggest that wild-type hiPSCs generated neurons have faster rate of maturation and excitability, firing more action potential in response to depolarizing current injection, when compared with RTT hiPSCs generated neurons. These results will contribute to the characterization and understanding of RTT during the early stages of development, before disease inception, by recapitulating the complex human neurodevelopmental defects. Moreover, this platform may also constitute a promising tool for disease modeling as well as for testing potential therapeutic drugs.

Funding Source
H2020-WIDESPREAD-01-2016-2017 – Teaming Phase2, DISCOVERIES CTR: The Discoveries Centre for Regenerative and Precision Medicine.

Keywords: Human induced pluripotent stem cells; Rett Syndrome; Forebrain organoids

033

MODELING FAMILIAL CEREBRAL CAVERNOUS MALFORMATIONS USING PLURIPOTENT STEM CELLS

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Familial cerebral cavernous malformation (FCCM) is a rare disease characterized by vascular malformations, mainly localized within the brain, that cause cerebral haemorrhage, seizures and strokes. Despite the severity of the lesions caused by loss-of-function mutations in any of three Ccm genes, the molecular mechanisms behind this dysregulated endothelial cell (EC) behaviour have not been elucidated. Here, we develop an improved model in vitro using pluripotent cells to decipher the role of each Ccm gene during endothelial cell-fate specification. Although we found that Ccm genes are dispensable for self-renewal of embryonic stem cells, their function is required for early differentiation towards endothelial lineage and vessel formation in 3D cell cultures. Our data indicates that absence of Ccm genes alters a conserved epigenetic landscape required for the transcriptional transition during mammalian EC development. These findings will allow us to identify novel druggable targets and therapeutic approaches for this severe, and so far, incurable, human disease.

Funding Source
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Keywords: FCCM disease; Endothelial Cell; Epigenetic landscape

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034

COMPARISON OF LARGE 1Q21.1 AND TARGETED NOTCH2NL DELETIONS IN CEREBRAL ORGANOIDS SUGGEST A MAJOR ROLE FOR NOTCH2NL IN 1Q21.1 DISTAL DELETION PATHOLOGY

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Cerebral cortex organoids (cerebral organoids) derived from human pluripotent stem cell lines provide a model system for studying how genetics can affect human brain development. We recently demonstrated that deletion of human-specific NOTCH2NL genes in human embryonic stem cell (hESC)-derived cortical organoids accelerates differentiation of neural stem cells resulting in smaller organoids with a higher proportion of cells expressing markers of excitatory projection neurons at early time points. This led to the hypothesis that NOTCH2NL expression in the human cortex increases the self-renewal capacity of neural stem cells. We also found that NOTCH2NLA and NOTCH2NLB flank a genomic locus (Chromosome 1q21.1) that is highly repetitive and is recurrently deleted or duplicated in patients with a variety of neurodevelopmental disorders including autism, schizophrenia, microcephaly and macrocephaly. Here we present our findings further exploring the phenotypes of NOTCH2NL deletions and larger deletion events that mimic those observed in patients with 1q21.1 distal deletions by comparing hESC-derived cerebral organoids with a variety of altered genotypes by single cell RNA-seq to determine how these alterations effect early brain development with respect to the proportions of cell types present and the transcriptional profiles of these resulting cell types. These studies will reveal the relative contributions of NOTCH2NL genes and the other genes in this genomic locus on cerebral organoid development and could provide a model system for exploring ways to mitigate the developmental effects of pathogenic genomic rearrangements at this locus.

Funding Source
Howard Hughes Medical Institute, Schmidt Family Foundation, NIH/NIGMS, CIRM.

Keywords: Neurodevelopmental disorders; NOTCH signaling; Cerebral cortex organoids

035

KIDNEY ORGANOIDS: DISEASE MODELING AND A PATH TO PERSONALIZED MEDICINE

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Human pluripotent stem cell (hPSC)-derived three-dimensional kidney organoid is an important model system to study kidney development, disease and a pre-clinical model for personalized therapeutics. However, human kidney organoid model requires a substantial improvement in terms of maturation of nephrons with functional vascularization. To achieve this objective, we generated renal subcapsular transplantation of hPSC derived kidney organoids or human kidney Organoid Transplants (HKOTs). Following 26 days post renal subcapsular transplantation, we performed comprehensive single cell atlas of HKOTs and demonstrated vascularization and maturation of nephrons. Our results showed that host-derived vascularization in kidney organoids significantly improved tubular and podocyte maturity at single cell resolution.

Keywords: Kidney Orgnaoids; Human kidney Organoid Transplants (HKOTs); Personalized therapeutics

036

MODELING CANCER STEM CELL AND DIFFERENTIATED CANCER CELL PHENOTYPES IN LIVER CANCER ORGANOIDS

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Cholangiocarcinoma (CCA) is a biliary-type liver tumor with a dismal prognosis, due to late diagnosis, high chemo-resistance and tumor heterogeneity. Recently, we established long-term 3D CCA organoids which retain the histological architecture, gene expression profile and genomic landscape of the original tumor and are amenable to drug screening approaches. CCA organoids represent a cancer stem cell (CSC)-like phenotype. CSC's are known to be resistant to chemo- and radiation therapy. However, the bulk of cancer cells in a tumor have a differentiated (non-stem cell) phenotype and are more therapy-sensitive. The aim of our study is to establish a model to study both the CSC and differentiated cancer cell phenotypes in CCA organoids and apply this in drug sensitivity screening. Organoids from CCA, non-tumorous adjacent liver and healthy liver tissue (all n=3-6) were differentiated by blocking cancer stem cell signaling pathways. At day 5, cell viability, proliferation, cell death and differentiation potential was tested on gene expression (qPCR) and protein (immunohistochemistry) level and compared to CSC-like organoids. Drug sensitivity of differentiated and CSC-like CCA organoids was tested with sorafenib. Live/dead staining revealed that the level of cell death in differentiated organoids was similar to CSC-type cultures. Differentiated CCA organoids had a reduced proliferative rate, as demonstrated by reduced EdU incorporation and downregulation of Ki67 gene expression ($p<0.001$). Even though proliferation is inhibited, metabolic activity was stable, indicating a higher metabolic activity per differentiated cell. Gene expression analysis showed that known CSC markers LGR5 ($p<0.001$), CD44 ($p=0.01$) and

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CD133 (p<0.01) were downregulated upon differentiation. Preliminary results from a drug sensitivity assay suggested that differentiation of CCA organoids increased their sensitivity to sorafenib. This study shows that it is feasible to differentiate liver tumor-derived organoids from a CSC-like phenotype towards a differentiated cancer cell phenotype. Differentiated CCA organoids are less proliferative, downregulate CSC markers and are more sensitive to sorafenib. More elaborate drug screenings are ongoing in order to more accurately identify effective compounds to treat CCA.

Funding Source
This project is financed with help of ZonMW - InnoSysTox projectnumber 40-42600-98-14017.

Keywords: Primary liver cancer; Cholangiocarcinoma organoids; Tumor stem cells

037

HUMAN INTESTINAL AND AIRWAY ORGANOIDS TO MODEL AND STUDY VIRAL INFECTIONS

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Human intestinal organoids, the first human organoid model derived from adult stem cells, can faithfully simulate the multi-cellular complexity and functionality of human intestinal epithelium. MERS-CoV has caused human respiratory infection since 2012, with a fatality rate over 30%. Airway exposure is intuitively assumed to be the infection route. However, various evidence implicates human may acquire MERS via gastrointestinal tract. We demonstrate that MERS-CoV can robustly replicate in human intestinal organoids. After direct intragastric gavage, the MERS-CoV permissive hDPP4 transgenic mice developed progressive intestinal infection. With the progression of enteric infection, inflammation, virus-infected cells and live viruses emerged in the lung tissues, indicating the development of sequential respiratory infection. Collectively, the results suggest that the human intestinal tract may serve as an alternative infection route for MERS-CoV. In order to study respiratory viral infection, we established long-term expanding human airway organoids which accommodate four types of airway epithelial cells: ciliated, goblet, club, and basal cells. We report differentiation conditions which increase ciliated cell numbers to a nearly physiological level with synchronously beating cilia readily discernible in every organoid. We also established improved 2D monolayer culture conditions for the differentiated airway organoids. To demonstrate the ability of differentiated airway organoids to identify human-infective virus, 3D and 2D differentiated airway organoids are applied to evaluate two pairs of viruses with known distinct infectivity in humans, H7N9/Ah versus H7N2 and H1N1pdm versus an H1N1 strain isolated from swine (H1N1sw). The human infective H7N9/Ah virus replicated more robustly than the poorly human-infective H7N2 virus; the highly human-infective H1N1pdm virus replicated to a higher titer than the counterpart H1N1sw. Collectively, we developed differentiated human airway organoids which can morphologically and functionally simulate human airway epithelium. These differentiated airway organoids can be applied for rapid assessment of the infectivity of emerging respiratory viruses to humans.

Keywords: Human intestinal organoids; Human airway organoids; Virus infection

038

USING INTESTINAL ORGANOIDS TO STUDY THE ROLE OF GROUP 3 INNATE LYMPHOID CELLS (ILC3S) IN INFLAMMATORY BOWEL DISEASE (IBD) PATHOGENESIS

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IBD is characterised by chronic mucosal inflammation. There are two types of IBD: Crohn's disease, where any part of the gastrointestinal tract can be inflamed and ulcerative colitis, which usually only affects the colon. In patients with IBD, mucosal T cells and ILCs respond to microbes by uncontrollably producing cytokines that can promote chronic inflammation. ILCs are divided into 3 groups based on transcription factor expression and cytokine production. ILC3s are further subdivided according to their expression of natural cytotoxicity receptors (NCRs). NCR+ ILC3s contribute to the maintenance of intestinal homeostasis. However, less is known about the role of NCR- ILC3s. To investigate the functional role of NCR- ILC3s the Tbx21-/- Rag2-/- Ulcerative Colitis (TRUC) model of disease was used, as T-bet deficiency results in failure of NCR+ ILC3 development and presence only of NCR- ILC3s. Interestingly, we found that in TRUC disease IL22 produced by colonic NCR- ILC3s had a pathogenic role. The IL22 receptor (IL22R1) is expressed in the epithelial cells of the colon, so to understand the mechanisms by which IL22 mediates colitis, we treated mouse colonoids with recombinant IL22 and conducted transcriptomic analysis. Comparison of microarray data from IL22-treated colonoids with data from the colon of TRUC mice revealed that IL22 responsive transcripts, as identified in IL22-treated colonoids, were significantly enriched in the colon of TRUC mice. Pathway analysis showed, as expected, changes in the expression of transcripts involved in microbial sensing, anti-microbial responses and barrier function. However, it also showed changes in the transcript levels of endoplasmic reticulum (ER) stress response genes, a pathway not previously linked to ILCs. Upregulation of ER stress response genes was confirmed by real time PCR. This effect was enhanced in the presence of IL17, another cytokine secreted by NCR- ILC3s. IL22-induced ER stress affected all colonic epithelial cells, not only stem cells, and caused ER stress-induced apoptosis. To conclude, our findings indicate that IL22 and IL17 co-produce by NCR- ILC3s can drive chronic colitis through induction of epithelial ER stress.

Keywords: IBD; IL22; ER stress

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039

SYNAPTIC DEFECTS AND IMPAIRED AUTOPHAGY IN A HUMAN IPSC-BASED MODEL OF FAMILIAL PARKINSON'S DISEASE

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Parkinson's disease (PD) remains an incurable neurodegenerative disorder with variable clinical characteristics, age of onset and course of progression. The hallmark of PD, whether sporadic or familial, is the deposition of protein aggregates, which are composed mainly of alpha-synuclein (αSyn). αSyn is a pre-synaptic protein with N-terminal binding to acidic lipids that can sense and generate changes in membrane curvature, suggesting its participation in presynaptic events, including endocytosis and exocytosis whilst its involvement in pre-synaptic organization has been postulated in mice. However, the mechanisms through which mutant αSyn affects synaptic organization in a human setting remain unknown. αSyn is the major gene linked to sporadic Parkinson's disease, while the G209A (p.A53T) αSyn mutation causes a familial form characterized by early onset and a generally severe phenotype, including non-motor manifestations. In this study, using cell reprogramming technologies, we have developed a robust induced pluripotent stem cell (iPSC)-based model of PD from patients harboring the p.A53T-αSyn mutation that faithfully simulates disease pathogenesis and uncovers novel disease-relevant phenotypes at basal conditions, including protein aggregation, compromised neuritic outgrowth and contorted axons with swollen varicosities containing αSyn and tau, as well as reduced synaptic connectivity. Global transcriptome analysis suggested defects in synapse formation and function. Electron microscopy of p.A53T neurons indicated impaired organization of synaptic vesicle pools, microtubule disorganization and a striking accumulation of autophagic vacuoles. In agreement, impaired autophagic activity and lysosomal protein degradation was shown by immunofluorescence and biochemical analysis. Finally, artificial synapse formation assay was used to study synaptogenesis of p. A53T neurons and monosynaptic rabies virus tracing to assess p.A53T neuronal circuitry. We aim to complement our investigations in 2D cultures with the development of a novel 3D model for synucleinopathy that will address how p.A53T affects the spatiotemporal organization of the neuronal network. The p.A53T-model can be used to answer fundamental questions for PD pathogenesis and serve as a new drug-testing platform.

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Keywords: Parkinson's disease; α-synuclein; Synaptopathy

040

HIGH-FIDELITY DISEASE MODELLING OF SKELETAL MUSCLE LAMINOPATHIES USING THREE-DIMENSIONAL HUMAN IPS CELL-DERIVED BIOENGINEERED MUSCLES

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Laminopathies are severe heterogeneous genetic diseases caused by mutations in A-type lamins, which are encoded by the LMNA gene. These proteins together with Lamin B1 and B2 form the nuclear lamina: a mesh-like structure located underneath the nuclear membrane which helps maintaining nuclear shape and regulating gene expression. Laminopathies affect multiple cell types and can be tissue-specific or systemic, with some subtypes affecting striated muscle, peripheral nerve and adipose tissue, while others cause multisystem disease with accelerated aging. Although several mechanisms have been proposed, the exact pathophysiology of laminopathies remains unknown; additionally, the rarity of the disorder and lack of easily accessible cell types for ex vivo studies negatively impact on therapy development. To overcome these hurdles, here we used induced pluripotent stem (iPS) cells from patients with skeletal muscle laminopathies such as LMNA-related congenital muscular dystrophy and limb-girdle muscular dystrophy 1B, to model disease phenotypes in vitro. iPSC lines from three skeletal muscle laminopathy patients were differentiated into skeletal myogenic cells and myotubes. Disease-associated phenotypes were observed in all genotypes, including abnormal nuclear shape and mislocalisation of nuclear lamina proteins. Notably, complex modelling in three-dimensional (3D) artificial muscle constructs resulted in recapitulation of nuclear abnormalities with higher fidelity than standard bi-dimensional cultures and identified nuclear length as a robust and objective outcome measure. Finally, we will present and discuss current efforts and future applications of this novel 3D organoid-like platform for therapy development and drug screening for skeletal muscle laminopathies. These results demonstrate that patient-specific iPS cells can model phenotypic readouts of skeletal muscle laminopathies with high fidelity upon 3D differentiation in vitro, laying the foundation for future therapy screening platforms for skeletal muscle laminopathies and other severe muscle disorders.

Funding Source
BBSRC, European Research Council, EU FP7 projects PluriMes & EBiSC, AFM, MDUK and National Institute for Health Research (the views expressed are those of the authors and not necessarily those of the NHS, NIHR or Department of Health).

Keywords: Artificial skeletal muscle; Disease modelling; iPS cells

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MODELLING IDIOPATHIC PARKINSONS DISEASE IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED MIDBRAIN ORGANOIDS

Monzel, Anna S.[1], Cardoso Pereira, Sandro L.[1], Badanjak, Katja[1], Wasner, Kobi[1], Antony, Paul[1], Bolognin, Silvia[1], Ghelfi, Jenny[1], Ouzren, Nassima[1], Treff, Antoine[1], Zagare, Alise[1], Krüger, Rejko[1], Diederich, Nico[2], Seibler, Philip[3], Klein, Christine[3], Grünewald, Anne[1], Schwamborn, Jens C.[1]

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Parkinson's disease (PD) is the most common age-related motoric neurodegenerative disorder. The motor deficits, including tremor, rigidity and bradykinesia, result from the loss of dopaminergic neurons (DNs) in the substantia nigra and the lack of the neurotransmitter dopamine in the striatum. Over the last decades, several genetic variants have been suggested to either cause or increase the risk of PD. Nevertheless, most cases are idiopathic and the underlying environmental triggers remain elusive. Our current understanding of PD suggests a multifactorial nature of the disease. Research on PD and other neurological disorders is limited by the lack of advanced experimental in vitro models that truly capture the complex nature of the human brain. Although animal models recapitulate some aspects of human physiology, they often fail to recreate specific pathogenic events, with low success rates in clinical trials. Thus, classical 2D cell culture approaches with isolated cell types poorly model human physiology due to distinct cellular microenvironments. In contrast, advanced human induced pluripotent stem cell (hiPSC)-derived 3D models represent patient-specific in vitro systems, which mimic certain aspects of the physiological situation and can reproduce specific features of human diseases. Here we present the usage of hiPSC-derived neuroepithelial stem cells (hNESCs), thereof derived midbrain dopaminergic neurons (mDNs) and complex 3D human midbrain organoids (hMOs) as idiopathic PD (IPD) in vitro models. Using these cell culture models of the different developmental stages, we investigate cellular hallmarks of PD such as mitochondrial dysfunction, oxidative stress and DN degeneration in a longitudinal fashion. Our studies include the evaluation of distinct mitochondrial DNA parameters, such as deletions, copy number and replication/transcription rate as well as proteomic and transcriptomic data and high-content image analysis. In the hMOs in particular we explore differential dynamics of neuronal dopaminergic specification in IPD patients. Overall, this project aims to capture the complex nature of PD and to clarify molecular mechanisms underlying the degeneration of DNs in IPD.

Funding Source
Fonds National de la Recherche Luxembourg.

Keywords: Parkinson's disease; Human midbrain organoids; Dopaminergic neurons

042

GENERATION OF DISEASE MODEL OF NEONATAL DIABETES MELLITUS WITH INS GENE MUTATION IN HUMAN

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One of the causes of diabetes in infants is the defect of the insulin gene (INS). The mutations that lead to hypoinsulinemia are usually heterozygous. The cause of the absolute deficiency of insulin in patients with heterozygous mutations is still not clear. Despite the presence of one normal allele of the insulin gene, the disease is manifested with the absolute deficiency in insulin in blood. We aimed to generate an isogenic system, i.e. a pair of insulin-producing cell lines with the same genome with the presence and the absence of a mutation in the INS gene. Patient specific iPS cell lines were generated from primary fibroblasts by non-integrative method of reprogramming from a patient with an intronic mutation of INS gene. The mutation leads to emergence of an ectopic splice site within the INS gene and clinically manifested at 6 months as a rare disease: permanent neonatal diabetes mellitus (PNMD). This mutation was edited by CRISP-Cas9 system by deletion of the mutant splice site. The editing led to the restoration of the RNA sequence of INS gene. IPSC lines with mutant INS and with edited INS were differentiated to the pancreatic beta cells to confirm the restored function in the edited cell line and to study the pathogenesis at the molecular and cellular level in an isogenic model.

Keywords: CRISPR-Cas9; iPSC; Permanent neonatal diabetes mellitus

043

EFFICIENT DIFFERENTIATION OF MACROPHAGES FROM HUMAN PLURIPOTENT STEM CELLS AND THEIR APPLICATION IN MODELING INFLAMMATORY DISEASES IN 3D ORGANOIDS

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Macrophages derived from human pluripotent stem cells (hPSCs) can be used to model inflammatory or autoimmune diseases, and may be genetically engineered for immune therapy. We established a monolayer, chemically defined culture system to induce hematopoietic differentiation and macrophage formation from hPSCs. We found that insulin-free medium allowed hPSC to leave pluripotency promptly and preferably enter the vascular lineage. Addition of insulin during later stage of differentiation was essential for the efficient induction of hemogenic endothelium and the emergence of large numbers of CD34+CD43+ hematopoietic stem/progenitor cells (HSPCs), while no insulin condition preferably permit endothelial differentiation. HSPCs generated from our protocol can be subsequently induced to form macrophages (iMacs) at high efficiency. Global

transcriptome profiling revealed that iMacs differentiated using our protocol was similar to macrophages developed in vivo. IMacs demonstrated robust phagocytic ability and upregulated inflammatory cytokines upon recombinant model pathogen stimulation. These iMacs are included in a range of 3D organoids as tissue residential immune cells to study inflammatory responses that implicated in many human diseases.

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Keywords: Human pluripotent stem cells; Differentiation; Macrophages

045

A BREAST CANCER ORGANOID INVASION MODEL IN A SYNTHETIC BIOELASTIC MATRIX

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Breast cancer invasion is a mechanically regulated process that critically depends on a subset of tumor cells with basal properties. During the malignant progression of breast cancer, these basal cells, expressing basal cell markers such as cytokeratin (CK) 14 are found at the invasive front of a tumor. Mammary tumor organoids derived from MMTV-PyMT mice grow with benign morphology under standard conditions in Basement Membrane Extract (Laminin 111 and Collagen IV) but transform into invasive tumors when transferred to a collagen I matrix. The transformation from benign to invasive form is accompanied by the appearance of CK14 positive basal cells in the invasive parts of the tumor. It is clear that adhesion-controlled changes in tumor stiffness are crucial in malignant transformation. However, which specific adhesive interactions are involved and how they could be targeted to inhibit tumor metastasis is not yet known. In this study, we set out to increase our understanding of such adhesive interactions, by using a synthetic, fully defined matrix based on polyisocyanopeptide (PIC) hydrogels. PIC hydrogels were chosen for this study since they, uniquely among synthetic hydrogels, exhibit the same strain-stiffening mechanical properties as collagen and other ECM proteins. In addition, they can be functionalized with various cell-binding peptides derived from ECM proteins. MMTV-PyMT mouse tumor organoids exhibited a benign morphology in most PIC hydrogels but transitioned to an invasive morphology with CK14 expressing cells at the tips of invasive outgrowths when grown in PIC matrices modified with collagen peptides and tuned to specific stiffnesses. Thus, we developed a tumor invasion model with increased control over adhesive interactions and mechanical properties for targeting adhesive interactions responsible for invasive behavior. The outcomes of our research will increase our understanding on the control over cellular mechanics, may drive or prevent malignant transformation, and ultimately help in guiding more effective therapeutic strategies.

Keywords: Invasion; Tumor Organoids; Hydrogels

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ORGANOID MODELING OF THE REPRODUCTION-CRUCIAL ENDOMETRIUM IN BOTH HEALTHY AND DISEASED STATE

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The endometrium is of crucial importance for mammalian reproduction and undergoes dynamic reiterative tissue remodeling during the menstrual cycle. Knowledge on the cellular and molecular underpinnings of endometrium biological remodeling is poor, as well as on the processes that go awry in endometrium pathogenesis. This limited understanding is primarily due to the lack of research models reliably recapitulating endometrium biology and disease in nature and heterogeneity. Therefore, we embarked on the development of organoid models from human healthy endometrium as well as from a wide spectrum of endometrial diseases. We established organoids from healthy endometrium biopsies which reproduced molecular, histological and cellular phenotype of the tissue's epithelium. The organoids phenocopied the physiological responses to reproductive hormones thereby mimicking the menstrual cycle at both morpho-histological and molecular level. Furthermore, the organoids showed long-term expansion capacity while remaining genomically, transcriptomically and functionally stable. Transcriptomic profiling advanced candidate markers of the still undefined endometrium epithelial stem cells. Long-term expandable organoids were also established from a broad range of endometrium pathologies, ranging from endometriosis and endometrium hyperplasia to low and high grade endometrial cancer. The organoids obtained recapitulated characteristics of the patients' diseased tissue and faithfully captured the clinical heterogeneity of the different pathologies regarding histological markers, differentiation status, genomic alterations and drug responses. Finally, endometrial disease organoids reproduced the original lesion when transplanted in vivo. Taken together, we established new organoid models for endometrium and a wide spectrum of endometrial diseases, thereby providing powerful tools to decipher the mechanisms underlying the biology (including embryo receptivity) and pathology of this key reproductive organ. The eventually generated organoid biobank will allow to in-depth explore genetics and pathway alterations in diverse forms of endometrium pathology, and at the same time serve as screening platform to test (new) drugs, even in a patient-personalized manner.

Keywords: Endometrium; Reproduction; Endometrial cancer

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047

IDENTIFYING THERAPEUTIC TARGETS IN THE INTRINSIC TUMOR IMMUNITY GENETIC PROGRAM USING PATIENT-DERIVED GLIOBLASTOMA ORGANOIDS

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Glioma research currently lacks patient-derived platforms that can effectively recapitulate gliomagenesis in vitro to guide the search for molecular vulnerabilities and screen for therapies. Tumor organoid cultures have been generated from several cancers; however, it is unknown whether organoids possess the tissue organization and genetic programs of glioblastoma multiforme (GBM), or if they can be leveraged to determine translational targets. Here, we describe a multi-level cellular, molecular discovery platform using cultures of patient-derived GBM organoids (GBMO) to recapitulate the unique histologic and genetic architecture of GBM and further understand GBM intrinsic immunity. We characterized GBMOs by comparing their cytoarchitecture with iPSC-derived organoids and molecular landscape with NSC-derived organoids. GBMO mimics stereotypic tumor architecture with distinct proliferative and apoptotic microenvironments but lacks the developmental self-organization recapitulated in iPSC organoids, despite hosting basal and outer radial glia. Targeted deep-parallel sequencing of GBMO validated a mutational load consistent with GBM, including ATRX and KDM6A mutations, while transcriptome data showed robust activation of cancer hallmarks, like the Warburg Effect. Notably, we devised a computational approach for identifying drivers of tumor immunity from transcriptome data to find an innate immunity landscape in GBMO enriched for aryl hydrocarbon receptor (AHR) genes, leading to the identification of an AHR-dependent program. Pharmacological and genetic inactivation of AHR revealed this program to be critical for glioblastoma stem cells (GSCs) and organoid evolution. Enhanced survival of mice intracranially injected with AHR-inactivated GSCs reinforces the in vivo relevance of our GBMO paradigm. Finally, we highlight the value of our organoid platform for discovering 41 prognostic candidate GBM biomarkers, 19 of which are novel, and expression signatures important for population-level survival by using advanced statistical models. Our work demonstrates, for the first time, the utility of patient-derived GBMO as a personalized-medicine approach toward in vitro pre-clinical trials for identifying molecular vulnerabilities and advancing therapy for this devastating tumor.

Keywords: Glioblastoma; Organoids; Radial glia

048

FRAGILITY OF DEVELOPMENTAL TRAJECTORIES OF HUMAN CORTICOGENESIS REVEALED BY SINGLE INFLAMMATORY PERTURBATION OF CEREBRAL ORGANOIDS

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Despite strong impetus from the recent Zika outbreak, resurgence of neurotoxic viral infections, and clear association to neurodevelopmental disorders, the fundamental principles by which maternal infection and prenatal inflammation impact human brain development remain unclear. Here we adopted a reductionist, systems neurobiology approach and perturbed developing human cerebral organoids derived from healthy donor iPSCs with proinflammatory cytokine interferon-γ (IFN-γ), the topmost hierarchal molecule of the antiviral response cascade in radial glia (RG), to unravel the downstream developmental consequences of a single perturbagen on a complex biological system. IFN-γ exposure restricts organoid growth across several concentrations and timepoints. Utilizing fluorescent DiD labeling, we visualized the RG scaffold in organoids to find marked underdevelopment of progenitor zones following IFN-γ administration. Subsequent immunostaining showed that IFN-γ exposure alters cell cycling of RG, disrupts adherens junctions of ventricular RG, and diminishes outer RG proliferation, leading to alteration of the prototypical radial unit. Likewise, RNA-sequencing revealed IFN-γ dysregulates the normal spatiotemporal expression pattern of the developing brain, activating postnatal programs while downregulating early neurogenic pathways. Importantly, inflammatory induction by IFN-γ also reshapes developmental hierarchies of organoid corticogenesis toward a ventral cell fate with increased GABAergic lineage output. This GABA-glutamatergic neuronal imbalance is accompanied by dysregulation of protein and ribosomal synthesis, both of which are implicated in autism spectrum disorder (ASD). Bioinformatic analyses further suggest the IFN-γ organoid transcriptome signature to be aligned with ASD and maternal infections, opposed to congenital neurodevelopmental disorders. Finally, mathematical modeling indicates IFN-γ perturbation affects nonlinear cell dynamics in a multilineage, parallel manner. Altogether, our results highlight the feasibility of a single perturbation organoid paradigm to model environmental, non-genetic determinants of human brain development and reveal a novel molecular and cellular link between neuroinflammation and ASD-like phenotypes.

Funding Source
OSU NRI, Nationwide Children's Hospital.

Keywords: Neuroinflammation; Infection; Neurogenesis

049

GENE CORRECTION REVERSES PHAGOCYTOSIS IN IHPSC-DERIVED RETINAL PIGMENT EPITHELIAL CELLS FROM RETINITIS PIGMENTOSA PATIENT

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Hereditary retinal dystrophies (HRD) are an important cause of blindness affecting mostly retinal pigment epithelium (RPE) and photoreceptors (PRs), with no effective treatments. Both cell types are highly specialized, interact mutually in retinal function and it is thought that the majority of the HRD are primary in one cell type leading to a secondary dystrophy in the other one. One of the monogenic primary RPE dystrophies includes mutations MERTK gene that has essential role in phagocytosis, one of the major functions of the RPE. One of the strategies to treat this rare disease is to replace diseased RPE with healthy autologous RPE tissue to prevent PR cell degeneration. Patient-derived induced pluripotent stem cells (ihPSCs) provide an unprecedented opportunity to create autologous stem cell source that is able to differentiate to virtually any human cell type including RPE and PRs. The major drawback of ihPSC cell therapy is that affected cells derived from ihPSC will carry the same MERTK gene mutation. Recently we reported the generation of a cellular model of MERTK-associated RP, which recapitulates the disease phenotype and efficiently genetically corrected MERTK. In order to determine whether gene corrected ihPSC is a faithful source for MERTK-associated RP in this study we efficiently differentiated ihPSC from corrected and patient´s ihPSC and showed that gene corrected ihPSC-RPE reverse the full expression of MERTK protein as well as reinstate phagocytosis of labelled photoreceptor outer segments. These findings represent a proof-of-principle the usefulness of gene correction in ihPSC to provide unlimited cell source for potential personalized cell therapy for these rare blinding disorders.

Keywords: Retinitis pigmentosa; Gene correction; Phagocytosis

051

MODELLING MICROENVIRONMENTAL MECHANICAL PROPERTIES IN DUCHENNE MUSCULAR DYSTROPHY IPSC-DERIVED CARDIOMYOCYTES

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Duchenne Muscular Dystrophy (DMD) is an X-linked disease affecting ~1:3500 boys per year that culminates in heart failure in early adulthood. DMD results from >200 possible genetic mutations on dystrophin. The lack of dystrophin disrupts the anchoring of the cell sarcomere to the extracellular matrix (ECM), affecting cardiomyocyte contraction. With disease progression, tissue increases in stiffness due to fibrosis and changes in ECM composition in accordance with a dilated cardiomyopathy phenotype. We hypothesize that this entails a positive feedback loop involving multiple mechanosensing pathways. Here, we use a single-cell platform to model the fibrotic remodeling in DMD. We measure the force production of single human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) on hydrogel substrates with a stiffness matching that of healthy (~10kPa) or fibrotic (~35kPa) tissue. In addition, single iPSC-CMs are patterned in an elongated 1:7 aspect ratio by using microcontact printing of ECM proteins. This enhances their intracellular structural maturity and enables standardized measurements on consistently oriented elongated contractile cardiomyocytes. We use traction force microscopy to computes the contractile strength as a function of bead displacement in the hydrogel substrate using Digital Image Correlation (DIC) and Fourier Transform Traction Cytometry (FTTC). We show that DMD hiPSC-CMs have a dramatically reduced ability to produce force on stiffer substrates compared to their isogenic controls. This loss of contractile function correlates with an increase in reactive oxygen species (ROS) and mitochondrial dysfunction. The effect of stiffness in this difference in contractile function uncovers a potent role of mechanosignaling mediated by the dystroglycan complex. This platform will increase our understanding of the biophysics underlying cardiomyocyte mechanosensing.

