SYStem

PROGRAMME

3–5 March 2021
Vienna, Austria

Symposium for the next generation of stem cell researchers

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Welcome to the Vienna BioCenter!

Dear SY-Stem attendees!
We are very happy to welcome you to the third SY-Stem Symposium!

It was almost exactly a year ago when the coronavirus situation forced us to cancel the 2020 Symposium. Since then, we all had to reshape much of our personal and professional lives and make many changes to the ways we spend our days. At the same time, we have learned so much about how virtual meetings and videoconferences can replace travel. In our 2021 SY-Stem virtual meeting, we are now **exploring new ways to connect** with participants from Europe and around the world to make this an exciting event for all of you.

We hope you like what we prepared for you and look forward to **welcoming you in the virtual space while hosting the conference in Vienna**, Austria.

For the 2021 sessions we selected a diverse area of stem cell-related research fields, ranging from topics like pluripotency, early development and gastrulation to organogenesis, disease and clinics.

On our first day keynote speaker **Amy Wagers** will share insights on advanced genome editing methods of therapeutically relevant stem cell populations, their in vivo applications in mouse disease models and the wealth of gene repair mechanisms thus made possible. The work presented by Wagers paves the way for genetic functional recovery and system-wide endogenous repair mechanisms through new gene edited stem cell therapeutics.

In addition to the talks by our invited speakers, we **opened the stage** for further talks by participants - from master student to PI - to present their work to the stem cell community.

During the **networking session** you will be able to catch up with old friends and colleagues and make new connections as well. A virtual networking space will allow all attendees to become part of the conversation. You will be able to **meet the speakers**, view and **discuss posters** as well as **connect with** our **exhibitors** and talk about latest technological solutions live with their delegates. Furthermore, there will be networking possibilities to discuss topics of common interest through chat channels as well as via social media. Make sure to follow the event hashtag on Twitter:
#SYStem_2021 !

Finally, we will conclude the symposium with a keynote lecture by **Lorenz Studer**. He will present strategies on the use of human pluripotent stem cells to model and treat neural diseases including neuroinflammation in neurodegenerative disease, Parkinson's disease (PD), as well as central and peripheral nervous system conditions. The translational aspect of Studer's work is emphasized by a recent FDA clearance for a clinical trial in PD patients.

Although we cannot bring to you the warm spring-like weather we currently have in Vienna, we will make every effort to give you a visual and acoustic taste of Vienna!

We are very happy to welcome researchers from around Europe and the world to this year's symposium, who join us online from **28 different countries**. You make this symposium a truly international event and we are confident you will **carry our message over to your home institutions**.

We would particularly like to thank our speakers who are contributing their knowledge and ideas to this symposium. We would also like to extend special thanks to the industrial sponsors of this year's symposium.

We hope you enjoy the conference and look forward to an exciting symposium!

Elly Tanaka, Juergen Knoblich, Uli Elling, Bon-Kyoung Koo & Sasha Mendjan

Programme

Wednesday, 3rd March 2021

13:00 **WELCOME AND INTRODUCTION**

Session 1 - Pluripotency, Early Development & Gastrulation

13:15 **CANTAS ALEV** (Kyoto University)
Towards reconstituting human somitogenesis in vitro

13:45 **GRAZIANO MARTELLO** (University of Padua)
Identification of novel regulators of pluripotency in human cells

14:15 **RUTH HORNBACHNER** (Medical University Vienna)
MSX2 cooperates with SWI/SNF complex to safeguard syncytiotrophoblast fate of human trophoblast stem cells

14:35 **ALEJANDRO AGUILERA CASTREJON** (Weizmann Institute of Science)
Ex utero Development of Mouse Embryos from Pre-Gastrulation to Advanced Organogenesis

14:55 *Networking break*

16:05 **SILVIA SANTOS** (The Francis Crick Institute)
Decoding cellular signals during cellular transitions

16:25 **EMIEL VAN GENDEREN** (Erasmus MC Rotterdam)
Linking morphogenesis and differentiation: a novel embryonic pluripotent state in between naïve and primed pluripotency

16:45 **CHRISTA BUECKER** (Max Perutz Labs)
Reshaping of the transcriptional and regulatory landscape during the exit from naïve pluripotency

Session 2 - Organogenesis

17:15 **SASHA MENDJAN** (IMBA)
Cardioids reveal self-organizing principles of human cardiogenesis

17:45 *break*

Keynote lecture

18:00 **AMY WAGERS** (Harvard Stem Cell Institute)
In vivo gene editing in tissues and tissue stem cells

19:00 *Networking*

Thursday, 4th March 2021

13:00 **MIKI EBISUYA** (EMBL Barcelona)
Human Time vs. Mouse Time with Recapitulated in vitro Systems

13:30 **GRAYSON CAMP** (Institute of Molecular and Clinical Ophthalmology Basel)
Human cerebral organoid development through the lens of single-cell genomics

14:00 **YEKATERINA MIROSHNIKOVA** (Helsinki Institute of Life Science)
Heterochromatin-driven nuclear softening protects the genome against mechanical stress-induced damage

14:20 **DENISE SERRA** (BRIC / University of Copenhagen)
Self-organization and symmetry breaking in intestinal organoid development

14:40 *Networking break*

15:50 **JENS PUSCHHOF** (Hubrecht Institute)
Organoids & Toxins - from snakebite to DNA-damaging bacteria

16:10 **GEORG BUSSLINGER** (Research Center for Molecular Medicine)
Human gastrointestinal epithelia of the esophagus, stomach and duodenum resolved at single-cell resolution

Session 3 - Disease

16:30 **MERITXELL HUCH** (MPI of Molecular Cell Biology and Genetics)
Unveiling liver regeneration and disease mechanisms using organoid cultures

17:00 **ÁBEL VÉRTESY** (IMBA)
Improved cell type resolution in cortical organoids by identification and removal of in-vitro stress

17:20 *break*

17:40 **JINWOOK CHOI** (Wellcome-MRC Cambridge Stem Cell Institute)
Inflammation shapes the lung regeneration

18:00 **KIM JENSEN** (BRIC / University of Copenhagen)
At the origin of intestinal epithelial stem cells

18:30 *Networking*

SYStem

Friday, 5th March 2021

13:00 **FLORIAN MERKLE** (University of Cambridge)
Modelling obesity with human hypothalamic neurons

13:30 **GERALD SCHWANK** (ETH Zurich)
Translational genome editing: From ex vivo to in vivo

14:00 **ALBA TRISTÁN-NOGUERO** (Fundació Sant Joan de Déu)
Stem Cell Modeling of Tyrosine Hydroxylase Deficiency Recapitulates Patient Phenotypes and Reveals Altered Neuronal Morphology

14:20 **ENZO POIRIER** (The Francis Crick Institute)
A novel isoform of Dicer protects mammalian stem cells against RNA viruses

14:40 *Networking break*

15:50 **KARL KÖHLER** (Boston Children's Hospital/Harvard Medical School)
Sensory organoids for modeling development and disease

16:20 **ALICE ROSSI** (King's College London)
A new evolutionarily conserved mechanisms of regulating neural stem cell quiescence

16:40 **ELISAVET TIKA** (Universite Libre de Bruxelles)
Spatiotemporal regulation of multipotency during prostate development

Session 4 - Clinics

17:00 **NICOLA VALERI** (Institute of Cancer Research)
Cancer organoids for forward and reverse translation

17:30 *break*

Keynote lecture

17:45 **LORENZ STUDER** (Memorial Sloan Kettering Institute)
Modeling and treating neural disease using human pluripotent stem cells

18:45 **CLOSING REMARKS**
19:00 **NETWORKING**

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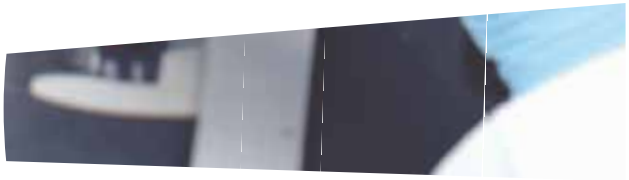

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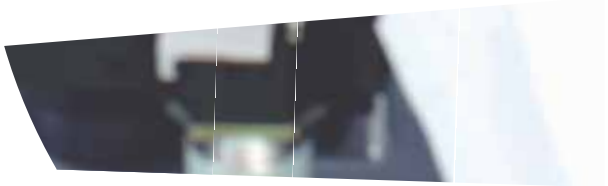

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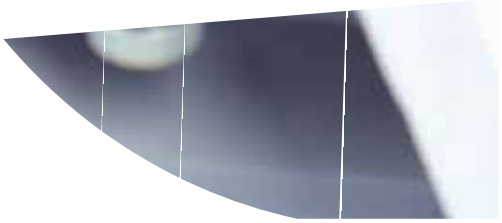

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Keynote lecture 1

In vivo gene editing in tissues and tissue stem cells

Amy Wagers
Harvard University / Harvard Stem Cell Institute

Effective functioning of the body's tissues and organs depends upon the maintenance of proper cell numbers (homeostasis) and replacement of damaged cells after injury (repair), both processes that require proper functioning of tissue stem cells. My laboratory is working to advance strategies for genome editing of therapeutically relevant stem cell populations, using experimentally engineered programmable nucleases. Application of this system in a mouse model of Duchenne Muscular Dystrophy (DMD) has shown simultaneous gene targeting in multiple organs of therapeutic interest, with restoration of the mutated Dystrophin protein reading frame, recovery of muscle function, and establishment of a pool of modified muscle stem cells capable of participating in subsequent muscle regenerative events. Further adaptation of this approach suggests the capacity to access additional mechanisms of gene repair in muscle stem cells, to achieve more precise gene editing outcomes, and to target multiple, distinct stem cell populations in different anatomical niches, providing a new experimental alternative to conventional transgenic/knockout mouse models and ex vivo transduction approaches that should allow for high throughput interrogation of gene functions in stem cells within their native niches. Taken together, this work opens novel avenues for experimentally manipulating stem cell function and suggests new strategies for therapeutic intervention to achieve functional recovery of disease-relevant gene products and promote endogenous repair activity across organ systems.

Keynote lecture 2

Modeling and treating neural disease using human pluripotent stem cells

Lorenz Studer
Memorial Sloan Kettering Cancer Center

Human pluripotent stem cells (hPSCs) present a powerful new avenue for studying neural disease and for developing cell-based therapies. Our group has established strategies to coax human PSCs into myriads of specific cell types on demand and at scale. Here, I will discuss new strategies to use human PSCs for neural disease modeling including the study of neuroinflammation in neurodegenerative disease and for studying questions of cell competency as a driver of disease susceptibility. Furthermore, I will present an update on our work on the translation of hPSC technology for treating Parkinson's disease (PD) including the studies that led to the recent FDA clearance for our first in human clinical trial using off-the shelf hPSC-derived dopamine neurons in PD patients. Those studies also provide a template for translating additional hPSC-based cell products for central (CNS) and peripheral (PNS) nervous system disease including our efforts to develop a cell-based therapy for treating Hirschsprung's disease. After nearly two decades of human PSC research the field is at an exciting stage where human testing has been initiated, and with PD being just one of many potential disease targets in the CNS and PNS.

Session 1 - Pluripotency, Early Development & Gastrulation

Session speakers

Towards reconstituting human somitogenesis in vitro

Cantas Alev
Kyoto University

Somitogenesis, a core developmental process during which the segmented vertebrate body plan is laid out via the controlled emergence of epithelial somites from presomitic mesoderm (PSM), has been extensively studied using model organisms such as mouse, zebrafish or chick, but remains largely elusive and poorly understood when it comes to human. Using embryonic development-inspired induction of human PSM from pluripotent stem cells (PSC), we recently succeeded to recapitulate key features of the human segmentation clock in vitro. We could visualize and quantify oscillation (~ 5h period) as well as traveling wave-like gene expression of key segmentation clock genes in human in vitro derived PSM. We furthermore identified novel species-specific as well as evolutionary conserved oscillating genes and associated signaling pathways for both human and mouse PSC-derived PSM (Matsuda M, Yamanaka Y et al., Nature, 2020). Building upon these findings we then asked whether we could not only reconstitute the segmentation clock but also recapitulate the actual process of human segmentation in vitro. Utilizing induced pluripotent stem cells (iPSCs) as starting material and following the guiding principles of the embryo we succeeded to reconstitute and analyze core features of human segmentation and somitogenesis in vitro. Our established novel model system provides a promising approach to study 3D axial development & disease in human and other non-model organisms.

Reshaping of the transcriptional and regulatory landscape during the exit from naïve pluripotency

Christa Buecker
Max Perutz Labs

Development is characterized by a series of highly ordered and controlled cell fate transitions, that ultimately build the embryo. During each transition, an existing gene expression program is dismantled, and a new cellular identity has to be established. One such transition is the exit from naïve pluripotency: mouse embryonic stem cells cultured under defined 2i+LIF condition pass naïve pluripotency on indefinitely, however, when the inhibitors of differentiation are removed, the cells transition irreversibly into the next closely related state often referred to as formative pluripotency.

Even though the core gene regulatory network of naïve pluripotency has been well established, formative pluripotency establishment and regulation is less understood. Despite extensive screening for factors required for exiting naïve pluripotency, so far not a single factor has been identified which completely abrogates differentiation ability. Interestingly, mutations that affect the transition ability of embryonic stem cells lead to a delay in the overall exit with a prolonged expression of pluripotency markers.

By combining single cell technologies, fine-tuned differentiation time courses and a variety of differentiation impaired mutants, we explored the nature of the exit from naïve pluripotency and the causes and consequences of differentiation delay.

Identification of novel regulators of pluripotency in human cells

Graziano Martello
University of Padua

Human pluripotent stem cells (hPSCs) have been derived from somatic cells via reprogramming or from the pluripotent epiblast of early embryos, with whom they share the capacity to give rise to all differentiated cells of the adult. TGF-beta is used routinely for expansion of undifferentiated hPSCs as flat epithelial colonies expressing the transcription factors POU5F1/OCT4, NANOG, SOX2. We performed a global analysis of the transcriptional program controlled by TGF-beta followed by an unbiased gain-of-function screening in multiple hPSC lines to identify factors mediating TGF-beta activity. We identified a quartet of transcriptional regulators promoting hPSC self-renewal including ZNF398, a C2H2-type zinc-finger transcription factor, which induces the expression of pluripotency and epithelial genes in hPSCs. Mechanistically, ZNF398 binds active promoters and enhancers together with SMAD3 and the histone acetyltransferase EP300, enabling transcription of TGF-beta targets. In the context of somatic cell reprogramming, ZNF398 inhibition abolishes activation of pluripotency and epithelial genes and iPSC colony formation. Thus, ZNF398 is a human-specific mediator of pluripotency and epithelial character downstream of TGF-beta.

Speakers from abstract

Ex utero Development of Mouse Embryos from Pre-Gastrulation to Advanced Organogenesis

Alejandro Aguilera-Castrejon & Jacob Hanna
Dept. of Molecular Genetics, Weizmann Institute of Science, Israel

Establishment of the mammalian body plan occurs shortly after the embryo implants into the maternal uterus, and proper development of the embryo is dependent on its interaction with the uterine environment. While methods for in vitro culture of pre- and peri-implantation mouse embryos are routinely utilized, approaches for robust culture of post-implantation embryos from egg cylinder stages until advanced organogenesis remain to be established. We develop herein highly conducive ex utero post-implantation mouse embryo culture platforms, that enable appropriate development of embryos before gastrulation (E5.5) until the hind limb formation stage (E11). Late gastrulating embryos (E7.5) are grown in 3D rotating bottles settings, while extended culture from pre-gastrulation stages (E5.5 or E6.5) requires a combination of novel static and rotating bottle culture protocols. Histological, molecular, and single cell RNA-seq analysis validate that the ex utero developed embryos recapitulate precisely in utero development. This culture system is amenable to introducing a variety of embryonic perturbations and micro-manipulations that can be followed ex utero for up to 6 days. Establishment of a system to robustly grow normal mouse embryos ex utero from pre-gastrulation to advanced organogenesis represents a flexible tool to investigate mammalian embryogenesis, eliminating the uterine barrier to mechanistically interrogate morphogenesis and tissue specification in mammals.

MSX2 cooperates with SWI/SNF complex to safeguard syncytiotrophoblast fate of human trophoblast stem cells

Ruth Hornbachner, Andreas Lackner, Sandra Haider, Martin Köfler, Paulina A. Latos
Medical University Vienna

Human placenta contains a progenitor population that gives rise to extravillous trophoblast and syncytiotrophoblast, the actual site of exchange between the mother and the embryo. The majority of placental pathologies are thought to arise from failures in precursor differentiation, yet transcriptional mechanisms regulating trophoblast cell fate determination are poorly understood. Here we use the human trophoblast stem cell model to elucidate the function of the transcription factor MSX2 in placental specification and development. We show that depletion of MSX2 results in activation of the syncytiotrophoblast transcriptional program and conversely, forced expression of MSX2 blocks it. A large proportion of affected genes are directly bound and regulated by MSX2, as demonstrated by chromatin immunoprecipitation. To gain molecular insights into MSX2 mode of action, we determined its interactome using mass spectrometry, and identified components of the SWI/SNF complex as strong interactors. Thus, MSX2 cooperates with the SWI/SNF complex and safeguards trophoblast progenitors from a premature syncytiotrophoblast commitment. Our findings uncover the pivotal role of MSX2 in cell fate decision that govern human placental development and function.

Decoding cellular signals during cellular transitions

Borzo Gharibi[1], Emanuel Gonçalves[2], Buhe Nashun[3,4], Petra Hajkova[3], Pedro Beltrao[5] and Silvia D. M. Santos[1]

[1.] The Francis Crick Institute, London, UK [2.] Welcome Sanger Institute, Hinxton, Cambridge, UK [3.] MRC-London Institute of Medical Sciences, Imperial College London, London, UK [4.] School of Life Sciences, Inner Mongolia University, Hohhot, China [5.] European Molecular Biology Laboratory – European Bioinformatics Institute, EMBL-EBI, Hinxton, CA, UK

Naïve pluripotency is a transient state during mammalian development that can be recapitulated indefinitely in vitro by inhibition of the mitogen-activated protein kinase (MAPK/Erk) signalling and activation of STAT and Wnt pathways. How Erk is inhibited in vivo to promote naïve pluripotency remains largely unknown. By combining live cell imaging and quantitative proteomics we found that FGF2, a known Erk activator and pro-differentiation cue, induces instead long-term Erk inhibition in both ES cells and mouse embryos. We show that Erk inhibition results from a FGF2-induced incoherent feedforward loop. Importantly, we see that FGF2 induces up-regulation of naïve pluripotency factors, down-regulation of DNA methylation by suppression of de novo DNA methylases thereby helping maintain naïve pluripotency. We show that FGF2 is expressed maternally and propose that integration of signals from the embryo's niche may contribute to the generation of embryonic lineages with the right cell proportions. We suggest that feedforward regulation may play a role driving transient, reversible developmental transitions.

Linking morphogenesis and differentiation: a novel embryonic pluripotent state in between naïve and primed pluripotency

Emiel van Genderen, Alex Neagu, rene Escudero, Lucas Verwegen and Derk ten Berge

Department of Cell Biology, Erasmus MC, Rotterdam, The Netherlands

Mouse embryogenesis encompasses a pluripotent phase starting at the blastocyst stage and ending with lineage commitment at gastrulation. During this phase, pluripotency proceeds from a naïve to primed state while epigenetic remodeling events reset the genome and prepare it for differentiation. Simultaneously, the blastocyst implants, the epiblast cells become organized into a rosette structure in which subsequently a central lumen forms. How these morphogenetic changes are linked to differentiation remains largely unknown. We find that downregulation of WNT signals upon implantation of the blastocyst induces transcription factor OTX2, which drives both rosette formation as well as transition to a novel pluripotent state. Subsequent activation of MEK signals in the rosette induces lumenogenesis and the transition to primed pluripotency. Using this signaling logic, we established Rosette Stem Cells (RSCs) in vitro, which enables us to study the naïve-primed transition in a way previously unattainable. We show that RSCs represent a naive-primed intermediate pluripotent state, with an epigenetic landscape poised to primed pluripotency and extensive remodeling of heterochromatin. Our work shows that pluripotency progression is inextricably linked to embryonic morphogenesis because both processes are regulated by the same signaling pathways. Moreover, we demonstrate the first genuine intermediate pluripotent state representing a defined embryonic stage. We aim to further elucidate the molecular mechanisms for these morphogenetic changes and their link to differentiation during the naïve to primed transition.

Session 2 - Organogenesis

Session speakers

Human cerebral organoid development through the lens of single-cell genomics

Grayson Camp
Institute of Molecular and Clinical Ophthalmology Basel

I will present how we are applying single-cell genomic tools to illuminate uniquely human brain development, malformation, and evolution. First, we use single-cell transcriptomics (scRNA-seq) to analyze the cell composition and reconstruct differentiation trajectories over the entire course of human cerebral organoid development from pluripotency, through neuroectoderm and neuroepithelial stages, followed by divergence into neuronal fates within the dorsal and ventral forebrain, midbrain and hindbrain regions. We find that brain region composition varies in organoids from different iPSC lines, yet regional gene expression patterns are largely reproducible across individuals. We then use a combination of scRNA-seq and accessible chromatin profiling (scATAC-seq) to explore gene regulatory changes that are specific to humans. We analyze chimpanzee and macaque cerebral organoids and find that human neuronal development proceeds at a delayed pace relative to the other two primates. Through pseudotemporal alignment of differentiation paths, we identify human-specific gene expression resolved to distinct cell states along progenitor to neuron lineages in the cortex. We find that chromatin accessibility is dynamic during cortex development, and identify instances of accessibility divergence between human and chimpanzee that correlate with human-specific gene expression and genetic change.

Human Time vs. Mouse Time with Recapitulated in vitro Systems

Miki Ebisuya
EMBL Barcelona

Different species have different tempos of embryonic development: larger animals tend to grow more slowly than smaller animals. My group has been trying to understand the molecular basis of interspecies differences in developmental time by using in vitro segmentation clock as a model system.

The segmentation clock is the oscillatory gene expressions that regulate the timing of body segment formation from presomitic mesoderm (PSM) during embryogenesis. We have recently succeeded in inducing PSM from both human iPS cells and mouse ES cells, recapitulating the oscillation and traveling wave of segmentation clock in vitro. Interestingly, the oscillation period of human segmentation clock was 5-6 hours while that of mouse was 2-3 hours. Taking advantage of our in vitro system, we measured several biochemical reaction parameters of the core gene of the oscillation mechanism, Hes7, finding out that the degradation and production processes of Hes7 are 2-3 times slower in human PSM cells compared to mouse cells. Our mathematical model quantitatively explained how the slower biochemical reactions in human cells give rise to the longer oscillation period in the human segmentation clock.

Cardioids reveal self-organizing principles of human cardiogenesis

Sasha Mendjan
Institute of Molecular Biotechnology

Organoids capable of forming tissue-like structures have transformed our ability to model human development and disease. With the notable exception of the human heart, self-organizing organoids have been reported for all major organs. Here, we established self-organizing cardioids from human pluripotent stem cells that intrinsically specify, pattern and morph into chamber-like structures containing a cavity. Cardioid complexity can be controlled by signaling that instructs the separation of cardiomyocyte and endothelial layers, and by directing epicardial spreading, inward migration and differentiation. We find that cavity morphogenesis is governed by a mesodermal WNT-BMP signaling axis and requires its target HAND1, a transcription factor linked to developmental heart chamber defects. Upon cryoinjury, cardioids initiated a cell type-dependent accumulation of extracellular matrix, a pathological hallmark of heart disease. Thus, human cardioids represent a powerful platform to mechanistically dissect self-organization, congenital heart defects, and serve as a foundation for future translational research.

Human gastrointestinal epithelia of the esophagus, stomach and duodenum resolved at single-cell resolution

Georg Busslinger[1,3], Bas L.A. Weusten[2], Auke Bogte[2], Harry Begthel[1], Lodewijk A.A. Brosens[2] and Hans Clevers[1]

[1] Hubrecht Institute, Utrecht, the Netherlands [2] UMC Utrecht, the Netherlands [3] CeMM, Vienna, Austria

The main functions of the gastrointestinal (GI) tract are food digestion, nutrient uptake, microbe defense and hormone production. The GI tract consists of multiple organs that act together in a coordinated manner and that are lined by specialized epithelia. In order to best assist with the organ-specific tasks, these cell layers have quite diverse epithelial architectures. The esophagus is lined by a multi-layered stratified epithelium, which is best suited to withstand constant abrasion due to food transport. The gastric epithelium is single-layered with glandular appearance and is mainly responsible for the secretion of hydrochloric acid and pre-digestive enzymes whereas the crypt to villus morphology of the small intestine facilitates nutrient absorption. Here, we performed single-cell RNA sequencing of biopsies from patients with healthy human epithelia from the esophagus, stomach and duodenum. In addition to a more in-depth molecular characterization of known cell types, we identified a quiescent COL17A1high KRT15high stem/progenitor cell population in the most basal cell layer of the esophagus. In the stomach, we detected substantial gene expression differences between identical cell types of the human and mouse stomach. In the duodenum, a rare cell type was characterized by the selective expression of BEST4, CFTR, guanylin and uroguanylin, which we referred to as BCHE cells. This cell type likely mediates high-volume fluid secretion due to continual activation of the CFTR channel by guanylin/uroguanylin-mediated autocrine signaling. We also compared enteroendocrine cells between the stomach and the duodenum. Serotonin-producing enterochromaffin cells in the antral stomach significantly differ in their gene expression pattern from duodenal enterochromaffin cells. Unexpectedly, we discovered that the human histamine-producing enterochromaffin-like cells in the oxyntic stomach express also the luteinizing hormone, yet another member of the enteroendocrine hormone family. Lastly, as transporters mediate food uptake, we systematically analyzed the expression of transporter genes along the human upper gastrointestinal tract.

First Author Session

Heterochromatin-driven nuclear softening protects the genome against mechanical stress-induced damage

Yekaterina Miroshnikova
Helsinki Institute of Life Science HiLIFE

Cell and tissue homeostasis require maintenance of functional integrity under stress. A central source of stress is mechanical force that acts on cells, their nuclei and its chromatin content, but how the genome is protected against persistent mechanical stress is unclear. We show that mechanical stretch deforms the nucleus, which cells initially counteract via a calcium-dependent nuclear softening driven by loss of H3K9me3-marked heterochromatin proximal to the nuclear lamina. The resulting changes in chromatin mobility, viscoelasticity, and architecture are required to insulate genetic material from mechanical force. Failure to mount this nuclear mechanoresponse results in DNA damage. Persistent, high-amplitude stretch induces supracellular alignment of tissue to redistribute mechanical energy before it reaches the nucleus. This slow, tissue-scale mechanoadaptation functions through a separate pathway mediated by cell-cell contacts, and allows cells/tissues to switch off nuclear mechanotransduction to restore initial chromatin state. Our work identifies an unconventional role of chromatin in altering its own mechanical state to maintain genome integrity in response to nuclear deformation.

Organoids & Toxins - from snakebite to DNA-damaging bacteria

Jens Puschhof
Hubrecht Institute

Organoids are emerging tools to study homeostatic function and disease of human organs. This technology has now for the first time been extended to reptiles by establishing cultures of the snake venom gland. Venom gland organoids from a panel of species produce active toxins and allow for the study of multiple aspects of venom such as gene regulation and evolution. These cultures could form the basis for a novel strategy of anti-venom production and bioprospecting.

In the second part of the talk, a co-culture of human intestinal organoids with genotoxic Escherichia coli is presented. Long-term exposure of organoids to these bacteria leads to accumulation of a specific mutational signature in the human cells. These specific mutations are also found in the genomes of colorectal tumours, highlighting for the first time the induction of specific mutations by bacteria in human cancer.

Self-organization and symmetry breaking in intestinal organoid development

Denise Serra
BRIC / University of Copenhagen

Intestinal organoids are complex three-dimensional structures that mimic cell type composition and tissue organization of the intestine by recapitulating the self-organizing capacity of cell populations derived from a single stem cell. Crucial in this process is a first symmetry-breaking event, in which only a fraction of identical cells in a symmetrical sphere differentiate into Paneth cells, which in turn generates the stem cell niche and leads to asymmetric structures such as crypts and villi. In our work we combine a quantitative imaging approach with single-cell gene expression to characterize the development of mouse intestinal organoids from a single cell. We show that intestinal organoid development follows a regeneration process driven by transient Yap1 activation. Cell-to-cell variability in Yap1, emerging in symmetrical spheres, initiates a Notch/Dll1 lateral inhibition event driving the symmetry-breaking event and the formation of the first Paneth cell. Our findings reveal how single cells exposed to a uniform growth-promoting environment have the intrinsic ability to generate emergent, self-organized behavior resulting in the formation of complex multicellular asymmetric structures.

Session 3 - Disease

Session speakers

Unveiling liver regeneration and disease mechanisms using organoid cultures

Meritxell Huch

Max Planck Institute of Molecular Cell Biology and Genetics

In vitro 3D cultures are emerging as novel systems to study tissue development, organogenesis and stem cell behavior ex-vivo. My lab and I, we have developed organoid cultures from healthy and diseased, human and mouse, adult and embryonic tissues for a range of organs including stomach, liver and pancreas. These have allowed, for the first time, the long-term expansion of adult (stomach, liver and pancreas) and embryonic (liver) tissue into 3D-epithelial structures that we have termed organoids, since these (1) self-assemble and can be clonally expanded, (2) resemble the corresponding tissues-of-origin and (3) allow the study of some aspects of tissue physiology in a dish. Here, I will present our liver organoid work and summarize our findings that our organoid culture system recapitulates many aspects of liver regeneration in a dish, specially the activation of adult differentiated liver cells into proliferating progenitors. At the molecular scale, we have found that progenitor activation from differentiated cells occurs through a transient, genome-wide remodelling, of the cells' transcriptome and epigenome (DNA methylome/ hydroxymethylome), both during organoid initiation and in vivo, following tissue damage. At the tissue scale, absence of this epigenetic remodeling results in loss of regenerative potential and fibrosis. Our results argue in favour of the remodelling of genomic methylome/hydroxymethylome landscapes as a more general mechanism by which differentiated cells exit a committed state in response to damage and initiate the regenerative response.