Funding Source
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Keywords: Human induced pluripotent stem cells; Duchenne Muscular Dystrophy cardiomyopathy; Mechanosensing

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MODELLING CANCER IMMUNOMODULATION USING EPITHELIAL ORGANOID CULTURES

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Immune escape has been recognised as one of the hallmarks of cancer. Overcoming this immunomodulatory process by tumour cells has become a major therapeutic target. Suitable model systems are missing to study this process in culture and test possible drugs. Organoid technology using epithelial cells has emerged as a powerful tool for the ex vivo modelling of tissue morphogenesis, cell differentiation and cancer biology. Yet, epithelial (cancer) organoid cultures lack all non-epithelial components of the tissue microenvironment such as immune cells. However, using transcriptional profiling and flow cytometry, we found that human colorectal cancer (CRC) organoids maintain differential expression of immunomodulatory molecules present

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in primary tumours. We hypothesised that this may allow us to study immune escape using CRC organoid cultures. To assess anti-tumour immunity and possible cancer immunomodulation, we developed a method of co-culturing CRC organoids with antigen-specific cytotoxic T cells. In this proof-of-principle assay, CRC organoids presenting specific peptides were readily killed by these cytotoxic T cells expressing matched T-cell receptors (TCRs). We did not observe significant CRC organoid cytotoxicity with a peptide-TCR mismatch. By applying the cytokine interferon gamma, we found that upregulation of immune checkpoints by CRC organoids suppressed T-cell mediated killing in the co-culture. By application of immune checkpoint inhibitors, we could reinstate T-cell mediated killing of CRC organoids, demonstrating that this method can be used to model cancer immunodulation and test cancer immunotherapy drugs. Our co-culture system may further serve as a first step towards rebuilding the tumour microenvironment in vitro.

Keywords: Organoids; Colorectal cancer; Cancer immunotherapy

053

EMERGENCE OF CELLULAR HETEROGENEITY IN LIVER METASTASES OF HUMAN COLORECTAL CANCERS IS ESSENTIAL FOR SUSTAINED GROWTH

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Accumulating evidence suggests that tumors are caricatures of normal tissues, where cancer stem cells are believed to be the driving forces of tumor growth. To identify intestinal stem cells (SCs) in the organoid system, the Snippert group has developed the SC reporter STAR, a minigene that reports activity of the transcription factor ASCL2, the master regulator of intestinal stem cell fate. Previous experiments in the Snippert group have shown that single colorectal cancer cells grow into organoids regardless of their STAR positivity. Intriguingly, while monitoring stem cell activity over time, we noticed that emergence of cellular heterogeneity is essential for tumor growth since complete stem cell organoids remained small and unsuccessful. Strikingly, the strict interdependency between cancer stem cells and neighboring support cells that underlies tumor growth seems to be independent of the mutational background of the organoids. In particular, this also holds true for highly mutated organoids with 'so-called' metastatic niche-independency. Importantly, these 'symmetry breaking' phenotypes are in agreement with the initial growth of liver metastases of a murine CRC model (VillinCre-ERT2; APCfl/fl; KRASLSL-G12D/+; P53KO/KO; R26R-Confetti; Lgr5DTR-eGFP), where emergence of heterogeneity between stem and non-stem cells correlates with the transition between micro- and macrometastases. In conclusion, we identified the existence of a universal signalling mechanism between cancer stem and non-stem cells – a cross-talk that is essential for metastatic tumor growth. We will combine our expertise in human organoid technology, high-resolution live imaging of tumor organoids and in vivo mouse models for in-depth characterization of the signalling crosstalk that support cancer stem cell function, especially in metastatic situations.

Keywords: heterogeneity; cancer stem cells; metastases

054

TOWARDS AN IN VITRO MODEL OF WILMS TUMOR: GENERATING TUMOR-ASSOCIATED MUTATIONS IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED KIDNEY ORGANOIDS

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Wilms tumor (WT) is the most common pediatric kidney cancer. Blastemal-predominant tumors represent the most malignant and chemotherapy-resistant form of WT. This tumor subtype expresses markers found only within the nephrogenic niche of the fetal kidney, suggesting it arises from a malignant transformation of these cells. Sequencing of blastemal-predominant tumors has identified a recurring single amino acid mutation, Q177R, within the conserved DNA binding domain of either of the closely related transcription factors SIX1 and SIX2. These factors are critical for the maintenance of nephron progenitors during mammalian kidney development. Therefore, we hypothesize that this recurring mutation disrupts the regulatory networks controlled by SIX1/2 resulting in aberrant gene expression, an inability of nephron progenitors to fully differentiate, and their malignant transformation. Efforts to model WT in mice have proven challenging and differences in gene expression dynamics between mouse and human nephrogenesis, including the temporal expression of SIX1, necessitates a novel system in which to investigate the tumorigenic potential of the Q177R mutation. HiPSC-derived kidney organoids recapitulate key stages of human kidney development in vitro, resulting in the generation of complex nephron-like structures. Based on previously developed protocols, we have established our own minimal, modified 3D protocol to reproducibly generate kidney organoids for use as a model system. With a focus on SIX1, ChIP-qPCR revealed proper targeting of SIX1 to its canonical DNA targets beginning by day 5 with significantly increased binding through day 8 of the protocol, recapitulating the in vivo activities of SIX1 during human nephrogenesis. Utilizing the Sleeping Beauty transposon system, we have exploited the defined differentiation timeline of our kidney organoid protocol to express the mutant SIX1 protein at critical developmental time points to elucidate the tumorigenic program initiated by the Q177R mutation. Our results will shed light onto the etiology of WT, opening up the possibility of new, targeted therapies for this disease.

Funding Source
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Keywords: Organoids; Tumorigenesis; Transcription

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AN ALPHA-ACTININ REPORTER CELL LINE TO MODEL SARCOMERIC DYNAMICS IN HUMAN HESC-DERIVED CARDIOMYOCYTES RELATIVE TO SUBSTRATE STIFFNESS

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Cardiovascular disease is a major cause of morbidity and mortality in modern society, being responsible for 37% of all deaths in the EU in 2017 and an overall estimated cost to the EU economy of 210 billion euro annually. Cardiac disease is often accompanied by remodeling of the heart wall (for example following myocardial infarction), resulting in cardiomyocyte hypertrophy and increased extracellular fibrosis in response to stress or injury. Although cardiac remodeling may be an adaptive response at first, cardiomyocyte hypertrophy and cardiac fibrosis, resulting in an increased wall stiffness, may ultimately lead to end-stage heart failure. Previous work demonstrated that cardiomyocytes are less effective in applying strain on stiff substrates than on physiologically elastic substrates, leading, in combination with the increased stiffness itself, to diastolic heart failure. Understanding the underlying mechanisms of cardiomyocyte adjustment to increased wall stiffness may provide a better insight in how to preserve or restore heart function. We generated an α-actinin-mRubyII (fusion protein) fluorescent reporter line in a previously documented cardiac NKX2.5-GFP human embryonic stem cell (hESC) reporter line using CRISPR-Cas9 technology. This α-actinin-mRubyII-NKX2.5-GFP-hESC double fluorescent reporter line allows visualization and live monitoring of z-disks during cardiomyocyte contraction. Using this reporter cell line, we analyzed sarcomeric dynamics in cardiomyocytes cultured on fluorescent bead-laden polyacrylamide gels with a stiffness ranging from below physiological (4 kPa) heart conditions to pathologically high (>100 kPa) levels. We found that absolute sarcomeric shortening, but not contraction time, depends on substrate stiffness. In addition, sarcomeric elongation during relaxation is more rapid on stiff substrates rather than compliant substrates. Furthermore, we will compare sarcomeric contraction and relaxation to contractile strain applied on the substrate by the cardiomyocyte. For this, we will determine the applied strain by quantifying bead displacement in the acrylamide gel, as previously described. With this data, we aim to demonstrate the validity of an α-actinin fluorescent cell line in a model for myocardial stiffness.

Keywords: heart failure; sarcomere dynamics; reporter cell line

056

HEMATOPOIETIC DIFFERENTIATION POTENTIAL OF INDUCED PLURIPOTENT STEM CELLS DERIVED FROM OSTEOPETROSIS PATIENTS' PERIPHERAL BLOOD MONONUCLEAR CELLS

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Malignant infantile osteopetrosis (MIOP) is a rare inherited disease characterized by increased bone mass and density resulting from defects in osteoclast formation and/or function. Only available therapeutic option is allogeneic stem cell transplantaion. Disease modeling with patient-derived IPS cells for better understanding of disease biology could pave the way for development of new therapies. Aim of this study is to evaluate hematopoietic differentiation potential of osteopetrotic-iPSC carrying TCIRG1 mutation, one of the most common mutations identified in autosomal recessive form, and their functionality in in vitro culture system. Banked peripheral blood MNC samples were used as a cell source because of ease of obtaining cells with a minimally invasive method. Erythroid progenitor cells (EPC) were expanded using erythroid expansion medium. Expression of CD45, CD34, CD36, CD235a and CD3 surface marker was checked prior to reprogramming. Enriched PB-EPC of three patients and one healthy donor were reprogrammed by using CytoTune-IPS 2.0 Sendai (SeV) kit. iPSC colonies which started to appear around day 12-14 were picked, manually passaged and expanded in matrigel-coated plates. Colonies were characterized (≥ 3 clones/line) by colony morphology, flow cytometry, alkaline phosphatase (AP) and immunofluorescence (IF) staining. Expression of pluripotency genes, detection of SeV were evaluated with PCR. In vitro pluripotency was assessed by embryoid body (EB) assay. iPSC lines exhibiting typical ESC-like colony morphology were shown to express pluripotency markers (OCT4, SSEA-4, SOX2, TRA-1- 60) by IF staining. 85-99% of the cells were found positive for SSEA-4 and Oct3/4 and negative for CD36 and CD235a with flow cytometry. All iPSC lines expressed pluripotency related genes (OCT4, SOX2, c-Myc, Klf4, Nanog, DNMT3B, CDH1, UTF1, REX1, TERT). Loss of SeV genome was observed at later passages. Fluorescence staining and lineage specific gene expressions (α-SMA, MAP-2 and SOX17) of EB and EB-based hematopoietic differentiation experiments of iPSC are in progress. In conclusion, results of this study would provide valuable information regarding the impact of osteoclast defect on the cellular components of hematopoietic niche specifically hematopoietic stem compartment in patients with MIOP.

Keywords: Osteopetrosis; induced pluripotent stem cells; hematopoietic differentiation

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CRISPR-INDUCED 'SECOND HIT' MUTATIONS IN APC CAUSE DIFFERENTIATION OF HESCS DERIVED FOLLOWING PREIMPLANTATION GENETIC DIAGNOSIS FOR FAMILIAL ADENOMATOUS POLYPOSIS

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Inactivation of adenomatous polyposis coli (APC), a major controller of Wnt signaling, is one of the most important genetic alterations in the development of colorectal cancer (CRC). Familial adenomatous polyposis (FAP) patients carry a single mutated APC allele, which invariably leads to CRC at a relatively young age due to sporadic loss of the remaining functional allele. Our goal in this study is to establish the role of APC in human embryonic stem cells (hESCs) and their derived colon organoids in order to understand its contribution to CRC carcinogenesis. Three FAP-hESC lines carrying different germline mutations in the APC gene were derived in our lab from blastocysts following preimplantation genetic diagnosis (PGD) from three unrelated FAP-affected families. We used CRISPR with specific gRNAs to target the APC gene in these FAP-hESC lines in order to induce the somatic mutation in two sites in the second (wild-type) allele of the gene. A total of 59 clones were isolated, and 11 (19%) of them were identified by NGS as carrying CRISPR-induced mutations. Interestingly, all clones were found by western blot analysis to express the full-length APC protein despite harboring frameshift mutations in one allele indicating that the targeted allele was the germline mutant in all cases. A follow-up time course FACS analysis of FAP-hESCs carrying a β-catenin GFP reporter that were subjected to CRISPR mutations in the APC gene revealed that the proportion of APC double-mutated cells (GFP+) declined significantly with time, a result that was also confirmed by NGS. RNA extracted from double mutant cells isolated by FACS sorting demonstrated relatively high expression of the endodermal markers, CDX2 and FOXA2, and low expression of the pluripotent genes, Nanog and Oct4, indicating that their loss during long-term culture is due to differentiation. In conclusion, APC knockout in hESCs results in Wnt signaling activation and loss of pluripotency, highlighting the central role of this pathway for hESC self-renewal. We are now generating colon organoids from these hESCs in order to further understand the role of APC in CRC. Inducing the APC mutations in the differentiated cells of the organoids will allow further clarification of the roles of APC in more clinically relevant cells.

Funding Source
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Keywords: Adenomatous polyposis coli (APC); Familial adenomatous polyposis (FAP); Human embryonic stem cells (hESCs)

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DIGEORGE SYNDROME; MODELING A COMPLEX DISORDER

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Results describing molecular mechanisms underlying complex disorders are limited by currently available model systems. Human iPSC-derived cell cultures are new, promising models for studying disease related phenotypes in vitro especially in cell types which cannot be investigated directly and maintained in long-term cultures (such as neural or cardiovascular cells) or when appropriate animal models are not available (such as psychiatric diseases). Studying the entire differentiation process may provide insights to the pathomechanism and disease development. Our aim is to investigate in vitro cellular phenotypes of DiGeorge syndrome, a genetically well-determined, multiorgan (cardiovascular, nervous and immune systems) disease caused by a microdeletion in 22q11.2. For our investigations we have chosen a family with inherited form of the syndrome and blood was taken from the index individual, a severely affected child, her mother and grandfather (all carriers of the deletion) and from father and grandmother as healthy controls. Reprograming of peripheral blood mononuclear cells to pluripotent state was performed by forced expression of four transcription factors – Oct3/4, Sox2, Klf4 and c Myc – using Sendai virus vectors. Stabilized iPSCs lines were genetically analyzed and subsequently differentiated into cell types relevant to the diseases by mimicking the in vivo developmental program. We performed cardiac and neural differentiation and disease-affected cells were compared to the control cells. Our data show impaired neural development and altered beating activity in patient-derived cells.

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Keywords: induced pluripotent stem cells; DiGeorge syndrome; directed differentiation

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CEREBRAL ORGANOIDS AS A NEW WAY TO MODEL ADHD PATHOPHYSIOLOGY: A LOOK AT MOLECULAR, CELLULAR AND CONNECTIVITY DEFICITS

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Attention deficit hyperactivity disorder (ADHD) is a heterogeneous neurodevelopmental disorder with a devastating impact on the quality of life of millions of children, adolescents and adults. While ADHD is thought to be highly heritable with unknown etiology, it is is likely to involve a combination of environmental factors and the contribution of multiple genes defects. To understand the molecular underpinnings of ADHD, we hypothesize that 3D neuralized structures (organoids) derived from patient-specific induced pluripotent stem cells (iPSCs) can be used as a potential platform. In particular, the Prefrontal Cortex (PFC) is emerging to be of central relevance to the neural pathways of ADHD, as it connects extensively to sensory and motor cortices, as well as to the basal ganglia and cerebellum. These areas are intricately interconnected and modulated by a mesh of neurons that in ADHD display heavy deficits in dopaminergic and noradrenergic transmission. Thus, it is critical to understand the molecular influences modulating PFC's function in order to develop novel medications for patients afflicted with the disorder. We have started to generate and characterize iPSC-derived cortical organoids from ADHD patients and healthy sibling controls to study the molecular and cellular differences in corticogenesis between diseased and control brains. Particularly, we propose that the root cause of the PFC's smaller structure involves a limited progenitor pool and impaired radial migration. To achieve these long-term goals, we attempted to use our novel and non-viral reprogramming methods to generate high quality control and ADHD-iPSC lines, to optimize in vitro organoid generation. Our approach will facilitate examination of how disease risk is translated at the cellular and tissue levels through comparative studies of processes such as progenitor cell proliferation, migration and connectivity during development.

Funding Source
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Keywords: ADHD; Corticogenesis; Progenitor

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DYNAMIC HYDROGELS TO MODEL FIBROSIS IN VITRO

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Despite the ubiquitous role of fibrosis in tissue dysfunction arising from aging and disease, no adequate in vitro model of the fibrotic microenvironment exists. Fibrosis is characterized by excess extracellular matrix (ECM) deposition that leads to a progressive stiffening of the cellular microenvironment. Thus, to model fibrosis in vitro, cell culture substrates that permit dynamic tuning of matrix stiffness and composition would be ideal. To date, existing hydrogel culture platforms do not readily enable real-time, cytocompatible manipulation of these properties wherein a single cell can be visualized as matrix properties change. Instead, cell populations are compared after culture on either elastic or stiff hydrogels. To address this need, we have developed a system that employs bioorthogonal chemical reactions to increase hydrogel crosslink density and stiffen the matrix in situ. Hydrogels are synthesized from chemically-functionalized multi-arm poly(ethylene glycol) (PEG) macromers and characterized by rheometry. Control static materials are designed to possess mechanical properties approximating both healthy tissue (soft, elastic modulus (E) ~ 1-10 kPa) and fibrotic tissue (stiff, E ~ 20-50 kPa). Dynamic materials are prepared that allow user-controlled stiffening to mimic fibrosis. Cell adhesion to the materials is controlled by chemically-coupling extracellular matrix (ECM) components (such as laminin and fibronectin) to the hydrogels. The hydrogel platforms are amenable to characterization techniques such as live-cell time lapse microscopy and immunostaining. These materials enable tracking of real-time, single-cell responses to alterations in substrate stiffness to probe the temporal changes in mechanotransduction during fibrosis.

Keywords: fibrosis; extracellular matrix; biomaterials

061

PHENOTYPIC EVALUATION OF FRESHLY-ISOLATED AND CRYOPRESERVED HPSC-CMS

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have emerged as a powerful platform for in vitro modelling of cardiac diseases, safety pharmacology and drug screening, and in vivo cardiac regenerative therapies. All these applications require large quantities of hiPSC-CMs. To facilitate this, cryopreservation of hiPSC-CMs without altering their biochemical and functional phenotype is essential. Not only does the ability to cryopreserve hPSC-CMs make the generation more cost and time effective, but is also enables the same batch of hPSC-CMs to be evaluated in multiple functional assays to investigate a disease phenotype. For this

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reason, this study aimed to functionally and molecularly compare freshly-derived and cryopreserved hiPSC-CMs generated from two different hiPSC lines. While the cryopreserved hiPSC-CMs exhibited poorer recovery than their freshly-derived counterparts, no significant differences in terms of cardiac-specific marker expression and contractility were observed. Similarly, one hiPSC line showed no difference in action potential parameters, although a second hiPSC line did exhibit a prolongation in action potential duration. These results provide evidence that cryopreservation does not compromise the molecular, physiological and mechanical properties of hiPSC-CMs, thereby enabling large quantities of hiPSC-CMs to be stored for prolonged periods and allowing the same batch of hiPSC-CMs to be used for multiple applications and evaluations.

Funding Source
This work was funded by an ERC-stG and a VIDI fellowship from the Netherlands Organisation for Scientific Research (NWO).

Keywords: hPSC-CMs; Cryopreservation; Electrophysiology

062

GENERATION OF ASTROCYTE SUBTYPES FROM HUMAN AND MOUSE INDUCED PLURIPOTENT STEM CELLS TO MODEL VANISHING WHITE MATTER

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Astrocytes have become an important player in research into various neurological diseases, such as demyelinating disorders. Drug screening experiments and regenerative studies on rare leukodystrophies would be greatly facilitated by the generation of astrocytes from induced pluripotent stem cells. As such, it has become the focus of various studies. Whereas human models are likely to provide especially useful insight, high variability and low sample size impede mouse-based models to a lesser degree. We have developed two astrocyte differentiation protocols for derivation from both mouse and human induced pluripotent stem cells to model the leukodystrophy Vanishing White Matter. By using fetal bovine serum or ciliary neurotrophic factor, associated with grey and white matter respectively, we obtained two different astrocyte subtypes. Both expressed astrocyte-associated markers, showed appropriate morphology, and displayed reactivity when challenged. The obtained white matter-like and grey matter-like astrocytes differed in size, process arborization, and expression profile. White matter-like astrocytes were found to be selectively vulnerable to Vanishing White Matter mutations. Human astrocytes of both subtypes derived from Vanishing White Matter iPSC lines and control iPSC lines were subjected to whole cell RNA sequencing. The analysis indicated white matter-like astrocytes were more affected than grey matter-like astrocytes. To compare human and mouse data, whole cell RNA sequencing of white matter-like astrocytes derived from Vanishing White Matter or control mouse iPSC lines was performed. In mouse, differentially expressed genes were related to the extracellular matrix and the immune system (based on Gene Ontology). Human but not mouse Vanishing White Matter astrocytes additionally showed differentially expressed genes involved in neuronal functioning, vasculature, and cell development. The stimulatory effect of hyaluronidase-treated astrocyte conditioned medium on oligodendrocyte progenitor maturation was also specific to human Vanishing White Matter astrocytes. These human- and mouse-based models of astrocyte subtype differentiations can underscore disease mechanisms when combined, and are useful in disease modeling in vitro and regenerative applications in vivo.

Funding Source
This research is funded by a ZonMw VIDI research grant (91712343; VMH), a European Leukodystrophy Association (ELA) Research Grant (2014-012L1; VMH), an E-Rare Joint Call project (9003037601; VMH), and an NWO Spinoza grant (MSvdK).

Keywords: iPSC differentiation; astrocyte subtypes; Vanishing White Matter

063

SCREENING CHEMICALS FOR RECOVERING IMPAIRED OSTEOGENESIS IN CFC SYNDROME-IPSCS

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Cardiofaciocutaneous (CFC) syndrome is a type of RASophathy, which is mainly caused by the germline mutations in BRAF gene. Among various symptoms of CFC syndrome, skeletal phenotypes, including short stature, bone growth delay, and low bone mineral density are characterized in the majority of CFC patients. We previously reported that CFC-induced pluripotent stem cells (CFC-iPSCs) showed impaired osteogenesis. In fact, CFC-mesenchymal stem cells (CFC-MSCs) differentiated from CFC-iPSCs represented aberrant alkaline phosphatase activity and mineralization during osteogenic differentiation in vitro. In addition, TGF-beta signaling was activated and BMP signaling was downregulated in CFC-MSCs compared to wild-type (WT)-MSCs. Based on these results, we screened potential chemicals to rescue defective osteogenic differentiation of CFC-MSCs using para-Nitrophenylphosphate (pNPP) assay on MSCs-plated 384-wells. Among 2261 clinical compounds (provided by Korea Chemical Bank), 10 potential compounds such as signaling inhibitors and nucleoside analogues were screened. Those chemicals also revealed pharmacological effects on the recovery of defective osteogenesis in CFC-MSCs. Our findings provide novel insights on the pathological mechanism and therapeutic targets in CFC syndrome.

Keywords: Drug screening; CFC syndrome; Osteogenesis

064

DISEASE MODELING FOR FABRY DISEASE USING PATIENTS-DERIVED INDUCED PLURIPOTENT STEM CELLS

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Fabry disease (FD) is an X-linked inherited lysosomal storage disorder which is caused by α-galactosidase(GLA) deficiency. Mutated GLA results in immoderate globotriaosylceramide (Gb3) accumulation in various cell types, thereby causing to progressive complications with age. In particular, accumulation of Gb3 in endothelium causes life-threatening complications such as ischemic stroke, hypertrophic cardiomyopathy, and renal failure at the terminal stage of FD patients. However, cellular mechanisms whether GB3 accumulation leads to these vasculopathies in FD are poorly understood. To study cellular modeling in vitro, we generated induced pluripotent stem cells (iPSCs) from four Fabry patients. Although FD-iPSCs exhibited low a-galactosidase activity and excessive Gb3 accumulation in undifferentiated state, they could differentiate vascular cells such as endothelial cells (ECs) and smooth muscle cells. Accumulated Gb3 was transiently cleared by treatment with alpha-galactosidase recombinant protein (Fabrazyme®) during endothelial differentiation of FD-iPSCs. Nonetheless, FD-ECs showed a lower expression of angiogenic factors such as ANG2, VEGF than normal ECs. These results demonstrate that endothelial dysfunction is associated with low activity of angiogenic factors in Fabry disease.

Keywords: human iPSCs; Fabry disease; disease modeling

065

CEREBRAL ORGANOIDS REVEAL AN IMPAIRED DEVELOPMENT OF CORTICAL NEURONS IN MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA

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According to the neurodevelopmental theory of schizophrenia (SZ), pathologic changes contributed by genetic and environmental factors start taking place during the first trimester of pregnancy, even though the onset of SZ with drastic symptoms (hallucination, delusion, social dysfunction) appear typically only in young adulthood. If one of the identical twins has SZ, the risk of illness for the other one is about 50% despite of the shared genome and childhood environment. We have generated human induced pluripotent stem cell (hiPSC) derived cerebral organoids from monozygotic twin pairs discordant for SZ and unrelated healthy controls to examine gene expression and morphological changes in cortical development. On the first 60 days of cerebral organoid development, RT-qPCR showed gradually increased expression in DCX and MAP2 gene markers for neuron progenitor cells and mature neurons, respectively. Interestingly, the expressions of both genes were increased in affected twins at all measured time points compared to non-affected twins and controls. Furthermore, we detected an increased expression in markers of various neuronal subtypes: glutamatergic (SLC17A7, FC>20), dopaminergic (TH, FC>1000) and GABAergic neurons (GABRA1 and GAD2, FC>5), all known to be implicated in SZ. The developing organoids derived from discordant pairs were smaller than organoids derived from control patients. Morphologically, the organoids of affected twins lacked ventricle-like structures and displayed disorganized rosette formation at 20 days of organoid differentiation, indicating malformation of the progenitor zone. In summary, the disruption of cortical neuronal programming suggests network dysfunction and contribution of several neuronal subtypes to SZ phenotype already at early stage development. Further investigation studies will focus on neuronal connectivity and signaling.

Keywords: schizophrenia; induced pluripotent stem cell; cerebral organoid

066

FUNCTIONAL EVALUATION OF CRISPR/CAS9 EDITED KCNQ1 MUTATIONS IN CARDIOMYOCYTES DERIVED FROM IPS CELLS OF LONG QT SYNDROME PATIENTS

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Congenital Long QT Syndrome Type-1 (LQTS-1) is a common form of cardiac arrhythmia that is characterized by a prolongation of the QT interval on an electrocardiogram. LQTS-1 is associated with mutations in the KCNQ1 gene encoding a voltage-gated potassium channel. Here, we reported to recapitulate the LQTS-1 disease phenotype in vitro by using cardiomyocytes derived from patient-specific iPSCs, to correct KCNQ1 gene mutation by CRISPR/Cas9 and to evaluate the electrophysiological phenotype of cardiac tissue following genome editing. First, we have reprogrammed venous blood cells of a LQTS-1 patient and his healthy family member to iPSCs via Sendai virus encoding pluripotency markers. After reprogramming, iPSC identity was confirmed by expression of pluripotency genes by qRT-PCR, immunocytochemical staining and teratoma assay. To correct the heterozygous point mutation at pore region of KCNQ1 gene, patient-specific iPSCs were co-electroporated with guide RNA, Cas9 enzyme and GFP encoding plasmid together with ssDNA carrying wild-type sequences. GFP expressing iPSCs were single-cell sorted by FACS. From the screened 476 individual iPSC colonies, 26 colonies showed CRISPR/Cas9 genome editing revealed by Sanger sequencing. Using a similar strategy, the disease causing mutation was generated in KCNQ1 gene of healthy control iPSCs with 3% efficiency. To electrophysiologically evaluate the cardiac phenotype, cardiac tissue generated from the healthy, patient-derived and gene-edited iPSCs were analysed

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for Ca2+ currents by Fluo-4 analysis and field potential by the multielectrode system. While we have observed a significant prolongation of QT interval in cardiomyocytes derived from LQTS-1 patient compared to healthy control, KCNQ1 gene correction shorten the QT interval and functionally alleviated the in vitro disease symptoms. Collectively, we have showed a direct correlation of electrophysiological cardiac phenotype with KCNQ1 gene sequence, sheding light on the disease mechanism and the potential corrective use of gene-editing in therapy. We are in the process of generating healthy, patient-derived and genome edited iPSC-derived cardiac organoids for drug screens in the future.

Funding Source
This study is supported by The Scientific and Technological Research Council of Turkey (TUBITAK) under 1003 Scientific and Technological Research Projects Funding program with Project number 213S192.

Keywords: Induced pluripotend stem cells; cardiac disease modeling; genome editing

067

SIMULTANEOUS MULTI-PARAMETER FUNCTIONAL ANALYSIS OF HUMAN PLURIPOTENT STEM CELL-CARDIOMYOCYTES USING FLUORESCENCE-BASED IMAGING

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Pharmacological compounds and genetic variants can affect different and sometimes multiple aspects of cardiac physiology, such as the cardiomyocyte (CM) action potential (AP), intracellular calcium transients, and contractility. Simultaneous and higher throughput modelling of these effects in human pluripotent stem cell (hPSC)-derived CMs can be achieved optically, using complementary voltage- and calcium-sensitive fluorescent indicators together with membrane labelling. Using an imaging platform capable of fast simultaneous recording in three separate fluorescence channels, we have determined the effects of a KCNH2 mutation, known to affect IKr current, on these three functional parameters using organic dyes in the mutant hPSC-CMs compared to the isogenic control. As an alternative to organic dyes, we are investigating genetically encoded voltage and calcium indicators (GEVIs and GECIs), since they may mitigate potential toxicity issues associated with the use of organic dyes. We find transfection of in vitro transcribed (IVT) mRNA to be efficient and gentle in hPSC-CMs. Comparison of two GEVIs, ASAP2f and ArcLight1, indicate that although ASAP2f has a weaker signal, faster kinetics mean that AP morphology is more similar to that of organic voltage sensitive dyes (VSDs). Two GECIs, GCaMP6f and jRCaMP1b, also exhibit a robust signal for Ca2+ transients. Together with an advanced imaging platform, combinations of fluorescent organic dyes or GEVIs and GECIs can facilitate bona fide and multi-parameter modelling of disease mutations or drug effects in hPSC-CMs.

Funding Source
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Keywords: cardiac disease modelling; optical measurements; optogenetics

068

MODELLING SUCCINIC SEMIALDEHYDE DEHYDROGENASE DEFICIENCY USING PATIENT IPSC-DERIVED CEREBRAL ORGANOIDS

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Succinic semialdehyde dehydrogenase deficiency (SSADHD) is a monogenetic autosomal-recessive neurological disorder caused by variants of the ALDH5A1 gene. Clinical symptoms develop during early childhood and include intellectual disability, developmental delay, behavioral problems, impaired motor coordination and seizures. Malfunction of the SSADH enzyme results in high concentrations of the neurotransmitter gamma-amino butyric acid (GABA) and its degradation product gamma-hydroxybutyrate (GHB). To date, it is unclear how altered concentrations of GABA and GHB could contribute to the disease etiology of SSADHD. Furthermore, little is known about the extra-synaptic role of these neurotransmitters in early brain development. Using patient-derived induced pluripotent stem cells (iPSCs) and isogenic control lines from two separate families harboring different genetic alterations in ALDH5A1 we generated a cerebral organoid model to study the underlying pathophysiology of SSADHD. Our first immunohistochemical analyses of the organoids at early stages revealed an increased signal and diffuse spreading of the neuronal marker MAP2 in patient-derived organoids with simultaneous reduction of SSADHD neuronal progenitor cells. Moreover, we investigated mitotic activity of organoid-resident progenitors, showing a strong reduction of M-Phase cells in SSADHD organoids, which may point to an overall decreased stem cell pool and/or premature cell cycle exit of symmetrically dividing stem cells. Currently, we are further studying the cellular composition of the SSADH organoids at later stages following aggregation. Moreover, the concentration of GABA and GHB in the supernatant of SSADHD and control organoids are determined using HPLC and additional analysis also include electrophysiological activity of SSADHD organoid-resident neurons. We propose an unprecedented role of GABA and GHB as extra-synaptic, non-neurotransmitter related trophic factors, contributing to the pathophysiology of SSADHD.