At the origin of intestinal epithelial stem cells

Kim Jensen

University of Copenhagen

The intestine is essential for digestion and absorption of nutrients, and the epithelium, which lines the luminal surface, constitutes a barrier that protects our body from gut microbiota. Adult intestinal stem cells located at the bottom of crypts of Lieberkühn are responsible for the life-long replenishment of the epithelium. Within the adult intestine, stem cells reside in specialised niches surrounded by secretory Paneth cells, basement membrane proteins and fibroblasts. Recent evidence does, however, suggest that stem cell identity is an induced rather than a hard-wired trait and that cell fate in this sense is dynamically regulated to cater for the immediate needs of the tissue. Using a combination of in vitro and in vivo studies from mouse models, we have outlined how cell fate and stem cell identity is first established during development, and how cell fate changes, when the adult epithelium is challenged both genetically and upon induction of inflammation. In order to address which molecular mechanisms that underpin changes in cellular identity we have now performed extensive genomics analysis of different cellular states in combination with functional studies. This provides unprecedented insights into establishment of the adult intestinal stem cell compartment.

Sensory organoids for modeling development and disease

Karl Köhler

Boston Children's Hospital/Harvard Medical School

The human body contains a variety of epithelia to transduce stimuli of the external world into sensory information underlying our ability to hear, balance, taste, smell, and touch. Although different in some aspects, the various sensory organs share certain cellular components and morphologies: a cell sheet with mechano- or chemo-sensory cells (e.g. inner ear hair cells, olfactory receptor cells, taste receptor cells) or appendages (e.g. hair follicles, teeth, fungiform papillae) that transmit signals to the brain via sensory neurons. For proper development, sensory epithelia require physical and chemical signaling between epithelial cells and mesenchymal cells. Our current understanding of how epithelial, mesenchymal, and neural precursor cells self-assemble and initiate function during development remains incomplete. Moreover, sensory organs have been difficult to reconstitute from adult stem cells using organoid culture techniques that have worked for other organs, such as the liver, intestines, and lungs. My laboratory has developed a three-dimensional organoid system that produces complex inner ear and skin organoids that including epithelial, mesenchymal, and neuro-glial cellular components. In this talk I will highlight our recent work on skin organogenesis, in which we have defined an approach for generating transplantable hair-bearing skin tissue equipped with somatosensory nerves. I will highlight our use of single-cell genomics to uncover novel—and potentially therapeutic—intermediate progenitor states for the various cellular compartments of the skin. In addition, I will discuss what this work reveals about the general mechanisms of tissue patterning that lead to assembly of organs during fetal development.

Modelling obesity with human hypothalamic neurons

Florian Merkle

University of Cambridge

Obesity is a disease with many adverse consequences that affects approximately a third of adults in Western countries. It is caused largely by excessive food intake controlled by specific cell populations in the brain, especially neuron populations in the hypothalamus. In order to mechanistically connect genotype to phenotype and identify new drug targets, we developed methods of generating human hypothalamic neurons from human pluripotent stem cells. These cells resemble their counterparts in the mouse brain by their transcriptional fingerprints, their responsiveness to metabolically relevant hormones, and their production and secretion of appetite-regulating peptides. To aid the further development of these and other disease modelling efforts, we carried out a large-scale whole genome sequencing analysis of commonly-used human embryonic stem cell lines. These studies revealed a diverse array of disease-associated genetic variants, an excess burden of large copy number variants, and unique polygenic risk score profiles for each cell line. Together, these findings and resources enable the rational selection of cell lines for modelling diseases such as obesity from genetic data.

Translational genome editing: From ex vivo to in vivo

Gerald Schwank

ETH Zurich

Programmable CRISPR-Cas9 nucleases are powerful genome editing tools. However, they rely on homology-directed repair (HDR) to install precise edits, making them inefficient and error-prone in most somatic cell types. Base editors (BEs) are CRISPR-based genome editing tools, where nuclease-impaired Cas9 directs a deaminase to the locus of interest in order to install single nucleotide conversions. Importantly they enable precise genome editing independent of HDR, and therefore also operate with high efficiency and accuracy in somatic tissues in vivo. I will present our recent efforts in establishing transient base editing approaches in the liver by delivery of lipid nanoparticle (LNP) encapsulated RNA. This approach enables up to 90% on-target editing in vivo in hepatocytes without generating off-target deamination on DNA or RNA. Furthermore, I will introduce BE-DICT, a machine learning tool trained on high-throughput datasets and capable of predicting base editing efficiencies and outcomes on any given target sequence, including disease-causing loci. Overall, the results I will present hint towards safety and efficacy of in vivo base editing to treat genetic liver diseases.

Speakers from abstract

Inflammation shapes the lung regeneration

Jinwook Choi

Wellcome – MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge, UK

Tissue regeneration is a multi-step process mediated by diverse cellular hierarchies and states that are also implicated in tissue dysfunction and pathogenesis. Here, we leveraged single-cell RNA sequencing in combination with in vivo lineage tracing and organoid models to finely map the trajectories of alveolar lineage cells during injury repair and lung regeneration. We identified a distinct AT2-lineage population, Damage-Associated Transient Progenitors (DATPs), that arises during alveolar regeneration. We found that interstitial macrophage-derived IL-1β primes a subset of AT2 cells expressing Il1r1 for conversion into DATPs via a HIF1α-mediated glycolysis pathway, which is required for mature AT1 cell differentiation. Importantly, chronic inflammation mediated by IL-1β prevents AT1 differentiation, leading to aberrant accumulation of DATPs and impaired alveolar regeneration in chronic human lung diseases. Together, this step-wise mapping to cell fate transitions shows how an inflammatory niche controls alveolar regeneration by controlling stem cell fate and behaviour.

A novel isoform of Dicer protects mammalian stem cells against RNA viruses

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Stem cells play a fundamental role in the maintenance of adult tissue architecture and integrity by providing a pool of differentiated cells through asymmetric division. They must therefore be shielded from exogenous threats such as viral infections. Early antiviral responses in mammals rely on the expression of type I and type III interferons (IFN), which act on differentiated cells in an autocrine and paracrine manner to promote the transcription of the interferon stimulated genes (ISGs) encoding antiviral effector proteins. This protection conferred by the IFN pathway is however severely compromised in embryonic and adult stem cells, which lack key components of the pathway. This may ensure that stem cells are protected from the cytostatic effects of IFN, such as antiproliferative and proapoptotic effects. Despite their IFN unresponsiveness, stem cells are largely resistant to viruses, which can be attributed in part to the steady-state expression of ISGs and other factors restricting infection. Nonetheless, immune responses in stem cells remain poorly characterised. In invertebrates and plants, antiviral immunity relies on RNA interference (RNAi), which is initiated by the cleavage of viral double-stranded RNA (dsRNA) by a Dicer protein, generating small interfering RNAs (siRNAs) which guide the degradation of viral RNA. Irrespective of infection, RNAi also has a role in regulating cellular gene expression via micro RNAs (miRNAs) produced by Dicer through cleavage of pre-miRNAs. Organisms that use RNAi both as an antiviral response and as a way of regulating mRNA translation with miRNAs have several Dicer genes, the product of which are dedicated to processing either dsRNA or pre-miRNAs. In mammals, a single Dicer gene has been described, which encodes a Dicer protein that generates miRNAs but cleaves dsRNA only poorly. Whether antiviral RNAi exists in mammalian cells and is relevant to immunity remains highly controversial. In this work, we identify a new Dicer isoform produced from the human and mouse Dicer gene that is better able to process dsRNA than canonical Dicer. This isoform, termed antiviral Dicer (aviD) is generated by alternative splicing of Dicer mRNA and lacks the central Hel2i domain of the helicase present in canonical Dicer. aviD expression is enriched in stem cells within adult tissues, including small intestine, hair follicle and brain. Using a model of brain organoid infected with Zika virus or SARS-CoV-2, we demonstrate that aviD protects adult stem cells from RNA viruses by mounting a canonical antiviral RNAi response. This work highlights the composite nature of antiviral immunity in mammals, tailored to the differentiation status of the cell.

A new evolutionarily conserved mechanisms of regulating neural stem cell quiescence

Alice Rossi[1,2], A. Coum1, M. Madelenat[1], L. Harris[2], A. Miedzik[1], S. Strohbuecker[2], A. Chai[1], H. Fiaz[1], R. Chaouni[1], P. Faull[2], W. Grey[2], D. Bonnet[2], E.V. Makeyev[1], A. P. Snijders[2], G. Kelly[2], F. Guillemot[2], R. Sousa-Nunes[1]

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Quiescence is a cellular state characterised by reversible cell-cycle arrest and diminished biosynthetic activity that protects against environmental insults, replicative exhaustion and proliferation-induced mutations. Entry into and exit from this state controls development, maintenance and repair of tissues plus, in the adult central nervous system, generation of new neurons and thus cognition and mood. Cancer stem cells too can undergo quiescence, which confers them resistance to current therapies. Despite clinical relevance, quiescence is poorly understood and is defined functionally given lack of molecular markers. Decrease of the most resource-intensive cellular process of protein synthesis is a feature of quiescence, controlled across species and cell types by inhibition of the Target of Rapamycin pathway. Here, we combine Drosophila genetics and a mammalian model to show that altered nucleocytoplasmic partitioning and nuclear accumulation of polyadenylated RNAs are novel evolutionarily conserved hallmarks of quiescence regulation. These mechanisms provide a previously unappreciated regulatory layer to reducing protein synthesis in quiescent cells, whilst priming them for reactivation in response to appropriate cues.

Spatiotemporal regulation of multipotency during prostate development

Elisavet Tika, Marielle Ousset, Anne Dannau, Cedric Blanpain

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The prostate is formed by a branched glandular epithelium composed of basal cells (BCs) and luminal cells (LCs). Multipotent and unipotent stem cells (SCs) mediate the initial steps of prostate development whereas BCs and LCs are self-sustained in adult mice by unipotent lineage-restricted SCs. The spatiotemporal regulation of SC fate and the switch from multipotency to unipotency remain poorly characterized. Here, by combining lineage tracing, whole tissue imaging, clonal analysis, proliferation kinetics and gene expression analysis we uncover the cellular dynamics that orchestrate prostate postnatal development and the molecular profile of SCs. We found that at the early step of development, multipotent basal SCs are located throughout the epithelium, and are progressively restricted at the distal tip of the ducts, where together with their progeny set up the different branches and the final structure of prostate. In contrast, pubertal development is mediated by unipotent lineage-restricted SCs. Last, trascriptomic analysis and comparison of multipotent and unipotent SCs revealed the molecular signature of these cell populations. Our results uncover the spatiotemporal regulation of the switch from multipotency to unipotency and highlight the molecular profile of multipotent SCs during prostate development.

Stem Cell Modeling of Tyrosine Hydroxylase Deficiency Recapituales Patient Phenotypes and Reveals Altered Neuronal Morphology

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Tyrosine Hydroxylase deficiency (THD) is an inherited metabolic disorder caused by a defect in the TH enzyme, which catalyses the rate-limiting step in the biosynthesis of dopamine (DA). Two clinical phenotypes have been described: i) "Type A" which refers to a progressive hypokinetic-rigid syndrome and dystonia with an onset in infancy or childhood and L-Dopa responsiveness; ii) "Type B" which produces a severe early-onset encephalopathy, mental retardation, oculogyric crises and parkinsonism with sub-optimal L-Dopa response. We established lines of induced pluripotent stem cells (iPSCs) from fibroblasts derived from one THD -Type A patient , one THD -Type B patient, two healthy young individuals and one isogenic control obtained by CRISPR/Cas9 technology. Upon DAn differentiation, neurons harbouring TH mutations (Types A and B) exhibited THD-related phenotypes such as: decreased tyrosine hydroxylase immunoreactivity, decreased TH protein expression, reduced levels of DA metabolites and altered expression levels of DA-related genes compared to control iPSC- derived neurons. In addition, in this spontaneous THD human model, both type A and B cultures presented a reduction in the total neurite length. Moreover Type B shows an impairment in TH neuronal arborization whereas Type A TH+ expressing neurons had an impaired neuronal transport of TH. L-Dopa + Carbidopa treatment in THD A derived neurons, normalized TH protein expression, DA metabolites levels and neuronal phenotypes. However, the treatment did not rescue neuronal deficits in THD B derived neurons, thus suggesting that early pathological events in THD B mutant neural cells may be crucial for the pathogenesis of the disease. This human iPSC-based model mimics not only the phenotype observed in THD patients but also the response to the existent treatment, highlighting new possible molecular mechanisms of the disease that can disclose new opportunities for future preclinical studies.

Improved cell type resolution in cortical organoids by identification and removal of in-vitro stress

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IPS based stem cell technology is at the forefront of research on human diseases. Organoids are 3D stem cell cultures that recapitulate complex and structured tissue in vitro. Alongside these recent breakthroughs, it became clear that in vitro conditions can adversely affect cellular metabolism and signaling. A particularly relevant issue in 3D culture are the effects of limited oxygen and nutrient supply to the center of the tissue, which is due to the lack of vascularization in these models. Cortical organoids are among the most complex and physically largest organoids, and thus are most affected by such limited supply. These in vitro factors have recently been reported to lead to a general stress, and a consequently impaired cell-type fidelity and maturation in cortical organoids. We analyzed patterning, maturation, identity, metabolic state, hypoxia, ER- and dissociation-stress in ~500.000 single cells from published and new cortical organoid datasets. We found that only an identifiable and bioinformatically separable subset of cells show cellular stress, therefore it is not a global effect. Cellular stress signature is also correlated with tissue quality across samples, and we also found that progenitors express genes with known stress function higher than neurons in vitro & vivo highlighting that genes have multiple functions in different contexts. Importantly, the presence of stressed subpopulation did not seem to affect cell-type fidelity nor maturation of other cells. To tackle this issue, we developed an in-silico tool to score single-cell RNA-seq samples for tissue quality and patterning and filter out stressed cells. The stress-decontaminated samples show higher similarity to in vivo neurons and show greater maturation. As neural maturity is crucial to expand organoid's time window to correctly model neuropsychiatric diseases, we analyzed experimental conditions to improve maturity and recommend a new media formulation.

Session 4 - Clinics

Session speaker

Cancer organoids for forward and reverse translation

Nicola Valeri
Institute of Cancer Research, London

Limits in the predictive power of molecular profiling and shortcomings of some pre-clinical models used in drug development represent important obstacles hampering the success of personalized medicine and drug discovery.

LGR5+ stem cells can be isolated from a number of organs and propagated as epithelial organoids in vitro. Mouse and human organoids have been used to study the physiology and neoplastic transformation of the liver, pancreas, bowel and prostate among other organs.