Funding Source
This research was supported by the SSADH-Defizit e.v. parental association and the Dietmar Hopp Foundation (St. Leon-Rot, Germany).

Keywords: neurotransmitters; SSADHD; neuronal development

069

ZOOMING IN ON CRYOPRESERVATION OF HIPSCS AND NEURAL DERIVATIVES: A DUAL-CENTER STUDY USING ADHERENT VITRIFICATION

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Human induced pluripotent stem cells (hiPSCs) are an important research tool and efficient cryopreservation is a major challenge. The current gold standard for hiPSCs is slow-rate freezing in suspension, but low recovery rates are limiting immediate post-thawing applicability. We tested whether the switch from slow-rate freezing to ultra-fast cooling by vitrification improves post-thawing survival in a selection of hiPSCs and small molecular neural progenitor cells (smNPCs) from Parkinson's disease and controls. In a dual-center study, we compared the results by immunocytochemistry (ICC) and fluorescence-activated cell sorting analysis. Moreover, RNA-sequencing (RNA-seq) before and after freezing was performed. Adherent vitrification was achieved in the so-called TWIST substrate, a device combining cultivation, vitrification, storage, and post-thawing cultivation. Vitrification resulted in preserved confluency and significantly higher post-thawing cell numbers and viability at day one after thawing, while results were not significantly different at day four after thawing. RNA-seq and ICC of hiPSCs revealed no change in gene expression and pluripotency markers after cryopreservation, indicating that physical damage after slow-rate freezing disrupts the cellular membranes. Scanning electron microscopy revealed preserved colony integrity and intact cell-cell adhesions by adherent vitrification. Experiments in smNPCs demonstrated that adherent vitrification is also applicable to neural derivatives of hiPSCs. Our data suggest that, compared to the state-of-the-art slow-rate freezing in suspension, adherent vitrification is an improved cryopreservation technique for hiPSCs and derivatives.

Keywords: Cryopreservation; Adherent vitrification; Neural derivatives

070

GENERATION OF A MIDBRAIN DOPAMINERGIC REPORTER LINE FOR MODELLING DOPAMINERGIC DEVELOPMENT AND PARKINSON'S DISEASE

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iPSC-derived midbrain dopaminergic (mDA) neurons are an invaluable tool for studying mDA development and neurodegenerative disorders as Parkinson's disease (PD). However, cellular heterogeneity is significant confound in these cultures and a human mDA lineage reporter line that enabled cell type sorting would significantly aid cell-type purity. LMX1A is a transcription factor with a fundamental role in mDA development and provides an early marker for this neuronal lineage. We designed a strategy to generate an LMX1A Blue Fluorescent Protein (BFP) reporter line for tracking and purifying early mDA progenitors and their neuronal derivatives. A silent BFP expression unit is firstly targeted into the AAVS1 safe harbour by CRISPR/Cas9 assisted homologous recombination in the KOLF2 human iPSC line and then Cre will be knocked in to the LMX1A locus of the derived AAVS1-BFP cells. A pilot study carried out in HEK293 demonstrated strong BFP expression following co-transfection of AAVS1-BFP and a Cre expression vector. We can expect strong expression of BFP in all LMX1A expressing cells and their derivatives (ie. committed mDA neural progenitors and their differentiated progeny) upon excision of the floxed stop signal upstream of BFP by the LMX1A driven Cre in our reporter cells. This reporter cell line will serve as a powerful tool both for in vitro modelling of PD and associated risk genes, as well as for developing PD cell therapies.

Funding Source
UK Dementia Research Institute.

Keywords: midbrain dopaminergic neurons; Parkinson's disease; reporter line

071

CARDIOMYOCYTES FROM ISOGENIC HUMAN IPSC LINES HARBORING MUTATIONS IN KCNH2 EXHIBIT PROLONGATIONS IN ACTION – AND FIELD-POTENTIAL DURATION

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Congenital long QT syndrome type 2 (LQT2) is one of the most common genetic channelopathies in the heart leading to life-threatening cardiac arrhythmias and sudden cardiac death (SCD). A broad range of phenotypes are associated with this cardiac channelopathy and nearly 500 variants identified in the gene KCNH2 have been associated with this disease. This phenotype variability impacts the management strategy of patients as it complicates the task of identifying individuals at risk. Patient-derived human induced pluripotent stem cells (hiPSCs) can be used to investigate the pathogenicity of these mutations, however interline variability complicates the use of these models to reflect genotype-phenotype relationships. To overcome this issue, we have created isogenic hiPSC by introducing KCNH2 mutations into a well-established and validated wild-type hiPSC line using Crispr/Cas9 technology. Preliminary analysis of cardiomyocytes derived from these isogenic lines show a prolongation of both action potential and field potential duration when compared to wild type. The response of these hiPSC-CM lines to different pharmacological compounds is currently being examined.

Funding Source
ERC – European Research Council; NWO – Netherlands Organization for Scientific Research

Keywords: Long QT syndrome; hiPSC; disease model

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STEM CELL DERIVED HUMAN BRAIN ORGANOIDS, A PROMISING MODEL TO STUDY ENTEROVIRUS INFECTION AND DISEASE PATHOGENESIS

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Picornaviruses commonly cause mild disease but can lead to severe CNS infection in children. Mainly due to lack of suitable model systems, their pathogenesis is poorly understood. New models to study picornavirus infection in the brain are needed to understand the underlying mechanisms. Brain organoids have increasingly proven to be useful to study human specific disease. Brain organoids are self-organizing 3D cultures derived from human stem cells that recapitulate the organ microenvironment. These brain organoids have the advantage of complex morphology over transformed cell lines and the advantage of its human origin over animal models. From induced pluripotent stem cells, over the course of 40 days, brain organoids were formed. Organoids were characterized by immunostaining. Beta tubulin 3 (Tuj1) and paired box protein 6 (PAX6) were stained to respectively visualize neurons and neural progenitors. Besides the presence of neural cell types, the organoid morphology was studied. After characterization, brain organoids were transduced with an adeno associated virus expressing GFP. In the future, the organoids will be infected with HPeV3, EV71 and E68, members from picornavirus family known to cause CNS infection. We can establish the iPSC derived human brain organoid model. Organoids expressed GFP, which allowed for tracking infection in brain organoids. We are able to establish brain organoids to study viral infection of the brain. This model will enable us to our knowledge on picornavirus infection leading to CNS damage.

Keywords: Brain Organoids; Virology; Picorna viruses

073

BUILDING AN ORGANOID-BASED MODEL FOR OVARIAN CANCER

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Over the last few years several studies led to a significant change in the field of ovarian cancer and it is currently believed that the fallopian tube and not the ovary surface epithelium (OSE) is the main origin of high-grade serous ovarian cancer (HG-SOC). Nevertheless, due to the lack of unique markers and adequate model systems the relative contribution of each tissue is not yet clear and the notion that OSE has a role in HG-SOC development was not cast aside altogether. In this work we have established novel organoid systems derived from both mouse OSE and oviduct (Ovi, the equivalent of human fallopian tube). These systems recapitulate their tissue of origin and demonstrate differences in medium requirements as well as gene expression. To establish comparable tumor progression models for both OSE and Ovi we used CRISPR-Cas9 technique and targeted commonly mutated genes in ovarian cancer (Trp53, Brca1, Nf1 and Pten). Thus, we were able to establish clones with different combinations of mutations. Histological, metaphase spread and gene expression analysis of the mutated organoid clones from both OSE and Ovi demonstrated different degrees of deviation from their wild type counterpart. This deviation became more evident as the amount of introduced mutations increased. Preliminary transplantation experiments showed that Ovi triple mutants (Trp53, Brca1 and Pten or Trp53, Brca1 and Nf1) are able to give rise to solid tumors. Further analysis will reveal what are the differences of ovarian tumors derived from distinct origins. Taken together, in this study we present the first comparable Ovi/OSE research platform that enables addressing questions related to origin and early stages of HG-SOC development.

Keywords: ovarian cancer modeling; organoids; CRISPR-Cas9

074

CARDIAC FIBROSIS IN A DISH – A PLATFORM FOR IDENTIFICATION OF ANTI-FIBROTIC COMPOUNDS

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Heart failure is a major public health problem, being described as an epidemic with a worldwide prevalence of 23 million affected individuals. A key feature in the pathogenesis of heart failure is cardiac fibrosis, but currently treatments specifically targeting cardiac fibrosis are not available. This is to a large extent due to the fact that informative in vitro models permitting high-throughput screening of chemical libraries for anti-fibrotic effects are lacking. Here, we describe a platform of cell-based assays aimed at identifying compounds that interfere with extracellular deposition of collagen from human cardiac fibroblasts. We provide evidence that these assays correctly identify chemical compounds with known anti-fibrotic effects, and further describe compound cultures, composed of human pluripotent stem cell-derived cardiomyocytes and cardiac fibroblasts, for further validation of results in a more complex organoid system. Taken together, we envision that these assays will provide a powerful tool for drug screening of anti-fibrotic chemical compounds and disease modelling of cardiac fibrosis.

Keywords: Cardiac fibrosis; Disease modelling; Organoid culture system

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DNAJB6, A KEY FACTOR IN NEURAL STEM CELL RESISTANCE TO POLYGLUTAMINE PROTEIN AGGREGATION

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Spinocerebellar ataxia type 3 (SCA3) is a neurodegenerative disorder caused by the expansion of polyglutamine (polyQ)-encoding CAG-repeats in the ATXN3 gene. The CAG-repeat length is proportionally related to the aggregation propensity of the ataxin-3polyQ protein. Although the protein is ubiquitously expressed, it only causes toxicity to neurons. To better understand this neuronal hypersensitivity, we generated iPSC-lines from three SCA3 patients. iPSC generation and neuronal differentiation is unaffected by the expression of the ataxin-3polyQ. No spontaneous aggregate formation is observed in the SCA3 neurons. However, upon glutamate treatment, aggregates form in SCA3 neurons but not in SCA3-derived iPSCs or iPSC-derived neural stem cells (NSCs). Analysis of chaperone proteins expression reveals a drastic reorganization of the chaperone network during differentiation, including an almost complete loss of expression of the anti-amyloidogenic chaperone DNAJB6 in neurons. Knockdown of DNAJB6 in iPSC and NSC derived from patients leads to spontaneous aggregation of the polyQ proteins. Moreover, DNAJB6-knockout cells are hypersensitive to polyQ aggregation, which is prevented by DNAJB6 re-expression. Our data show that downregulation of DNAJB6, which occurs upon neuronal differentiation, is directly linked to neuronal toxicity of polyQ aggregation.

Keywords: IPSCs; HTT and SCA3; Protein aggregation

076

MODELLING GENETICALLY COMPLEX DISEASES USING IPSCS

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The discovery of induced pluripotent stem cells (iPSCs) has provided researchers with a new tool to study neurodevelopmental disorders in previously sparsely accessible human cells, such as neurons. These iPSCs are of particular interest for studies concerning genetically complex diseases with a strong genetic component, such as schizophrenia (SCZ) and autism. Since iPSCs are genetically identical to the donor, the need for gene editing is eliminated and the complex genetic architecture such diseases is recapitulated. Past research has focused on the role of neurons in the pathology of SCZ. However, recent evidence shows an important glial component in this disease of both astrocytes and oligodendrocytes. We would like to investigate the impact of such glial components on neuronal functioning. In the current research we first investigated how we could obtain the best statistical power to investigate this glial component in schizophrenia. Schizophrenia is a genetically complex disorder, involving single nucleotide polymorphisms (SNPs) and copy number variations (CNVs), which makes this disease very heterogeneous. This heterogeneity in turn creates variability, lowering the statistical power of iPSC studies because participants will have varying causal variants and varying genetic backgrounds. In order to designs a study that has enough power and allows us to claim causality, we have strategically selected patients using genetic information. We based our selection on polygenic risk scores (SNPs) and the penetrance of CNVs. Furthermore, considering the limitations in sample sizes, we have validated our model in order to determine the best research pool using iPSCs: should we use multiple clones from a smaller number of individuals or should we use one clone and opt for a large number of individuals? In order to answer this question we performed parallel differentiation of nine clones from three individuals (3 clones each) in order to determine the variability at different level.

Funding Source
This work was funded by The Netherlands Organization for Scientific Research (NWO VICI 453-14-005).

Keywords: schizophrenia; variability; modelling

077

A 3D HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURAL CULTURE MODEL TO STUDY CELLULAR AND BIOCHEMICAL ASPECTS OF ALZHEIMER'S DISEASE IN VITRO

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Alzheimer's disease (AD) is the most prevalent form of dementia with no cure yet. The pathophysiology is characterized by accumulation of Abeta oligomers forming plaques and hyperphosphorylation of Tau forming tangles. The major advantage of using Induced pluripotent stem cell (iPSC) is that mature neurons could be generated in vitro from AD patients with inherited mutations thereby allowing patient-specific insight into biochemical and pathophysiological nature of AD. 3D culture models provide a physiologically relevant spatial microenvironment aiding better differentiation and maturation of cells in vitro. In this study, we aim to develop a 3D neural model derived from AD patients and control iPSC

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wherein cells can differentiate, self-organize and mature to replicate the pathophysiological mechanisms of AD. For this study, neural progenitor cells derived from AD-iPSCs with Presenilin1 mutation (L286V, M146L, and A246E) and age-matched controls were differentiated in matrigel for 18 weeks in vitro. Characterization of cell morphology, protein profile and synaptic functions were performed using immunofluorescence and electrophysiology studies. Western blotting was used to determine tau isoform expression and disease associated changes. In this study, we found iPSCs-derived NPCs differentiate into neurons and astrocytes, which self-organize into 3D structures by 3 weeks of differentiation in vitro. These heterogeneous 3D cultures express astrocytic (GFAP), neuronal (beta-3-tubulin, MAP2), pre-synaptic (Synapsin 1) and glutamatergic neuronal (VGLUT1) markers after differentiation. The foetal 3Rtau and adult 4Rtau isoforms were detected at 6 and 18 weeks respectively. There is evidence of action potential in these cultures at 12 weeks. At 6 weeks, 3D cultures express Abeta oligomers as well as hyperphosphorylated tau (PHF1) in AD-iPSC derived 3D cultures. In conclusion, we have developed an in vitro human 3D iPSCs derived neural model with mature neurons. In the AD-derived cells, we have also shown presence of hyperphosphorylated 4R tau and Abeta oligomers. This model recapitulates the early disease biochemical features and can be a relevant platform for studying early cellular and biochemical changes for identification of drug targets.

Funding Source
ARUK south coast network to SWM, Commonwealth Scholarship to PP, Marie Curie to SC.

Keywords: iPSCs; 3D cultures; Alzheimer's disease

078

INVESTIGATING THE CELLULAR ORIGIN OF GLIOBLASTOMA USING DROSOPHILA MELANOGASTER

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Glioblastoma is the most common malignant glioma in adults. Patient survival has not improved since the 1980s and is limited to approximately one year. Developing effective diagnostic tools and treatments for glioblastoma is reliant upon understanding the early molecular events of tumour initiation. Central to this quest is determining the cell of origin for distinct tumour types. Differentiated glial cells are one possible cell of origin of glioblastomas, but the identification of adult neural stem cells (NSCs) with glial identity also implicated NSC lineages as alternative potential cells of origin. Studies in mice have shown that neural lineages can give rise to glioblastomas but it is not known from which cells tumours arise. Drosophila melanogaster has proved a useful model for investigating the diverse molecular mechanisms that underlie tumourigenesis in vivo. Importantly, 60 % of genes associated with human cancers are found in the Drosophila genome, many with extremely high conservation. Current Drosophila glioblastoma models have focussed on the glial cell of origin only and have not considered NSCs as a potential route to tumourigenesis. We are investigating the role of NSC lineages in glioblastoma initiation. We have analysed the effect of expressing oncogenes associated with glioblastoma in Drosophila NSC lineages. To identify the tumour cell of origin, we restricted expression to specific cells within NSC lineages and assessed tumourigenic transformation. Strikingly, we found that the Drosophila and human counterparts of glioblastoma genes had highly comparable tumourigenic capacities in NSC lineages and appear to act through conserved mechanisms. We used Targeted DamID to map the genome-wide binding sites of transcriptional regulators involved in tumour initiation in NSCs in vivo to determine their direct target genes and downstream effectors. Our results show that tumours resulting from expression of Drosophila or human genes have common cellular origins and the molecular pathways involved are consistent with mammalian studies.

Keywords: Tumourigenesis; Neural stem cell; Brain development

079

DISEASE MODELING OF ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY IN HUMAN INDUCED PLURIPOTENT STEM CELLS

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Developmental commitment of cells and tissues is governed by combinations of lineage-specific transcription factors that act as master switch genes defining and reinforcing cell type-specific gene expression patterns. While it is widely accepted that mechanical cues contribute to cell fate decisions during differentiation of stem cells, little is known about how mechanical inputs at cell–cell or cell–matrix adhesions translate into intracellular signals to maintain cell identity or direct fate switch. Using an induced pluripotent stem cell (iPSC) model of arrhythmogenic right ventricular cardiomyopathy (ARVC), we show that in human developing cardiomyocytes, cell-cell contacts at the intercalated disk connect to remodeling of the actin cytoskeleton by regulating the RhoA-ROCK signaling to maintain an active MRTF/SRF transcriptional program essential for cardiomyocyte identity. Genetic perturbation of this mechanosensory pathway in ARVC leads to progressive loss of myocytic identity and acquisition of an adipocyte phenotype in response to adipogenesis-inducing signals. We also demonstrate by clonal analysis of cardiac progenitors that cardiac fat and a subset of cardiac muscle arise from a common precursor expressing Isl1 and Wt1 during heart development, suggesting related mechanisms of determination between the two lineages.

Keywords: ARVC; Cardiac progenitors; Lineage conversion

080

COMMON DEVELOPMENTAL ORIGIN OF CARDIOMYOCYTES AND CARDIAC FAT

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a disease characterized by fibro-fatty replacement of cardiomyocytes predominantly in the right ventricle. Mutations in genes encoding desmosomal proteins account for the majority of ARVC cases. To date, it remains still not completely understood which cell type and which molecular mechanisms are responsible for the fibro-adipose substitution. Using induced pluripotent stem cells from ARVC patients our group could recently show that a spontaneous conversion of a subset of cardiomyocytes into fat is the underlying pathomechanism of the disease and uncover the signaling linking cell-cell contacts to changes of transcriptional programs driving this pathological cell fate switch. Based on these findings we hypothesized that myocyte-to-adipocyte transdifferentiation could occur if both lineages are closely developmentally related. Indeed, by means of in vivo fate mapping we identified a common Isl1+/Wt1+ expressing precursor giving rise to cardiac muscle and cardiac fat. Furthermore, we demonstrated that a single Isl1+/Wt1+ progenitor can differentiate into both cardiomyocytes and adipocytes in vitro, which ultimately suggest related mechanisms of determination between the two lineages.

Keywords: ARVC; Cardiac Progenitors; Lineage Conversion

081

AGGRESSION IN A DISH: A HUMAN MODEL FOR BRUNNER SYNDROME REVEALS INCREASED NEURONAL NETWORK ACTIVITY OF DOPAMINERGIC NEURONS

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Brunner syndrome (BS) is a very rare genetic psychiatric disorder caused by mutations of MAOA at X-chromosome and featured as mild mental retardation and impulsive aggression in the male patients. MAOA encodes monoamine oxidase A which is an important enzyme to catalyze the degradation of dopamine, noradrenaline and serotonin that are neurotransmitters in the brain modulating movement, emotion and cognitive functions. However, how the MAOA mutation contribute to the abnormal behavior of BS patients is largely unknown. Using the induced pluripotent stem cell (iPSC)-derived dopaminergic neurons from three BS patients and two unaffected independent controls along with the isogenic lines generated by CRISPR-Cas9, we investigated how the MAOA mutation affected the neuronal electrophysiology, morphology, and the expression profile of neurotransmitter receptors. Compared to the neurons differentiated from healthy controls, the MAOA mutant neurons showed higher neuronal activity, especially at the network level. And the increased neuronal network activity could be attenuated by correcting the mutation of MAOA in mutant neurons. No significant difference in terms of synaptic strength and synapse density were found at the single neuron level. Morphologically, the neurons from one of the MAOA mutant lines had a significantly more complex morphology than the controls, but there seems to be heterogeneity of the consequences of the different mutations in terms of morphology. At the molecular levels, first results suggest differences regarding to the NMDA but not AMPA receptors of the neurons (suggesting alterations in glutamatergic signalling), but these need to be further investigated in detail. Our findings provide a human cellular model for investigating pathogenic mechanisms underlying BS and identifying the potential therapeutic targets.

Keywords: iPSC Model; Brunner syndrome; CRISPR-cas9

082

DISEASE MODELING OF CARPENTER SYNDROME IN HUMAN IPSC AND RAB23 MOUSE MUTANTS REVEALS CILIOPATHY

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The non-motile primary cilium is an organelle found on the surface of nearly every cell. It serves as a signaling hub to transduce extracellular growth signals such as Sonic Hedgehog (Shh), Wnt and several GPCR signaling pathways. Defective cilia lead to hereditary disorders collectively called ciliopathy, which is an expanding spectrum of at least 35 diseases involving multiple genes mutations. Resolving the intricate regulatory networks for cilia biogenesis and signaling is critical for the understanding of disease pathogenesis. Rab23 belongs to the family of small GTPases, which genetic loss-of-function mutations underlie Carpenter syndrome (CS). CS patients exhibit profound clinical features that overlap with other ciliopathies. This suggests that RAB23 mutation may cause dysfunctional primary cilia, and consequently contributes to the pathogenesis of CS. Given this hypothesis, we aim to elucidate the disease mechanisms of CS, and its potential relationship with ciliopathy disorder. A human disease model of CS has been established from CS patient-derived induced-pluripotent stem cells (iPSC). In line with our hypothesis, patient iPSCs-derived neurons showed reduced ciliation, strongly suggesting the clinical relationship between CS and ciliopathies. Moreover, mouse models of Carpenter syndrome were established by global deletion and conditionally knock-out Rab23 in the central nervous system (CNS) using Actin-cre (Actin-CKO) and Nestin-cre (Nes-CKO) driver lines respectively. Indeed, our data show that Rab23-null and brain-specific knockout mouse mutants of Rab23 exhibited perturbed cilia formation and neurological impairments bearing some similarities with CS and ciliopathies. This is the first in vivo evidence revealing novel roles of Rab23 in ciliogenesis and neurophysiology. Taken together, our data uncover novel in vivo function of Rab23 in ciliogenesis. This suggest RAB23 as a causative

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gene for ciliopathy, further indicating Carpenter syndrome as a ciliopathy disorder.

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Keywords: Carpenter Syndrome; Primary cilia; Central Nervous System Development

083

EFFECT OF MESENCHYMAL STEM CELLS THERAPY ON THE LEVELS OF SERUM BIOMARKERS IN A RAT MODEL OF OSTEOARTHRITIS

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Osteoarthritis (OA) is a chronic disease of the joints characterized by progressive degeneration of connective tissues. OA treatment are mainly aimed to alleviate pain and limitations in movement. Mesenchymal stem cells (MSCs) therapy appear to have a great therapeutic potential, given their differentiation potential and immunomodulatory properties. The present study evaluated the effects of intra-articular injection of BM-MSCs in a rat model of knee OA and its relation to serum biomarkers. Ethical approval was obtained from the Bioethics Committee of King Abdulaziz University for this study. R-BM-MSCs were derived, established in culture and characterized. Sixty Sprague Dawley rats were divided into four groups (n=15/group) as follows: Group I -normal saline controls, Group II – Normal rats that were given MSCs alone; Group III- Knee OA with no treatment; and Group IV- Knee OA + MSCs (1x106 cells in 25ul PBS). Knee OA was induced by injection of sodium-monoiodo acetate (MIA, 2mg in 25ul vehicle) into the femo-rotibial joint space of the left hind limb. Five rats from each group were sacrificed at 10, 20 and 30 days for histological assessment following rBM-MSCs therapy and evaluation of the serum biomarkers namely, hyaluronic acid (HA), N-terminal telopeptide of type I collagen (NTX-1), nerve growth factor (NGF) and calcitonin gene-related peptide (CGRP) by ELISA. FACS analysis showed that the rBM-MSCs were positive for MSC related CD markers. Histological assessment showed near normal restoration of cartilage structure in the BM-MSC treated group compared to the normal. All serum biomarkers analyzed showed significantly decreased levels in the rBM-MSC treated group compared to the untreated controls. The mean levels of HA were 4.66, 7.49, 37.37, 15.81 ng/ml; NTX-1 were 31.56, 40.89, 90.78, 64.97 ng/ml; NGF were 568.54, 32.81, 1644.13, 1351.68 pg/ml and CGRP were 106.26, 124.36, 256.34, 189.92 pg/ml for the Groups I-IV respectively. In conclusion, treatment of induced knee OA in rats with rBM-MSCs helped in structural recovery of the damaged cartilage and the levels of the biomarkers correlate with the disease status. Therefore, these biomarkers can be useful to determine the burden of the disease and/or the disease prognosis.

Keywords: Osteoarthritis; in vivo; Biomarkers;

084

MODELING HEREDITARY RETINAL DYSTROPIES BY PATIENT SPECIFIC IPSC-3D RETINAL ORGANOIDS

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Hereditary retinal dystrophies (HRD) are clinically diverse group of disorders which are characterized by progressive degeneration of light sensing cells, photoreceptors. Here have set out to develop unique human model of HRD by generating retinal organoids via patient-specific iPSCs. These native retina mimics display inner and outer retinal structure with the presence of correctly layered major retinal cell type repertoire including photoreceptor cells, featuring highly differentiated rods and cones. We evaluated the differentiation of healthy and disease 3D organoids by immunofluorescence detection of molecules associated with differentiation and function of retinal cell types. Differentiation and maturation was further evaluated at the ultrastructural level by transmission electron microscopy. We show that this patient specific disease model recapitulates molecular aspects of the disease phenotype.

Keywords: Inhereted retinal dystrophies; Retinal organoids; Patients' iPSCs

085

DISEASE MODELING IN A DISH USING HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSCS): PROCESSES AND CONSIDERATIONS FOR SCALE UP AND QUALITY CONTROL

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Technological advancements in human iPSC cell culture and reprogramming methods have resulted in widespread adoption of disease modeling in a dish using iPSCs and their derivatives. By using patient-derived, disease-specific cells, iPSC models have the potential to recapitulate important aspects of disease states that present in vivo. Although iPSC models have led to many breakthroughs, current processes and methodologies lack consistent metrics of quality control and reproducibility. Here we describe a process to generate iPSCs at high throughput without compromising differentiation capabilities or inherent pluripotency. We use several genetically distinct iPSC lines to highlight the importance of monitoring genetic drift and karyotype instability, genetic identity, and pluripotency.

Keywords: disease modeling; quality control; human pluripotent stem cell

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ATRX SPECIFIES STEM CELL IDENTITY AND NEUROGENIC POTENTIAL IN HUMAN NEURAL STEM CELLS

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The prevalence of human diseases caused by mutations in chromatin remodeling genes underpins the importance of chromatin structure in gene regulation. One such disease, Alpha-thalassemia X-linked intellectual disability (ATR-X) syndrome, is caused by mutations of ATRX gene. This syndrome is characterized by mental retardation with severe developmental delay, craniofacial and urogenital abnormalities, as well as mild anemia. Previous studies have reported the role of ATRX in α-thalassaemia and cancer; however, the function of ATRX during human brain development remains unknown. In addition, multiple organ defects observed in ATR-X syndrome patients, and the identification of ATRX mutation as the sole genetic cause of ATR-X syndrome has raised the question of how disruption of single factor can lead to multiple phenotypes. In the present study, we found that ATRX regulates stemness and neurogenic property of human neuroepithelial (NE) cells. Dysregulation of ATRX results in the loss of NE identity and the aberrant acquisition of neural crest properties. We also generated chromatin landscapes of ATRX-bound genomic regions and found that ATRX displayed correlation with repressive heterochromatin (H3K9me3), as reported previously. In addition to its well-established repressive role, we found that a small but significant fraction of ATRX-bound regions was associated with active histone marks. Such an association was undetectable in other somatic cells, indicating the dual function of ATRX is exclusive to neural lineage. Thus, the function of ATRX as a transcription activator in the regulation of cell identity during human brain development certainly warrant further study.

Keywords: ATRX syndrome; chromatin remodeling; epigenetics

087

GENERATION OF MICE CARRYING HUMAN NEURONS BY EMBRYONIC INJECTIONS TO MODEL HUMAN NEUROLOGICAL DISORDERS

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Animal models are limited for understanding human neurological disorders. Modeling neurological disease with cultured human induced pluripotent stem cells (hiPSC) presents several biases, including high inter-batch variability and the fact that cell culture conditions do not recapitulate an in vivo environment. We made the hypothesis that placing neurons derived from hiPSC in in vivo conditions, i.e. culturing them in a living organism, might represent a model closer to physiological conditions and allow to study neuronal properties such as neuronal morphology that are hard to study in in vitro models. Published studies and our results show that transplantation of human neural precursor cells (hNPCs) in the neonatal mouse brain leads to the migration of the cells away from the injection site into different brain regions of the brain. These cells differentiate into several neural cell types including neurons. However, neural progenitor cells mostly differentiate into excitatory neurons and fail to generate a significant amount of inhibitory neurons. Also, in our hands, the survival of neurons generated via neonatal injections decreases over time, limiting analysis at later timepoints. Here, we hypothesized that transplanting hNPC prenatally, within the time window of neurogenesis, at embryonic day 13.5, might provide more adequate cues for the development and integration of neurons in the mouse brain circuitry, and lead to more robust integration of the neurons and increased survival. Our data suggests that embryonic engraftment increases neuronal integration into the network compared to neonatal engraftment and represents a viable in vivo approach to model human neurological disorders.

Keywords: human-mouse chimera; neuron; neurological disorders

088

PHENOTYPIC FUNCTIONAL IN VITRO SCREENING OF PATIENT IPSC-DERIVED MOTOR NEURONS USED FOR IN VITRO HTS DISEASE MODELING WITH AI-BASED ANALYSIS OF MICRO ELECTRODE ARRAY DATA

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Patient-derived iPSC models have been designed for various indications promising higher physiological relevance and thus, better translation to the in vivo situation. Their application eventually may decrease attrition rates in drug discovery and development. We focused on investigating motor neuron diseases (MND) such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), both causing loss of motor neurons and associated symptoms. Here, we phenotypically describe the consequence of the genetic variation present in ALS and SMA patient iPSC-derived motor neurons on the functional activity and network connectivity. We further elucidated how functional ALS and SMA phenotypes separated from controls during network establishment to enable compound testing to rescue the disease phenotypes. We cultured patient iPSC-derived motor neurons (BrainXell) and controls on multi-well micro-electrode arrays (MEA, Axion Biosystems) for several weeks to analyze their functional network activity patterns by multi-parametric analysis (NeuroProof). Our results showed reproducible spontaneously active motor neuron networks with synchronized activity. We identified disease-specific functional phenotypes and showed how reference compounds can affect them. In conclusion, we show that hiPSC-derived motor neurons are able to produce functional in vitro phenotypes which can be associated with known motor neuron diseases. By using artificial intelligence-based multivariate MEA data analyses combined with reproducible physiologically relevant iPSC neuron models we provide a functional phenotypic assay platform for high throughput compound screening.

Keywords: In Vitro Screening; iPSC-Derived Neurons; motor neuron disease

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GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CORTICAL ASTROCYTES IN FRAGILE X SYNDROME

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Fragile X syndrome (FXS) is the most common form of familial intellectual disability and a variant of autism. The syndrome is caused by the lack of functional fragile X mental retardation protein (FMRP) due to epigenetic silencing of the Fmr1 gene. FMRP is crucial for the early neural development and its absence leads to changes in the synaptic plasticity and imbalance of excitatory and inhibitory networks. Recently, an increasing number of studies have shown that astrocytes also play a role in the FXS pathology. In this study, we generated frontal cortex astrocytes from induced pluripotent stem (iPS) cells to model FXS in human astrocytes. The induction of neural differentiation was accomplished using dual SMAD inhibition combined with Dickkopf-Related Protein 1- and cyclopamine treatments to inhibit hindbrain and midbrain patterning, respectively. Generated progenitors were directed towards astrocyte lineage with ciliary neurotrophic factor. During the differentiation, the cells were monitored for the expression of neuronal progenitor and astrocyte markers. Generated astrocytes were positive for the selected astrocyte markers and were responsive to environmental stimuli. The differentiation protocol, therefore, allows the generation of functional patient-derived astrocyte cultures that can be used to study the mechanisms underlying impaired astrocyte function in FXS.

Funding Source
The Arvo and Lea Ylppö Foundation and the Academy of Finland

Keywords: Neurodevelopmental disease; astrocyte; induced pluripotent stem cells

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THE CAUDATE NUCLEUS IN SCHIZOPHRENIA, FROM COMPUTATIONAL FINDINGS TO POSTMORTEM BRAIN TISSUE AND IN VITRO FUNCTION

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Schizophrenia (SZ) is a devastating neuropsychiatric disorder unique to humans that affects ~1% of our population. The disorder is characterized by distortions in thinking, perception, emotion, and behavior. Decades of research have offered many insights, including evidence for a strong genetic component, but we have yet to identify the disease causing molecular pathways. Advancing human genetic findings to reveal the mechanisms of SZ pathogenesis can identify potential therapeutic targets. A recent genome-wide association study (GWAS), which tests for associations between common genetic variants and traits, identified 108 loci with modest contribution to SZ risk; however, the association reveals little about the role of these loci in SZ. Gene expression studies to elucidate the function of GWAS risk alleles have focused on cortical areas, even though other regions have been implicated. For example, dopamine was the first neurotransmitter implicated in SZ, and dopaminergic receptors in the caudate nucleus are the primary targets of antipsychotic drugs. Therefore, we carried out a comprehensive analysis of the genetic and transcription landscape of SZ in postmortem caudate nucleus samples from the Lieber Institute for Brain Development (LIBD) brain repository for neuropsychiatric disorders; the largest of its kind. The BrainSEQ™ Consortium led by LIBD has used the repository to generate and analyze vast amounts of genomic data including RNA-seq data from SZ and health controls for dorsolateral prefrontal cortex (N = 500), hippocampus (N = 450), and caudate nucleus (N = 438) - and genotypes. We identified expression quantitative trait loci (eQTL) in caudate nucleus and differentially expressed genes in schizophrenia. We find strong evidence that the caudate nucleus is a primary site of SZ pathogenesis. Within the 108 loci risk SNPs, 6 of 6 gene-SNP pairs have concordant expression changes in SZ and a heritable genic risk that alters expression in the same direction. These genes are likely involved in SZ pathogenesis. Therefore, we are currently investigating RNA and protein levels of these genes in postmortem brain tissue, as well as, by studying the functional implications of their altered expression in cerebral and striatal organoids from dura-derived human induced pluripotent stem cells (iPSCs).

Funding Source
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Keywords: schizophrenia; caudate nucleus; bio-computation

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MODELLING HUMAN NEPHROGENESIS USING HUMAN PSC-DERIVED PATTERNABLE AND FUNCTIONAL KIDNEY ORGANOIDS

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The incidence of end-stage renal disease (ESRD) is increasing at an alarming rate, but treatment options remain unchanged over the past 70 years. Directed differentiation of human pluripotent stem cells (hPSCs) into kidney organoids offers an unprecedented opportunity to study human kidney development and pathogenesis, for performing drug screening, and ultimately to generate functional kidney tissue for replacement therapy. Despite recent advances in generating kidney organoids form hPSCs, the ability to modulate various cellular components within kidney organoids has not yet been illustrated. Here, we report the establishment of a highly efficient protocol for differentiating hPSCs into kidney organoids comprised of segmented nephron epithelium, vascular endothelium and interstitium. Through stringent temporal modulation of canonical WNT-signalling pathway, we could preferentially modulate the choice of proximal versus distal lineage, as well as the vascular compartment in the kidney organoids. Within hPSC-derived kidney organoids, we identified that KDR+ vascular progenitors originated from a subpopulation of SIX2+SALL1+ cells that further differentiate and mature into CD31+ endothelial cells in response to VEGF-A secreted by podocytes. Following renal capsule implantation into an immunodeficient host mouse, kidney organoids acquired significant structural maturation, as represented by the formation of Bowman's capsule space surrounding glomerular capillary turfs of a human origin. The implanted kidney organoids exhibited size-selective dextran filtration and reabsorption; and the accumulation of putative filtrate within tubules, demonstrating functional maturation. Our work represents an advanced version of kidney organoids that promise to offer extensive utility in both basic science and clinical practice.

Funding Source
Y.X. is supported by Nanyang Assistant Professorship Grant, LKCMed-NTU; Ministry of Education grant (2017-T1-001-086); National Medical Research Council grant (NMRC/OFIRG/0076/2018) and NTU internal grant (JNT-14/2016-CG).

Keywords: Kidney organoid; Patterning; Pluripotent stem cells

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CHARACTERIZATION AND TRANSPLANTATION OF CD73-POSITIVE PHOTORECEPTORS ISOLATED FROM HUMAN IPSC-DERIVED RETINAL ORGANOIDS

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Photoreceptor degenerative diseases are a major cause of blindness, for which there are currently no effective treatments. For stem cell-based therapy using human induced pluripotent stem cells (hiPSCs) is crucial to obtain a homogenous photoreceptor cell population. We previously showed that the cell surface antigen CD73 is specifically expressed in photoreceptors in hiPSC-derived retinal organoids. Flow cytometry analysis in dissociated cells from retinal organoids indicated that the percentage of CD73+ cells increased with organoid maturation, with CD73+ cells representing more than 60% of cells at day 180 of differentiation. Targeting of CD73 by Magnetic-Activated Cell Sorting (MACS), led to enrichment to 90% of CD73+ cells in the positive sorted fraction. Nanostring analysis on CD73-sorted cells confirmed expression of photoreceptor-specific genes, while showing downregulation of genes expressed in other retinal lineages compared to dissociated retinal cells before MACS. We confirmed that CD73 targeting by MACS is an effective strategy to separate a homogenous population of photoreceptors by using a fluorescent Cone rod homeobox (Crx) reporter hiPSC line. Freeze-thawing of whole retinal organoids resulted in a source of viable cells and did not affect MACS effectiveness. Finally, transplantation studies in a rat model of photoreceptor degeneration demonstrated the capacity of CD73-sorted cells to survive and mature in close proximity to host inner retina during several weeks. Human cells were identified by a combination of human-specific cytoplasmic and nuclear markers, morphological features and specie-specific fluorescence in situ hybridization (FISH) probes, excluding the occurrence of cytoplasmic material exchange between donor human cells and recipient rat cells. Functional analysis by full-field electroretinogram (ERG) recording failed to detect an improvement of the visual function in transplanted eyes compared to controlateral uninjected eyes. In conclusion, although the ability of donor cells to establish functional synaptic connections and mediate a significant rescue of the visual function remains to be assessed, these data demonstrate that CD73+ photoreceptor precursors hold great promise for the development of a future clinical translation.

Keywords: photoreceptors; organoid; transplantation

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DEVELOPMENT OF A HUMAN 3D VASCULARIZED SKIN ORGANOID MODEL

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Stem cell-derived organoids provide self-organized 3D tissue models, representing the ex vivo organ physiology but frequently lacking stromal vascular support. Here, we aimed at developing suitable metabolic conditions for iPSC-derived as well as adult skin cell re-organization that support functionality of the different cell types in a 3D skin organoid model. Adult interfollicular epidermal skin keratinocytes (KC), skin fibroblasts (FB) for stromal support, and endothelial cells (EC) for prevascularisation, were isolated and propagated in 2D under animal serum-free conditions. Umbilical cord blood-derived induced pluripotent cells (iPSC) were differentiated into iPS-KC, -FB and –EC in addition. Cell viability, identity and purity was confirmed by flow cytometry and clonal assays indicated their stem/progenitor potential. Triple cell type organoid formation required optimized media selection and human platelet-derived growth factors to support the regenerative and angiogenic metabolic condition. 3D confocal microscopy revealed viable skin organoids with a size of 150 µm after 4 days in culture. Using fluorescent probes and life cell tracking, the cell organization process was monitored. Live cell imaging revealed sequential organoid assembly starting from stromal-vascular aggregation and followed by the superficial anchorage of KC, indicating an organized structure already 50h after cell seeding. These data indicate that optimized metabolic conditions required for skin regeneration, potentially promoting skin re-organization and angiogenesis in vivo, can be determined in a 3D organoid model that may be suitable for drug development as well as establishing novel skin transplantation strategies.

Keywords: skin organoid; stem cell self-organization; skin regeneration

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MAYO CLINIC'S HIGH-RISK MAMMARY GLAND-FALLOPIAN TUBE 'LIVING' ORGANOID BIOBANK FOR WOMEN'S CANCER RESEARCH

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A woman's life-time risk for breast cancer and ovarian cancer are more than 10% and 1.5% respectively. This risk is highly elevated in patients with strong family history of such cancers and/or deleterious mutations in genes such as BRCA1/2. A significant proportion of these high-risk patients succumb to their disease due to lack of effective therapies. The first malignant cells of highly aggressive triple negative breast cancer and high-grade serous ovarian cancer are believed to be from epithelial cells lining the mammary gland and fallopian tubes. The prevention strategy currently available to these patients involve highly invasive and least desirable surgical debulking of breast and/or ovaries/fallopian tubes. Patient tissue-organoids and isolated epithelial stem cell derived-orga noids generated from high-risk patients are vital resource to study tissue regeneration and track disease origin. With a vision to boost women's cancer prevention research at Mayo, we have established the first and largest clinically and genetically-annotated patient-derived organoid biobank for mammary gland and fallopian tube tissues from average and high-risk patients undergoing surgeries at different sites of Mayo Clinic. We have viably frozen >1000 vials of mammary tissue-organoids from 100 patients including carriers of BRCA1/2 and other breast cancer-associated mutations who underwent prophylactic mastectomy, reduction mammoplasty or autopsy, and >16,000 individual fallopian tube stem cell derived organoids from 14 patients who underwent salpingectomy. This continuing effort to create largest and well-annotated 'living' organoid biobank for women's cancer prevention program, attendant challenges in its establishment, preliminary characterization studies of epithelial stem cells and resource sharing plan will be discussed in detail.

Funding Source
This work is partly supported by grants to N.K. from Mayo Clinic's Breast Cancer SPORE and Ovarian Cancer SPORE.

Keywords: Organoid Biobank; Mammary gland; Fallopian tubes

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BMP ANTAGONISTS SECRETED BY MSC INDUCE COLONIC ORGANOID PROLIFERATION: APPLICATION TO SIDE EFFECTS OF RADIOTHERAPY TREATMENT

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Radiation therapy is used in at least 50% of cancer patients and plays a critical role in 25% of cancer cures. Many cancer patients have undergone radiation therapy of tumors in the abdomen and the bowel is an organ at risk due to the presence of some parts of it in the irradiation field. Delayed bowel radiation toxicity is a highly important issue for long term cancer survivors. It is a progressive condition with substantial long-term morbidity and mortality. Today, there is no unified approach for the assessment and treatment of this disease. The aim of this study is to use cultured adult stem cell from colon (CSC) to reduce colonic injuries induced after colorectal irradiation. We also try to improve the therapeutic outcome by co-injection with mesenchymal stromal cells (MSC) involved in the function of niche and widely used in clinical trials. In mice model reproducing radiation-induced histological damage observed in patients suffering from severe side effects after radiotherapy, epithelial cells from colonic organoids of C57/Bl6 mice expressing GFP were locally injected. We determined that CSC in vitro amplified, implant and proliferate in irradiated colonic mucosa. CSC were readily detectable in the colon until 21 days after injection. We also highlighted that CSC injections improve colonic regeneration as determined by a reduction of the ulcer size on histologic slides. Moreover, we also determined the benefit of using CSC in association with MSC. First of all, in vitro analysis demonstrated that co-culture of MSC with CSC increase the number, proliferation and size of colonic organoids in normal and irradiated conditions. Gene expression analysis on MSC revealed huge expression of two BMP-antagonists, and inhibition using siRNA demonstrated the involvement of these molecules in organoids formation. Then, we co-injected CSC with MSC in irradiated colon. This study provides evidence of the potential of CSC to limit radiation effects on the colon and open perspectives on combined strategies to improve their therapeutic benefit.

Keywords: Colonic organoid, mesenchymal stromal cells; Treatment, Cell therapy; Irradiation, pelvic radiotherapy side effect

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HUMAN THYROID-DERIVED ORGANOIDS GENERATE HORMONE-PRODUCING GLANDULAR TISSUE

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Treatment of hypothyroidism with thyroid hormone replacement often results in unbalanced thyroid hormone levels which results in fatigue, constipation, weight gain and potential cardiovascular diseases or osteoporosis severely compromising the quality of life of patients. Organoid transplantations could be used to regenerate and restore tissue functionality. We isolated and cultured primary murine and human thyroid gland tissue as single cells to form thyroid organoids. Transcriptomic analysis of these human thyroid gland organoids demonstrated the expression of several stem cell markers, such as Sca-1, CD133 and EpCAM, and the thyroid-specific markers Nkx2-1, thyroglobulin and T4. After prolonged passaging, upregulation of stem cell markers, FUT4 and SOX2, and proliferation markers, PCNA and Ki67 was observed. Induced differentiation of murine and human organoids in vitro induced the development of tissue-resembling mini-glands that abundantly expressed thyroid gland markers and produced T4 hormone. (Xeno-) transplantation of dissociated organoids underneath the kidney capsule of athyroid mice resulted in the generation of hormone-producing murine and human thyroid-resembling follicles, and increased survival. These studies provide the first proof of principle that primary thyroid gland-derived organoids can be cultured, and are capable of developing into a functional mini-gland in vivo, thereby suggesting a promising applicability for thyroid gland regeneration.

Funding Source
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Keywords: Hypothyroidism; Hormone-producing thyroid gland organoids; T4 hormone

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IDENTIFYING HUMAN SALIVARY GLAND STEM CELLS SIGNATURE THROUGH A MOLECULAR NETWORK BASED APPROACH

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Radiotherapy treatment for head and neck cancer increases patient survival but comes with the frequent cost of xerostomia. This is caused by radiation-induced hypo-salivation, resulting from sterilization of the salivary gland stem/progenitor cell compartment. Therefore, salivary gland stem cell therapy could hold great potential to restore tissue functionality. Although we previously showed that salivary glands contain a pool of cells exhibiting stem/progenitor cell properties, the slow turnover of the salivary gland epithelium, the scarcity of tissue specific stem cells and their niche complexity have made their characterization until now particularly challenging. We

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performed bulk RNA sequencing and weighted gene co-expression network analysis to define the molecular signature of human salivary gland stem cells and the molecular cues that control their self-renewal and differentiation. Using salivary gland organoid, we generated samples corresponding to a heterogeneous human salivary gland stem/progenitor population, a more pure stem cell population (by adding CHIR99021 and Valproic acid (CV)), and a more differentiated population showing budding and branching features. Stem cell potency was assessed by organoid forming efficiency (OFE). RNA profiling data were used to construct a molecular network where modules of co-expressed genes were directly correlated with OFE,CV treatment and differentiation potential. The analysis allowed us to identify 3 modules strongly correlated with high OFE and CV treatment and weakly correlated with differentiation ability, indicating that these modules could be associated with a more primitive stem cell profile, showing the involvement of the Hippo and Wnt pathways and several unique genes expressed in the salivary gland stem cell compartment. In particular, YAP overexpression in salivary gland organoid cultures showed increased OFE revealing a key role of YAP in maintenance of salivary gland stem cells. Functional high-throughput genetic screens will be used for the validation of driver genes in these modules, and could potentially lead to stem cell surface markers. These markers could be used to isolate the most potent stem cell population and therefore could be used in clinical trials for patients suffering from radiation-induced hypo-salivation.

Funding Source
Dutch Cancer Society Grant RUG2013-5792.

Keywords: Salivary Gland Stem Cells; Organoid; Weighted Gene Co-Expression Network Analysis

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BILE AS A NON-INVASIVE SOURCE OF BILE CHOLANGIOCYTE ORGANOIDS FOR DEVELOPING PATIENT-SPECIFIC DISEASE MODELS

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Bile duct related diseases are the leading cause for pediatric liver transplantation and adult re-transplantation of a liver graft. Studying biliary diseases has long-term been hampered by the inability to culture bile duct lining cholangiocytes long-term. Recently was shown that Extra-hepatic Cholangiocyte Organoids (ECOs) that are derived from extra-hepatic bile duct (EHBD) tissue can be long-term expanded in culture. However, with the current limitation for disease modeling or personalized regenerative medicine applications highly invasive bile duct biopsies are required to obtain these ECOs from individual patients. Therefore the aim of the current study is to investigate whether ECOs can be cultured from less invasively obtained bile fluid. Bile-derived ECOs were cultured, according to the previous published protocol, either from gallbladder bile obtained from donor livers for transplantation, or from bile obtained by endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic cholangiography drain (PTCD). Next, ECO's were initiated from three different patients and compared to bile-derived cholanchiocyte organoids (BCO's) on the genetic level (qrt-PCR), protein level (either immunohistochemistry), immunofluorescence or western blotting) and functional level by testing the cholangiocyte specific transporter channels (Ussing chamber). Cultures were initiated from 1 ml of bile obtained from all different sources. ECOs could beeffectively (8/9 attempts) expanded from all sources of bile from patients with a variety of diseases. Bile-derived ECOs expressed similar cholangiocyte markers on gene and protein level as tissue-derived ECOs and both lacked either stem cell- or hepatocyte markers. Furthermore, these cells expressed and responded similarly to stimulation and inhibition of different cholangiocyte ion-channels. Interestingly, bile-derived ECOs from a patient with cystic fibrosis (CF) clearly lacked CFTR channel activity, showing that ECOs can be used as a disease model to study biliary diseases. Our study showed that bile provides a novel and less-invasive source of patient-specific ECOs. This creates new opportunities to study autologous bile duct regeneration and develop patient-specific disease models.

Keywords: Bile duct; Disease model; Bile cholangiocytes

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HIGH-THROUGHPUT MICROFLUIDIC PLATFORM FOR STUDYING VASCULARIZATION OF IPSC-DERIVED KIDNEY ORGANOIDS

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Kidney organoids derived from human induced pluripotent stem cells (iPSCs) represent a powerful in vitro model for studying kidney development, disease mechanisms and drug testing. Despite the great level of structural complexity reached in vitro, these kidney organoids are immature possibly due to the lack of a functional vascular system. Transplantation of kidney organoids under the kidney capsule of a mouse can significantly improve their maturation. However, alternative approaches are valuable for studying these processes in vitro. Microfluidic techniques show great potential in bridging the gap between 2D in vitro cultures and animal models. Here, we present the use of a high-throughput in vitro 'grafting' platform which allows co-culture of vessels with kidney organoids. One unit of the Mimetas Organoplate® Graft is made of two microfluidic channels in which endothelial cells can be patterned against ECM. Presence of a tissue chamber allows endothelial cell co-culture with 3D tissues. When kidney organoids are used, extensive vascular remodeling occurred with formation of a complex 3D network of angiogenic sprouts growing towards the tissue. Moreover, vessel stabilization can be monitored overtime by real time imaging and perfusion with 150 kDa Dextran. The established kidney organoid-on-a-chip system provides a promising platform for drug testing and disease modeling.

Keywords: Kidney Organoids; Microfluidics; Vascularization

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HUMAN BILE DUCT-DERIVED ORGANOID CULTURES FOR STEM CELL CHARACTERIZATION AND DISEASE MODELING

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Integrity of the biliary tree is imperative for liver function. Though evidence suggests that peribiliary glands harbor stem cells that contribute to bile duct homeostasis and repair during disease or injury, these are not well characterized. Therefore, the aim of our study is to expand and characterize human biliary stem cells using 3-dimensional organoid. For this, human extrahepatic bile ducts and paired liver biopsies (n=40) were collected from donor liver grafts at time of liver transplantation. Bile duct organoid cultures were initiated and propagated by weekly passaging using similar conditions as described for liver biopsies. The bile duct-derived organoids were characterized and compared to paired liver-derived organoids by phenotypic (EM, light sheet microscopy), genomic (q-PCR, RNAseq) and proteomic (MassSpec, immunohistochemistry) analysis. Growth characteristics and genetic stability was determined and functional transporter channel function was measured using Ussing chamber technology and Forskolin Induced Swelling (FIS) assays. In addition, the ability of hepatocyte and cholangiocyte differentiation was studied. Organoids were efficiently grown from the bile ducts and expanded for >9 months. These organoids stained positive for biliary cell markers CK19, EpCAM and MUC1. RNAseq analysis demonstrated expression of stem cell markers LGR5, PDX1 and Sox9. Functional transporter channels activity was detected for CFTR and AE2. Although bile duct-derived organoids were less prone to differentiate towards hepatocyte lineage as compared to their liver-derived counterparts, they had good differentiation capacity towards cholangiocyte lineage. To demonstrate that these bile duct organoids could be used for disease modeling, we observed lack of CFTR channel activity in bile duct organoids from a cystic fibrosis patient. In conclusion: This study showed the presence of LGR5-positive stem/progenitor cells in human extra hepatic bile ducts which can be expanded long-term as bile duct organoids. These organoids express hepato-biliary genes and proteins, and show functional transporter channel activity and can be used for disease modeling and tissue engineering applications.

Keywords: Bile duct-derived organoids; Disease modeling; Extrahepatic bile duct

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THE RENIN-ANGIOTENSIN SYSTEM IS PRESENT AND FUNCTIONAL IN IPSC-DERIVED KIDNEY ORGANOIDS

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The intrarenal renin-angiotensin system (RAS) arises early during kidney development and is important for proper nephrogenesis. The lack of a suitable human model to study the intrarenal RAS hampers investigation into the relevance of this system. Recently protocols for the in vitro generation of induced pluripotent stem cell (iPSC)-derived kidney organoids were developed. In this study we investigated the presence and functionality of the RAS in iPSC-derived kidney organoids. 4 human iPSC lines were grown on Geltrex and treated with CHIR99201 and fibroblast growth factor 9, after which the cells were transferred to a transwell membrane. The resulting organoids showed a 10- to 40-fold increase in mRNA for kidney-specific markers after 25 days of culture, including a marker for renin-producing stromal cells, FOXD1. Renal structures were observed using immunohistochemistry. Moreover, there was a 10-fold increase in the expression of the organic anion transporters OAT1 and OAT3, suggesting tubular function. Interestingly, the mRNA level of angiotensinogen (AGT) increased more than 100-fold as early as day 7 of the culture in comparison to iPSC and remained stable until day 25. Also, angiotensin receptor type 1 and type 2 mRNA expression increased and remained highly expressed throughout the culture, while high levels of ACE were maintained in kidney organoids. Finally, a 10- to 100-fold increase in the mRNA expression of renin was observed at day 25. The use of an indirect enzyme-kinetic assay revealed the functionality of renin in the kidney organoids at day 25, as measured by the conversion of exogenously administered AGT to angiotensin I. Moreover, analysis of the medium harvested from kidney organoid cultures at day 25 exhibited varying amounts of renin activity, ranging from 12 to 200 ng angiotensin I/ml per hour. The addition of the cyclic AMP-elevating agents forskolin and dibutyryl cyclic AMP to the culture for 24 hours increased the mRNA expression of renin drastically (up to 1000-fold), indicating that the production of renin in the kidney organoids may be a regulated and inducible process. In summary, we demonstrate the presence and functionality of components of the RAS in human iPSC-derived kidney organoids. This provides the opportunity to study the intrarenal RAS and its regulation in an in vitro human model.

Keywords: Renin-angiotensin system; Induced pluripotent stem cells; Kidney organoid

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CHARACTERIZATION OF THE IMMUNOGENICITY OF IPSC-DERIVED KIDNEY ORGANOIDS

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There is an increasing interest in iPSC-based therapies for kidney regeneration. Recently protocols for the in vitro generation of kidney organoids have been developed. For successful implementation into clinical practice, immunological acceptance of the iPSC-derived cells is crucial. Therefore, our aim was to study the immunogenicity of iPSC-derived kidney organoids. Three human iPSC lines were grown on Geltrex and treated with CHIR99201 and fibroblast growth factor 9. The resulting organoids showed a 10- to 40-fold increase in mRNA for kidney-specific markers after 25 days of differentiation. Immunostaining confirmed that organoids contained essential renal structures. The kidney organoids were cultured together with peripheral blood mononuclear cells from two healthy donors for 7 days. Subsequently, a >100-fold increase in the mRNA expression of the leukocyte marker CD45 was observed in the organoids. Immunostaining confirmed the presence of infiltrating CD45+ cells in the organoids. The response appeared to be T-cell mediated as there was a 10-fold mRNA increase in CD4 and CD8, while macrophage marker CD68 was also highly expressed. At the protein level, T-cells predominantly clustered around glomerular structures while macrophages were diffusely distributed throughout the organoid. A mixed pattern of macrophages could be observed, as both pro-inflammatory (M1) and anti-inflammatory (M2) macrophage markers were substantially increased at the mRNA level. Even though a 10-fold mRNA increase of inflammatory factors such as TNFα and Granzyme B was suggestive of a pro-inflammatory response, immunofluorescence showed that the infiltrating cells did not proliferate as observed by the absence of Ki-67+CD45+ cells. The mRNA expression of kidney differentiation markers remained stable throughout the co-culture. Yet, immunohistochemistry revealed that the expression of WT1, a podocyte marker, was decreased 4-fold in comparison to control organoids, indicating that specifically podocytes may be a target of the immune response. Although further characterization of the immune response to kidney organoids and its influence on differentiation is required, these preliminary results offer novel insights into the in vitro interaction of immune cells with iPSC-derived kidney organoids.

Keywords: Immunogenicity; Induced pluripotent stem cell; Kidney organoid

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THE ARYL HYDROCARBON RECEPTOR PATHWAY DEFINES THE TIME FRAME FOR RESTORATIVE NEUROGENESIS

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Zebrafish have a high capacity to replace lost neurons after brain injury. New neurons involved in repair are generated by a specific set of glial cells, known as ependymoglial cells that represent stem cells in the zebrafish telencephalon. We analysed changes in the transcriptome of ependymoglial cells and their progeny after injury to infer the molecular pathways governing restorative neurogenesis. We identified the aryl hydrocarbon receptor (AhR) as a regulator of ependymoglia differentiation towards post-mitotic neurons. In vivo imaging showed that high AhR signalling promotes the direct conversion of a specific subset of ependymoglia into post-mitotic neurons, while low AhR signalling promotes ependymoglial proliferation. Interestingly, we observed the inactivation of AhR signalling shortly after injury followed by a return to the basal levels 7 days post injury. Interference with timely AhR regulation after injury leads to aberrant restorative neurogenesis. Taken together, we identified AhR signalling as a crucial regulator of the neurogenic fate of ependymoglia and of the timing of restorative neurogenesis in the zebrafish brain.

Keywords: Regeneration, Stem Cells; Neurogenesis, AhR signalling; Differentiation, proliferation

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3D CARDIAC ORGANOIDS FOR STUDYING MULTINUCLEATION OF HUMAN CARDIOMYOCYTES DERIVED FROM INDUCED PLURIPOTENT STEM CELLS LACKING HEME OXYGENASE-1

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During myocardial infarction up to 25% left ventricle cardiomyocytes (CMs) are lost. Non-functional scar tissue is formed in damaged area which weakens the heart muscle and might lead to heart failure. Therefore it is reasonable to understand the mechanisms of CMs proliferation. To date, mechanisms of CM proliferation were investigated mainly in murine model. However, there are several limitations of this model. One of them is different proportion of multinucleated CMs. In mice percentage of terminally differentiated, non-dividing multinucleated CM is up to 90%, whereas in human it's 25-60%. This might be linked to higher energy demand of mice hearts, as their heart beating rate is approx. 10x faster than in human. High energy production is linked with elevated ROS production, which in turn can damage DNA and result in cell cycle arrest and formation of multinucleated cells. Due to mentioned differences, molecular pathways responsible for proliferation in murine model might be different in human. CMs derived from human induced pluripotent stem cells serve as a platform for investigating CMs proliferation mechanisms in human. By modulating Wnt/β-catenin pathway using small molecular inhibitors, virtually pure (95%) CM population is obtained. Expression of cardiac markers i.e Cardio Troponin T and I and action potential measured by patch clamp confirmed their cardiac properties. What is important, we also detected multinucleated CMs. CRISPR/Cas9 mediated knock down of cytoprotective enzyme heme oxygenase-1 (HO-1), crucial for embryonal heart development, resulted in less mature phenotype of CMs, basing on shortened action potential and lower expression of ion channels. To partially mimic in vivo process of heart development, we have modified 2D culture protocol. At different days of differentiations, cells were formed into organoids and cultured in 3D up to 45 days. Interestingly, CMs from organoids, compared to 2D cultured cells were characterised by more mature phenotype, basing on expression pattern of Titin N2BA and N2B and increased expression of potassium channel KCNH2. Spatially-patterned CMs in organoids might facilitate formation of multinucleated cells, thus are more suitable platform for investigating multinucleation and proliferation of CMs, compared to 2D culture.

Funding Source
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Keywords: Cardiomyocytes; Organoids; Multinucleation

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HUMAN PLURIPOTENT STEM CELL DERIVED LIVER ORGANOIDS

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Human pluripotent stem cell derived hepatocytes have immense potential to revolutionise the field of hepatotoxicity and represent a paradigm shift for the treatment of liver disease and regenerative medicine. Unfortunately, hPSC derived hepatocytes do not fully recapitulate the full cellular and metabolic repertoire of a fully functional, adult liver. With the advent of hPSC derived organoids marking a critical turning point in this regard. Current liver organoid models, while useful, generally provide the opportunity to study one or two cell types of the liver (traditionally hepatocytes and/or cholangiocytes). While these model systems can be used to interrogate drug metabolism, liver tissue regeneration, and generate large number of hepatocytes, the lack of functional and cellular equivalency when compared to the liver sinusoid makes them insufficient to accurately model liver toxicity. In order to address these shortcomings we have developed a 3D liver organoid model system from hPSCs that accurately present with all the expected cell types of the liver (hepatocytes, cholangiocytes, stellate, LSECs, endothelial cells etc). The hPSC derived liver organoids demonstrate significantly enhanced drug metabolism activity and inducibility, and critically this enhanced function can be maintained during long-term culture. This simple and robust method of generating large number of liver organoids is of potentially great interest to the field as it is readily scalable at low cost due to the exclusive use of small molecules as the drivers of differentiation.

Funding Source
This work was supported by the Research Council of Norway project 247624 and partially supported by the Research Council of Norway through its Centres of Excellence scheme, project number 262613.

Keywords: Liver Organoids; Human pluripotent stem cells; Small molecule driven procedure.

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THREE-DIMENSIONAL BIOPRINTING IPSCS TO FORM ORGANOIDS TO RECAPITULATE HUMAN LIMB BUD DEVELOPMENT – AN OSTEOARTHRITIS DISEASE MODEL

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Cartilage lesions that can develop into degenerated osteoarthritis (OA) cartilage are a worldwide burden. As a prospective treatment for such lesions, we have previously shown that human-derived induced pluripotent stem cells (iPSCs) can be 3D bioprinted into cartilage-mimics. The advantages by using an established iPSC line developed from chondrocytes are unlimited, immortal characterized cell source with a differentiation bias towards cartilage. Designing protocols that generates hyaline cartilage from pluripotent cells in vitro is still a challenge, due to that joint formation are late in development and far from the pluripotent state. There are recent protocols for hyaline-like cartilage generation from iPSCs. Herein our 3D differentiation of bio-printed iPSC line or organoids experience that resemble limb bud formation will be discussed. Also, results from screening by use of a molecular library on our genetically modified iPSC-line expressing green fluorescence protein (GFP) controlled by an OA-marker will be presented.

Funding Source
Financial support was received from Swedish research council VR 2015-02560, granted Stina Simonsson.