During my talk, I will highlight opportunities, limitations and potential clinical applications of patient-derived organoids in personalised oncology, emphasising strengths and hurdles in the use of the organoid technology in forward and reverse translational cancer research. In particular, I will stress the importance of patient-derived organoids as pre-clinical tools to define mechanisms of drug resistance and to design novel drug combinations.

Sponsor Talks

CELLINK

Harness the power of 3D cell cultures in stem cell research

Isabella Bondesson
Field Application Specialist at CELLINK

CELLINK Life Sciences provide technologies that enables manufacturing of 3D tissue constructs in laboratory settings and large-scale, automatic production. With our complete workflow and solutions in cell line development, 3D cell culture and non-contact liquid handling we arm scientists with the tools to create the future of medicine. The wide application of 3D tissue models includes the development and validation of pharmaceutical and cosmetic products, screening of drug therapies, understanding of tissue development and cellular processes, and in the future translation to use in clinical applications.

Join Isabella Bondesson, Field Application Specialist at CELLINK, for an insightful discussion to learn more about:

- The benefits and impacts of 3D Cell Culture
- How 3D bioprinting is a revolutionizing technology for advancing and accelerating the field of tissue engineering and regenerative medicine
- How to develop 3D models for any cell type and establish improved pharmaceutical workflows

GenScript

Stable Cell Line Catalog Products for Immune Checkpoints Drug Discovery

Vevian Zhang

Inhibitors of immune-checkpoint proteins, such as PD1, PD-L1, VISTA, Tim3 and Lag3, have indicated broad and diverse opportunities to increase antitumor immunity by enhancing T cell activity with the potential to produce durable clinical responses. To meet these needs, we have developed stable cell line products expressing the most popular immune checkpoints to accelerate your immunotherapy discovery and clinical translation. All these stable cells are developed for screening high affinity antibodies or compounds against immune checkpoint proteins.

Stable Cell Line Catalog Products for Immune Checkpoint Drug Discovery are generated with advanced technologies of lentiviral transduction and antibiotic selection system, carefully screened for single clone with high performance, and fully quality assured.

Stemcell Technologies

Generation of microglia from human pluripotent stem cells for neurodegenerative disease modeling

Jeanne Chan[1], Melanie Kardel[1], Alym Moosa[1], Allen C. Eaves[1,2], Sharon A. Louis[1], and Erin Knock[1]

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Microglia are critical modulators of neurodegenerative disease. As microglia are of mesodermal origin, current human pluripotent stem cells (hPSC)-derived neuroectodermal differentiation models do not give rise to this critical cell type. We attempted to standardize microglia differentiation by developing microglia differentiation and maturation media, which efficiently differentiate hPSC into functional microglia based on the publications of Abud et al. (2017) and McQuade et al. (2018). Human pluripotent stem cells maintained in mTeSR™1 were differentiated into CD43-expressing hematopoietic progenitors using the STEMdiff™ Hematopoietic Kit for 12 days. The resulting hematopoietic cells were maintained using the microglia differentiation media and supplements for 25 days and followed by the maturation media and supplements for 4-10 days. At the end of maturation stage, the cells were characterized by expression of CD11b and CD45 through flow cytometry, and for microglia-specific gene and protein expression by qPCR. Functional characterization by phagocytosis assays were performed with pHrodo™ Red Zymosan Bioparticles™. Finally, we tested several microglia densities in co-culture with cerebral organoids produced using the STEMdiff™ Cerebral Organoid Kit. Flow cytometric analysis reveals that 96.3% ± 0.6% of the resulting population co-express CD11b and CD45 (mean ± SEM, n=7; 3 ESC and 4 iPSC lines). They also express the microglia-specific markers TMEM119, P2RY12, TREM2, and IBA1- by qPCR. The microglia are functional, with the ability to phagocytose pHrodo™ Red Zymosan Bioparticles™ beads. Adding between 250-500,000 microglia was optimal for integration into 40 day old cerebral organoids. These data demonstrate robust differentiation of functional microglia across multiple cell lines using the optimized microglia culture system. Using these microglia in co-culture with cerebral organoids will provide a useful tool for modeling neuroinflammation in disease.

Miltenyi Biotec

Pluripotent stem cell research: fundamentals of cultivation and differentiation

Viola Stella Palladino

Global Product Manager at Miltenyi Biotec for Pluripotent Stem Cells Research and Cardiovascular Research

Pluripotent stem cell (PSC) research is a continuously evolving field that beholds great promises for the future. The great application potential of PSCs ranges from in vitro disease modeling and drug screening to translational research as a first step for cellular-based therapeutic approaches.

To guarantee a successful experimental application, it is pivotal to follow good practices when cultivating and maintaining PSC. At the same time, ensuring a consistent and highly efficient differentiation is key point for experimental reproducibility.

The webinar will present an overview of the fundamentals in PSC culture and differentiation and solutions for high-quality PSC research.

Poster Abstracts

Hypoxia conditioned mesenchymal stem cell-derived extracellular vesicles induce increased in vitro vascular tube formation

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Mesenchymal stem/stromal cells (MSCs) display relevant therapeutic effects, such as migration to injured and induction of angiogenesis, particularly under oxygen-reduced (hypoxic) conditions. MSCs are known to exert their therapeutic effects via the secretion of paracrine factors and stimulation of host cells. Increasing evidence suggest that some of these effects are mediated by MSC-derived extracellular vesicles (EVs), which are central mediators in a number of physiological processes, including intercellular communication and maintenance of tissue homeostasis. However, the current knowledge on MSC-EVs from hypoxic conditions is very limited. In this context, adipose-derived MSCs from 6 donors were cultivated for 6 days under normoxic (21 % O2) and hypoxic (5 % O2) conditions. The cell count and viability was determined every other day. EVs in MSC culture supernatants were analysed every other day by flow cytometry using lactadherin (LA) as a marker of phosphatidylserine (PS) expressing EVs, as well as with MSC surface markers (CD73, CD90, CD63 and CD81). Nanoparticle tracking analysis (NTA) was performed to determine particle concentration and size distribution. The angiogenic properties of EVs were assessed by a tube formation assay using hTERT-immortalized human umbilical vein endothelial cell (HUVEC) line. Although proliferation and viability were higher under hypoxic conditions, the number and size distribution of EVs were similar in both conditions. An increase of EV production was observed over time but no significant difference in the EV concentration between normoxic and hypoxic conditions was observed. Furthermore, we observed a significantly increased tube formation in EVs from hypoxic conditions compared to normoxic EVs or the corresponding supernatants from both groups. Therefore, the tube formation seems to be mainly facilitated by EVs rather than by secreted soluble factors. This study demonstrates that MSC-EVs play a role in mediating the angiogenic effects that are generally observed in MSCs cultivated in hypoxic conditions.

Distinct and Sequential Functions of PRC2 in Radial Glia Lineage Progression

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Radial glial progenitor cells (RGPs) generate different populations of neocortical projection neurons, glia and adult neural stem cells. We recently uncovered sequential temporal transcriptional fingerprints in RGPs correlating with their lineage progression during cortical neurogenesis, and RGP proliferation behavior appears to be controlled by PRC2-mediated H3K27me3. Yet, the mechanism how PRC2 instructs RGP lineage progression in vivo remains elusive. Here we utilized Mosaic Analysis with Double Markers (MADM) to genetically dissect the cell-autonomous function of the PRC2 core component Eed. Our genetic loss-of-function approaches show that global tissue loss of Eed results in precocious depletion of RGPs and strong microcephaly. However, we reveal that Eed does not regulate RGP behavior and neuron output in a cell-autonomous manner at single cell level. Furthermore, we discover a novel cell-autonomous Eed function which is essential for cortical astrogliogenesis. On the transcriptional level, absence of PRC2 activity from astrocytes correlates with downregulation of genes implicated in proliferation and synapse formation. Accordingly, cell-autonomous loss of PRC2 function results in reduced proliferation rates, impaired surface expansion and reduced complexity of mutant astrocytes. Altogether, our data reveal distinct sequential requirements of Eed and thus PRC2-mediated H3K27me3 in RGP lineage progression during cortical development and an essential role in cortical astrocyte production and maturation.

Defining alternative human naïve pluripotency conditions devoid of MEK/ERK inhibitors

Jonathan Bayerl, Muneef Ayyash, Tom Shani, Noa Novershtern, Sergey Viukov and Jacob H. Hanna

Weizmann Institute of Science

In mouse and human naïve pluripotency conditions, the use of MEK/ERK inhibition is the major mediator for inducing global DNA hypomethylation which in turns leads to sporadic erosion of imprinting that becomes more severe with extended passaging. In mice, using alternative naïve conditions that do not employ MEK/ERK inhibitors allows isolating genetically and epigenetically stable murine PSCs with all features of naivety. The latter murine cells are fully naïve and are capable of generate all-iPS mice with contribution to the germline, and thus provide a safer route for exploiting defined mouse naïve PSCs. However, such alternative naïve conditions have not been described so far with human cells. Here we engineer reporter systems that allow functional screening for conditions that can endow both the molecular and functional features expected from human naive pluripotency in the absence of MEK/ERK inhibitors. We establish defined alternative naïve conditions in which MEK/ERK inhibition is substituted with inhibition for a novel signaling pathway that allow obtaining alternative human naïve PSCs with diminished risk for loss of imprinting and deleterious global DNA hypomethylation. Furthermore, following refinement of this growth condition we are able to overcome limited propensity for in vitro developmental potential in human primordial germ cell commitment and enhance interspecies-chimaerism competence following microinjection into mouse morulas for in vivo differentiation tracing. Our findings set a new framework for the signaling foundations of human naïve pluripotency and may advance its utilization in future translational applications.

Utilising equine adult and embryonic stem cell-derived tenocytes to model the impact of interleukin 1 beta on inflammatory signa

Ross Beaumont[1], Emily Smith[1], Alyce McClellan[2], and Deborah Guest[1]

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Tendon injuries are common in thoroughbred racehorses and exercising humans. Tissue healing occurs via the formation of biomechanically inferior scar tissue, resulting in high re-injury rates. Post-injury, excessive interleukin 1 beta (IL-1β) production is suggested to impair tendon regeneration in vivo. Adult equine tenocytes exposed to IL-1β in vitro elicit altered expression of extracellular matrix remodelling and tendon-associated genes in 2D culture and impaired 3D collagen gel contraction. Conversely, embryonic stem cell (ESC)-tenocytes are protected from the negative effects of IL-1β, possibly due to differential activation of NF-κB signalling. However, it is unknown if IL-1β differentially activates other inflammatory pathways in equine adult and ESC-tenocytes. Such findings could identify novel signalling pathways as therapeutic targets to promote better tendon regeneration in vivo. In 2D, primary equine tenocytes were exposed to IL-1β (0.01, 0.1 and 1 nM) for 20 minutes, followed by immunocytochemical analysis of NF-κB nuclear translocation. Subsequently, tenocytes were exposed to IL-1β (1 nM) for 5 minutes to 24 hours, with immunocytochemistry analysis of NF-κB and JNK nuclear translocation profiles. For 3D culture, equine tenocytes were seeded in collagen gels and stimulated with IL-1β (1 nM) for 14 days. Daily gel contraction was examined along with qPCR analysis of tendon associated and extracellular matrix remodelling genes at day 14. Dose-response immunocytochemistry revealed that IL-1β at 1 nM enhanced NF-κB cytosol-nuclear shuttling after 20 minutes, with peak translocation at 60 minutes. IL-1β had no effect on JNK nuclear translocation at any timepoint. IL-1β reduced 3D gel contraction and increased the expression of matrix metalloproteinases at day 14. Finally, the tendon associated genes Scleraxis, Mohawk and Cartilage oligomeric matrix protein were attenuated by IL-1β. These findings demonstrate that IL-1β enhances NF-κB, but not JNK, cytosol-nuclear translocation in equine adult tenocytes. Furthermore, IL-1β inhibits equine tenocyte function and modulates the expression of tendon associated and extracellular matrix remodelling genes after 14 days in 3D culture, suggesting negative consequences for tendon regeneration in vivo. This supports recent studies demonstrating that enhanced activation of the NF-κB pathway in mouse tendon elicits impaired mechanical properties and prolongs healing at the site of injury. Interrogating specific components of the NF-κB pathway differentially activated by IL-1β in adult and ESC-tenocytes could identify key signalling factors as novel therapeutic targets capable of promoting better tendon regeneration in vivo. Therefore, while additional inflammatory pathways and cytokines may be involved in facilitating the negative effects of inflammation on equine tendon degeneration, pharmacological modulation of the IL-1β-NF-κB pathway during the initial inflammatory phase post-tendon injury might be a promising strategy to improve long-term tendon healing in horses.

Syndecan-4 Modulates Cell Polarity and Migration by Influencing Centrosome Positioning and Intracellular Calcium Distribution

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Efficient cell migration requires cellular polarization, which is characterized by the formation of leading and trailing edges, appropriate positioning of the nucleus and reorientation of the Golgi apparatus and centrosomes towards the leading edge. Migration also requires the development of an asymmetrical front-to-rear calcium gradient to regulate focal adhesion assembly and actomyosin contractility. Here we demonstrate that silencing of syndecan-4, a transmembrane heparan sulfate proteoglycan, interferes with the correct polarization of migrating mammalian myoblasts (i.e., activated satellite stem cells). In particular, syndecan-4 knockdown completely abolished the intracellular calcium gradient, abrogated centrosome reorientation and thus decreased cell motility, demonstrating the role of syndecan-4 in cell polarity. Additionally, syndecan-4 exhibited a polarized distribution during migration. Syndecan-4 knockdown cells exhibited decreases in the total movement distance during directional migration, maximum and vectorial distances from the starting point, as well as average and maximum cell speeds. Super-resolution direct stochastic optical reconstruction microscopy (dSTORM) images of syndecan-4 knockdown cells revealed nanoscale changes in the actin cytoskeletal architecture, such as decreases in the numbers of branches and individual branch lengths in the lamellipodia of migrating cells. Given the crucial importance of myoblast migration during embryonic development and postnatal muscle regeneration, we conclude that our results could facilitate an understanding of these processes and the general role of syndecan-4 during cell migration.

Characterization of human-specific cell cycle regulators in hNSCs using CRISPRi.