Keywords: iPSC; Organoid; Osteoarthritis

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EXPLORING THE HOMING POTENTIAL OF HEMATOPOIETIC STEM CELLS THROUGH BONE MARROW ORGANOIDS

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A crucial initial step for the successful transplantation of hematopoietic stem cell (HSCs) is their 'homing', the trafficking of the stem cells and the subsequent engraftment in their bone marrow niches. Several approaches are available to improve the homing ability of HSCs, including the pre-treatment with drugs that increase the expression of CXCR4, a surface marker mediating homing. Rodent models are routinely used to functionally test the homing capacity of human HSCs. However, these models are very costly, time-consuming and only poorly recapitulate the human bone marrow niches. Here we explored the possibility of building a 3D bone marrow-like culture system that could be used to quantify the homing capacity and maintenance of cord blood (CB)derived-HSCs in a more tractable in vitro setting. To this end, we established a bioengineered microtissue platform in which human mesenchymal stem/progenitor cells (hMSPCs) and human endothelial cells (hECs) can be aggregated and co-cultured with high reproducibility and throughput. The two cell types were found to form compact spheroids that expanded over time in culture and maintaining key phenotypic markers of the two cell types. Intriguingly, hECs self-organized into interconnected, vessel-like networks within the hMSPCs environment. A small fraction of CB-HSCs showed the capability of migrating into compact spheroids and colonized mostly in regions close to the vasculature network. Interestingly, CB-HSCs of different donors showed clear varieties of their homing capacity. These data suggest that the bone marrow organoids hold potential for assaying stem cell homing in vitro. Ongoing experiments aim at validating these initial findings by manipulating the molecular machinery that controls homing in vivo, by capturing stem cell dynamics through time-lapse microscopy, and by characterizing interactions between CB-HSCs and their putative in vitro niches.

Keywords: Homing; Hematopoietic stem cells; Bone marrow niche

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THE ROLE OF GRAVITY MECHANOTRANSDUCTION IN REGULATING STEM CELL TISSUE REGENERATIVE POTENTIAL AT THE SINGLE CELL EXPRESSOME LEVEL

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Forces generated by gravity in load-bearing tissues such as bone marrow promote stem cell-based tissue-regenerative processes by increasing proliferation and differentiation of tissue progenitors. Conversely, in microgravity, mouse bone marrow mesenchymal and hematopoietic precursors down-regulate differentiation markers and up-regulate stemness maintenance genes. In-vitro, mechanical loading of mesenchymal stem cell-derived osteoprogenitors on a collagen matrix also promotes proliferation and differentiation leading to increased mineralized tissue via a p21/CDKN1a-regulated mechanism. In this work we sought to test the hypothesis that gravity mechanotransduction regulates stem cell tissue regenerative processes by modulating stem cell proliferation and differentiation fates at specific cell cycle stages. To do this we subjected clonally-derived mouse embryonic stem cell cultures on a collagen matrix to either a 60 min pulse of gravity mechanotransduction, or no stimulation. Six hours post-stimulation, we used a 10X Genomics Chromium/Single Cell controller to generate bar-coded single cell Illumina libraries and sequenced expressomes for 5,000 1g control cells, and 5,000 cells pulse-stimulated with 50g, simulating running/jumping bone marrow hydrostatic pressures. Initial analyses of unstimulated cells show seven major graph-based clusters of cells, corresponding to different cell cycle stages, with cluster seven showing a unique highly over-expressed (3-5 log2 fold) pattern of cytoskeletal and lipid membrane traffic genes including actin, and actin regulating proteins, cytokeratins and annexins (Acta2, Tagln, Ker8, Ker9, Ker19, Anxa2, Anxa3, Anxa5). Mechanostimulation of cells in

cluster seven also resulted in maintenance of the cytokeratin/annexin pattern with additional highly elevated expression levels of differentiation markers for neuroblasts and muscle as well as pH/chloride regulation (Ahmak, Cald1, Lgals3, Sct, Clic1), with other clusters showing much smaller or no alterations. The results suggest that gravity mechanostimulation results in increased cell proliferation and differentiation, and that these effects at the expressome level are most notable on a specific cell-cycle cluster encompassing 10% of the total mouse embryonic stem cell population.

Funding Source
NASA Space Biology and NASA grant NH14ZTT001N-0063 to E. Almeida.

Keywords: gravity; mechanotransduction; mouse embryonic stem cells

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METABOLIC REGULATORY PATHWAYS IN KIDNEY ORGANOID DIFFERENTIATION

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The role of metabolites in regulating differentiation of pluripotent stem cells to kidney organoids is a field of growing interest. Mapping these intrinsic regulatory pathways can aid in understanding embryonic development, as well as provide insight to improve the directed differentiation process for tissue and organ engineering. We performed a screening assay to assess which amino acids play a regulatory role in the development of kidney organoids. Advanced RPMI media was generated that lacked each of the 22 distinct amino acid components, and directed differentiation to nephron progenitor cells was performed using these media on pluripotent stem cells. Deprivation conditions for arginine, cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine or valine were incompatible with cell survival from pluripotency to day 3 of the differentiation process. Deprivation of alanine, asparagine, aspartate, glycine, glutamine, glutamate, hydroxyproline, or proline allowed for cell survival until completion of the protocol at the end of day 9. All conditions displayed expected morphologic development demonstrated six2 expression. Most notable were the deprivation conditions for glutaminergic pathway. Deprivation of members of the glutaminergic pathway advanced in vitro differentiation more rapidly than complete media. These data further illustrate the differing effects of discrete metabolic pathways in regulating differentiation of pluripotent stem cells to nephron progenitor cells.

Funding Source
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Keywords: kidney organoid; metabolism; directed differentiation

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HUMAN IPSC-DERIVED CARDIAC ORGANOIDS MIMIC EARLY EMBRYONIC HEART DEVELOPMENT

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The development of cardiac organoids is crucial to model human cardiac development and disease. Heart morphogenesis initiates from a simple structure and composition, which evolves into a more complex organ through the temporal emergence of distinct cells populations that contribute to heart substructures. Human induced pluripotent stem cells (hiPSCs) are a valuable cell source, due their ability to model ex vivo aspects of human cardiogenesis. Previous efforts to study cardiac development with hiPSCs lack the co-emergence of multiple cardiac cell populations, whose temporal differentiation, growth, morphogenesis and maturation processes are inherent to heart development. In order to generate heterogeneous, self-organized, functional cardiac organoids from hiPSCs,- cardiac mesoderm progenitor cells were specified by modulation of Wnt signaling prior to aggregation and 3D suspension culture thereafter in hydrodynamic conditions. By day 10 of differentiation, organoids consisted of a cardiomyocyte (CM) core surrounded by stromal cells embedded within a proteoglycan-rich extracellular matrix and an outer layer of Tbx18+ epicardial-like cells. The organoids grew significantly with culture duration, from ~250μm to >1mm in diameter by day 100 and survive for more than 1 year. Gene expression and patch clamp analysis revealed that the organoids were comprised of >70-80% atrial/nodal-like CMs and dissociated CMs exhibited greater sarcomere alignment and more elongated morphology than typical hiPSC-CMs. After 40 days of differentiation, CD31+ endothelial cells were interspersed among CM and increased in number by day 100. As early as day 10 of differentiation, organoids exhibited spontaneous contractility and calcium flux transients which became more responsive to external field stimulation over time. After 100 days, organoids exhibited little spontaneous beating but were capable of being paced up to 4-6 Hz, revealing an increased functional maturation state. Overall, these results describe the first cardiac organoid from human iPSCs that recapitulates the co-emergence and self-organization of multiple cardiac cell populations as well as physiological heart function, thereby establishing an in vitro model to examine mechanisms regulating human embryonic morphogenesis and cardiac maturation.

Funding Source
CIRM.

Keywords: Organoids; cardiogenesis; stem cell-derived cardiomyocytes

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REGENERATION OF FULL THICKNESS SKIN AND HAIR FOLLICLES IN WOUNDS TREATED WITH AN AUTOLOGOUS HOMOLOGOUS SKIN CONSTRUCT IN PRECLINICAL PORCINE MODEL

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Standard of care for cutaneous full-thickness wounds is limited by incomplete healing, graft failure, scarring and contraction, donor site morbidity and lack of appendage formation. A novel approach using an autologous homologous skin construct (AHSC) (SkinTETM, PolarityTE, Salt Lake City, UT) was evaluated for full-thickness skin regeneration in a pre-clinical porcine model. AHSC, which is comprised of minimally polarized functional units created from a piece of full thickness skin involving a process of micro-wounding that activates the endogenous stem cells that can generate all components of the skin and are returned immediately to the wound bed to expand within the wound itself and not ex vivo. 36 full-thickness wounds on the dorsum of female Yorkshire swine were treated with AHSC or split-thickness skin graft (STSG). For 6 months, healing was documented with digital single-lens reflex (DSLR) and stereoscopic imaging. Excised defect areas were evaluated by brightfield, confocal, and scanning electron microscopy (SEM). Molecular composition of wounds was assessed with Raman spectroscopy. Gene expression was analyzed using stem cell, apoptosis, and extracellular pathway PCR arrays. Wounds treated with the AHSC demonstrated improved healing, decreased contracture, and development of hair follicles and dermal appendages on macroscopic and fluorescent imaging. Collagen organization characterization via SEM was consistent with native skin. Raman intensity profiles demonstrate similar bond energy peaks corresponding to collagens in native and AHSC-treated wounds. RNA extracted from AHSC-treated wounds demonstrated that stem cell, apoptotic, extracellular matrix and adhesion gene expression is similar to native tissue. Stereoscopic, fluorescent, and SEM imaging of hair follicles from AHSC-treated areas have morphology, organization, and nuclear content consistent with hair follicles from native skin. Raman intensity profiles have correlative peaks among native and AHSC regenerated hair. Preclinical application demonstrated complete healing with appendage and pigmentation development along with improved functional outcome compared to STSGs suggesting AHSC is capable of regenerating fully functional skin in pre-clinical models.

Keywords: autologous homologous skin construct; wound healing; functional full thickness skin regeneration

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CARTILAGE MICRO-TISSUES MIMIC BONE DEVELOPMENT AND REGENERATE LONG-BONE DEFECTS

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A major limitation in Tissue Engineering is the ability to control complexity within 3D engineered constructs. Diffusion limitations lead to the development of uncontrolled or even adverse environments leading to inappropriate stem cell fate decisions and cell death. Recently, bottom-up strategies have been introduced advocating the use of smaller tissue modules as building blocks for the formation of larger implants. This strategy in combination with recapitulation of the developmental process of bone formation; endochondral ossification, where a cartilage intermediate is transformed into bone may possess great potential for bone regeneration. Human periosteal derived progenitor cells were seeded on non-adherent agarose surfaces containing microwells. This allowed initial condensation and the formation of µ-aggregates with controlled-size. Chondrogenic differentiation resulted in 3D cartilage intermediate µTissues, positive for safranin-o, indicating deposition of mature cartilaginous extracellular matrix while RNA sequencing analysis revealed the onset of hypertrophy following signaling patterns analogous to endochondral ossification. When single µTissues were implanted subcutaneously, they developed into µBone organs exhibiting a central compartment with blood vessels. Interestingly, when these µTissues were fused via self-assembly into larger implants (2mm Ø) in vitro they formed one larger bone organ containing a cortex and a prominent bone marrow compartment after subcutaneous implantation. As control we used implants formed by cells cultured in macro-pellet format in the same media formulation and containing the same amount of cells as the bottom-up assembled implant. In contrary to the fused µTissues, the macro-pellet demonstrated a large fibrotic tissue domain at the center of the implant. Finally, using fused µTissues, we evidenced reproducible successful healing of a murine critical-sized bone defect with presence of implanted human cells after 8 weeks. Our findings provide a first step in establishing a biomanufacturing pipeline for robust production of fracture callus-like cartilage tissue intermediates for bone regeneration. Although demonstrated for a skeletal application, the strategy presented could be applied for the manufacturing of a variety of tissues.

Funding Source
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Keywords: Developmental Tissue Engineering; Endochondral ossification; Fracture healing

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DEVELOPMENT OF SUBSTRATE PLATFORMS FOR GENERATING HUMAN PLURIPOTENT STEM CELL-DERIVED OLIGODENDROCYTES

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Oligodendrocyte poses a crucial role to facilitate transmission of electrical signals by forming myelin on neurons in central nerve system (CNS). Thus, defects of oligodendrocytes cause critical neurodegenerative disease, cognitive and behavioral disorders. Despite the important roles of oligodendrocyte in CNS, there have been few studies to develop effective culture platforms for scalable production of functional oligodendrocytes from stem cells. In this study, we developed a novel substrate platform to efficiently generate oligodendrocytes from human induced pluripotent stem cells (hiPSCs). Through a suspension culture of hiPSC-derived oligodendrocyte progenitors on the engineered substrate platform, we could shorten time required for oligodendrocyte differentiation and maturation, and produce a large quantity of functional oligodendrocytes. Oligodendrocytes generated from our substrate platforms showed increased expression of several oligodendrocyte markers including myelination binding protein (MBP), one of the most important indicators of oligodendrocyte maturation and myelination functionality. Here we may conclude that our novel culture platform enables efficient and scalable production of oligodendrocytes from stem cells, which would be applied for demyelination disease model and regenerative medicine for the treatment of demyelination diseases.

Funding Source
This research was supported by a grant (2015M3A9B4071076) from the Bio & Medical Technology Development Program of the National Research Foundation of Korea funded by the Korean government, MSIT and Brain Korea 21 plus program.

Keywords: Human induced pluripotent stem cell; Oligodendrocyte; Culture substrate

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ESTABLISHMENT OF THREE-DIMENSIONAL IN VITRO DIGESTIVE SYSTEM WITH A TRI-CULTURE OF ORGANOIDS

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Recently, there is an increasing interest on the researches of the digestive systems which play important roles in the human pathophysiology. The development of three-dimensional (3D) organoid culture technology enables to establish in vitro models that possess cellular complexity and resemble native organs, leading to more precise in vitro studies of the digestive systems. Despite promising aspects of organoid technology, a single organoid culture is insufficient to reconstitute the dynamic communications and interactions between various digestive organs in the body. Therefore, here we developed a multi-organ cultivation platform of digestive systems with stem cell-derived gastric organoids, intestinal organoids, and hepatic organoids. We confirmed that phenotypes of each organoid were maintained during the tri-culture. We also observed cross-talk between organoids in this digestive system such as bile acid-mediated regulation of the hepatic enzyme for bile acid synthesis in co-culture of intestinal organoids and hepatic organoids. Our in vitro digestive system with multiple organoid culture can recapitulate in vivo-like complex phenomena and interactions more precisely than a single organoid model.

Funding Source
This research was supported by Brain Korea 21 plus (BK-21PLUS) program, and a grant (2018M3A9H1021382) from National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (MSIT), Republic of Korea.

Keywords: organoid; digestive system; tri-culture

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CULTIVATION OF HUMAN BRAIN-LIKE TISSUE MODELS IN A THREE-DIMENSIONAL PRINTABLE MINIBIOREACTOR

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Standard three-dimensional (3D) tissue-like in vitro cultivation involves agitation-mediating devices such as orbital shakers or stirred-tank bioreactors, which facilitate oxygen and nutrient uptake and help forming compact spheroids. However, these devices do not support parallelization while allowing minimal condition testing only. Here we show a customized, 3D-printed mini-bioreactor that can be utilized for brain-like tissue cultivation in up to 48 (12-well format) or 96 (24-well format) conditions in parallel due to its stackable 4-levels setup. We find that our mini-bioreactor in combination with a modified cultivation protocol for cerebral organoids is beneficial for standardized growth, shape and nutrient supply. These optimized conditions result in the formation of brain-like tissues with an overall more physiological, non-spheroid morphology as compared to standard agitation cultivation. Moreover, we show that human neuroblastoma-derived spheroids profited from cultivation in the mini-bioreactor judged by an average 2-fold increase in diameter. We anticipate our optimized procedure to be of high value for cost-efficient and robust realization of in vitro 3D models of brain and tumor development needed to parallelize drug screening at small scale.

Keywords: Organoid; 3D-printing; Neuroscience

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ENTEROENDOCRINE CELL-DERIVED HORMONE A IS INVOLVED IN THE INTESTINAL HOMEOSTASIS BY DIRECTING DIFFERENTIATION OF THE MOUSE INTESTINAL ORGANOID

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Organoids can be utilized as a modeling system for the investigation of stem cell biology, organ development and disease progression, as well as for drug discovery.
Here, we isolated mouse small intestinal crypts and cultured intestinal epithelial organoids, one of the most well-established organoid system. It develops a highly organized structure with both adult stem cell niches and fully differentiated populations, which enable us to study the nature of stem cell homeostasis. Interestingly, as the largest endocrine system in the body, enteroendocrine cells (EECs) produce the highest level of hormones and bioactive molecules despite that they comprise only 1% of the intestinal epithelium. In this study, we focused on EEC-derived endogenous signals to evaluate their impact on the intestinal homeostasis. It was noted that one of EEC-secreted hormone A was impeded the normal generation of intestinal organoids; upon treatment of A, organoid growth was retarded and the typical budding pattern was almost disappeared, resulting in the round to oval shaped-organoids. The epithelial lining was intact and budding ability was restored after A withdrawal, suggesting that A did not induce epithelial cell death. We found that the morphology of A-treated organoid was similar to that of IWP-2(Wnt inhibitor)-, DAPT (Notch inhibitor)-and U0126(MEK inhibitor)-treated organoid. Also, re-budding process after A removal was accelerated with Chir99021 and Epiregulin, which activates Wnt and EGF pathway, respectively. Since those signaling are important to maintain ISC population, we performed qPCR to screen the ISC and differentiated intestinal cell markers. Importantly, both active ISC and proliferation markers are down-regulated, while secretory lineage markers such as Neurogenin3, defensin-a and Gob5 were increased upon A treatment. Immunohistochemistry analysis also revealed that lineage-specific differentiation was induced after A treatment. These data suggest the novel endogenous impact of EEC-derived hormones on ISC maintenance, differentiation and intestinal homeostasis.

Funding Source
This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. NRF-2018R1A5A2023879) and and partially supported by a grant from Pusan National University.

Keywords: intestinal organoid; enteroendocrine cell; wnt signaling

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IN VITRO MODELING OF UROLOGICAL DISEASES BY THREE-DIMENSIONAL PRINTED MINIATURE BLADDER

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Despite tremendous efforts for studying in vivo physiology and disease modeling with organoid system, organoids do not faithfully recapitulate in vivo tissues because of the lack of niche or microenvironment. Here, we first established a novel in vitro co-culture platform that structurally and functionally similar with in vivo tissues, normal bladder or bladder tumor. We demonstrated that urological diseases, such as urinary tract infection (UTI) or bladder cancer, can be modeled in our co-culture system, recapitulating in vivo phenomena. Our 3D model provides an advanced tool representing in vivo tissues with implication of potential use for personalized medicine and disease modeling.

Keywords: Organoid; Co-culture; Disease modeling

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DEVELOPMENT AND CHARACTERISATION OF CHOROID PLEXUS ORGANOIDS

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The choroid plexus is a highly conserved and surprisingly understudied secretory tissue in the brain. This tissue is enlarged in humans proportionally to our brain size and displays a number of important functions in the brain such as forming a protective epithelial barrier and secreting the cerebrospinal fluid (CSF). The CSF is important for the maintenance of physiological levels of nutrients in the brain, for the transport of signalling molecules and growth factors and for its protective role in the regulation of intracranial pressure. This fluid regulates several aspects of development in two fundamental ways: by exerting ventricular pressure that may be necessary for driving brain expansion and by regulating neural stem cell proliferation and differentiation in the brain. To explore the role of the choroid plexus-CSF system in early stages of human brain development, we recently established a protocol to generate choroid plexus organoids using a combination of signalling molecules that are physiologically present during the stages of development of this tissue. More interestingly, not only do these organoids develop the choroid plexus but they also recapitulate fundamental functions of this tissue, namely secretion and formation of a tight epithelial barrier. We detected the presence of choroid plexus specific water channels and

transporters localised on the apical brush border of the choroid plexus epithelium by histological and EM analysis. These tissues displayed tight junctions forming the epithelial barrier, and we noticed the formation of large fluid-filled cysts protruding from the organoids, the contents of which, analysed by mass spectrometry, highly resembles human embryonic CSF. In conclusion, we believe this system represents an excellent tool to study the role of the choroid plexus-CSF system in human brain development.

Keywords: Organoids; Choroid plexus; Cerebrospinal Fluid

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TBI MODELING THROUGH SELF-ORGANIZED HUMAN FORE-BRAIN ORGANOIDS FROM SINGLE CELL SOURCE

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Three-dimensional structure brain organoid represents human brain in vitro models that can be usefully utilized to study early neuronal development, neurodegenerative disease, and human based drug screening. However, long-term culture and heterogeneous generations of brain organoid are challenges to overcome. Moreover, in vitro traumatic brain injury model has not been previously reported in human brain organoids. Here, we generate self-organized human forebrain organoid from adult dermal fibroblast-derived neural stem cells, which can recapitulate traumatic brain injury model using a 3D cell culture system. For homogenous brain organoid generation, similar-sized organoids were produced using same number of cells embedded in matrigel and maintained in stationary culture. These organoids were characterized by apical-basal polarity and self-formation of neuroepithelium, which display premature neuronal development. For maturation of the organoids, self-organized neuroepithelium was transferred to spinner flask and maintained in agitated culture.
To analyze these fore-brain organoids, we performed immunohistochemistry and quantify cell composition, division rate, and relative gene expression level. Based on our data, cortical plates in mature fore-brain organoids were self-organized, displaying two distinct layers; inner layer (ventricular zone) and outer layer (early and late cortical-plate zone). In addition, we applied our fore-brain organoid to in vitro traumatic brain injury model system. In this model, we confirmed cell apoptosis and microglia activation in the traumatic lesions of the organoids.
We also investigated neuroregenerative patterns in fore-brain organoids during recovery. Overall, this study suggests that human fore-brain organoids generated from neural stem cells can provide new opportunities to develop drug screening platforms and personalized disease modeling.

Keywords: Fore-brain; NSC; TBI

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FUNCTIONAL DIFFERENCES AND SIMILARITIES BETWEEN HIPSC- AND PERIPHERAL BLOOD-DERIVED MONOCYTES AND MACROPHAGES

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The ability to obtain high quality, functionally characterized monocytes and macrophages from human induced pluripotent stem cells (hiPSCs) would be of great benefit for modelling diseases in which inflammation plays an important role. Here we developed a fully defined, efficient differentiation protocol to derive monocytes and macrophage subtypes from multiple hiPSC lines. We compared hiPSC-macrophages functionally and for gene expression and cytokine production with primary peripheral blood-derived monocytes/macrophages (PBDMs) and found similarities but also important differences. hiPSC-macrophages showed higher endocytotic activity for AcLDL and efferocytosis than PBDMs but similar bacterial phagocytosis. Interestingly, hiPSC-macrophages also showed high tumor cell phagocytosis activity indicating potential value of these cells for drug screening in cancer immunotherapy. In summary, hiPSC-macrophages and PBDMs showed significant similarities but some functional differences, indicating that they could be complementary to PBDMs in many functional assays in vitro but with the additional advantage that they could be derived from patients with inflammatory diseases of interest to understand the nature of inflammatory defects.

Keywords: macrophages; hiPSCs; phagocytosis

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INDUCED PLURIPOTENT STEM CELLS (IPSC) GENERATED FROM ERYTHROID CELLS OF POLYCYSTIC KIDNEY DISEASE 1 (PKD1) PATIENT DIFFERENTIATED INTO THREE GERM LAYERS: POTENTIAL SOURCE FOR RENAL ORGANOIDS

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Human iPSC (hiPSC) can be obtained from any somatic cell. Here we showed that it is possible to generate hiPSC from circulating erythroid progenitors cells of patients with PKD1. The choice of erythroid cells for reprogramming is due minimally invasive procedure, genomic integrity and epigenetic memory. From these hiPSC it will be possible to generate renal organoids. Blood samples was donated by a patient with PKD1 and by a control health person, according to approved institutional procedures. Erythroid progenitor cells were separated from whole blood and expanded in vitro. Erythroid cell colonies were identified by expression of the transferrin receptor (CD71) and Glycophorin A (235A). The

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efficiency of the method was evaluated as 28.6% according to GFP expression, being 50% CD71 positive) and 67% positive for 235A. Dedifferentiation was induced by transfection (electroporation) with episomal vectors containing 5 reprogramming factors (Oct-4, Sox2, Lin28, L-Myc and klf4). After transfection, typical images of iPSCs-like colony began to appear as large size, tight cell packing and well defined borders. After 10 passages, endogenous pluripotency was assessed by Imunofluorescence using specific markers as Nestin, ATX2, GATA4 and SOX2. hiPSC were able to differentiated into the three layers, ectoderm, mesoderm and endoderm. Each layer was characterized by the expression of specific markers for endoderm (GATA4 and SOX147), mesoderm (CXCR4) and ectoderm (PAX6) detected by RT-PCR. Additionally, through immunostaining technique, differentiated cells of each layer were labeled by specific markers, including FOXA2 and OTX2 for endoderm, smooth muscle actin (SMA) and Brachyury (T) for mesoderm and Nestin for ectoderm. These results indicate that the easily obtained circulating erytroid progenitor cells from either, a PKD1 patient and a health person, could be reprogrammed to reach a pluripotency phase. As a perspective, from these hiPSC it will be possible to generate organoids, which similar architecture to the organ of interest as for example the kidney. The success in generate organoids from PKD1 patients will rise the possibility to better understand the PKD1 pathophysiology and the mechanisms of renal cysts formation

Funding Source
Research supported by FAPESP and cNPQ.

Keywords: iPS cells; erythroid cells; organoids

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SIMPLIFIED DIFFERENTIATION OF TRANSPLANTABLE NEURONAL CELLS FROM HES-CELLS

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Human embryonic stem cells are an attractive renewable source of various cell-types for transplantation and mechanistic studies. The existing protocols for making neuronal cells from stem cells are however mostly inefficient, time-consuming, intricate, pricey and/or giving low yields - resulting in low availability of relevant cells. Here we show how human embryonic stem cells can be differentiated into aggregates of neuronal cells of spinal cord identity by simple means – resulting in efficient, rapid differentiation of sufficient functional transplantable cells for downstream applications. As this gives around 3 million cells for under 30$ and 3h of hands-on time, purpose-specific optimisation should ensure its usefulness regarding both research and clinical applications relevant to pathological conditions affecting the spinal cord.

Keywords: human embryonic stem cells; organoids; spinal cord

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MODELLING REACTIVE GLIOSIS IN HUMAN RETINA ORGANOIDS

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Retinal organoids derived from human induced pluripotent stem cell (hiPSC) are a potential experimental model system for studies on neuronal degeneration and regeneration. Here, we sought to develop a human retinal organoid model to study reactive gliosis, a glial response commonly associated with neurodegenerative diseases of the mammalian central nervous system and potentially related with regenerative capacity, at least in some types of vertebrates. We established that the major types of retinal neurons, rod and cone photoreceptors, and glia, the radial Müller glia, develop and become postmitotic by culture day 150. Of note, human photoreceptors and Müller glia in retina organoids under baseline culture conditions did not present hallmarks of cell death or reactive gliosis, e.g. glial fibrillary acidic protein (GFAP), respectively. In contrast, reactive gliosis is well-known to be strongly induced upon organotypic culture of primary animal and human retina and brain tissues. Thus, we hypothesized that the retinal organoid system might provide a powerful tool for disease modeling. To test this hypothesis, we challenged retinal organoids with signaling factors previously associated with retinal damage, reactive gliosis and glial derived neuronal regeneration, at least in animal models. Thereby, we established a human retinal organoids model replicating several histopathologies that develop at the same time dynamically within 10 days (N>4 experiments, > 20 oragnoids per N in >3 different hiPSC lines): Of note, we observed acute and severe loss of photoreceptor neurons, hallmarks of reactive gliosis – including GFAP expression and cell proliferation of Müller glia; as well as impaired retinal stratification. In sum, our data suggest that acute, severe and complex histopathologies can be modeled in human retinal organoids. We conclude, that the organoid system offers a powerful model system and opportunity to study potential mechanisms of retinal degeneration, reactive gliosis and regeneration.

Keywords: organoid; disease model; retina

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CHARACTERIZATION OF HUMAN PANCREAS ORGANOIDS FOR CELL-BASED THERAPY OF TYPE 1 DIABETES

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Organoids are three-dimensional in-vitro-grown cell clusters that recapitulate many key features of the native organ. In the groundbreaking European project LSFM4LIFE (www.lsfm4life.eu) directed towards the therapy of type 1 diabetes (T1D) with human pancreas organoids (hPO). The aim is the isolation and the therapeutic-scale manufacture of hPO for cellular therapy of T1D. In this context, starting from discarded pancreatic tissues we developed a large-scale process in order to obtain large quantities of undifferentiated organoids and we set up a standardized protocol for passaging and culturing the hPO skipping enzymatic digestions and operator-dependent hPO progenitor picking. To give a new identity to our hPO we defined an experimental strategy aimed to deeply characterize our product. We first have designed a quality control gene card containing expression profiles for each developmental and differentiation pancreatic stages. We analyzed pancreatic markers for the bipotent progenitors (PDX1, SOX9, HNF1B), endocrine progenitors (NGN3, NEUROD1), adult epithelial stem cells (LGR5), acinar cells (AMY), ductal cells (MUC1, KRT19, EPCAM) and mature endocrine cells (INS, GCG, IAPP, SST). Concerning the immunophenotype we have determined the identity of the starting material and the hPO purity during passages by flow cytometry. We tested relevant markers of acinar (UEA-1), ductal (MUC1, CXCR4, EPCAM), endothelial (PECAM, MCAM) and mesenchymal (CD90, CD73) compartments. To address the healthy/quiescent status of hPO during passages, we performed a RT2 Profiler PCR-array till passage 11. In conclusion, we have been able to define a new hPO profile at the cellular and molecular level that will pave the way to defining GMP quality controls.

Keywords: Pancreas Organoids; Diabetes; GMP quality control

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P21WAF1/CIP1, MUSASHI-1, AND KRÜPPEL-LIKE FACTOR 4 REGULATE ACTIVATION OF MOUSE RESERVE INTESTINAL STEM CELLS AFTER GAMMA RADIATION-INDUCED INJURY IN VIVO

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The intestinal epithelium is a rapidly self-renewing tissue, maintained by active intestinal stem cells. After injury, BMI1 expressing reserve intestinal stem cells (rISCs) serve as a source of regeneration, but the molecular mechanisms regulating rISC remains unclear. Previously, we showed that Krüppel-like factor 4 (KLF4) plays a radioprotective role in the intestinal epithelium by modulating BMI1+ cells' response to radiation injury. Here, we investigated the mechanisms by which p21Waf1/Cip1, Musashi-1 (MSI1) and KLF4 coordinate to regenerate the intestinal epithelium. Bmi1CreER;Rosa26eYFP and Bmi1CreER;Rosa26eYFP;Klf4fl/fl mice were exposed to 12 Gy total-body γ-irradiation (TBI). Tamoxifen was administered 48 h before or 24 h after TBI to induce recombination in BMI1+ cells for lineage tracing (YFP+ cells). Small intestine was collected 0, 6, 48, 72 or 96 h after TBI and examined by immunofluorescent stain. We observed that during apoptotic phase (0-48 h post-TBI), surviving rISC-lineage YFP+ cells were located around +4 position and in transit-amplifying zone. Shortly after injury YFP+ cells started expressing p21 with a peak at 48 h post-TBI at nearly 60% of YFP+ cells. During regenerative phase (48-96 h post-TBI) p21 expression started to diminish while 70% of YFP+ cells co-expressed MSI1. RT-qPCR analysis of FACS-isolated YFP+ cells confirmed the inverse relationship between Cdkn1a and Msi1 expression. Luciferase assay showed that mouse MSI1 inhibits p21 translation, which was further confirmed by western blot analysis of irradiated HCT116 cells with or without human MSI1 overexpression. Furthermore, we observed that 96 h post-TBI KLF4 expression was increased, whereas MSI1 started to diminish following regenerative phase. Using luciferase assay, we showed that mouse KLF4 negatively regulates Msi1 transcription, suggesting KLF4 plays an important role in the initiation of the normalization phase. Collectively, these data suggest that p21, MSI1, and KLF4 are key factors that regulate survival, proliferation, and normalization of rISC-lineages during regeneration of the intestinal epithelium.