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Subtle genomic (1.2%) and epigenomic (3.5%) differences between humans and non-human-primates (NHP) are enough to translate into remarkable cognitive differences. Human brains have higher complexity, connectivity and proliferative capacities that facilitate neocortical expansion. Nonetheless, the study of the genetic modulation behind these differences has been challenged by ethical, legal, and methodological limitations. This project attempts to surpass these limitations by using state-of-the-art technologies like neural stem cells (NSCs) derived from human induced pluripotent stem cells (hiPSCs), gene editing, and single cell RNA sequencing. Specifically, the aim is to characterize the function of human-specific genes responsible for the proliferation and differentiation of hNSCs. For example, among the human-specific genes replicated in the literature, we found ANKRD20A2, ARHGAP11B, and NOTCH2NL. The last one been associated with the expansion and neuronal output of cortical progenitors though Delta/Notch regulation. Not surprisingly, some of the genes of interest are associated with aging and neurodevelopmental conditions like Autism Spectrum Disorder and Schizophrenia. Candidate genes like these will be knocked down in hNSCs with Clustered Regularly Interspaced Short Palindromic Repeats interference (CRISPRi), a gene editing variation that represses gene expression with the binding of dCas9-KRAB to the Transcription Start Site of the gene targeted. The resulting transcriptome of perturbations will be analyzed in high-resolution with the implementation of direct-capture perturb-seq, a screening approach that enables sgRNAs sequencing alongside single-cell transcriptomes. Lastly, RNAi will be used to further validate the effect of the MYC family genes in self-renewal and differentiation of hNSCs. Recently, we identified MYC genes to be responsible for re-entering cell cycle after dormancy in embryonic stem cells (ESCs). Both approaches will enable to analyze to which extend the downregulation of candidate genes will result in decreased proliferation, aberrant differentiation, and decreased connectivity. The findings of this project will not only contribute to unravel the mechanisms behind the specializations of the human brain, but can have implications in disease modelling, regenerative medicine, and aging processes.

Plasticity of oesophageal epithelial stem cells: A model of ectopic niche regeneration

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Epithelial tissues have evolved to respond to environmental challenges, displaying great adaptability and regenerative capacity. At certain developmental stages, epithelial cells can switch their identity upon exposure to ectopic mesenchymal signals. Yet, it remains unknown whether such responsiveness to environmental cues is retained in adult tissues and, if so, what molecular regulators define this flexibility. Here, we explore adult cell plasticity by exposing the epithelium of the mouse oesophagus to a foreign environment, that of the skin stroma (the dermis). Using a 3D organ culture system, we cultured oesophageal epithelium over skin-free dermis, bearing niches for skin appendages such as the hair follicle. We found that oesophageal-derived cells efficiently reconstitute hair follicle-like and sebaceous gland-like structures. After 10 days, de novo formed hair follicle-like structures start expressing markers typical of these skin appendages. Remarkably, heterotypic transplantation of oesophagus-skin constructs led to the formation and long-term maintenance of these structures in vivo. Single-cell RNA sequencing and trajectory analysis revealed the relevance of regenerative signals in the temporality of cell identity transitions. Our work suggests that niche signals can rewire cell identity in adult epithelial cells in vitro and identifies potential molecular mechanisms driving cell fate conversion in adult progenitor epithelial cells.

Induced neurons to evaluate psychiatric risk for Alzheimer's disease development and progression

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The number of elderly humans is growing, and with it the incidence of yet untreatable age-dependent neurodegenerative diseases such as sporadic Alzheimer's disease (AD) is on a path to dramatically burden our societies. The situation is particularly alarming, because the development of disease-modifying treatments is hampered both, by an incomplete understanding of the age-dependent pathogenic mechanisms of AD, as well as the unavailability of early diagnostics to design appropriate clinical trials. Identification of early disease signatures and pathways is essential to develop future treatment and prevention strategies. Aging is the by far most significant risk factor for the development and progression of dementia. Additionally, the impact of late-life psychiatric diseases on the progression of a healthy cognitive state to a neurodegenerative one is coming into the limelight of AD research. Recent studies have demonstrated compelling evidence that patients suffering from severe psychiatric illnesses, including bipolar disorder, major depressive disorder, and post-traumatic stress disorder, show a significant acceleration of various systemic and brain-specific aging signatures. These observations prompt the question to what extent late-life psychiatric diseases may contribute to the initiation and progression of neurodegeneration through the acceleration of biological aging pathways. Human induced neurons (iNs) can be directly generated from skin fibroblasts. They present an attractive model system to study age-related epigenetic alterations in patients-specific adult-like neurons. To illuminate the interface between psychiatric and neurodegenerative pathways in neurons, we generated and characterized iNs from a neurodegeneration trajectory cohort. This cohort ranges from cognitively healthy individuals, to individuals suffering from mild cognitive impairment (MCI), and further to cognitively impaired AD patients of diverse clinical stages. The MCI patients have been stratified to show varying degrees of psychiatric illness, and we further include well-defined cognitively normal psychiatric patients. Thus far, we have generated and performed basic characterization of iNs from over 70 subjects. To reconstruct the neurodegeneration trajectory, and to evaluate its relationship with psychiatric disease signatures that are captured in iNs, we performed paralleled genome-wide transcriptomic and epigenomic analyses. Transcriptome analysis revealed distinct signatures in several subgroups of the cohort, and epigenetic aging analysis indicates that iNs indeed capture signs of epigenetic age acceleration associated with a psychiatric disease state. Prospective studies will include multi-omics analyses, integrative meta-analyses with post-mortem AD data, as well as functional assays to validate and better understand the underlying cellular and molecular mechanisms relevant to the psychiatric risk factors for neurodegeneration.

Systematic dissection of an extended naïve pluripotency gene regulatory network

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How do pluripotent stem cells define their identity to go further into proper development? To answer this question the exit from naïve pluripotency model has been extensively studied, nevertheless the involving gene regulatory networks (GRNs) still remain not fully elucidated. With the purpose of studying an extended naïve GRN, a set of 496 naïve pluripotency associated genes, hereafter named NAGs, have been identified as tightly associated to known core pluripotency markers in mouse. This gene set involves not only transcription factors but also epigenetic factors and genes not previously reported as naïve pluripotency-related. Furthermore, the NAGs follow a similar expression dynamic in vitro and in vivo during epiblast transition, suggesting in vivo relevance. Moreover, these genes exhibit similar regulation in human and macaque, suggesting potential relevance across mammalian species. These aspects make the NAGs an interesting gene set to be studied. To identify which genes within the NAGs have an actual and important role in the naïve state, we propose to perform both CRISPR activation and interference-based screens. To reach this goal, dCas9 fused to transcriptional effectors and a customized pooled gRNA library will be delivered into the cell line reporter "Rex1-GFP". This cell line loses GFP signal under cell differentiation making it possible to monitor the exit from naïve pluripotency in high resolution. Two kind of screens will be performed: in 2D, by stablishing a monolayer culture of embryonic stem cells, and in 3D by employing single-cell derived embryoid bodies to study genetic cooperation of the NAGs. By setting up this this genetic screen we expect to identify novel gene roles that can contribute to a better understanding of the underlying mechanisms involved in the naïve pluripotent state.

Development and Homeostasis in sea anemones and jellyfish: a multi-faceted approach to characterize Cnidarian stem cells

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Stem cell research in the Cnidaria is well established in the hydrozoan genus Hydra, where multi-potent interstitial cells (i-cells) give rise to a variety of differentiated cell types including all neurosecretory cells. However, i-cells have not been described outside of the Hydrozoa, and may therefore not represent the ancestral condition. The anthozoan Nematostella vectensis also demonstrates regenerative abilities and shows no signs of senescence, suggesting the presence of a multipotent stem cell population that maintains tissue homeostasis. To identify these putative stem cells in Nematostella, we generated unbiased single cell transcriptome libraries from different developmental stages. In parallel, we conducted an in situ hybridization screen of genes whose homologs are known to have a conserved role in stem cell biology in both vertebrates and Hydra, and generated transgenic lines expressing fluorophores under the control of gene-specific promoters for a number of these candidate genes. From these data we identify a population of neurosecretory progenitor cells in the sea anemone that give rise to a similar cell-type complement as the Hydra i-cells. To further address the evolution of this stem-cell type, we generated similar single cell transcriptomic data from the scyphozoan moon jelly (Aurelia aurita) and from the medusa of the hydrozoan Clytia hemispherica. Comparisons between the three datasets identify putatively homologous cell types amongst the neurosecretory derivatives. Our data show that the combination of unbiased single cell transcriptomes and gene-directed validations permit the identification of stem- and progenitor- cell populations, and their corresponding differentiation trajectories, in the absence of prior morphological knowledge. Further comparisons amongst species will reveal conserved gene regulatory networks underlying stem cell biology in metazoans.

A novel 3D screening approach to define transcriptional regulatory circuitries that regulate endo-mesodermal lineage commitment

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Acquisition of definitive cell identity is one of the most important events during embryogenesis. It results in the segregation of the three primary germ layers, endoderm, mesoderm, and ectoderm. One of the fundamental open questions of developmental biology is how the three primary germ layers are generated from the homogeneous pluripotent cell population of the epiblast. We currently lack a comprehensive understanding of regulatory interactions between regulators and their potential targets that instruct individual cells to acquire all the cell fates necessary to build complex embryonic structures. The main focus of this study is to identify the key players involved in mesendoderm induction, segregation between endoderm and mesoderm, and acquisition of definitive endoderm, and to delineate their molecular mode of action. Although many aspects of endo-mesodermal lineage commitment have been revealed, including how extracellular signals and downstream transcription factors control its formation, the key drivers which govern response to differentiation-cues and regulators of lineage defining gene regulatory networks remain unknown. To identify lineage defining factors, I will perform CRISPR based genetic screens utilizing endo-mesodermal double lineage reporter (EMdR) mouse embryonic stem cell line. Beside screening methods that utilize monolayer cell cultures, I will develop and use a novel three-dimensional (3D) approach that will allow high-throughput screening with complex molecular readouts. Mutations that skew lineage decision and lead to aberrant lineage reporter activity will be screened by a large particle flow sorter. Causative gRNAs and thereby the relevant genes with potential role in lineage commitment will be identified by next generation sequencing and statistical analysis. Utilizing the EMdR cell line and following differentiation along a differentiation trajectory towards endoderm allow me to distinguish between gene-function in mesendoderm induction, segregation, and endoderm specification in the same experimental system within a single genetic screening setup that provides a significant advantage over strategies which independently assay differentiation towards endo- or mesodermal identity. Furthermore, the utilization of a 3D large particle sorter will enable the exact quantification of differentiation defects during the screen and therefore allow me to directly correlate phenotypic strength to a mutation. Moreover, the novel 3D approach allows the efficient detection of skewed differentiation at the single embryoid body level, which will provide a significant improvement over the sensitivity provided by pooled 2D screens in mixed populations. A key deliverable of this project will be a powerful in vitro 3D model system to study early developmental events. The basic rules of germ layer specification will be applicable to and important for many cell fate decisions. For instance, there is great interest in understanding the molecular mechanisms that regulate the generation of definitive endoderm, since endoderm-derived organs, such as liver or pancreas are potential targets for stem cell-based therapy. Hence, it is clear that understanding the lineage decisions taken during gastrulation will be a milestone in efficiently directing proper lineage choice in medically relevant settings.

Erythropoietin directly remodels the clonal composition of murine hematopoietic multipotent progenitor cells

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The cytokine erythropoietin (EPO) is a potent inducer of erythrocyte development and one of the most prescribed biopharmaceuticals. The action of EPO on erythroid progenitor cells is well established, but its direct action on hematopoietic stem and progenitor cells (HSPCs) is still debated. Using cellular barcoding, we traced the differentiation of hundreds of single murine HSPCs, after ex vivo EPO-exposure and transplantation, in five different hematopoietic cell lineages, and over different timespans, and observed the transient occurrence of high-output Myeloid-Erythroid-megaKaryocyte-biased and Myeloid-B-cell-Dendritic cell-biased clones. Single-cell RNA sequencing analysis of ex vivo EPO-exposed HSPCs corroborated our lineage tracing results, revealing that EPO induced the upregulation of erythroid associated genes in a subset of HSPCs, overlapping with multipotent progenitor (MPP) 1 and MPP2, and not long-term hematopoietic stem cells. Collectively, our data show that EPO does act directly on HSPCs independent of the niche, and modulates fate by remodeling the clonal composition of the MPP pool.

A human tissue screen identifies a regulator of ER secretion as a brain-size determinant

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Primary microcephaly is a developmental disease, characterized by patients' small brains at birth and intellectual disabilities, oftentimes caused by monogenetic mutations. Human cerebral organoids can model key aspects of microcephalic phenotypes, offering the opportunity to study this disease in a human model system. Over one hundred genes have been linked to microcephaly based on clinical case reports but only a handful of these are experimentally verified and characterized leaving a huge discrepancy between clinical sequencing and biological experimental data. Here, we present CRISPR-LIneage tracing at Cellular resolution in Heterogenous Tissue (CRISPR-LICHT), a method to perform genetic loss-of-function screening in human 3D tissue based on CRISPR-Cas9 technology to rapidly assess many genes' functions. To overcome current sensitivity limitations, we employ an extensive DNA barcoding strategy allowing us to faithfully track and compare thousands of wildtype and KO stem cell lineages in human cerebral organoids. We use this methodology to systematically test microcephaly candidate genes and reveal 23 new genes to be involved in both known and novel pathways causative for the disease. We characterized Immediate Early Response 3 Interacting Protein 1 (IER3IP1) regulating the unfolded protein response (UPR) and extracellular matrix (ECM) protein secretion crucial for tissue integrity, with dysregulation resulting in microcephaly. We show that human tissue screening technology can identify microcephaly genes and mechanisms involved in brain size control, with the potential to be used to tackle other biological processes in human brain development.

Modeling CBFA2T3-GLIS2-driven acute megakaryoblastic leukemia using human induced pluripotent stem cell differentiation

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Acute megakaryoblastic leukemia (AMKL) is a rare form of myeloid leukemia characterized by exacerbated self-renewal and proliferation as well as blocked differentiation of thrombocyte progenitors. The CBFA2T3-GLIS2 fusion, generated by a cryptic inversion of chromosome 16, is the most common chimeric oncogene identified in pediatric non-Down syndrome AMKL and accounts for about a quarter of the cases. This genetic subtype of AMKL is associated with a dismal outcome even when treated with high-intensity therapy, emphasizing the need for the development of novel therapeutic strategies. We therefore aimed at establishing a novel disease model system by conditionally expressing the oncogenic fusion in human induced pluripotent stem cells (hiPSCs) and subjecting them to in vitro hematopoietic differentiation, in order to investigate the underlying oncogenic pathways.

For this purpose, we employed precise CRISPR/Cas9 genome editing and via an in trans paired nicking approach inserted the fusion part encoding GLIS2 into one endogenous CBFA2T3 allele of hiPSCs to ensure cell type-specific physiological expression levels. We added an FKBP12/F36V-degron tag, which facilitates fusion protein depletion using the heterobifunctional compound dTAG-13, and a monomeric Kusabira-Orange (mKO2) fluorescence reporter to monitor locus activity. During hematopoietic differentiation the genetically modified cells start to simultaneously express wild-type CBFA2T3 and the CG fusion protein in the hemato-endothelial compartment, which we traced on the single cell level via mKO2 fluorescence or immunofluorescence staining. The derived CG-expressing hematopoietic stem and progenitor cells display a significantly higher clonogenicity in methylcellulose semisolid medium as compared to isogenic parental hiPSCs or dTAG-13-treated knock-in cells. Furthermore, they exhibit an increased propensity to differentiate toward the megakaryocytic lineage in MegaCult assays and are capable of continuous growth in liquid media but remain cytokine-dependent. However, CG expression is essential for sustained cell growth and survival suggesting that in this model system cells become addicted to the immortalizing potential of the CG oncogene.