Funding Source
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Keywords: mouse reserve intestinal stem cells (rISCs); gamma radiation-induced injury; KLF4, MSI1, p21

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EFFECTS OF L-TYPE CALCIUM CHANNEL REGULATORS ON CHONDROGENIC POTENTIAL OF 3D CULTURED HUMAN MESENCHYMAL STEM CELLS UNDER MECHANICAL LOAD

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Osteoarthritis (OA) is characterized by the progressive deterioration and loss of articular cartilage and is actually one of the most common, costly, and disabling forms of articular diseases, while there is no effective treatment for it. Under physiologic conditions, moderate physical activity reduces the risk of cartilage deterioration, while in an altered biochemical and/or biomechanical setting, mechanical factors can lead to the OA development. One of the earliest chondrocyte responses to extracellular mechanical input is a transient increase in intracellular Ca2+ levels. Voltage-dependent Ca2+ channels that are regulated by different physical signals play critical role in controlling the intracellular Ca2+ responses of in situ chondrocyte in the loaded cartilage. Arterial hypertension is a comorbidity in more than 75% of patients with OA. Long-term consumption of antihypertensive drugs L-type Ca2+ channel blockers may lead to the altered performance of chondrocyte chanellome and modulate Ca2+ oscillations, resulting in altered synthesis of extracellular matrix (ECM). We analyzed the effects of abundantly used anti-hypertensive agents – Ca2+ channel blockers on human mesenchymal stem cell (hMSC)-derived organoids in hydrogel scaffolds under chondrogenic conditions, and compared them to the effects in human OA cartilage explants. Mechanical compression was applied using Flexcell Fx500 for 1 hour daily. Increased production of ECM has been determined by immunohistochemistry in the 3D cultured hMSCs in the presence or absence of Nifedipine or Bay K8644. MSC organoids and OA cartilage explants differentially responded to the mechanical compression and Ca2+ regulators. Mechanical compression in the presence of Nifedipine resulted in modulated expression of Sox9, MMPs and other genes related to chondrogenesis and catabolism. These data are important for consideration of chondroprotective approach in patients having comorbidities of cardiovascular diseases and osteoarthritis.

Funding Source
This research is funded by the European Social Fund according to the activity 'Improvement of researchers' qualification by implementing world-class R&D projects' through Measure No. 09.3.3-LMT-K-712; 2017-2021.

Keywords: Chondrogenesis of MSCs; L-type Ca2+ channel regulators; Osteoarthritis

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HUMAN ATRIAL TISSUES FOR TESTING ATRIAL-SPECIFIC PHARMACOLOGICAL COMPOUNDS OR MODELING ATRIAL FIBRILLATION

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Atrial fibrillation (AF) is the most common condition with abnormal atrial rhythm affecting more than 33 million patients worldwide. Unfortunately, most antiarrhythmic drugs are non-selective for the atria and risk fatal ventricular pro-arrhythmic events. Thus, it is very important to improve our understanding of underlying causes of AF and to develop more advanced models to predict atrial selectivity. By modulating retinoic acid signaling during differentiation of human embryonic stem cells (hESCs) towards cardiomyocytes (CMs), we generated atrial-like and ventricular-like CMs. Expression of atrial-specific genes, including the ion channel genes KCNA5 (encoding Kv1.5 channels) and KCNJ3 (encoding Kir 3.1) was strongly enriched in hESC-atrial CMs when compared to their ventricular counterparts. These ion channel genes were regulated by atrial-enriched chick ovalbumin upstream promoter (COUP)-TF transcription factors I and II. Importantly, these hESC-derived atrial CMs could predict atrial-selectivity of pharmacological compounds.
To generate pure populations of atrial- and ventricular-like CMs, we then targeted mCherry to the COUP-TFII genomic locus in hESCs expressing GFP from the NKX2.5 locus. This dual atrial NKX2.5eGFP/+-COUP-TFIImCherry/+ reporter allowed identification and selection of GFP+/mCherry+ atrial CMs from heterogeneous cultures following cardiac differentiation. These GFP+/mCherry+ cells exhibited transcriptional and functional properties of atrial CMs, whereas GFP+/mCherry- CMs displayed ventricular characteristics. To develop a more advanced model for drug testing or modeling AF, in a next step, we now generated 3-dimensional atrial and ventricular cardiac tissues which allow measurement of absolute contraction force. These atrial and ventricular tissues not only offer the possibility to compare functional aspects of pure cardiac tissues with cardiac tissue composed of CMs and non-CMs like endothelial cells and fibroblasts, but also allow functional testing of atrial-specific drugs, such as carbachol or DPO-1. Preliminary functional data on atrial versus ventricular tissues will be presented. In the future, it will be of interest to generate tissues using CMs differentiated from mutated hESC or hiPSC lines to model genetic facets of AF.

Keywords: Atrial cardiac tissue; Drug testing; Atrial disease modeling

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RETINAL ORGANOIDS: AN ACCELERATED METHOD OF GENERATING GANGLION CELLS FOR DISEASE MODELS

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Retinal diseases, like glaucoma and age-related macular degeneration, affect more than 253 million people worldwide. Since there is no effective cure for most of these disorders, they are becoming an increasingly large problem in global health. A subset of patients rely on treatments that only slow down the progression of the disease, but do not stop the impending visual impairment. Only recently, new experimental treatments such as gene-, stem cell-, and small molecule therapies are being developed. A complicating factor in the development of such treatments is that the various retinal diseases are associated with different cell types. Lately, a lot of research is focused on the development of a new type of in vitro model: retinal organoids. These retinas-in-a-dish contain all retinal cell types and are useful for investigating multiple disorders. Although the basic protocol has been established, methods are still continuously being improved. During our glaucoma research, we have developed a more rapid and efficient protocol for retinal organoid differentiation, focused on ganglion cell generation. Most published retinal organoid protocols initially use a floating embryoid body culture, and develop ganglion cells within 34-50 days of differentiation, promoted by certain factors that modulate developmental pathways. For research purposes, a faster and more robust protocol is desirable. We replaced the floating method with an encasement in a 3D matrigel matrix, which gives an initial boost to the differentiation of stem cells towards a retinal fate. With this method, we have managed to generate ganglion cells within 28 days of differentiation, without the addition of other external factors to direct cells towards a retinal fate. We have also been able to generate all other retinal cell types, confirmed by sq-PCR and immunohistochemistry using both developmental and cell specific markers. These cells include retinal pigmented epithelium and photoreceptors. Utilizing this protocol will not only allow us to study the development and morphology of the emerging eye with different degenerative disease models, but also gives us the opportunity to generate multiple different cell types that can be used for transplantations in a variety of animal disease models and ultimately, human transplantations in the future.

Funding Source
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Keywords: Retina; 3D-matrix; Organoids

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HYDROGEL ENCAPSULATION OF MESENCHYMAL STEM CELL USING TWO-PHASE SYSTEM FOR CHONDROGENIC DIFFERENTIATION

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In this study we optimized the encapsulation of mesenchymal stem cells (MSC) with hydrogel to enhance chondrogenic differentiation. We encapsulated MSC with alginate and gelatin using two phase system. Micro-beads were passed through a mineral oil to form uniform size aggregates using syringe pump and micro size needle uniform size. Cell encapsulated micro-beads were precipitated by gravity force and crosslinked with crosslinking agent (ex. Ca2+, Mg2+). We further confirmed the survival and production of extracellular matrix of micro-bead cultured cells were enhanced. These results implicated that hydrogel encapsulation provides useful platform technology for chondrogenic differentiation of MSC and enhanced cell survival after transplantation and consequently can be applied for cartilage defect repair.

Funding Source
We would like to acknowledge the financial support from the Korea Healthcare technology R&D Project (HI16O1002 & HI18C2186), Ministry for Health, Welfare & Family Affairs, Republic of Korea.

Keywords: Hydrogel; encapsulation; micro-beads

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GENERATION OF HAIRY-SKIN ORGANOIDS FROM PLURIPOTENT STEM CELLS

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Over one hundred million people worldwide suffer from injury or loss of skin due to burns, diseases, or genetic defects. Skin is essential for protecting body by regulating fluid retention and temperature, guarding against external stresses, and mediating touch and pain sensation. Human skin develops from coordinated interactions between multiple cell lineages and is vulnerable and difficult to be reconstructed once damaged. Despite repeated attempts for decades, however, a method of reproducing the full cellular diversity of skin in tissue cultures or in bioengineered skin equivalents has been elusive. Here we report a skin organoid culture system that generates complex skin from human pluripotent stem cells and recapitulate key features of skin development. We found that skin organoids are composed of stratified epidermis, fat-rich dermis, dermal condensate, pigmented hair follicles equipped with sebaceous glands and bulge stem cells, and sensory neuronal cells forming synapses with Merkel cells in organoid hair follicles, mimicking

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human touch circuitry. Furthermore, the skin organoids are comparable to human fetal facial skin and capable of reconstituting hairy skin in a xenograft mouse model. Together, our results demonstrate that the skin organoids produced in our culture system are the most functional and fully equipped human skin tissue that can be generated in vitro, to date. We anticipate our study provides a foundation for using skin organoids in studying skin development, modeling skin disease processes, or supplying cell source for skin regeneration and transplantation.

Funding Source
Ralph W. and Grace M. Showalter Trust (to Karl R. Koehler).

Keywords: Skin; Organoids; Hair Follicles

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IDENTIFICATION AND CHARACTERIZATION OF MATURE DOPAMINERGIC NEURON SUBTYPES AT SINGLE-CELL RESOLUTION IN HUMAN VENTRAL MIDBRAIN-PATTERNED ORGANOIDS

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Parkinson's disease (PD), the most common neurodegenerative disorder, is characterized by progressive loss of dopamine (DA) neurons in midbrain. Although the relatively focal degeneration in PD makes it a good candidate for cell-based therapies, the inaccessibility of functional human brain tissue and the inability of two-dimensional in vitro cultures to recapitulate the complexity and function of dopaminergic circuitries have made the study of human midbrain functions and dysfunctions challenging. Despite intensive research efforts in recent years, the molecular mechanisms controlling the developmental program and differentiation of DA neuron subtypes remain largely unknown. In this study, we designed a method for differentiating human pluripotent stem cells into three-dimensional (3D) dopaminergic organoids, which mimic features of human ventral midbrain (VM) development by recreating authentic and functional DA neurons. Immunolabelling-enabled 3D imaging of solvent-cleared organs (iDISCO) of whole organoids provides an anatomical perspective useful for reconstructing regional identities, spatial organization and connectivity maps. By combing CRISPR-Cas9 gene editing – used for generation of transgenic tyrosine hydroxylase (TH)-Cre reporter cell line – with unbiased transcriptional profiling at single-cell resolution, we showed that TH+ neurons exhibit molecular and electrophysiological properties of mature DA neurons expressing functional receptors of A9 and A10 neurons, which are severely affected in PD. Importantly, we also conducted a direct comparison with fetal VM organoids, which may serve as a valuable reference for creating the optimal conditions to differentiate pluripotent stem cells into human midbrain organoids, underscoring developmental similarities and differences.

Keywords: Stem cell differentiation; Dopaminergic Organoids; Parkinson's disease (PD)

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USING OLIGOCORTICAL SPHEROIDS TO STUDY DEVELOPMENT AND DISEASE OF OLIGODENDROCYTES IN THE BRAIN

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Cerebral organoids provide an accessible system to examine developmental aspects of cell specification, cell interactions and organization. Most research has focused on generating neurons and astrocytes in cerebral organoids, but we have focused on generating oligodendrocytes, the myelinating glia of the central nervous system. We have reproducibly generated oligodendrocytes and myelin in human pluripotent stem cell-derived "oligocortical spheroids". We have used this newly developed system in conjunction with single cell RNA sequencing and molecular characterization to study the development of oligodendrocytes within these organoids. Additionally we have used this system to study new aspects of leukodystrophies such as Pelizaeus-Merzbacher disease.

Keywords: Cerebral organoids; Oligodendrocyte; Disease modeling

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THREE-DIMENSIONAL SPHEROID CULTURE WORKFLOW USING PURE NEURONAL SUB-TYPES DERIVED FROM HUMAN IPSCS

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Human induced pluripotent stem cells (iPSC) derived neurons are now considered a more relevant in vitro model system for psychiatric and neurological diseases. They can be used for the development of physiological cell models, human disease models, and drug library screening. Three dimensional (3D) cultures are also being pursued as a more physiologically relevant system because they provide a microenvironment, cell-to-cell interactions, and biological processes that better represent in vivo conditions. The implementation of 3D spheroid culture plays an important role as an alternative approach for drug development and therapeutic applications in central neural system (CNS) disorders. To develop a neuronal 3D spheroid culture system, we tested iPSC-derived human motor neurons and cortical glutamatergic neurons using the S-BIO PrimeSurface Ultra Low Attachment Micoplates. After 2 hours, plated neurons started to settle down at the bottom of the well and form large clusters. On day 3, 3D spheroids could be seen clearly under phase contrast. On day 7, the spheroids were more condensed. The size of the 3D spheroids was proportional to the number of neurons seeded per well. In addition to the morphological assessments, a cytotoxicity assay and MEA assay were performed to demonstrate the suitable of 3D spheres as a platform for various applications. In both assays, the spheroids yielded expected results. The results presented here demonstrate the feasibility of generating uniform and reproducible spheroids using human neurons and the potential application for neurotoxicity studies.

Keywords: Spheroids; iPSC-Derived Neurons; 3D

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COMBINING NGN2 PROGRAMMING AND DEVELOPMENTAL PATTERNING TO RAPIDLY GENERATE CORTICAL ORGANOIDS

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The current models for investigating psychiatric or neurodegenerative diseases rely on animal and/or neuronal cell culture based models. While these formats have been integral for elucidating many underlying disease mechanisms, this often leads to a failure to translate into effective therapeutics. Many of these failures can be attributed to the biological differences between human disease and animal models, or the lack of proper neuronal development/maturation in vitro (e.g. dendritic spine formation). Our lab has pioneered a rapid and robust protocol for differentiating human stem cells into functional excitatory cortical neurons, providing a species-specific model for investigating human disease. The recently published protocol (Nehme et al. 2018) combines twopotent neuralizing components: expression of the transcription factor NGN2, and small molecule patterning (DUAL SMAD and Wnt inhibition). Here we apply the neuronal differentiation protocol to 3D cultures (e.g organoids) and characterize their composition. The patterned NGN2-organoids show high expression of multiple upper layer cortical neuronal markers (e.g. Cux1, Brn2), few deep layer neuronal marker (Ctip2), as well as GFAP expressing cells. Interestingly, the spheres also do not require embedding in Matrigel droplets, as they naturally keep their structural integrity. These results show that the coupling of the 3D organoid protocol with NGN2/patterning allows for easy, rapid formation of neural organoids that could be used as a cortical model. However, further analysis (e.g. single cell RNA-seq) is necessary for determining the absolute identity of the cells within the spheres, as well as understanding the maturation stage of the neurons.

Keywords: NGN2; Organoid; Cortical

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INDUCED 2C EXPRESSION AND IMPLANTATION-COMPETENT BLASTOCYST-LIKE CYSTS FROM PRIMED PLURIPOTENT STEM CELLS

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Soon after fertilization, the few totipotent cells of mammalian embryos diverge to form a structure called the blastocyst (BC). Although numerous cell types, including germ cells and extended pluripotency stem cells, have been developed from pluripotent stem cells (PSCs) in-vitro, generating functional BCs only from PSCs remains elusive. Here we describe induced self-organizing 3D BC-like cysts (iBLCs) generated from mouse PSC culture. Resembling natural BCs, iBLCs have a blastocoel-like cavity and were formed with outer cells expressing trophectoderm (TE) lineage markers and with inner cells expressing pluripotency markers. iBLCs transplanted to pseudopregnant mice uteruses implanted, induced decidualization, and exhibited growth and development before resorption, demonstrating that iBLCs are implantation-competent. iBLC precursor intermediates required the transcription factor Prdm14 and concomitantly activated the totipotency-related cleavage stage MERVL reporter and 2C genes. Thus, our system may contribute to understanding molecular mechanisms underpinning totipotency, embryogenesis, and implantation.

Keywords: primed pluripotent stem cell germ; 2C totipotency MERVL early embryo genesis; reprogramming cell identity

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CELL TYPES OF THE HUMAN RETINA AND ITS ORGANOIDS AT SINGLE CELL RESOLUTION: DEVELOPMENTAL CONVERGENCE, TRANSCRIPTIONAL IDENTITY, AND DISEASE MAP

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Organoids are stem cell-derived artificial organs that mimic aspects of organ development, function and disease. How closely cell type diversity and cell type maturation in human organoids recapitulate that of their target organ is not well understood. We performed histochemistry and sequenced the RNA of 163,971 single cells from improved human retinal organoids at different developmental stages and from donated healthy adult human retinas and choroid. Cell types in mature organoids had morphologies and transcriptomes that resembled their adult equivalents. Remarkably, organoids developed at a similar rate to the fetal retina and the transcriptome trajectory of cell types contained a progression of key developmental markers. Mapping disease-associated genes to cell types revealed cellular targets for studying disease mechanism in organoids and performing targeted repair in adult retinas.

Keywords: Retinal Organoid; Single-cell RNA sequencing; Cell type developmental convergence

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UNCOVERING REGULATORS OF HUMAN EMBRYONIC STEM CELL DIFFERENTIATION BIAS TOWARDS DEFINITIVE ENDODERM

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Recently launched clinical trials aim to use human pluripotent stem cell (hPSC) mesendodermal (ME) derivatives to treat patients with heart failure and type 1 diabetes. As individual hPSC lines can significantly vary in their ME differentiation efficiencies, acquiring a deeper understanding about this phenomenon and the development of tools to rapidly screen hPSC differentiation bias is of great value to the field of regenerative medicine. In this study, we used five karyotypically normal human embryonic stem cell (hESC) lines to investigate molecular mechanisms of hESC differentiation bias towards definitive endoderm (DE). We quantified DE differentiation efficiency using both a classic adherent differentiation and an in vitro model of early gastrulation-associated fate patterning in geometrically-confined micropatterned colonies, and identified VUB04 as a hESC line with significantly lower DE differentiation efficiency when compared to the other lines. As our DE differentiation protocol employs a strong activator of WNT signalling, we hypothesize that differentially expressed genes in VUB04 prevent its proper activation for endodermal specification. Bulk mRNA-sequencing at the undifferentiated stage showed that the main pluripotency genes were expressed a t comparable levels between hESC lines, however, pathway enrichment analysis of differentially expressed genes in VUB04 pointed at MAPK/ERK signalling. GO-term analysis of mRNA-sequencing samples 6 and 24 hours after the onset of DE differentiation showed that VUB04 fails to upregulate genes responsible for gastrulation, endoderm formation and BMP signalling. We are currently modifying expression levels of candidate genes selected from a list of 120 genes which were differentially expressed in VUB04 at all three mRNA-sequencing timepoints, and investigating if they have regulatory effects on early lineage specification to DE. Our work provides preliminary insight into the molecular mechanisms of how hESC may manifest DE differentiation bias and provides experimental validation of an in vitro platform that can be employed for high-throughput screens of hPSC differentiation propensities.

Keywords: Differentiation bias; Definitive endoderm; Micropatterned culture

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MODELING IN VIVO KIDNEY CELL FATE SPECIFICATION PROCESSES BY REACAPITULATING KIDNEY ORGANOGENESIS IN VITRO

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Recent progress in developmental biology identified metanephros, the embryonic kidney, develops by the interaction of nephron progenitors (NPs), ureteric bud (UB), and stromal progenitors, each of which finally gives rise to distinct cell types. However, the cell fate specification processes before and after the anlage formation remains largely unknown, in part due to the limited time resolution of genetic studies and difficulty to address the in vivo signal redundancy. We recently took advantage of in vitro directed differentiation approach and identified distinct signal requirement for the differentiation of NPs and UB from pluripotent stem cells (PSCs). Assembly of the PSCs-derived NPs, UB together with mouse embryonic stromal progenitors recapitulated "higher-order kidney organogenesis" in a dish, confirming the reliability of NPs and UB induction signals. We further addressed the cell fate specification process during the post-metanephros formation stage. NPs differentiate into 5 types of nephron segments, including parietal epithelial cells, podocytes, proximal tubules, loop of Henle and distal tubules, which form along the proximo-distal axis in this order. Here we focused on the podocytes differentiation process, which are one of the major targets in the kidney disease research field. We first cultured embryonic nephron progenitors in the presence of growth factors/inhibitors and identified the differentiation stage dependent signal requirement including, mesenchymal-to-epithelial transition, proximalization and podocyte specification. Further application of these cues to our human PSC-derived NPs enabled the highly-selective (more than 90% purity) induction of podocytes, which is much higher than the unbiased nephron differentiation method, in which podocytes consisted 5-20% of the kidney organoid. The induced podocytes exhibited comparable global signature gene expressions to those in adult human podocytes and had podocyte morphological features including foot process-like and slit diaphragm-like structures, which suggested identified signals were sufficient to induce podocyte fate. Taken together, in vitro directed differentiation approaches not only produce organotypic kidney organoid but also give novel insights into cell-type specification processes in vivo.

Keywords: Kidney organoid; Cell fate specification; Pluripotent stem cell

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MOUSE FETAL ORGANOIDS: NEW MODEL TO STUDY INTESTINAL EPITHELIAL MATURATION FROM SUCKLING TO WEANING

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During the suckling-to-weaning transition the intestinal epithelium adapts to the change in diet from milk to solid food by altering its gene expression profile. Previous studies have suggested that at least part of this transition is intrinsically programmed. In this context, we used mouse fetal intestinal organoids as a model and aimed to determine whether fetal intestinal organoids in vitro mimic the in vivo gut epithelial maturation process that takes place from birth to weaning. Organoids were cultured from fetal intestinal epithelial cells (E19) for one month, with adult intestinal organoids as control. Global gene expression profiles of fetal and adult organoids at day 3 and day 28 of culture were identified by micro-array and compared to gene expression profiles of fetal and adult intestinal tissue. Expression of specific maturation markers was evaluated weekly in fetal organoids by qPCR, enzyme activity assay and immunohistochemistry. To investigate whether gut maturation in fetal intestinal organoids can be modulated, organoids were treated with dexamethasone (DEX), a factor known to stimulate gut maturation. Global gene expression profiles showed an overall shift from fetal towards adult epithelium in fetal organoids cultured for 28 days, compared to day 3 of culture. Markers of neonatal intestinal epithelium could be detected in fetal organoids at day 3 but were progressively lost in time and were completely absent at day 28 of culture as well as in adult control organoids. In contrast, markers characteristic for the adult intestinal epithelium were absent in the fetal organoids during the first two weeks of culture and gradually increased to adult levels after 4 weeks of culture. Results were confirmed at enzyme activity level indicating that the organoids develop a functional adult brush border over time. Finally, DEX accelerated certain aspects of in vitro maturation in fetal intestinal organoids. Our data show that mouse fetal intestinal organoids mature into adult epithelium in vitro in a process that recapitulates the hallmarks of in vivo intestinal epithelial maturation. Fetal intestinal organoids can therefore be used to elucidate the molecular mechanisms of postnatal epithelial development and identify novel factors that influence the timing of epithelial maturation.

Keywords: Intestinal epithelium; Maturation; Fetal organoids

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3D IN VITRO MODEL FOR HUMAN BRAIN DEVELOPMENT IN AN ENVIRONMENT OF BRAIN VASCULATURE AND SYSTEMIC MICRO-ORGANOIDS NEURONAL DEVELOPMENT

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Compared to 2D neuronal cultures, brain organoids provide a more relevant model of in vivo neural development and neurological disorders. However, 3D brain organoids developed using current protocols lack critical cell types of brain such as mature astroglia and endothelial cells, or include a combination of the separately differentiated cell types, and thus is not a perfect representation of natural neural development. Further, interactions among different organs also play an important role in the condition of the neural system, thus asking for better models involving multiple micro organs. To address these critical issues, we determined if blood vessel building endothelial cells may play a critical role in organ development. We first made embryoid bodies from human iPSCs derived from CD34 cells, then initiated endothelial cell differentiation using a combination of small molecules and growth factors Activin A, BMP-4 and VEGF in a 3D environment consisting of Matrigel. The resulting spheroids were further cultured in media to induce neural development. The resulting organoids were collected at 2 weeks and 8 weeks and characterized by immunofluorescent staining for endothelial and neuronal markers. Gene expression was studied by Real-time PCR and RNA-Seq analysis. The organoids thus developed showed a spectacular morphology consisting of a vesicular sac and dense mass within. Immunostaining showed that the organoids were positive for the endothelial cell marker CD31, mostly in the outside layer and neuronal marker βIII-tubulin mostly in the mass. RT-PCR and RNA-Seq confirmed the existence of both arterial and venous endothelial cell markers and mature neural cells, as well as glial cell markers such as GFAP and MBP. Furthermore, transcripts specific for other organs such as kidney, liver and lung were also detected, indicating tracks of multiple organ development in the sac, which makes it a model for multiple organ interactions. This model holds great promise for a more in vivo relevant 3D model, suitable for applications for studying organ development, disease modeling, drug screening and development.

Funding Source
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Keywords: 3D organoid; Neuronal development; Multiple organ interactions

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CO-DEVELOPING HUMAN HEART MUSCLE AND NEURONAL STRUCTURES IN BIOENGINEERED HEART MUSCLE

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Recent work in our lab showed the ability of human pluripotent stem cells (hPSC) to self-assemble into a force generating bioengineered heart muscle (BHM) with spontaneous co-development of sympathetic neuron-like structures in long-term cultures (day 60). We hypothesized that the neuronal co-development in BHM can be guided by directed differentiation of neurogenesis, resulting in a complete heart-neuron tissue with enhanced contractile performance. BHM was generated from hPSCs (5x105 cells/BHM) under stage-specific directed cardiac differentiation. Mechanical loading was introduced on day 13 followed by defined neuronal induction from culture day 29 onwards. Isometric force measurement was performed on day 60 to assess BHM contractility under defined conditions. Enzymatic dispersion of BHMs was performed to determine total cell count and composition via flow cytometry. Neuronal gene expression was confirmed using qPCR and immunohistochemistry. Previous results revealed that BHMs closely mimic key steps of in utero heart development with the induction of mesoderm, followed by specification towards cardiac lineages. At culture day 60, BHMs subjected to neuronal induction (8 days), neuronal expansion (7 days) followed by neuronal differentiation (14 days), showed improved contractility (maximal force of contraction in mN: 0.38±0.05 vs 0.25±0.03 for Control; n=75; p<0.05). Flow cytometry results revealed significantly higher survival of cardiomyocytes in the neuro-enhanced BHM group (% α-actinin+ cells: 19±3 vs 10±2 for Control; n=27/30; p<0.05). On the molecular level, neuronal markers such as PAX6 and MAP2 were highly upregulated in neuro-enhanced BHM. Detailed morphological studies confirmed MAP2 and NF positive staining, presence of neuronal rosettes and fine neuronal outgrowths, suggesting innervation in day 60 as well as day 90 neuro-enhanced BHMs. Taken together, our study provides first evidence for pro-survival effects and enhanced contractility in human BHM with an engineered neuro-cardiac interface. The engineered co-development of cardiac mesoderm and ectoderm appears to recapitulate the complex process of autonomic innervation of heart muscle during fetal heart development.

Keywords: Pluripotent Stem Cells; Cardiac Differentiation; Neuronal Differentiation

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MTOR AND LIF SIGNALING IN HUMAN CORTICAL DEVELOPMENT

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The cerebral cortex, the folded exterior of the brain, is expanded in humans and is important for our cognitive abilities. Abnormal cortical development is a leading cause of epilepsy and developmental delay and can lead to malformations where the brain does not fold appropriately or grow as in Lissencephaly, Microcephaly, and Megalencephaly. The cortex expands by directing appropriate proliferation of its resident neural stem cells, radial glia cells. There are multiple distinct types of radial glia, and the outer radial glia (oRG) population is dramatically expanded in humans, suggesting its contribution to human cortical expansion. Currently we have a limited understanding of the developmental processes that regulate oRG generation and maintenance. We used single cell RNA sequencing and discovered an association between oRG cells and mTOR signaling. Interestingly, the Lif/Stat3 pathway, which regulates pluripotency in a variety of stem cell populations, is also transcriptionally associated with oRG cells. We assessed the functional role of mTOR signaling in oRG formation and the relationship between mTOR and Lif signaling utilizing two human-specific models of cortical development: ex vivo culture of developing human cortical tissue and in vitro culture of human induced pluripotent stem cells (iPSCs) directed toward cortical organoids. We found that Lif activity is sufficient to increase the number of oRGs and also activates mTOR signaling in these models of human cortical development. Manipulation of mTOR signaling using small molecules resulted in a decrease in oRG basal fiber length and a reduction in the number of oRGs. These studies provide insight into developmental mechanisms guiding the generation of the oRG population and have implications for the treatment of cortical disease.

Keywords: Cortex; Development; Signaling

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MEX3A DELETION IMPAIRS LGR5+ STEM CELL MAINTENANCE IN THE MOUSE GUT

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Intestinal stem cells (ISCs) fuel the lifelong self-renewal of the intestinal tract and are paramount for epithelial repair. Genetic studies have defined the Wnt signalling as a crucial regulator of ISC identity. One of the most reliable ISC markers is the Wnt/β-catenin pathway member LGR5. Still, the effort to reveal ISCs identity and regulatory networks remains a challenge. It is becoming clear that RNA-binding proteins (RBPs) and the post-transcriptional mechanisms they rule underpin stem cell fate decisions in response to different stimuli. We have generated the first Mex3a knockout mouse model and report that the function of this RBP is crucial for the in vivo maintenance of the Lgr5+ ISC pool and for efficient epithelial turnover during intestinal postnatal development. Mex3a null mice exhibit growth retardation and postnatal mortality due to impaired epithelial turnover, underlined by a dramatic decrease in Lgr5+ ISCs and KI67+ cells. Transcriptomic profiling revealed that Mex3a ablation induces activation of the peroxisome proliferator-activated receptor (PPAR) signalling at the crypt level, with a concomitant loss of the Lgr5+ stem cell signature. Furthermore, we provide evidence that PPARgamma is a target ofMEX3A-mediated repression, thus uncovering a new layer of post-transcriptional regulation operating in ISCs that critically contributes to intestinal homeostasis.

Funding Source
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Keywords: Lgr5+ intestinal stem cells; RNA-binding protein MEX3A; Intestinal homeostasis

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HUMAN IPSC DERIVED 3D-NEUROSPHERE ASSAY TO STUDY DEVELOPMENTAL NEUROTOXICITY

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Neuronal stem cells (NSCs) are considered as a multipotent and self-renewing pool of cells of the mammalian central nervous system (CNS) and have the capacity to differentiate into all neuronal cell types, as well as glial cells. They are able to form spherical, free-floating 3D cell clusters, the neurospheres, and differentiate into neurons, glial cells and oligodendrocytes, forming a complex tissue, mimicking the events of early neurodevelopment. NSCs can be differentiated from pluripotent stem cells, provide an attractive in vitro tool for studying CNS disorders or for drug development purposes. Here, we present a 3D human induced pluripotent stem cell (hiPSC)-based in vitro toxicology assay that can be used to test developmental neurotoxicity. Human iPSCs derived neurospheres grown in 3D culture were characterised timewise to monitor their complexity and homogeneity over a 7-weeks-long period using immunocytochemistry and electron microscopy. 3D neurospheres were exposed to 10 different toxicants (e.g. Paraquat, VPA, acrylamide, mercury chloride) activating different toxicity pathways. Samples were examined at different developmental time points (21, 28 and 42 days after plating), representing different developmental stages and maturity, with an ATP-based cell viability assay optimised for 3D-tissues in 96-well plate format. Concentration-responses were investigated after acute (72 hours) exposure and the effect of toxicants were determined by histology as well. In addition, Transcriptional activity of major developmental, structural and cell type specific markers was investigated at weekly intervals. The results demonstrated that the acute exposure to different classes of toxicants resulted in distinct cell susceptibility profiles in different developmental stages, indicating that hiPSC-based in vitro neurodevelopmental models might be used effectively to evaluate developmental neurotoxicity. This will open new avenues for 3Rs replacement of animal models with in vitro assays in various academic and pharma-, chemical- and cosmetics industry applications.

Funding Source
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Keywords: Induced pluripotent stem cells; 3D neurospheres; Developmental neurotoxicity

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DEVELOPMENTAL NEUROTOXICITY (DNT) OF GAMMA-RADIATION IN A 3D EARLY BRAIN DEVELOPMENTAL MODEL GENERATED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSC)

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Due to increasing medical imaging, radiation therapy and long-distance flights humans are increasingly exposed to ionizing radiation (IR). The sensitivity of cells to IR differs a lot between different cell types. Neural progenitor cells are known to have low radiation resistance. Low dose IR may induce permanent damage with potential consequences for the developing brain. However, data on impact of IR on very early human brain development is scarce. We had previously published a three dimensional (3D) model from hESC recapitulating the very early stages of predominantly fore-brain development. Altered neural marker gene expression after exposure to known DNT chemicals validated the 3-D DNT model. Here we established the same model from human iPSCs and investigated the effects of IR (1Gy X-ray). To evaluate the capacity of self-renewal of the surviving, irradiated neural stem/progenitor population neurosphere (NS)-forming assays were performed. We also evaluated long-term effects by measuring the diameter of NS over time. In addition, the expression levels of 19 different marker genes crucial for normal neural development such as patterning, structure, neural/neuronal development and stress response genes were determined 2 and 9 days post IR (p.IR) by qRT-PCR. NS formation was reduced after irradiation and NS were found to have significantly smaller diameters starting 16 days p.IR. Significant changes were also present at the molecular level. Most analyzed marker genes were reduced 2 days p.IR but interestingly went back to control levels by day 9 p.IR. 7 genes were however not expressed at control levels 9 days p.IR, including 3 markers for stress. Among the 6 reduced markers were also genes known to be involved in neurodevelopmental syndromes (microcephaly, Mowat-Wilson syndrome, growth abnormalities). We were thus able to identify a significant impact of radiation in the surviving cells. The detected reduction in marker expression points towards potential detrimental effects of IR on early brain development and contradict the long held hypothesis that in development irradiated cells die, or survive with no later appearing major damage. Our data suggest that IR, even at low doses could have a negative neurodevelopmental impact on the human brain.

Funding Source
Federal Ministry for Education and Research Germany (BMBF); Ministry for Science, Research and Arts of Baden-Württemberg.

Keywords: Irradiation; Neurogenesis; Neurospheres

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TOWARDS IN VITRO MODELING OF EARLY MOUSE DEVELOPMENT

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In-vitro differentiation approaches of mouse embryonic stem cells (ESC) have attempted to generate cell types that occur during embryonic development. However, early mouse development is a complex system comprising interaction of various cell types in a spatially and temporally controlled manner. Therefore, such differentiation approaches failed to faithfully recapitulate early mouse development in vitro. Recently, it became possible to generate self-organizing multicellular tissue constructs termed organoids. While important aspects of the complex in-vivo organization have been recreated in these organoid systems, most of these studies have focused on the recapitulation of organogenesis, not of the early embryonic development. In this study, we aim to study certain features of the developing mouse embryo by using an embryonic organoid model called 'gastruloids'. Gastruloids, small aggregates of mouse embryonic stem cells, demonstrate symmetry breaking, axial elongation and germ layer specification in a reproducible manner. Moreover, gastruloids are capable of establishing anterior-posterior (A-P), dorso-ventral (D-V) and medio-lateral (M-L) axes in vitro. Surprisingly, full implementation of spatial and temporal colinearity of Hox genes is recapitulated in these self-organizing aggregates of mESCs. We hope that this model would be useful to provide an alternative way to study development and to generate rare cells types that arise during embryogenesis in vitro.

Keywords: Gastruloids; Self organisation; Embryonic development

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EFFECTS OF MEDIUM SUPPLEMENTS ON EXPANSION AND CHONDROGENIC POTENTIAL WITH CAPACITY TO UNDERGO ENDOCHONDRAL OSSIFICATION OF MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) offer a promising source of cells for bone regeneration by mimicking the process of endochondral ossification. However, their chondrogenic differential potential and stem cell properties decreases during in vitro expansion. In this study, we examined effects of various medium supplements on MSC self-renewal and chondrogenic differentiation potential with a capacity to undergo endochondral ossification. Human bone marrow-derived MSCs (passage 3, P3) were cultured until confluent and split continuously until reaching senescence. Medium supplementation including fibroblast growth factor (FGF)-2, transforming growth factor (TGF)-β1, and dexamethazone (Dex) increased cell numbers before reaching senescence (from P3 to P8), which was associated with loss of chondrogenic differentiation potential in pellet culture for 3 weeks. However, for maintaining chondrogenic differentiation potential up to P8, two other supplements were required in addition to these supplements. The MSCs (P6) expanded with 5 supplements were subjected to chondrogenic differentiation for 3 weeks and hypertrophic maturation for 2 weeks. The resultant MSCs formed bone ossicles, including blood vessels, when subcutaneously implanted into nude mice for 8 weeks, indicating that the long-term cultured MSCs retained the capacity to undergo endochondral ossification. We are currently performing further experiments to confirm these observations.

Funding Source
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Keywords: Mesenchymal stem cells; chondrogenic differentiation; endochondral ossification

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WNT1/B-CATENIN SIGNALING REGULATES SUBSET-SPECIFIC MIDBRAIN DOPAMINERGIC NEURON DIFFERENTIATION IN A DOSE DEPENDENT MANNER IN THE MOUSE

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Mesodiencephalic dopaminergic (mdDA) neurons, which comprise the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA), play significant roles in the neural circuitry responsible for movement, cognition, and emotion. Considering their critical functions in neuropsychiatric and neurodegenerative disorders, such as Parkinson disease (PD), considerable attention has been paid to the development of mdDA neurons. A growing body of literature has revealed the importance of the WNT1/b-catenin signaling pathway in the differentiation and survival of these neurons and showed that a disrupted activation or inhibition of the WNT1/b-catenin signaling pathway leads to an incorrect specification or differentiation of the mdDA neurons. This study aims to unravel the identity of the cells responding to this pathway in the murine ventral midbrain (VM) and the precise mechanism of WNT/b-catenin action in these cells. We found that the WNT/b-catenin-responsive cells constitute only a fraction of all mdDA progenitors, precursors, and neurons in the murine VM. Located mostly in the Wnt1+, Rspo2+ and Lef1+ lateral floor plate of the medial and caudal midbrain, these WNT/b-catenin-responsive cells develop preferentially into caudomedial (VTA) mdDA neurons. We also show that the differentiation of WNT/b-catenin-responsive mdDA progenitors into mature mdDA neurons is inhibited by a strong activation of the WNT/b-catenin signaling pathway via RSPO2, a WNT/b-catenin agonist, and LEF1, a nuclear effector of this pathway. Furthermore, we show that this inhibition is due to the repression of the murine Pitx3 gene promoter via conserved LEF1/TCF binding sites in this promoter. Our results indicate that the correct differentiation of mdDA progenitors into specific mdDA neuron subsets, particularly into SNc DA neurons, relies on the attenuation of WNT/b-catenin signaling in these cells and provide new means for stem cell-based regenerative therapies of PD and in vitro models of neuropsychiatric diseases.

Keywords: WNT1/b-catenin signalling pathway; Mesodiencephalic dopaminergic neurons; Pitx3

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IDENTIFICATION OF KEY REGULATORS DURING THE ESTABLISHMENT OF MOUSE INTESTINAL ORGANOID FROM EMBRYONIC STEM CELLS

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Direct lineage specification of pluripotent stem cell is one of the valuable systems for studying cell fate decision as well as developmental process. We successfully established a robust methodology for intestinal organoids formation from mouse embryonic stem cells by treatment of specific growth factors and inhibitors. During this cell lineage process, we sought key regulators responsible for the cell fate determination in embryonic stem cells, definitive endoderm, mid-gut and intestinal organoid by single cell transcriptomic analysis. Also we compared the temporal-spatial single-cell transcriptome landscapes of our mouse intestinal organoid with that of the public human fetal digestive tract to understand the cellular mechanism of stemness, differentiation, and cell-to-cell communication. From this study, we can provide a gene regulation network focused on the master regulators at a single cell resolution during the differentiation.

Keywords: Embryonic stem cell; Intestinal organoid; transcriptomics

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CARDIAC ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS RESEMBLE KEY FEATURES OF HUMAN HEART DEVELOPMENT

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Organoids are three-dimensional (3D) cellular aggregates that better resemble features of native organs regarding functionality and morphology compared to conventional cell culture. They can be used as in vitro models for organ development and diseases, drug development, and potentially for future regenerative therapies. Although comprehensive organoids have already been published for a wide range of tissues including small intestine, kidney and brain, advances in the cardiovascular field are limited. Ideally, cardiac organoids should resemble heart morphology at early developmental stages. This should include proper formation of the three heart layers (epi-, myo-, and endocardium) and an organ-typical tissue composition, in particular cardiomyocytes, myofibroblasts and endothelial cells. However, up to date such cardiac organoids do not exist and recent approaches failed to mimic the human embryonic heart properly. Therefore, the aim of this project is to overcome these prior limitations. We have established a protocol, which leads to the highly reproducible generation of cardiac organoids from human pluripotent stem cells, which contain at least two heart layers in an organized 3D pattern. We show that these structures are composed of all cell types expected for the heart, including the formation of endothelial networks. Moreover, we show that these organoids could be used as an informative model for drug screening e.g. in a teratogenicity assay and for genetic disease modeling as well. Together, we provide evidence that these novel organoids represent a superior in vitro model for human embryonic heart development, open new perspectives in pharmacological research and are potentially of interest for organ-specific lab-on-chip approaches.

Keywords: Heart model; Cardiac organoid; Heart development

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DEFINING THE SINGLE-CELL DEVELOPMENTAL LANDSCAPE OF HUMAN INNER EAR ORGANOIDS

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Inner ear development requires the complex interaction of numerous cell types arising from multiple lineages. Most of what is known about inner ear development has been derived from animal studies; thus, the cellular and molecular idiosyncrasies of human inner ear development are unknown. Our group recently showed how to recapitulate human inner ear organogenesis in vitro using 3D self-organizing stem cell cultures. Our method promotes development of an entire sensorineural circuit, including hair cells, inner ear-like neurons, and Schwann cells. Although we have characterized many of these cell types, we have not fully defined the process by which individual cells commit to lineages represented in inner ear organoids. Here, our goal was to reconstruct a time-based map of the origin of cell lineages during inner ear organoid development. We analyzed inner ear organoids using single-cell RNA sequencing at various time points during the first two weeks of our protocol. We identified expression patterns of inner ear organoids as they progress from undifferentiated stem cells to surface ectoderm then to otic placode following treatment with FGF, BMP, and WNT signaling modulators. The first major cell group identified was surface ectoderm, indicated by TFAP2A and ECAD. At a later time point, we observed lineage commitment to posterior cranial placode (PAX8, SOX2, TFAP, ECAD and NCAD). Further along differentiation, we observed cells expressing markers specific to otic placode (PAX2, PAX8, SOX2, SOX10, JAG1). Interestingly, we also mapped a population of cells with expression patterns similar to cranial neural crest (TFAP2A, SLUG, SOX10), which bifurcated into mesenchymal and neuroglial cells. By elucidating organoid developmental lineages, we have created a platform upon which to perturb developmental signaling mechanisms. We plan to use this platform to model genetic disease using gene editing and test new drug targets for hearing loss and balance disorders.

Funding Source
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Keywords: inner ear; organoid; lineage trajectory

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THE RNA METHYLTRANSFERASE TRDMT1 COORDINATES EARLY EMBRYONIC STAGE CELL-FATE SPECIFICATION

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Proper cytosine methylation of nucleic acids to give rise to 5-methylcytosine is vital for the regulation of developmental processes, but the molecular underpinnings of this epigenetic modification in RNA species remain poorly understood. tRNA aspartic acid methyltransferase 1 (Trdmt1), which acts by catalyzing tRNA methylation, is the most conserved and enigmatic member of the DNA methyltransferase family. In this study we attempt to understand Trdmt1 functions during early embryo development using mouse embryonic stem cells (ESCs) stimulated to undergo gastrulation-like events. For this purpose, we employed combinations of RNAi and CRISPR/Cas9 approaches for Trdmt1 loss-of-function in pluripotent stem cells. Remarkably, we found that while Trdmt1 is dispensable for the maintenance of the pluripotent cell state, the absence of this protein impairs the establishment of the three germ layers (ectoderm, mesoderm and endoderm) and therefore hampers proper gastrulation process in vitro. At the molecular level, we show that absence of Trdmt1 in in vitro organoids affects transcriptional landscapes required to modulate early cell fate decisions during embryo developmental processes. Taken together, our results reveal an unanticipated role of an epitranscriptomic writer, Trdmt1, in coordinating gene expression programs that are essential for exiting pluripotency.

Funding Source
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Keywords: RNA methylation; Organoids; Differentiation

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HISTONE AMINOTRANSFERASE 1 IS A NOVEL PLAYER GOVERNING EARLY ECTODERM LINEAGE COMMITMENT

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Proper epigenetic regulation of gene expression programs is critical for orchestrating the cell-fate transitions occurring in every organism during embryonic development. Although histone acetylation is well known to play a central role in many nuclear processes such as transcriptional competence, the specific mechanisms by which histone acetyltransferases regulate developmental processes remain to be addressed. Here, using 3D in vitro cultures we identified the Histone aminotransferase 1 (Hat1) as a new key regulator of bona fide pluripotency commitment. We show that both short and stable Hat1 loss-of-function significantly alters the histone acetylation-dependent control of gene expression in mouse embryonic stem cells. Importantly, we found that Hat1 ensures accurate expression of ectoderm lineage regulators during early stages of in vitro gastrulation process. Thus, our data suggest that Hat1 plays an essential role through the establishment of histone acetylated landscapes that are required for exiting pluripotency. Overall, our findings link Hat1 as a histone acetyltransferase enzyme that exerts a fundamental role in ectodermal cell specification. Furthermore, our results may open new avenues to understand developmental diseases linked to faulty development of the ectoderm during embryogenesis.

Funding Source
This research was funded by the the Agencia Estatal de Investigación (BFU2016-80899-P) (AEI/FEDER, UE) and the Consellería de Cultura, Educación e Ordenación Universitaria (ED431F 2016/016) to M.F.

Keywords: Histone Acetyltransferase 1; development; gastrulation

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GENERATION AND CONFIRMATION OF HIGH THROUGHPUT MODELED CEREBRAL BRAIN ORGANOIDS AS A FUTURE SCREENING MODEL

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Generating brain organoid have allowed researchers to understand actual human developments. Upon the onset of brain organoids, many new possibilities have been opened, such as ZICA virus, Alzheimer, Parkinson's, and many more models. Furthermore, brain organoids have given us a new option to replace PDX mouse models. As PDX mouse models are expensive, time consuming and irrelevant to actual humans, brain organoids offer far more understandings and choices. Even though all seems great, brain organoids are still yet to be generated as a high throughput model. Here, a new method of generating brain organoids as a high throughput model will be introduced. By culturing cerebral organoids without Matrigel on an ultra-non-adhesive well plates, size controlled, quantifiable, and identical cerebral brain organoids were grown. Confirming with the immunofluorescence data, not only do MAP2 and TUJ1 well show the recapitulation of cortical plate in the cerebral cortex area, but PAX6 also shows the ventricular zone in the brain. Furthermore, when the electrical cues were applied to the brain organoids made in high throughput manners, various signals such as action potential, LFP waves, burst waves and random waves that of in vivo brain's were all detected; assuring that the brain organoids cultured in a high throughput method well represent actual in vivo cerebral cortex. In the future, it is believed that high throughput cerebral brain organoids will be utilized in many areas such as disease modeling, screening, transplanting and many more.

Funding Source
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Keywords: high throughput; cerebral organoids; Matrigel

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IMPACT OF SOX11 ON HUMAN EARLY NEURODEVELOPMENT

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The SoxC family of transcription factors controls a number of key processes in murine CNS development including neuronal fate determination, migration, neurite growth, neuronal survival and synapse development. While the function of SoxC in mice has been well characterized, the role of SoxC members in human neurodevelopment remains largely unknown. Heterozygous missense mutations or deletions in SOX11 have recently been identified as the underlying genetic cause in children with a Coffin-Siris Syndrome (CSS) like syndrome. Hallmarks of CSS include developmental disability, skeletal abnormalities, and characteristic facial features. The involvement of multiple organ systems in CSS, indicate that SOX11 fulfills critical functions in the development of different germ layers. Here, we investigate the function of SOX11 in human development using human embryonic stem cells (hESCs) as a model system. Homozygous SOX11-deficient hESCs lines with a SOX11 frameshift mutation were generated using CRISPR/Cas9 genome editing. In recent years different approaches have been optimized to study the development of human brain: 2D hPCS (human pluripotent stem cells) neural differentiation models, which mimics the environment that produces the neuroectoderm though embryoid body formation, and hPCS- derived 3D brain organoids. In both models we found that Sox11 deficiency was associated with decreased expression of Nestin, Pax6 and Sox1 upon induction of neural differentiation. Moreover, we observed that Sox 11 deficiency resulted in strong impairment in the generation of neurons. Collectively, our data point at an essential role for Sox11 during early stages of human neural development.

Funding Source
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Keywords: SOX11; human neurodevelopment; organoid

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TRANSCRIPTIONAL REGULATION OF CELL STATES IN THE DEVELOPING MOUSE INTESTINAL EPITHELIUM

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The processes that govern the development of functional organs, such as the gastrointestinal tract, are complex and require accurate regulation of cell fate decisions. Once formed, a series of events ensure that fetal tissues transition into mature organs that are subsequently maintained throughout life. One of the significant differences that discriminate tissues during homeostasis versus development is the balance between gain and loss of cells, whereas the developmental state is characterized by unbalanced cell fate decisions with limited natural loss of cells in order to fuel constant tissue growth. Stem cells are well characterized in the adult intestinal epithelium, but the mechanisms that regulate the transition of the cells from a fetal to an adult state remain largely unknown. By utilizing the organoid culture system, we aim to identify gene regulatory networks that control the transition between fetal and adult stem cells states in the intestinal epithelium. We have previously demonstrated that intestinal epithelial cells derived from the fetal epithelium are distinct from their adult counterpart when cultured in vitro. When cultured in a 3D matrix fetal epithelial cells form enterosheres that resemble the fetal state in vivo with respect to differentiation states. Moreover, fetal enterospheres retain the capacity to mature into adult epithelium upon transplantation. Transcriptional analysis reveal that more than 3000 genes are differently expressed between the fetal and adult organoids, suggesting that distinct gene regulatory networks control the different cellular states. In order to identify key gene regulatory networks, we are currently performing a CRISPR/Cas9 KO screen for transcription factors and epigenetic regulators differentially expressed between fetal enterospheres and adult organoids. Identified factors will subsequently be characterized for their role in maturation of the intestinal stem cells in vitro and in vivo in order to provide molecular insight into the mechanisms that govern tissue maturation of the intestinal epithelium.

Keywords: Intestinal development; CRISPR/Cas9; Organoids

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UNCOVERING THE ROLE OF PLANAR CELL POLARITY DURING MOUSE INTESTINAL MORPHOGENESIS

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The mammalian intestine is lined with millions of finger-like projections, termed villi. These villi are critical for maximizing nutrient absorption, digesting food and serving as a barrier from the harsh luminal environment. As such compromised villi can lead to serious diseases including malabsorption, short bowel syndrome, celiac and others. Therefore, understanding how intestinal morphogenesis occurs is essential for regenerative therapies for these diseases. Although villi are precisely patterned by a network of signalling pathways during embryogenesis, most notably Hedgehog (Hh), it remains unclear as to how these signals translate into distinct morphogenetic transformations. Using the mouse model, our RNA-seq analyses coupled with GLI2 (Hh-transcriptional activator) ChIP-seq reveal that planar cell polarity (PCP) genes such as Fat4, Dchs1 and Vangl2 are direct targets of Hh in the gut mesenchyme. Notably, mice deleted and/or mutated for these genes exhibit severe villus fusions and fail to form mesenchymal clusters, demonstrating for the first time the importance of PCP in villification. Furthermore, genetic interaction studies reveal that the core-PCP axis (Vangl2) acts in parallel to the atypical cadherin axis (Fat4, Dchs1) in maintaining PCP. By utilizing live light-sheet fluorescence microscopy, we have visualized and tracked stromal cell behavior during villification ex vivo and have identified perturbed cell migration and orientation upon Fat4 knockout. Additionally, we have been able to model this stromal behavior in vitro using 3D-culture based approaches. Together, we introduce Hh-activated stromal PCP as novel mechanisms required for morphogenetic cell behaviour and subsequent epithelial rearrangement, critical for villification.

Keywords: Intestinal Morphogenesis; Planar Cell Polarity; Live imaging and 3D-culture systems

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TARGETING DECISION NODES DURING DIRECT LINEAGE REPROGRAMMING

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Ectopic expression of defined transcription factors can force direct cell fate conversion from one cell lineage to another in the absence of cell division. Several transcription factor cocktails have enabled successful reprogramming of various somatic cell types into induced neurons (iNs) of distinct neurotransmitter phenotypes. However, the intermediate stages bridging starter cell and iN population have been largely unstudied. We addressed this aspect by studying the transcriptional landscape during the course of pericyte-to-neuron conversion by using single cell RNA-sequencing (scRNA-seq). We could thereby recently show that successful reprogramming of adult human brain pericytes into functional iNs by Ascl1 and Sox2 (AS) encompasses activation of a neural stem cell-like gene expression program which is characterized by the expression of a particular set of genes referred to as switch genes. Building up on this work, we show here using scRNA-seq, that modulating the neural stem cell-like intermediate stage by manipulating signaling pathways specifically active in this switch-state greatly impacts on the reprogramming outcome. Our findings suggest a novel unanticipated entry point to navigate the reprogramming trajectory towards specific target cell populations. These results for the first time provide evidence for the possibility to specifically target an intermediate neural stem cell-like state during AS-mediated reprogramming allowing the modulation of the reprogramming outcome. The investigation of cellular intermediates during iN reprogramming will provide handles to improve lineage conversion towards therapeutically relevant cell types.

Keywords: direct lineage reprogramming; neural stem cells; scRNA-sequencing

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LEPTIN RECEPTOR IDENTIFIED MESENCHYMAL STEM CELL IN ENDOCHONDRAL BONE FORMATION AND FRACTURE REPAIR

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Endochondral ossification is an important process for both skeletal development and bone repair. In the long bones, cartilage anlage is first formed by mesenchymal condensation, followed by chondrocyte differentiation and maturation and subsequently bone mineralization. A common molecular marker for mesenchyme progenitors has not been fully identified. Here, by lineage-tracing experiments in mice, we discover that leptin receptor (Lepr+) cells distribution during endochondral bone formation from fetus to adult. Pp2a is cell-autonomously required for the maintenance of MSCs. To assess the consequences of ppp2r1a deletion from MSCs, we generated Lepr-cre; ppp2r1a fl/fl mice. As expected, YAP phosphorylation increased in LepR+ stromal cells isolated from Lepr-cre; ppp2r1a fl/fl mice. Pp2a is thus cell-autonomously required to positively regulate YAP activation and to maintain normal numbers of quiescent Lepr+ MSCs in adult bone marrow. To investigate the contribution of Lepr+ cells to the repair of perforated cartilage, we created a hole at diaphysis of the femur to observe fracture healing in Lepr-cre; tdTomato mice. Six days following perforation, the injured region of the diaphysis was covered Lepr+ cells infiltration. Thus, Lepr marks mesenchymal progenitors responsible for both normal bone formation and fracture repair.

Keywords: leptin receptor; mesenchymal stem cell; endochondral ossification

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THE EFFECT OF DIFFERENT OXYGEN CONDITIONS ON MITOCHONDRIAL DYNAMICS IN THE EARLY STAGE OF DEVELOPMENT OF HUMAN CEREBRAL ORGANOID

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Biomimetic in vitro conditions of 3D culture together with lowering oxygen level to 5% (physiological normoxia) were used to study mitochondrial dynamics and neuronal differentiation during early development using human cerebral organoids model. In this report we will elucidate whether the constant 5% O2 conditions or the short-term changes (pulses) in the oxygen level affect cortical development and mitochondrial dynamics in cerebral organoids. For that purpose cerebral organoids were generated from induced pluripotent stem cells (hiPSC) in feeder-free conditions and cultured either in 21% (control) or 5% O2. For short-term pulse of 5% O2 2-week cerebral organoids were transferred from 21 to 5%O2 and cultured for one week, followed by transition back to the 21% O2 for an additional week. Next, cerebral organoids were fixed and processed for immunohistochemistry (IHC) with mitochondrial and neural-specific antibodies and transmission electron microscopy (TEM). To quantify mitochondrial dynamics and neuronal networks in selected Regions of Interest (ROIs) within ventricular zone (VZ) and cortical zone (CZ) of cerebral organoids imaging software and Mitochondrial Network Analysis toolset were applied. Data analysis revealed significant changes of mitochondrial number and branches in mitochondrial networks in different regions of cerebral organoids in all tested groups, suggesting that lower level of O2 may affect mitochondrial dynamics. The number of individual mitochondria in cortical zone was lowered in the cerebral organoids cultured in 21%O2. Staining against non-glycosylated protein of mitochondria surface membrane showed differences in their fluorescence intensity between ventricular and cortical zones in different oxygen conditions. IHC detection of neuronal processes with Pan Neuronal Marker showed reduced fluorescence intensity in cortical zone in 5% O2 as compared to control. Analysis of TEM images suggests that short-term pulses of low O2 may induce neuronal differentiation within cerebral organoids. Further studies are ongoing to compare mitochondrial dynamics and structure between VZ and CZ in organoids cultured in constant physiological normoxia conditions. The cerebral organoid model offers a unique opportunity to study early human neural development.

Funding Source
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Keywords: Cerebral organoids; neurogenesis; mitochondrial dynamics

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NONVIRAL REPROGRAMMING OF HUMAN ASTROCYTES TO DOPAMINERGIC NEURONS

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Parkinson's disease (PD) is currently treated with dopaminergic drugs or deep brain electrical stimulation. However, these treatments are symptomatic, do not slow the progression of disease, and their clinical benefits inevitably decline as the loss of dopaminergic neurons progresses. Disease modifying strategies such as transplantation of stem cell-derived midbrain dopaminergic cells or direct reprogramming of resident glia to dopaminergic neurons are currently being developed. We recently reported that astrocytes can be converted into neurons, both in vitro and in vivo, using viral delivery of transcription factor genes that recapitulate developmental expression patterns. Now, we have developed a virus-free, nanoparticle-based reprogramming protocol that eliminates the risk of deleterious genetic integration associated with viral methods. We delivered cocktails of transcription factor gene and microRNA constructs packaged in polymer nanoparticles derived from the synthetic bioreducible poly(amidoamine) p(CBA-ABOL). Programmed degradation of the polymer backbone releases the payload efficiently, while diminishing toxicity of the carrier so as to enable repeated application without compounding cytotoxicity. Using a serial dosing protocol, we could efficiently convert human astrocytes to Tuj1+/TH+ cells. Conversion efficiencies were assayed using high content imaging and automated analysis, which also allowed us to quantify neuronal morphometrics including neurite number, length, and branching. These nonvirally-induced dopaminergic neurons (NiDAs) were further validated for expression of MAP2, DAT, synaptophysin, and synapsin I. We next aim to model the nonviral gene delivery and reprogramming processes in an organoid model. A virus-free strategy to produce NiDAs from human astrocytes may facilitate a safe and effective translation of direct reprogramming of resident glia to dopaminergic neurons.

Keywords: Cell Reprogramming; Neurodegenerative Disease; Nonviral Gene Delivery

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HUMAN IPSC COLONY MORPHOLOGY VARIATIONS ASSOCIATED WITH DISTINCT PROTEOMIC SIGNATURES

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Human induced pluripotent stem cells (hiPSC) are of high interest for both scientists and clinicians due to its capacity to differentiate into a whole range of different cell types. In theory, after a successful reprograming hiPSC has the capacity for long term culturing in a healthy undifferentiated state. However, some hiPSC lines spontaneously differentiate and change cellular phenotype and colony morphology. The variation is unpredictable, and not fully understood. In our study, we assess the molecular differences on the proteome level between 20 hiPSC lines classified in 3 distinct colony morphology groups (classic, intermediate and monolayer). All 20 hiPSC lines were analysed with global label-free proteomics, which yielded ~5000 identified and quantified proteins for each samples. We find distinct proteomic signature for the different colony morphology groups and our results indicate an EMT event induced by TGFB signalling for one of the colony morphology groups.

Funding Source
The Western Norway Regional Health Authority; Bergen Research Foundation.

Keywords: HiPSC; Colony morphology; Proteomics

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TESTES-ON-CHIP MODEL FOR IN VITRO SPERMATOGENESIS

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Testes evolved as a bifunctional (steroid and gamete producing) organ, consisting of basal, intraepithelial and adluminal compartments. Endocrine regulation of the testes is controlled by the HPG-axis via gonadotropins. Spermatogenesis occurs within the long convoluted structures known as seminiferous tubules. The tubular compartment comprises primarily of germ cells and somatic Sertoli cells. Sertoli cells anchor the germinal epithelium, creating a blood-testis-barrier (BTB) separating the basal and the adluminal compartments. Undifferentiated mitotic germ cells lie in the basal region, while the differentiating meiotic cells are intraepithelial and the spermatids are in the adluminal region. Conventional culture approaches failed to recapitulate the in vivo testicular structure and complex stem-cell niche microenvironment of the human testes. Niche-driven fundamental mechanisms guiding testicular stem cells to active or inactive (quiescent) states are largely unknown. To address this research gap, we aimed to create a controlled in vitro environment mimicking in vivo physiological and biological conditions in testes, and developed a tested-on-a-chip platform. Intact tubules were cultured to preserve the complex cellular (and clonal) arrangements, testicular stem cell niches and maintain the structural and functional integrity of seminiferous tubules. Our microfluidic device consisted of a chamber surrounded by perfusion channels. In contrast to classical approaches, our platform allowed continuous shear-free medium renewal while locally maintaining the tissue microenvironment with the microgrooves placed between the culture chamber and the perfusion channels. Devices were fabricated from PDMS using soft-lithography and a 3D-printed mold, and bonded to glass slides. Functionally regressed adult human testicular tissue (from Gender dysphoria patients) was loaded in the culture chamber and medium perfused at 90 µL/h for up to 11 days. On-chip live-cell imaging revealed maintenance of the tubule structural integrity, and active spermatogenesis during culture. Off-chip live-dead assays showed the tissues remain viable during the same period. This model will be further exploited to understand spermatogenesis regulation, spermatogonial stem cell expansion & differentiation.

Keywords: Testes; Spermatogenesis; Microfluidics

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A TUBULAR ORGANOID-DERIVED GUT-ON-A-CHIP MODEL BY PRESERVING THE STEM CELL NICHE OF LGR5+ INTESTINAL EPITHELIA

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Microfluidic techniques are increasingly recognized as an important toolbox to add physiologically relevant cues to traditional cell culture. These cues include long term gradient stability and continuous perfusion. Microfluidic technology allows patterning of cell layers as stratified co-cultures that are devoid of artificial membranes, in order to capture complex tissue architectures found in vivo. Previously, we have introduced the OrganoPlate® platform for growing human intestinal gut tubules in a membrane-free manner. Although suitable for toxicity studies, this model uses human intestinal cell lines, such as adenocarcinoma line Caco-2, which has limited differentiation capabilities and

harbors multiple gene mutations. In contrast, Lgr5+ intestinal organoids can develop crypt-villi morphology and form an epithelial barrier – features associated with gut epithelium. These organoids are usually grown as a polarized ball-like structures embedded in an ECM, with limited apical access. Here we show a human organoid gut-on-a-chip model which is composed of Lgr5+ gut epithelial cells grown inside of the microfluidic channels of the OrganoPlate®. We established a tubular shaped epithelial barrier model of the intestinal tract showing rapid cell polarization, tight junction formation and proper expression of intestinal markers. These gut tubules are suitable for high-throughput screening of compound effects through real time imaging of transport and barrier integrity. Moreover, the OrganoPlate® facilitates development of complex models of gut epithelial tubules co-cultured with endothelial vessels. These complex gut-on-a-chip models allow mimicking disease phenotypes such as inflammatory bowel diseases (IBD) and support screening for potential drug targets. Protocols have been established that allow automated readout of the barrier integrity, followed by image analysis and quantification. The combination of Lgr5+ gut organoids with the OrganoPlate® technology are a powerful combination to study physiology and disease mechanisms in patient specific gut models.