Single-cell RNA-sequencing revealed an excess of abnormal progenitors upon CG expression. These progenitors not only express typical megakaryocyte-specific genes encoding lineage-associated cell surface markers, e.g. ITGA2B (CD41) and ITGB3 (CD61) but also display deregulation of subtype-specific genes as already observed in CG-positive AMKL patients, such as high NCAM1 (CD56), BMP2, and ERG, but low PTPRC (CD45) expression. In contrast to wild-type cells, which primarily differentiate toward erythrocytes and granulocytes, GC-expressing cells mainly develop toward abnormal megakaryocytes. This population is however heterogeneous ranging from cycling early progenitors to continuously differentiating megakaryoblasts.

These data strongly suggest that our in vitro hiPSC model system recapitulates important aspects of the disease and can be utilized to gain novel insights into AMKL pathogenesis and therapy resistance. Ultimately, the model may prove useful for exploration of improved therapeutic options.

Overlapping Definitive Progenitor Waves Divide and Conquer to Build a Layered Hematopoietic System

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Adult innate immune cells are part of a layered hematopoietic system constructed from definitive hematopoietic stem and progenitor cells (HSPC) with diverse origins during development. One source of HSPC are fetal hematopoietic stem cells (HSC) that provide long-term reconstitution throughout life. However, the extent to which HSC produce mature cells in utero is only recently being uncovered. This is in part due to the added complexity of an overlapping wave of definitive progenitors that derive from yolk sac erythro-myeloid progenitors (EMP). HSC and EMP are generated from spatiotemporally distinct hemogenic endothelia, yet they both migrate to the fetal liver niche where they co-habitate and are presumed to reach their full potential. Delineation of the respective HSC and EMP pathways towards developmental immune cell differentiation has been confounded by challenges in ontogeny-specific cell labeling. In this study, in vivo inducible pulse chase labeling revealed that HSC contribute little to fetal myelopoiesis and that EMP are the predominant source of mature myeloid cells until birth. This is similar to what has been reported for the erythroid branch of hematopoiesis thereby establishing a developmentally-restricted privilege for erythro-myeloid differentiation from EMP compared to HSC. Tracing the origins of mature cells to the progenitor level by immunophenotyping and single cell RNA sequencing uncovered a dichotomy in the allocation of fetal liver EMP and HSC to myeloid progenitor subsets, both in timing and lineage bias. This has exposed an uncoupling between developmental granulopoiesis and monopoiesis from EMP and HSC pathways, and provides a framework for future studies of HSC-dependent and -independent hematopoiesis.

Neural stem/progenitor cells from the olfactory mucosa in the treatment of spinal cord cysts

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Background. The existing methods of treating chronic spinal cord injuries are ineffective since the regeneration of the nerve tissue does not occur. As a result, cysts are often formed in the spinal cord. They interfere with the circulation of cerebrospinal fluid, conduction and regeneration of nerve tissue. A promising trend in the treatment of spinal cord injuries is the use of neural stem/progenitor cells. Numerous in vitro experiments have shown that NSPC can differentiate into neurons and glial cells. The olfactory mucosa is a promising source of NSPK production. To build up and grow NSPCs, usually use the method of cultivating them in a three-dimensional culture as part of neurospheres. Neurospheres are heterogeneous structures of NSPC differentiation into neural and glial cells. The aim of this study is to explore the therapeutic effect of transplantation of the NSPC into a spinal cord cyst. Methods. To obtain the olfactory mucosa, Wistar rats were used (n=18). A primary culture of the olfactory epithelium was obtained from the tissue of the olfactory mucosa, then it was cultured for 3-5 days until the formation of a monolayer. After that, the cells of the olfactory epithelium were passaged for poly-L-lysine and cultured in serum-free medium. A small population of SOX2/Nestin+ cells from the olfactory epithelium adheres and begins to form neurospheres. To simulate spinal cord injury and cyst formation, we used Wistar rats (n=7). They underwent a laminectomy and received a contusion injury. As a part of our studies, cells in number of 200 thousand were transplanted as part of neurospheres in DMEM/F12 medium without FBS in a volume of 10 µl. As a control experiment, the animals were injected with a cell-free DMEM/F12 medium without FBS in a volume of 10 µl. To visualize spinal cord cysts before and after cell injection, an MRI study was performed. An MRI study was performed to visualize spinal cord cysts before and after cell injection. For cell survival research we use immunochemistry. Results. After cell injection, we observed a restoration of hindlimb motility in rats using weekly BBB scoring tests. There was also a decrease in cyst volumes by an average of 40.5% compared to the initial data. It has been shown that neurospheres can survive and retain their localization in the cyst area for 4 weeks after transplantation. Conclusions. For the first time, the effectiveness of transplantation of NSPCs of the olfactory mucosa for the treatment of post-traumatic spinal cord cysts has been shown. The procedure for obtaining an olfactory lining is safe for the patient and is performed with local anesthesia. As a result of such a procedure, it is possible to obtain autologous and tissue-specific cells for personalized treatment of a patient with a post-traumatic spinal cyst. This work was supported by the Russian Science Foundation: Grant No. 17-15-01133.

Robustness of adult neurogenesis maintains homeostasis and neutralises the early detrimental effects of intermittent fasting

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Adult stem cells contribute to tissue maintenance and to its regeneration upon injury. With age, stem cell numbers and function decline, compromising homeostasis. Adult neural stem cells (aNSCs) reside only in restricted areas of the adult brain such as the dentate gyrus of the hippocampus. aNSCs transition between quiescent and active states and give rise to newly born neurons that integrate into the hippocampal circuit and modulate memory and emotions. Intermittent fasting (IF), known to extend life and healthspan, has been proposed to halt neural stem cell decline and increase neurogenesis, and therefore holds great potential as a strategy to improve cognitive ability and promote a healthier aging. We fasted mice for 24 hours on alternate days for 3 months, and followed their neurogenesis using lineage tracing of aNSCs. Mice lost weight during the fasting day and returned to control levels after refeeding throughout the whole treatment. Despite these weight oscillations and contrary to previous reports, we found no differences in neurogenesis between IF and ad libitum fed mice that had unrestricted access to food: the total number of neurons generated throughout the treatment, ongoing neurogenesis and aNSCs maintenance and activity remained unchanged. Surprisingly, we observed that a shorter period of IF (1 month) reduced the number of new neurons and altered aNSC transitions between quiescence and activation. However, this early aNSC response didn't lead to persistent changes in adult neurogenesis. Overall, this data suggests that adult neurogenesis senses and reacts to environmental cues but is more robust than previously thought. We are currently investigating the long-term effects of IF and whether other adult stem cell niches are more responsive to systemic changes than the brain.

Introducing and restoring Craniosynostosis-related gene mutations using prime-editing

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Background: Craniosynostosis is a rare congenital condition where one or more calvarial sutures prematurely fuse together, resulting in aberrant skull and brain growth often accompanied with craniofacial deformities and intellectual retardation. As a rare bone disease and due to the multigeneity of Craniosynostosis, the condition lacks reliable cell model systems and establishing cell models based on the variety of causative mutations is extensive and difficult. Prime-editing is a new variant of CRISPR/Cas9 capable of inducing specific mutations in target DNA with high accuracy.

Aim: Using Prime-editing we aim to incorporate Craniosynostosis-related gene mutations in a mesenchymal stromal cell (MSC) line, in order to ultimately generate in vitro disease models of monogenic bone diseases that can be utilized for further research. Additionally, with the generation of patient-specific induced Pluripotent Stem Cells (iPSC), we aim to restore the causative mutation from the patient's genome using the same technique.

Methods: Custom designed Prime-editing guide RNAs (pegRNA) targeting FGFR2 are generated, and together with the Prime-editor Cas9, transfected into TERT immortalized MSCs (MSC-TERT). After 5 days of editing, genomic DNA (gDNA) is isolated and analyzed by Edit-specific PCR, restriction enzyme digestion analysis, and Sanger Sequencing.

Results: A 3 base-pair insertion in HEK3 was successfully incorporated in HEK293FT and MSC-TERT cells. Custom pegRNAs targeting FGFR2 were successfully generated, but require structural optimization in order to be functionally utilized in prime-editing.

Patient specific iPSCs (Crouzon Syndrome) were successfully generated illustrated by stem cell marker expression (OCT4, TRA-1-81, NANOG, and SSEA4), and were shown to differentiate into each of the three germ layers.

Conclusion: Prime-editing can be effectively used to introduce mutations in both HEK239FT and MSC-TERT cells. Functionality of pegRNA transcripts heavily rely on the secondary structures that are formed. Patient specific iPSCs are successfully generated.

Keywords: Craniosynostosis, Mesenchymal Stromal Cell, CRISPR, Prime-editing, iPSC.

Exploring a human 3D stem cell-derived model to study astrocyte activation during neuroinflammation

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Neuroinflammation is a pathogenic process leading to neurodegeneration, associated with disease and aging. One of the hallmarks of this process is glial cell activation with astrocytes acquiring characteristics of a neurotoxic (A1)-phenotype. These activated astrocytes undergo morphological, molecular, and functional changes, playing a major role in modulating the inflammatory process. Still, much remains unknown in what concerns the astrocyte activation process, such as the changes in secretory profile inducing microenvironment remodeling, as well as the consequent impairment of neuronal functionality. Experimental models in which the human neural extracellular space and its dynamic changes are represented will be key to study such processes. This work aims to develop a human cell model of neuroinflammation and characterize the changes in the neural microenvironment induced by astrocyte activation. We explored a three-dimensional (3D) neural cell model derived from human induced pluripotent stem cells (hiPSC) – the neurospheroid model, developed by our team. Neurospheroids composed of neurons, astrocytes, and oligodendrocytes are generated without resourcing to heterologous matrices and have been shown to recapitulate specific features of the brain microenvironment, such as brain extracellular matrix (ECM) deposition, and neuron-glia interactions. Neural progenitor cells derived from hiPSC cell lines were cultured as cell spheroids in stirred-tank bioreactors and differentiated into neurospheroids. To mimic a neuroinflammatory environment, neurospheroids were challenged with TNF-a, IL-a, and C1q, pro-inflammatory factors reported to be secreted by microglia and to induce the A1-subtype of astrocytes in mice models. After 72 h of challenge, the temporal kinetics of the transcriptional response of the activated astrocytes was evaluated, alongside the inflammatory cytokine secretion profile, and astrocyte functionality. Upregulation of neuroinflammatory genes (e.g., SERPINA3 and C3), previously associated with the A1 phenotype), concomitant with the secretion of pro-inflammatory cytokines, such as MCP-1 and IL-6, suggested that astrocytes triggered an inflammatory response upon cytokine stimulation. Challenged astrocytes displayed an impaired capacity to uptake glutamate and to synthesize/secrete glutamine in a glutamate-rich and glutamine-free environment, in comparison to the unstimulated control, suggesting functional impairment. By plating the neurospheroids on a laminin-rich matrix, we observed increased migration of b-III tubulin-positive neurons in comparison to the unstimulated control, suggesting ECM remodeling events. The results indicate that pro-inflammatory factors induce astrocyte activation within the human neurospheroids, recapitulating transcriptional changes and functional impairment previously described under neuroinflammation conditions on mice models. Therefore, the human neurospheroid model can be a useful tool to dissect neuroinflammatory mechanisms, including potential consequences on neuron-astrocyte signaling, paving the way to explore disease mechanisms. Currently, transcriptomic and proteomic profiling of neurospheroids is ongoing, aiming to uncover molecular mediators and signaling pathways underlying astrocyte activation and its consequences on the composition of the neuroinflammatory microenvironment. Acknowledgments: This work was supported by Fundação para Ciência e Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES), under the scope of the projects AstroReact (PTDC/BTM-ORG/29580/2017) and iNOVA4Health (UID/Multi/04462/2013), which was co-funded through national funds and FEDER under the PT2020 Partnership Agreement.

A standardized bone organ model of human hematopoiesis

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The lifelong blood production is maintained by the hematopoietic stem cells (HSCs), through balanced differentiation and self-renewal (Eaves 2015). HSCs are one of few stem cells with clinical application, in the form of bone marrow (BM) transplantation as a treatment for hematologic disorders (Weissman and Shizuru 2008). HSCs are primarily located within the BM, in specialized microenvironments, knowns as niches (Crane, Jeffery et al. 2017) in which mesenchymal cells have an indispensable role in maintaining and regulating their function (Calvi, Adams et al. 2003, Mendez-Ferrer, Michurina et al. 2010, Acar, Kocherlakota et al. 2015). To study the function of human HSCs and their niches remains challenging due to the lack of relevant models. The gold standard consists in the use of humanized mouse models expressing human cytokines, offering an improved engraftment of human HSCs and in vivo hematopoietic reconstitution. However, these models lack the specificity of the human BM niche. Towards this objective, recent studies, including our work, reported the possibility of engineering humanized bone organs as a novel promising strategy to overcome the limitation of mouse models (Dupard, Grigoryan et al. 2020) . Human BM-derived mesenchymal stromal cells (MSCs) can be primed to form cartilage in vitro. The resulting engineered cartilage naturally remodels into mature bone/bone marrow tissues upon ectopic implantation in mice, by following the developmental process of endochondral ossification (Salhotra, Shah et al. 2020). This "ossicle" was shown to support and maintain human hematopoiesis while reconstituting the human mesenchymal niche (Scotti, Piccinini et al. 2013, Serafini, Sacchetti et al. 2014, Reinisch, Thomas et al. 2016, Abarrategi, Foster et al. 2017, Fritsch, Pigeot et al. 2018), thus providing an ideal in vivo model to study interactions between human HSCs and their mesenchymal niche. So far, the process of humanized ossicle formation has revealed highly variable, largely depending on specific primary MSCs donors that display good chondrogenic capacity. To overcome this donor-dependent variation we have generated a unique mesenchymal cell line capable of forming standardized bone organs by priming them into osteogenic and chondrogenic differentiation in vitro (Bourgine, Le Magnen et al. 2014). Strikingly, our data reveal that these mesenchymal line-derived ossicles host and support long-term engraftment and lymphoid and myeloid balanced differentiation of human HSCs. This ossicle model thus represents a unique powerful tool to decipher human hematopoiesis in an advanced human setting.

Cultrex™ UltiMatrix BME–A Versatile New Matrix for Organoid and Stem Cell Culture

Yas Heidari, Sol Degese, Xi Lu, David Galitz, Susan Tousey, and Kevin Flynn

Bio-Techne

Organoid and stem cell-derived culture systems are evolving and enhancing our basic Understanding of developmental biology and improving the predictability of in vitro disease Modelling and drug screening. The quality and consistency of the adhesion matrix substrates, Such as mouse EHS tumor basement membrane extracts (BME), used to embed organoids And expand pluripotent stem cells can greatly impact model variability. This study describes An advanced BME designed to address current shortfalls of existing EHS-derived matrices. Cultrex™ UltiMatrix Reduced Growth Factor (RGF) BME is a soluble form of basement Membrane that provides high tensile strength, enhanced levels of entactin/nidogen, elevated Protein concentration, and robust clarity and purity. These compositional enhancements Translate into substantial performance benefits that make Cultrex UltiMatrix RGF BME an ideal Cell scaffolding matrix for stem cell and organoid cell culture

T-cell specific factor 4 is crucial for Wnt signaling-dependent intestinal stem cell homeostasis and tumorigenesis in mouse

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T-cell specific factor 4 (Tcf4) is the most intensively studied member of the conserved Tcf/Lymphoid enhancer-binding factor (Lef) family of transcription factors. Together with β-catenin coactivator, Tcf4 represents the prominent nuclear effector of canonical Wnt signaling in the intestinal epithelium. Regulation of Wnt-β-catenin signaling in intestinal stem cells is crucial for tissue homeostasis and tumor formation initiation. Up to date, several mouse models were generated to manipulate Tcf4 abundance or activity in vivo and dissect its function. Moreover, mutational screens and expression profiling of human colorectal tumors were carried out to disclose a contribution of TCF4 to tumor progression. However, subsequent studies brought conflicting results in relation to the potential of Tcf4 to activate or repress Wnt target genes and drive or inhibit cell proliferation. Here in this study, we present newly generated Tcf4flox5 mouse with a conditional Tcf4 allele that can be used to eliminate expression of Tcf4 from two alternative promoters of the gene. Using this mouse strain, we documented that Tcf4 loss led to the demise of intestinal stem cells (ISCs) and substantial reduction of cell proliferation in the crypts of the small intestine and colon. Moreover, Tcf4 ablation in APC-deficient intestinal adenomas reduced their growth considerably, despite limited presence of proliferating cells. In the Tcf4flox5 intestinal organoids, Tcf4 conditional knock out caused downregulation of Wnt-βcatenin target genes followed by the collapse of organoid structures. Furthermore, Apc deficient intestinal organoids exerted the same reaction. Based on these results, we proposed that Tcf4 is a crucial positive regulator of Wnt-β-catenin signaling in the intestinal epithelium and driver of proliferation in both intestinal homeostasis and tumorigenesis. However, our results from TCF4-deficient cell lines and tumors indicated that, upon transformation, cancer cell dependency on TCF4-mediated signaling could by bypassed by other transcription factors, namely the TCF1/LEF1-β-catenin complexes.

How human microglia shape developing neurons in retinal organoids

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Microglia are resident macrophages of the central nervous system. During embryonic development, they are infiltrating the brain from the yolk sac and are actively involved in regulating neurogenesis, interneuron positioning, axonal outgrowth, and neuronal wiring. These functional observations were mostly performed in mouse models, yet, little is known whether human microglia similarly shape their early neuronal environment. Neuronal organoids differentiated from human induced pluripotent stem cells provide a unique opportunity to investigate embryonic neuronal organization and connectivity. However, they are commonly lacking microglia.

Here, we established a model system in which we assembled microglial precursor cells into retinal organoids. First, we independently generated both components and then applied microglial precursor cells at selective time points to retinal organoids. Immunostaining for the microglia marker IBA1 showed that microglia integrated into retinal structures labeled with OTX2 and RECOVERIN but the time point of microglia application significantly impacted microglia density within organoids. Commonly, microglia integration was transient and organoids lost them within a couple of days. Incorporated microglia are actively screening their environment and form phagocytic cups as determined with live cell imaging.

Overall, the integration of microglia precursor cells into neuronal tissue depends on an optimal time window. For the next steps, we are exploring how microglia presence impacts neuronal function using calcium imaging. This will provide important insights into the role of microglia in neuronal patterning and -activity during early human brain development.

Tilorone improves glucose uptake in myoblasts by increasing BMP signaling and glucose transporter levels

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Satellite cells, the stem cells of skeletal muscle, are found between the sarcolemma and basal lamina of muscle fibres. Upon activation, they multiply rapidly to form a set of myoblasts that fuse together to form myofibres and repair them with damaged fibers. In addition to these physiological importance, myoblasts serve as a widely used model for in vitro skeletal muscle metabolism studies. The global prevalence of type 2 diabetes mellitus is continuously rising. Insulin resistance in skeletal muscle is manifested by decreased insulin-stimulated glucose uptake resulting from impaired trafficking and decreased amount of glucose transporter 4 (GLUT4). Here we show that tilorone, a low-molecular weight antiviral agent, improves glucose uptake of C2C12 myoblast cells. Tilorone increased BMP (bone morphogenetic protein) signaling; the transcription multiple BMPs (BMP2, BMP4, BMP7, and BMP14), and the amount of Smad4 and the phosphorylation of Smad1/5/8 transcription factors were increased. The activation of signaling cascade regulating GLUT4 trafficking also increased, as well as the amount of GLUT4 and GLUT1 glucose transporters, leading to enhanced 18F-fluoro-2-deoxyglucose uptake of the cells. However, this excess glucose is not translated into ATP formation by mitochondrial respiration, both the basal and ATP-linked respiration decreased. Our results help to open up new perspectives in the treatment of type-2 diabetes, as the number of drugs that regulate the amount or translocation of remains limited. This research was supported by the National Research, Development and Innovation Office of Hungary [grant numbers: GINOP-2.3.2-15-2016-00040 (MYOTeam), NKFI FK 134684 and NKFI K 132446], János Bolyai Research Scholarship of the Hungarian Academy of Sciences, and UNKP-20-5 -SZTE-162 New National Excellence Program of the Ministry for Innovation and Technology Sciences.

Self-organization of a signaling center in neural tube organoids

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Patterning is crucial for body-axis formation and organ specification in mammalian organisms. The molecular event that underlies patterning during early development is called symmetry breaking. While symmetry in biological systems can be broken upon receiving an external stimulus that provides spatial information, it can also form an asymmetric state spontaneously in the absence of spatial information. However, studying spontaneous symmetry breaking and patterning in vivo still remains particularly challenging due to the presence of external tissue that can influence pattern formation drastically. Thus, reconstituted in vitro systems provide a reliable alternative for tackling these mechanisms. Organoids have been previously proposed to represent suitable tools to study spontaneous symmetry breaking and patterning in vitro, due to the self-organizing properties of ESCs and their accessibility for large-scale perturbations. In this study, we use single mouse embryonic stem cell-derived neural tube organoids to study spontaneous symmetry breaking upon a global, spatially uniform application of the signaling molecule retinoic acid. Retinoic acid induces the expression of the transcription factor FoxA2 initially in a subset of cells in a scattered spatial distribution, which subsequently self-organize to a form a single signaling center within 48 hours that patterns the organoid. We show that clusters of FoxA2-expressing cells compete via long-range signaling interaction. Transcriptional analysis and large-scale pathway perturbations revealed a relevant importance for WNT and BMP pathways during self-organization. Additionally, we observed that larger organoids tend to have multiple clusters. Altogether, our results suggest a reaction-diffusion interaction orchestrating self-organization and patterning of neural tube organoids with FoxA2 as visible read-out.

Organoid modelling reveals virus-specific responses leading to microcephaly

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The recent pandemics of Zika virus (ZIKV) and Coronavirus (SARS-CoV-2) underscore the threats posed by viruses and highlight the need to better understand their unique pathogenesis. Stem-cells derived organoid models are emerging platforms for the study of viral pathogenesis in a human and physiologically relevant environment but to which extent organoids can recapitulate unique aspects of viral pathogenesis for multiple viruses remains unclear. Here we describe human brain organoids infected with Herpes Simplex virus 1 (HSV-1) and ZIKV, two viruses that severely impair foetal brain development and can cause microcephaly during pregnancy. We find that both viruses efficiently replicate in brain organoids and attenuate their growth by causing cell death. However, transcriptional profiling reveals that ZIKV and HSV-1 elicit distinct cellular responses and we show that HSV-1 uniquely impairs neuroepithelial identity. Furthermore, we demonstrate that while both viruses fail to potently induce the type I interferon system, the organoid defects caused by their infection can be rescued by distinct type I interferons. Importantly, these phenotypes cannot be seen in 2D cultures, underscoring the importance of the 3D environment for modelling viral spreading. Together, our work uncovers the ability of organoids to model unique aspects of viral pathogenesis in the human brain.

Stemness features of mesenchymal stem cells from exfoliated dedicious and permanent teeth: modulatory effects of IL-17 and bFGF

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Dental pulp tissue from primary/exfoliated deciduous teeth and adult/permanent teeth harbors mesenchymal stem cells (MSCs) populations termed as SHEDs and DPSCs, respectively. The comparative evaluation revealed certain functional diversity between SHEDs and DPSCs, which might suggest their distinct activity in presence of various environmental stimuli, including growth factors and cytokines. However, the data on distinct reactivity of these cells are scarce. In this study, we investigated if IL-17 and bFGF, as participants of inflammatory/regenerative pulp microenvironment, can modulate stem cell features and fate of dental pulp MSCs of different donor age by analyzing SHEDs and DPSCs proliferation, clonogenicity, pluripotency markers expression and differentiation capacity upon 7-day treatment with these factors. Our results revealed that IL-17 and bFGF differently affect SHEDs and DPSCs proliferation and clonogenicity, while both factors enhanced NANOG, OCT4 and SOX-2 expression in both types of dental pulp MSCs. Interestingly, different intracellular expression pattern of these pluripotency-associated markers depending on MSCs type was detected. As for the differentiation capacity, 7-day pre-exposure of SHEDs and DPSCs to IL-17 increased early osteogenesis, contrary to the strong inhibitory effect of bFGF, while neither IL-17, bFGF nor their combination affected late osteogenesis of pretreated cells. Also, no significant changes of chondrogenic differentiation of SHEDs and DPSCs pretreated for 7 days with examined factors were observed. Further analyses evidenced the potential of IL-17 and bFGF to stimulate IL-6 expression in both MSC types, indicating a possible role of IL-6 in manifested effects of IL-17 and bFGF. These findings clearly demonstrate intrinsic features of dental pulp MSCs dependent on donor age, manifested through their differential responsiveness to the IL-17 and bFGF, which should be considered particularly important in the context of dental pulp regeneration.

The Fgf/Erf/NCoR axis controls cell fate decisions in Trophoblast Stem Cells

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Mouse trophoblast stem cells (TSCs) recapitulate aspects of placental development and provide an excellent in vitro model to study differentiation into specialized placental cells types. The withdrawal of the self-renewal inputs Fgf/Erk and Nodal/Activin triggers exit from multipotency and TSC differentiation. However, the molecular mechanism of how attenuation of Fgf signalling drives transcriptional changes that result in TSC differentiation remains largely unknown. Global phosphoproteomic profiles of TSCs confirmed the Ets2 repressor factor (Erf) as an early Fgf/Erk phosphorylation target. Intriguingly, the Erf interactome at the exit from TSC multipotency revealed a specific interaction with components of the Nuclear Receptor Co-Repressor complex (NCoR). TS cell lines depleted for Erf and the NCoR component Tbl1x showed a differentiation defect and global gene expression analysis revealed a substantial overlap of deregulated genes, further corroborating the Erf-NCoR1/2 functional relationship. Many of the deregulated genes were co-occupied by Erf, Tbl1x, Ncor1 and Ncor2 strongly indicating transcriptional co-regulation. Surprisingly, Erf and Tbl1x KO TSCs showed impaired upregulation of the master regulator Gcm1, suggesting that Erf in cooperation the NCoR complex is involved in activation of the gene regulatory networks of the differentiating trophoblast. Ncor1 and Ncor2 were binding de novo to Gcm1 in differentiating TSCs as determined by ChIPseq. This recruitment is strongly impaired in Erf KO TSCs indicating the surprising possibility that Erf helps recruiting NCoR to de novo targets upon exit from multipotency. Overall, these findings shed light on the regulatory mechanisms of TSC differentiation and suggest an intriguing cooperation of Erf and the NCoR complex in regulation of TSC differentiation.

Novel surface markers to isolate human megakaryocyte progenitors

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Platelets are the cells responsible for clotting and every year in the UK, 280,000 platelet units are transfused into patients with a low platelet count, known as thrombocytopoenia, to prevent haemorrhage. Patients receive transfusions after road traffic accidents, surgery, cancer treatment, severe infection, immune-mediated platelet destruction, abnormal platelet distribution, hypercoagulation or inherited or acquired bone marrow deficiency. However, platelet units must be matched, putting a particular strain on the service when donations are reduced or when rare platelet units are required.

The cells which produce platelets are known as megakaryocytes (MKs) and these are large, multinucleated, polyploid cells which differentiate from haematopoietic stem cells in the bone marrow. Over the past few years, we have developed and refined a protocol which allows the differentiation of MKs from human pluripotent stem cells (iPSCs) in vitro, providing us with an on-demand source of universal platelets for transfusion. This protocol produces MKs in only 20 days, using only two cytokine combinations and driven by the overexpression of haematopoietic transcription factors GATA1, TAL1 and FLI1. Crucially, these MKs can produce functional platelets which can contribute to thrombus formation.

One of the key characteristics of the cultures is their ability to survive for up to 120 days, indicating the presence of cells capable of reconstituting the culture when the older MKs fragment to generate platelets. We hypothesised that the culture are maintained by highly proliferative MK progenitors (MKPs) and if we could identify and purify these MKPs, we could amplify their production at earlier culture stages, enabling much larger production efficiencies. In addition, isolating these cells would allow us to analyse the processes underpinning the progenitor state and to find culture conditions which counteract culture exhaustion.

During this project, we identified five cell surface markers which, when used together, allow us to reproducibly purify MKPs from mixed cultures. Through single cell RNA sequencing, we have mapped the entire differentiation trajectory from iPSC to MK and sequential rounds of 10X sequencing have allowed us to pinpoint the location of these progenitors along the timecourse and map their emergence. Isolating these progenitors then allowed us to compare the biological underpinnings of megakaryocyte forward programming to in vivo MK differentiation. We found the transcription factors underlying this state in vitro were strikingly similar to those found in human MKPs in vivo. Finally, we optimised culture conditions, varying oxygen and cytokine concentrations to allow us to produce more progenitors at early stages, prolonging cultures and maximising output, a step which will be key in the large-scale manufacturing of transfusable platelet units.

In conclusion, this project has enabled us to identify a surface marker panel specific to megakaryocyte progenitor cells, purify them from mixed cultures, compare them to human bone marrow MKPs and optimise the production of mature megakaryocytes from iPSC-derived cultures. This provides us with a crucial insight into processes taking place deep in the bone marrow and is a crucial step in the advance of iPSC-derived transfusion products towards the clinic, providing universal platelets for patient transfusions.

p57 is a gatekeeper that limits the injury response of gastric chief cells

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Adult stem cells constantly react on local changes to ensure tissue homeostasis. In the main body of the stomach, chief cells produce digestive enzymes while, upon injury, they undergo rapid cell cycle for prompt tissue regeneration. Here, we identified p57Kip2 (p57) as a molecular switch for the reserve stem cell nature of gastric chief cells. p57 expression is constantly on in chief cells in homeostasis, which rapidly diminishes after injury, followed by robust proliferation. Both single-cell RNA sequencing and dox-induced lineage tracing confirmed the sequential loss of p57 and activation of proliferation within the chief cell lineage. In corpus organoids, p57 overexpression induced long-term reserve stem cell state, accompanied with altered niche requirements and mature secretory characteristics. With a constitutive expression of p57 in vivo, chief cells showed an impeded injury response. In conclusion, p57 is a gatekeeper that limits the injury response of gastric chief cells in homeostasis.