Keywords: Microfluidics; Gut-on-a-chip; Intestinal organoids

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TOWARDS ENGINEERING HUMAN EXTRAHEPATIC BILE DUCTS FROM DECELLULARIZED BILE DUCT SCAFFOLDS AND BILE DUCT-DERIVED ORGANOIDS

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Bile duct-related complications are a common case of graft failure after liver transplantation and may require retransplantation. Tissue engineered extra hepatic bile duct (EBD) constructs might help to solve this problem. Using decellularization techniques, all cells can be removed from the EBD-tissue to generate bile duct extracellular matrix (ECM) which can be used as a bio-scaffold for tissue engineering purposes. The aim of this study is to establish a reproducible protocol for the decellularization of EBD tissue and to explore recellularization using human LGR5+ bile duct-derived organoids. EBD tissue is obtained from human livers, which are unsuitable for transplantation due to inferior quality (N=10). EBD tissue was treated with Trypsin-EDTA for 30 minutes and 10 times 30 minutes 4% Triton-x-100 + 1% NH4 to remove all cells. After decellularization, circular discs (12,5 mm^2) were created. Bile duct derived organoids were initiated from healthy EBD tissue and made single cells. The cell suspension (1×⟦10⟧^5 cells per 10µl) was incubated on the luminal side of the ECM surface and kept in culture for up to 3 weeks. EBD tissue was completely decellularized, while maintaining ECM proteins and architecture. After seeding with organoid-derived cells, a confluent epithelial layer was formed on the luminal surface of the EBD ECM. Further analysis showed that cells polarize similar to large cholangiocytes and that cholangiocyte-markers, such a cytokeratin-7 and 19, were detectable at genetic and protein level. In conclusion this study shows that decellularization and recellularization of EBD tissue with bile duct derived organoids is feasible. The complete surface of the ECM could be covered with a confluent epithelial layer, potentially restoring the barrier function. Furthermore, key cholangiocyte markers were present suggesting differentiation towards functional bile duct lining cells. Further study is required whether this bile duct engineering is suitable for clinical application after liver transplantation.

Keywords: Bile duct tissue engineering; Bile duct-derived organoids; Extrahepatic bile duct

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DIRECT GENERATION OF HUMAN NAÏVE INDUCED PLURIPOTENT STEM CELLS FROM SOMATIC CELLS IN MICROFLUIDICS

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Induced pluripotent stem cells (iPSCs) are generated by expression of transcription factors OCT4, SOX2, KLF4 and cMYC (OSKM) in somatic cells. In contrast to murine naïve iPSCs, conventional human iPSCs are in a more developmentally advanced state called primed pluripotency. Here we report that human naïve iPSCs (niPSCs) can be generated directly from less than 1000 primary human somatic cells without stable genetic manipulation by delivery of modified messenger RNAs with microfluidics. Expression of OSKM in combination with NANOG for 12 days generates niPSCs free of transgenes, karyotypically normal, and display transcriptional, epigenetic and metabolic features indicative of the naïve state. Importantly, niPSCs efficiently differentiate into all three germ layers. While niPSCs could also be generated at low frequency under conventional conditions, our microfluidics approach will allow robust and cost-effective production of patient-specific niPSCs for regenerative medicine applications, including disease modelling and drug screening.

Keywords: Human somatic cell reprogramming; Human naïve iPSCs; Microfluidics

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LARGE-SCALE PRODUCTION OF HUMAN LIVER ORGANOIDS WITH IMPROVED HEPATOCYTE DIFFERENTIATION

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Human organoids represent an exciting cell source to treat end-stage liver failure. However, establishing large numbers of organoids with current protocols is tedious, since organoids are cultured in droplets of Matrigel. Here we established a method for the expansion of large quantities of human liver organoids in a bioreactor. We observed rapid proliferation of the organoids in the bioreactors reaching an average of 40-fold cell expansion after two weeks. Despite their increased proliferation, the organoids did not form tumors in a xenograft model. When culturing the organoids in differentiation medium, expression levels of mature hepatocyte markers such as CYP3A4, MRP2, and ALB were highly upregulated and albumin secretion and midazolam metabolism reached levels equivalent to hepatocytes. Lastly, we showed that organoids from bioreactors were able to repopulate decellularized liver discs and formed liver-like tissue including cholangiocyte-like cells and hepatocyte-like cells on the extracellular matrices.In conclusion, we established a novel method to culture billions of liver organoid cells in a time-, work-, and cost-efficient way. This method paves the way for the application of organoids for cell therapy, in which large quantities of cells are needed.

Funding Source
This work was supported by the Dutch Research Council NWO TTW (15498) to B.S., Dutch Research Council NWO ZON/MW (116004121) to L.C.P. and B.S.; and K.F. Heinfonds to K.S.

Keywords: Human liver organoids; Tissue engineering; Bioreactor

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THE CRITICAL ROLE OF AUTOPHAGY AND MITOCHONDRIAL REMODELING IN ENDOTHELIAL CELL DIRECTED DIFFERENTIATION

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Vascular tissue derived from patient-specific induced pluripotent stem cells (iPSCs) suffers from premature replicative senescence, limiting clinical applications and creating a significant barrier to the advancement of organ and tissue bio-engineering. Autophagy plays a critical role in stem cell survival and directed differentiation, at least in part through selective autophagy of the mitochondria, i.e., mitophagy. While mitophagy also contributes to mediating cellular senescence, the mechanisms of premature senescence in reprogrammed iPSC-derived endothelial cells remain poorly understood. The iPSC lines ACS1028 and Y6 were subjected to directed differentiation over 6 days, and cells were then purified by positive selection for VE-cadherin. Autophagy marker microtubule-associated proteins 1A/1B light chain 3B (LC3) and mitochondrial matrix protein Tom20 were quantified through Western blotting. Mitochondria were evaluated with MitoTracker staining. Endothelial cell function was assessed through immunofluorescence of key markers and quantification of nitric oxide production. During directed differentiation from iPSCs to endothelial cells, mitochondrial morphology evolved from globular to filamentous. LC3 expression decreased by 50% in the middle phase of differentiation ($p<0.05$), correlating with significant mitochondrial remodeling, suggestive of fluctuating mitophagy. Mature iPSC-derived endothelial cells had minimal autophagy activity 5 days after purification, over which time nitric oxide production also declined by 60% ($p<0.05$) and key cell surface marker expression decreased. These iPSC-derived endothelial cells senesced within 2 weeks after purification. Mature iPSC-derived endothelial cells demonstrate a significant decline in autophagy activity that correlates with the loss of mature endothelial cell function, represented by decreased nitric oxide synthesis, which precedes the onset of premature replicative senescence. This supports the role of mitophagy in mediating cellular senescence of iPSC-derived endothelial cells, and renders autophagy induction a target for attenuating senescence.

Funding Source
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Keywords: endothelial cell; autophagy; mitochondria

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LYMPHATIC ENDOTHELIAL PROGENITOR CELLS AND VEGF-C LOADED WITH SELF-ASSEMBLING PEPTIDE NANOFIBERS PROMOTE LYMPHANGIOGENESIS IN INFARCTED MYOCARDIUM

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Lymphatic vessels play a crucial role in draining excess fluid and transporting macromolecular substances from extracellular spaces. Disfunction of lymphatic vessels may cause lymph edema and chronic inflammation, leading to fibrosis of the local tissue. This study investigated efficiency of transplantation of lymphatic endothelial progenitor cells (LEPCs) and sustained release of VEGF-C from self-assembling peptide (SAP) on promoting lymphangiogenesis after myocardial infarction (MI). CD34+VEGFR-3+ EPCs were isolated from rat bone marrow. Sustained release of VEGF-C from SAP nanofibers (SAPNs) was detected with ELISA. Compatibility of SAPNs with the cells was accessed with transmission electron microscopy and EB/AO staining. After rat MI models were established with ligation of the anterior descending branch of the left coronary artery, SAP carrying the cells and VEGF-C was injected at the border of the infarcted region. At four week after transplantation, the survival and differentiation of the cells labeled with GFP were examined, and repair of the infarcted myocardium was evaluated. Under induction with VEGF-C, CD34+VEGFR-3+ EPCs could differentiate into lymphatic endothelial cells. The cells spread well along SAPNs. SAPNs protected the cells from apoptosis in the condition of hypoxia, and released VEGF-C sustainedly. After transplantation, cardiac function was improved significantly. The number of the survived LEPCs increased, and some cells differentiated into lymphatic endothelial cells. Density of lymphatic vessels increased, and cardiac edema was reduced. Moreover, angiogenesis and myocardiac regeneration were enhanced. These results suggest that SAPNs load LEPCs and release VEGF-C effectively. VEGF-C released from SAPNs induces differentiation of LEPCs towards lymphatic endothelial cells. Loading stem cells and releasing growth factor with SAPNs is a promised strategy for MI therapy.

Funding Source
This work was supported by National Natural Science Foundation of China (81870215, 81770288, 81470385).

Keywords: Self-assembling peptide; Endothelial progenitor cells; VEGF-C

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COMBINATION OF THREE-DIMENSIONAL SCAFFOLDS AND INTERMITTENT HYDROSTATIC PRESSURE IN EX-VIVO EXPANSION OF HEMATOPOIETIC STEM/PROGENITOR CELLS

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Hematopoietic stem/progenitor cells (HSPCs) transplantation is used for the treatment of several hematologic or immunological diseases. Since successful outcomes required sufficient number of cells, many researchers have focused on ex vivo expansion of HSPCs. HSPCs reside complex niche including various factors such as secreted factors, extracellular matrix, mechanical environment and so on. It is well known that niches can modulate expansion, homing, migration, differentiation and others of HSPCs. In this study, we constructed an optimized 3-dimensional (3D) culture system to mimic niche by treating vitronectin with 3D hierarchical scaffolds for ex vivo expansion of HSPCs. Lattice was made of three layers using rapid prototyping technique and nanofiber was electrospun on each layer of three layered lattice using electrospinning technique. Intermittent hydrostatic pressure (IHP) was also applied to represent the in-vivo mechanical environment (4h/day, 20kPa). The group to which the stimuli was applied was divided into two pattern: P(1min/7min) and P(2min/13min). Trypan blue staining was performed to evaluate expansion of total cells and flow cytometry was conducted to confirm phenotypes of HSPCs (CD34+, CD34+CD38-). And clonogenic potential was also examined by colony forming cell(CFC) assay and long term culture-initiating cell(LTC-IC) assay. Total cell expansion was higher in vitronectin treated 3D scaffold compare to 2D culture system and untreated 3D scaffold. Moreover, the number of total cells was further increased under IHP. Maintenance of phenotype (CD34+, CD34+CD38-) was better under HP, especially P(2min/13min), and that it also had a greater colony formation ability by CFC assay and LTC-IC assay. As a result, the combination of 3D hierarchical scaffold and mechanical environment was superior to control the fate of HSPCs, and could be an useful model system mimicking niche.

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Keywords: Hematopoietic stem/progenitor cells; ex vivo expansion; 3D hierarchical scaffolds

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A PULSATILE FLOW BIOREACTOR ENHANCES MESENCHYMAL STEM CELLS DIFFERENTIATION INTO ESOPHAGEAL CELL LINEAGES IN THE ELECTROSPUN TUBULAR SCAFFOLD

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Tissue-engineered esophagus serves a potential therapeutic approach for esophageal replacement. We aimed to simultaneous differentiate human mesenchymal stem cells (hMSCs) into esophageal epithelial cells (EpCs) and smooth muscle cells (SMCs) on the electrospun tubular scaffold by adopting mechanical stimuli in vitro for esophageal tissue engineering. Briefly, a two-layered tubular scaffold was fabricated by electro-spinning and hMSCs were seeded onto the inner and outer layer of the scaffold. Then the constructs were placed in a self-designed hollow organ pulsatile flow bioreactor and stimulated with EpCs and SMCs induction medium in the inner and outer chamber for two weeks. Meanwhile, either 0.1dyne/cm2 of shear stress or pulsatile shear together with 50mmHg radius pressure and 5% circumferential stretch was engaged during the second week. Finally, esophageal epithelial and muscular differentiation level was tested by RT-PCR and immuno-fluorescence staining. As a result, electro-spun fibers were found randomly and circumferentially oriented on the inner and outer layer of the tubular scaffold. After 14 days of culture, scaffolds supported hMSCs attachment and esophageal cell lineage differentiation was found under specific chemical stimuli, i.e. EpCs differentiation markers were significantly increased in the inner layer while SMC markers were significantly increased in the outer layer. Furthermore, physiologic pulsatile flow which also resulted in shear stress further promote the differentiation level of epithelial and smooth muscle cells in vitro and the compliance of the construction was also slightly increased in the pulsatile flow group. In conclusion, we demonstrated a potential platform for tissue-engineered esophagus based on differentiation of hMSCs under chemical and mechanical stimuli in vitro. Selection of optimal differentiation conditions and further in vivo tests are required.

Funding Source
This work was supported by Priority Research Centers Program (2010-0020224, the Ministry of Education, ROK) and by the National Research Foundation of Korea (NRF) Grant (NRF-2015M3A9B6073643).

Keywords: esophageal tissue engineering; mesenchymal stem cells; pulsatile flow

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EFFICIENT GENERATION OF ARTIFICIAL VASCULARIZED LIVER USING MULTIPLE HUMAN CELL TYPES

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Liver transplantation is a well-established therapeutic strategy for end-stage liver disease. As the patients with severe liver failure have increased gradually, the demand for organs has also grown worldwide. However, due to donor organ shortage, liver tissue engineering has currently been considered as an alternative. Decellularized extracellular matrix scaffold, obtained by removal of cells from a donor tissue, has been known as a promising substrate for tissue remodeling. Due to preservation of its own structural and biological signals, the cells seeded into decellularized scaffold can migrate to the appropriate microenvironment. Especially, the liver is a very vascular organ, which receives about 25% of total cardiac output. Therefore, efficient re-endothelialization of the scaffolds is the key to successful reconstruction of the artificial liver. Based on this, we aimed to efficiently generate vascularized liver with multiple human cell types using decellularized rat liver scaffold. First, the scaffolds were coated with reagent A and subsequently repopulated with human umbilical vein endothelial cells (HUVECs). Injected endothelial cells were specifically captured by reagent A in an efficient manner, leading to the formation of confluent endothelium along the vascular channels. To evaluate the efficiency of endothelialization of the scaffold, we perfused the scaffolds with human blood and measured platelet counts. The results showed significantly reduced platelet aggregation in the perfusate of reagent A-coated scaffolds compared with non-coated scaffolds, indicating improvement of endothelialization and less thrombogenicity of the scaffolds. Moreover, we developed a new protocol for recellularization of liver parenchymal cells and non-parenchymal cells simultaneously. With this protocol, reagent A-coated scaffolds were successfully repopulated with HepG2, LX2 (hepatic stellate cell), HUVEC and umbilical cord blood-derived mesenchymal stem cell (UCB-MSC) which were properly localized to their original sites. To this end, our study provides a new strategy for fabrication of tissue-engineered liver, suggesting the possibility to reconstruct full-sized human artificial liver retaining elaborate histological architectures including endothelialization.

Keywords: Artificial Liver; Endothelialization; Decellularized extracellular matrix

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FIBROUS NEURAL ORGANOID USING CELL ENCAPSULATION TECHNIQUE

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Organoid formation from stem cell aggregates holds great promise for contributing development biology, regenerative medicine, drug development, and pathological modeling. Until now, various type of tissue including brain, intestine, and lung has been reconstructed by using organoid formation from human induced pluripotent stem cells (hiPSCs) or human embryonic stem cells (hESCs). The organoids reproduce a part of the developmental cell dynamics and three-dimensional (3D) structure of the developing tissues in vitro, and contribute for disease modeling and drug screening. However, in current methods for the organoid formation, most organoids only represent single or partial component of tissues. This is because it is often difficult to control architectures of the organoids, organization, cell-cell/cell-extracellular matrix (ECM) interactions within the methods. Recently, to overcome the limitation, engineering stem cell organoids has been focused. Here we generate fibrous neural organoids from hiPSCs using cell encapsulation technique. By using the microfluidic device system (cell fiber technology), singly dissociated hiPSCs were encapsulated into core-shell microfibers composed of Matrigel (core) and alginate hydrogel (shell). In the ECM-rich microenvironment of the core region of the fibers, the hiPSCs formed fibrous aggregates for a few days (hiPSC fibers). The hiPSC fibers were cultured N2B27 containing CHIR99021 and TGFβ inhibitor for several days to induce neuroepithelium, and then the fibers were differentiated in neuronal medium (neural organoid fibers). By controlling the flow condition for the cell encapsulation using the cell fiber technology, the neural organoid fibers ranging from μm to cm scale lengths was easily constructed. In the fibers, hiPSCs differentiated into neuroepithelial cells and formed 3D neural rosette structure, and a cortical layer-like structure was observed. Furthermore, we found that GSK3 signaling regulated neural cell expansion in the organoids fibers as well as in developing brains. These results indicate that the organoid engineering technique can be a powerful tool for steering the 3D architecture of the organoids and cell dynamics and organization in the organoids.

Funding Source
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Keywords: Organoid engineering; Neural organoid; Cell encapsulation

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REGULATION OF MOUSE IPS CELL PROLIFERATION AND CARDIOMYOGENIC DIFFERENTIATION ON POLYPEPTIDE MULTILAYER FILMS

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The mechanical properties of extracellular matrix play important roles in controlling cell adhesion, cell proliferation and cell differentiation. In this study, the thickness, stiffness and adhesiveness of polyelectrolyte multilayer films were modulated, then used to study the proliferation and cardiomyogenic differentiation of mouse induced pluripotent stem cells (iPSCs). Polypeptide multilayer films were built up by alternate deposition of cationic poly-L-lysine (PLL) and anionic poly-L-glutamic acid (PLGA), cross-linked with EDC/sulfo-NHS, and coated with gelatin. Quartz crystal microbalance and atomic force microscopy were used to certificate polypeptides depositing process and the granular structure of the film surface. The stiffness of the films were modulated by cross-linking with EDC/sulfo-NHS. The films were then coated with gelatin to enhance cell adhesion. The proliferation assay showed that iPSCs were growing well in six layers multilayered films coated with gelatin. Cardiomyogenic differentiation was monitored with electric cell-substrate impedance sensing (ECIS). Mouse iPSCs were cultured on gelatin-terminated PLL/PLGA multilayer-coated electrodes and cardiomyogenic differentiation was monitored with ECIS. The occurrences of beating cardiomyocytes were observed when iPSCs were cultured on gelatin-terminated PLL/PLGA multilayer-coated electrodes. In addition, the periodic fluctuations of measured impedance caused by cardiomyocyte beating were observed accordingly. These results demonstrated that the mechanical properties of the gelatin-coated PLL/PLGA multilayer films can enhance the efficiency of iPSCs differentiation into cardiomyocytes.

Funding Source
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Keywords: induced pluripotent stem cells; polypeptide multilayer film; cardiomyogenic differentiation

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PROCESS DEVELOPMENT FOR DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TOWARDS MESENCHYMAL STEM CELLS IN 3D SUSPENSION CULTURE

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Mesenchymal stem cells (MSCs) are multipotent cells which have the ability to differentiate in-vitro into mesodermal derivatives; adipocytes, osteocytes, and chondrocytes, and with a lesser extent into ectodermal or endodermal derivatives; neurons or hepatocytes respectively. In addition, these cells have special immunomodulatory properties and therefore are attractive candidates to the regenerative medicine and cell-based therapy fields, but only if the cell number barrier can be crossed. In addition, MSC derivatives can also serve as a model for tailored-made drug screening. 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 and often requires invasive procedures. Moreover, their expansion potential in-vitro is finite. Therefore, human pluripotent stem cells (hPSCs), either - embryonic or induced, can potentially provide alternative, unlimited and reproducible source of MSCs. Two-dimensional (2D) adherent culture has been traditionally used as the standard technique for in-vitro expansion of MSCs. Nevertheless, the number of cells that can be obtained in 2D adherent culture is limited and requires high costs and laborious work. Accellta has developed carrier-free suspension platform for culturing PSCs as cell aggregates (MaxellsTM) or single cells (SinglesTM), at high densities of 10 million cells per ml and above. This platform allows directed differentiation of PSCs towards ectodermal, endodermal and mesodermal progenitors in suspension. Thus, Accellta's unique technology provides the solution for mass production of cells needed for cells-based therapy in cost-effective manner. Here we present, novel, specific and efficient process for the directed differentiation of hPSCs towards MSCs in non-adherent, carrier-free suspension culture along with designated media. Following few days in differentiation media, our protocols generated bona fide MSCs with high-rate differentiation towards adipocytes, chondrocytes and osteoblasts in-vitro. Therefore, Accellta's carrier- free suspension culture may serve as a base for directed differentiation of PSCs into mini organelles, that can be utilized to the field of cell-based therapy as well as for a humanized model for drug screening.

Keywords: Mesenchymal stem cells, in-vitro; human pluripotent stem cells, drug screening; mini organelles, carrier-free suspension

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BEHAVIORS OF AXONS FROM MOTOR NEURON SPHEROID IN THE ECM ENVIRONMENT AND MICROCHANNEL

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Motor neurons in the central nervous system (CNS) plays role in downstream signaling to the effectors through their axons. The axons interact with their extracellular environment and navigate to reach to the final target. There are number of studies about axons or neurons to elucidate the mechanisms about the development of neural circuits or neural diseases. But in many cases, it is hard to distinguish the parts of neural circuit unit visually. To understand their interaction and build model of complex network, it is necessary to separate the cells and the axons in controllable manner. Here, we used a PDMS device with a microchannel and reservoirs to observe the behaviors of axons of motor neurons. The motor neurons were differentiated from small molecule neural progenitor cell(smNPC) spheroids, and the spheroids move to the device with Extracellular-matrix(ECM) environment. Axons were classified from the motor neuron-differentiated spheroids and protrude in Matrigel area. During protrusion, the axons were affected by guidance of PDMS structures and other axons. Axons which interact with other axons build the fascicles. And lastly, the protrusion was seemed to be affected by other spheroids with different manner. The cells of differentiated NPC spheroid and the axons were evaluated by immunofluorescence images and quantified.

Keywords: motor neuron; axon; guidance

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HUMAN PLURIPOTENT STEM CELL DERIVED NEURAL 3D CULTURES – BUILDING NEURAL TISSUE BLOCK FOR BODY-ON-A-CHIP PLATFORM

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The brain tissue, like many other tissues, is highly organized 3D structure. When aiming to build an in vivo-mimicking BoC platform, it is necessary to take into account tissue specific architecture and extracellular matrix (ECM). Traditional 2D cell culturing conditions fail to offer cells in vivo mimicking growth cues, leading cell adaptation to unnatural conditions and creating bias to gene and protein expression of the cells. Some of these problems could be overcome by 3D culturing, which can offer more in vivo-like cell to cell and cell to ECM interactions. Hydrogels as biomaterials are soft tissue ECM mimicking. For this reason, hydrogels are very prominent materials to be used as an artificial ECM in BoC platforms. Here we compared the properties of several hydrogels to find the best material to be used in brain tissue block. In this work, we used human pluripotent stem cell derived neural cells. Hydrogels use were PuraMatrix, Gellan gum, hyaluronic acid – poly(vinyl)alcohol (HA-PVA) and collagen 1. The main findings were: 1. When several biomaterials were compared, the mechanical properties of scaffold material did not correlate with neural cell growth inside the scaffold. 2. For culturing human neural cells inside hydrogel scaffold, it was beneficial to use multicomponent hydrogels by using collagen 1 as one component for making interpenetrating network scaffolds. During recent years, many different hydrogels have been promoted as the state of art scaffold materials for neural tissue engineering both in vivo and in vitro. Based on the literature it is difficult to evaluate what hydrogel would be most prominent to be used in BoC. Our results suggest that both the choice of cells and the choice of analysis methods have effect on which material has the best performance. Also, the usability of the biomaterial and reproducibility of the cultures are important aspects to take into account. It seems that some biomaterials are good for some cell lines but not for others, but at the same time it is possible to find materials that are able to support many cell lines, like the combination of HA-PVA-Collagen 1.

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Keywords: 3D culture; Hydrogel; Neural network

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TRANSPLANTATION OF CARDIAC PATCH LOADING TB4-OVEREXPRESSED MSCS PROMOTES REPAIR OF INFARCTED MYOCARDIUM BY ACTIVATING EPICARDIUM

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Recent studies suggest that the epicardium plays an important role in cardiomyogenesis during development, while it becomes quiescent in adult heart. Thymosin beta 4 (Tβ4) has an effect on activating the epicardium. However, effectiveness of Tβ4 administration is unsatisfactory. Therefore, this study prepared cardiac patch and investigated efficiency of activating the epicardium and repairing infarcted myocardium by Tβ4 released sustainedly from the cardiac patch. Mesenchymal stem cells (MSCs) isolated from bone marrow of rats and mice were transfected with Tβ4. Tβ4 release from the cells was determined with an acquity ultra-performance liquid chromatography system. For preparing of cardiac patch, the cells transfected with Tβ4 and Flag were seeded on PLACL/collagen membrane formulated by electrospinning. The survival and proliferation of the cells on the nanofibers were examined after treatment with hypoxia. In MI models of rats and W 1CreERT2/+,R26mT-mG mice, the patches were implanted on the epicardium of the infarcted region. In rat models, differentiation of the epicardium-derived cells (EPDCs) and the engrafted MSCs towards cardiovascular cells was examined by Wt1 immunostaining and Flag labelling. In transgenic mouse models, the activated EPDCs expressed GFP, so GFP could be used to trace fate of the EPDCs. At four week and six month after implantation of the patches, cardiac function was improved significantly, scar area in the infarcted region was reduced obviously. EPDCs increased in subepicardium and myocardium, and some Wt1+ cells and GFP+ cells expressed CD31, α-SMA or cTnT. Angiogenesis and lymphangiogenesis in the para-infarcted region were enhanced. At six month after implantation, lymphangiogenesis were obvious. Flag labelling showed that some engrafted MSCs migrated into subepicardium and myocardium. These results suggest that Tβ4 released from the transfected MSCs in PLACL/collagen nanofibrous patches may effectively attenuate left ventricular remodelling and improve cardiac function by activating the epicardial cells. Our finding provides a novel strategy for myocardial regeneration by enhancing the endogenous regenerative mechanisms.

Funding Source
This work was supported by National Natural Science Foundation of China (81270200, 81470385, 81770288).

Keywords: Tβ4; Epicardium; Cardiac patch

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THE ROLE OF NOTCH IN THE ACTIVATION OF A LATENT NEUROGENIC PROGRAM IN REACTIVE ASTROCYTES

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Astrocytes respond to injuries to the central nervous system (CNS) by reactivating and acquiring an undifferentiated state, which is characteristic of neural stem cells. Under in vitro conditions, these cells regain multipotency and long term self-renew, however, the molecular factors involved in astrocyte dedifferentiation are poorly understood. In this context, we investigated the role of Notch signaling as well as Galectin-3 (Gal3) in triggering a latent neurogenic program in reactive astrocytes in vitro. Astrocytes extracted from neonatal mouse cortices were cultivated in vitro and reactivated by mechanical lesion. Three days post lesion, cells were fixed, immunolabeled for NICD (Notch intracellular domain), Jagged1 (Notch1 ligand), Gal3 and GFAP, and analyzed by confocal microscopy and flow cytometry. Our results show that reactive astrocytes overexpress Gal3, which is a lectin related to the proliferative capacity of reactive astrocytes, and their potential to express neural stem cells markers in vitro. Astrocytes submitted to mechanical lesion also showed increased NICD in the nucleus, indicating that Notch signaling pathway was activated in reactive astrocytes. Co-localization analysis further revealed that a subpopulation of reactive astrocytes located in the border of the injury co-localized with Notch/Jagged (receptor/ligand) significantly more than astrocytes located far from the border, suggesting that Notch signaling pathway is mainly activated at the border of a lesion. We conclude that Notch signaling pathway has a main role in astrocyte reactivity, dedifferentiation response and activation of a latent neurogenic program. The study was approved by the Ethics Committee on Animal Use of UNIFESP (CEUA number 7740290318).

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Keywords: reactive astrocyte; neurogenic program; Notch

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NOVEL INSIGHTS IN HUMAN AXONAL DEVELOPMENT FROM IPSC-DERIVED NEURONS

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During neuronal development, a symmetry break occurs when one of the unipolar processes shows extensive growth and transforms into an axon. To date, most mechanistic insights of axon initiation come from non-human studies. However, it remains unknown to what extent these mechanisms can be extrapolated to human neurons, and human axonopathies (e.g. paraplegia, degenerative motor neuron diseases, etc.) remain yet unresolved. Here, we have mapped the molecular events during axonal development in human iPSC-derived neuron cultures using immunofluorescence and mass spectrometry analysis. We observed successful initiation of axon development in hiPSC-derived neurons, indicating that this event is driven by cell-autonomous processes. Consistent with human brain development in vivo, axon formation in hiPSC-derived neuron cultures occurred relatively slow (~7–14 days). Unexpectedly, we found that during this process the axon initial segment (AIS) proteins Trim46 and AnkyrinG first appear distally in axons, after which they accumulate at the proximal axon. Hence, we propose a working model where human axon development is accomplished by a two-stage process. First, axon formation starts with AIS proteins accumulating distally, possibly to promote accelerated outgrowth which may involve microtubule stabilization. Secondly, AIS proteins reorganize at the proximal axon to form the specialized AIS structure. Together, these data provide novel insights in the cell-autonomous processes driving human axonal development, and highlight the relevance of studying these mechanisms in a human model system in addition to the existing non-human model systems.

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Keywords: axon; development; human neurons

Poster Abstracts

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LONGITUDINAL DISSECTION IN BRAIN ORGANOIDS AT SINGLE CELL RESOLUTION UNCOVERS THE DEVELOPMENTAL ROLE OF GSK3 IN HUMAN CORTICOGENESIS

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The regulation of proliferation and polarity of neural progenitors is crucial for the development of the brain cortex, with modes and timings of cell division intimately related to the stereotypical acquisition of layer-specific neuronal identities. Animal studies have implicated glycogen synthase kinase 3 (GSK3) as a pivotal regulator of both proliferation and polarity, yet the functional relevance of its signaling for the unique features of human corticogenesis remain to be elucidated. Here we harness human cortical brain organoids to probe, at single cell resolution, the longitudinal impact of GSK3 inhibition through multiple developmental stages. Our results indicate that chronic GSK3 inhibition increases the proliferation of neural progenitors and causes massive derangement of cortical tissue architecture. Surprisingly, single cell transcriptome profiling revealed only a discrete impact on early neurogenesis and uncovered GSK3-specific neurogenic trajectories. Through this first single cell-level dissection of the GSK3 regulatory network in human corticogenesis, our work uncovers a remarkably specific conduit between the architecture of progenitor niches and lineage specification.

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Keywords: Brain organoids; Corticogenesis; GSK3

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HUMAN FETAL VENTRAL MIDBRAIN IN STANDARD CULTURE VERSUS ORGANOIDS

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Parkinson disease (PD) is characterized by a loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). The selective loss of this cell population makes PD a good candidate for cell-based therapies. Clinical trials using cells derived from human fetal ventral midbrain (hVM) have shown that dopamine release was restored to normal levels and in some PD patients produced substantial long-term clinical improvement. Although new sources of cells such as human embryonic stem cells (hESCs) and pluripotent stem cells (IPCs) are now been the focus of studies and new clinical trials, the hVM tissue represent the gold standard for cell based therapies and serve as important comparator for new cell sources. Therefore there is a need of an increased understanding and characterization of this tissue with new technologies such as organoid culture conditions and single cell sequencing. This study is designed to give a detailed characterization of human fetal VM tissue in standard culture conditions (2D) versus organoids (3D). We used immunocytochemistry to study the presence of ventral midbrain markers in 2D versus 3D condition together with single cell RNA sequencing to transcriptionally compare fetal VM tissue in standard or organoid cultures. Our analysis confirmed that the 3D condition preserve the neuronal composition of the tissue more than standard culture where instead, over a long period of time, the majority of the cells are non-neuronal. This suggest that 3D culture represent a better condition to maintain and study dopaminergic neurons in culture.

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