Deciphering the specification of posterior lateral plate mesoderm

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Mesoderm gives rise to a diverse range of tissues and the first stages of mesoderm specification are decisive to achieve this variety. Subtypes of mesoderm include cardiac, posterior lateral plate (a progenitor of limb mesoderm) and somitic mesoderm but the underlying mechanisms of early cell fate bifurcations that separate them remain unresolved. To dissect how posterior lateral plate mesoderm specification differs from its cardiac and somitic counterparts, we established developmentally-guided human pluripotent stem cells differentiation into hindlimb-specific TBX4+ and forelimb-specific TBX5+ posterior lateral plate progenitors. These stem cell-derived precursors were functional as they efficiently specified into endothelial cells and chondrocytes. We found that while the dosage of WNT and retinoic acid signalling patterned mesoderm into either cardiac, forelimb or hindlimb precursors, BMP had a key role in promoting limb over somitic mesoderm. Among other factors, we identified and are in the process of genetically validating MSX2 and SOX2 as possible direct targets of BMP signalling and found strong indications that these transcription factors are on top of the posterior lateral plate mesoderm specification hierarchy. Interestingly, despite profound differences, limb and cardiac mesoderm share a network of key transcription factors. As a future outlook, we will use our cardiac and limb differentiation platforms to molecularly decipher how mutations in these shared developmental regulators cause cardiac and limb malformations in human syndromes.

Mutational Processes in Intestinal Stem Cells in Response to High Fat Diet

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Mutational signatures provide a new way of investigating the evolution of cancer. However, many etiologies of known signatures remain unidentified, while endogenous processes involved in DNA damage and mutagenesis remain understudied. This project aims to study how genome integrity in intestinal stem cells is impacted in the obese condition by identifying and characterizing mutational signatures associated with high fat diet induced obesity. To do so, wild type C57BL/6J mice were placed on a normal or high fat diet and mutational signatures were tracked over time by sequencing clonally derived intestinal organoids. Analysis of the observed signatures reveals which mutational processes were at play and thus which DNA repair pathways were dysregulated in the obese condition. In the second part of the project, we will validate the dysregulation of predicted pathways and generate CRISPR-Cas9 mediated knockout organoids, targeting genes in candiate pathways. In this in vitro system we can investigate functional consequences of obesity and metabolic dysregulation on DNA damage repair and mutagenesis. Mutational signature analysis of knockout organoids will confirm the mechanistic connection between mutational process and resulting mutational profile. By studying the effect of endogenous stresses on the DNA damage response in the obese condition, we expect to bring new understanding to the molecular basis of obesity induced colon cancer development and identify new markers and targets for treatment of pre-cancerous lesions.

Human pluripotent stem cell-derived neurons as an in vitro model for studying neural stress and CDK5 signaling

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Human pluripotent stem cells (hPSCs), which include embryonic and induced pluripotent stem cells (hESCs and hiPSCs, respectively), can differentiate into a wide range of specialized cells, including neurons. Moreover, hPSCs-derived neurons are proposed as a model for studying neurodegeneration. Neurodegeneration is a complex multifactorial process that causes progressive loss of structure and function of neurons. CDK5/p35 complex is involved in neuronal homeostasis and development. However, under pathological conditions p35 can be cleaved by calpain into p25. The resulting complex CDK5/p25 engenders aberrant activity and phosphorylates a number of substrates abnormally. Although CDK5 signaling has been intensively studied in animal models, currently there are no-suitable in vitro models for studying its participation in human neuronal homeostasis and neurodegenerative processes. In this work we aimed to generate an in vitro human model for studying CDK5 signaling and neural stress based on the neuronal differentiation of hPSCs. For this purpose, first we derived neural stem cells (NSC) from hESCs (H9 line) and hiPSCs (FN2.1 line), which were further differentiated into neurons using a 2D-based protocol. NSC and neuron-like phenotype were validated by electrophysiology and expression of lineage specific markers (Sox-1, Sox-2, Pax-6 and Nestin for NSC; MAP5, MAP2 and Tuj-1 for neurons) by immunofluorescence microscopy and RT-qPCR. Then, CDK5 and p35 mRNA and protein expression levels were analyzed in hPSCs, NSC and neurons by RT-qPCR and western blot. Interestingly, we observed that while CDK5 is ubiquitously expressed, p35 mRNA and protein are mainly expressed in neurons. We next evaluated how different types of stress (rotenone, glutamate and calcium ionophore A23187) affected hPSCs-derived neurons viability. We determined the percentage of cell viability after a 24 hours treatment with increasing concentrations of rotenone and glutamate and a 2 hours treatment with A23187 using a XTT vital dye assay. Cell viability fell down significantly in the case of rotenone and A23187 treatments in a concentration dependent manner, which was not accompanied by an increase in cell death. However, only a slight effect was observed with glutamate treatment. Moreover, an increase in mitochondrial membrane potential (measured using Mitoprobe JC-1 Assay and morphologic changes (axonal spheroid appearance) were found in derived neurons upon rotenone (1uM for 24h) and A23187 (2uM for 2h) stressful stimuli. Further, rotenone and A23187 treatments induced p35 cleavage to p25, which was mediated by calpains as proteolysis was inhibited with a calpain inhibitor (ALLN, 50uM). In conclusion, hPSCs derived neurons emerge as a potential in vitro human model for studying the relevance of CDK5 signaling in neural stress as they responded to stressful stimuli inducing calpain-mediated cleavage of p35 to p25.

The Role of MTCH2-mediated Mitochondrial Fusion in Regulating the Quiescence of Hematopoietic Stem Cells

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Elucidating the mechanisms regulating the fate of hematopoietic stem cells (HSCs) is a central goal of ongoing research in the field. HSCs are capable of maintaining homeostasis and meeting demand for blood cell production through control of their cell cycle status and metabolism. Both the cell cycle status and metabolism are regulated by mitochondrial activity. Quiescent HSCs are believed to have less active mitochondria, but they can elevate their function upon stress or stimuli for proliferation. Mitochondrial carrier homolog 2 (MTCH2) was previously characterized in our lab as a negative regulator of mitochondrial function in HSCs. Conditional deletion of MCTH2 in the hematopoietic system (MTCH2F/F Vav1-Cre+ mice) resulted in increased mitochondrial oxidative phosphorylation (OXPHOS) and triggered HSCs to enter the cell cycle. Such an increase in cell cycle entry is usually associated with potential HSCs exhaustion. Therefore, we asked whether MTCH2 -/- HSCs can self-renew following transplantation. Despite the increased mitochondrial activity and higher cell cycle entry rates, MTCH2 -/- HSCs were capable of self-renewing and repopulating lethally irradiated mice. In addition, the ex-vivo studies of HSC division patterns revealed a higher percentage of symmetric self-renewing divisions in MTCH2 -/- HSCs. From these studies we concluded that HSCs lacking MTCH2 compensate for higher cycling rates by increasing their symmetric self-renewal to preserve the stem cell pool in transplanted animals. Nevertheless, the exact role of MTCH2 in preserving HSCs in quiescence remained elusive. Recently, we reported that MTCH2 is involved in regulating mitochondrial morphology/dynamics (fusion/fission of mitochondria), a critical mechanism that regulates mitochondrial function. Specifically, we found that MTCH2-mediated mitochondrial fusion drives exit from naive pluripotency in embryonic stem cells. Thus, we asked whether mitochondrial fusion is required for maintaining HSCs in quiescence. To address this question, we applied advanced microscopy techniques on the single cell level and found that HSCs' divisional activity correlates with mitochondrial morphology. HSCs' entry into the cell cycle was accompanied by short/fragmented mitochondria, whereas elongated mitochondria prevailed in quiescent HSCs. Therefore, the increased proliferation rate and exit from quiescence observed in the MTCH2 -/- HSCs is possibly due to a defect in mitochondrial fusion. In addition, mitochondrial morphology was assessed during forced in-vivo HSCs entry into the cell cycle using single 5-Fluorouracil (5-FU) administration. The HSCs from wild type and MTCH2 -/- mice showed mainly

fragmented mitochondrial morphology at the proliferative recovery phase (days 5 and 9), whereas at the end of the recovery phase (day 14), mitochondria started to elongate again. Overall, these findings suggest that mitochondrial fusion in HSCs may prevent excessive entry into the cell cycle (symmetric division) and may play an important role at the stage of re-entry into quiescence after acute stress.

Non-invasive online monitoring of stem cell aggregate cultivation in a stirred tank bioreactor

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Their regenerative potential and immunomodulatory effects have made mesenchymal stem cells (MSCs) prime candidates for use in tissue engineering and stem cell therapies. As the majority of these applications need large numbers of cells (approximately 1 – 2x10^6 cells/kg body weight), in vitro expansion of these cells is inevitable. Additionally, conversion of traditional 2D cell culture on plastic-ware towards GMP-compliant and up-scalable 3D expansion systems is essential to maintain stemness and improve genetic stability during cultivation. In this context, culture of scaffold-free 3D MSC-aggregates in dynamic cultures (e.g. stirred-tank bioreactors) represents a suitable in vivo-like environment and is easily adaptable for large-scale production-processes. However, due to non-uniform aggregate formation and risk of contamination, the development of built-in non-invasive monitoring systems is of particular importance. Here, we present an advanced stirred tank bioreactor system for the 3D expansion of MSCs in aggregates. For this we developed a non-invasive method to monitor aggregate size and shape in a time resolved manner. The design of our bioreactors allows for a closed transfer of the aggregates from the culture vessel to a channel microscopy slide. Images are further analyzed in ImageJ using a custom-adapted macro to measure particle size, number and roundness. Complementary to the aggregate characteristics, dissolved oxygen is measured online non-invasively via an optical sensor system. In parallel, glucose and lactate concentration in the culture medium is measured to gain more insight into the relationships between aggregate formation/compaction and cell metabolism. In the future, this monitoring system could help in predicting cell-aggregate maturation and determine optimum feeding and harvesting time points, making it a valuable tool for large scale stem cell expansion.

Biosafety assessment of intranasally delivered mesenchymal stem cells for the treatment of nervous system disorders

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Mesenchymal stem cell (MSC)-based therapy is a promising therapeutic approach for regenerative medicine, including brain healing. Previously, we demonstrated the therapeutic effects of human adipose-derived MSCs for neurological sequelae of radiotherapy using the intranasal route as a noninvasive delivery method. However, safety concerns should be comprehensively investigated before exposing patients to intranasally delivered MSCs. Here, we administrated repeated doses of MSCs (a weekly dose of 5 × 105 cells for 4 consecutive weeks) into the nostrils of immunodeficient mice, mimicking the dosing regimen in a clinical situation. Short- and long-term effects were evaluated by examining toxicity, tumorigenicity and biodistribution on weeks 12 and 24 post-transplantation. Non serious adverse events were reported in relation to toxicity effect (i.e. body weight and plasma biochemistry). Magnetic resonance imaging (MRI) and histological analysis did not reveal secondary tumors in mice receiving MSCs, whereas mice transplanted with positive control cancer cells developed malignant mass. Biodistribution of MSCs was restricted to the brain the days after transplant, but gradually disappeared over time. The mRNA of human specific GAPDH was not detected in any major organ examined on weeks 12 post-transplantation. Furthermore, animals were subjected to neurocognitive tests showing no differences in short- and long-term performance. Altogether, our preclinical data indicate that the intranasal application of MSCs is safe and feasible in mice and could be applicable in future clinical trials.

Exploring H3K27me3 dynamic at pericentromeric heterochromatin in mouse early embryo

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Early mouse development is characterized by structural and epigenetic changes at the chromatin level while cells progress towards differentiation. Especially, embryonic pluripotent cells progress from the naïve to the primed state concomitantly with the segregation of the Inner Cell Mass (ICM, naïve) then the epiblast at peri-implantation stages. Naïve and primed pluripotent states differ in their transcriptional and epigenetic features, such as DNA methylation and histones modifications. Here, we focus on pericentromeric heterochromatin, its enrichment in histone modifications, and its transcriptional status from the 2-cell stage to prestreack stage, in particular during the transition from naïve to primed pluripotency in embryo. We bring out that H3K27me3 localizes at chromocenters (clusters of pericentromeric heterochromatin) from their formation at the 2-cell stage and is then highly dynamic in the pluripotent ICM. This histone modification is at first enriched at chromocenters in the whole embryo and evolves into a diffuse distribution in epiblast cells during their regionalization from the Primitive Endoderm in the pre-implantation ICM. H3K27me3 is then weak and diffuse in primed epiblast as well as in extra-embryonic lineages at post-implantation stages. By contrast, only a few ESCs cultivated in 2i medium exhibit H3K27me3-marked chromocenters whereas being representative of the ground state of pluripotency as described from naïve ICM. Besides, a delayed loss of H3K27me3 at pericentromeric heterochromatin is observed during the conversion of ESCs into EpiSCs. Altogether, our results allow us to conclude that H3K27me3-labelled chromocenters are a physiological condition in the young embryo and that cell culture may influence the epigenetic profile of pericentromeric heterochromatin of ESCs and its dynamic during the transition to the primed state.

Understanding the genetic basis of fracture risk in Thoroughbred horses using a stem cell approach

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Bone fracture is a common and important cause of morbidity, health-care costs and an increased risk of mortality in humans. Osteogenesis maintains skeletal integrity through the function of osteoclasts (resorption) and osteoblasts (bone formation). Bone diseases associated with an imbalance between remodeling and bone formation lead to weakened bones and an increased risk of fracture. Bone fractures with non-traumatic origin occur in Thoroughbred racehorses and they are the main reason for euthanasia on the racecourse. Catastrophic fracture in horses is a complex condition with both environmental and genetic risk factors. Identification of the genetic factors and an understanding of the molecular pathways that they affect would allow the future development of new pharmaceutical and management interventions to prevent, and possibly treat, fractures. Comparison of horse and human chromosomes reveals strong shared synteny between these species. Furthermore, the detailed breeding records and large family sizes of Thoroughbred horses provide an advantageous route to better understanding the biology underlying complex diseases and their component traits. A previous genome-wide association study (GWAS) for catastrophic fracture in Thoroughbreds showed significant genetic variation for fracture risk on chromosomes 9, 18, 22 and 31. The GWAS identified four single nucleotide polymorphisms (SNPs), three on ECA18 (horse chromosome 18) and one on ECA1, which reached genome-wide significance (pgenome

In vitro differentiated epicardium interacts with self-organizing human cardioids

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Studying self-organizing organoids has transformed our ability to model and understand human development and disease. Using human pluripotent stem cells, we established a time- and developmentally matched system to study interactions between epicardium and self-organizing chamber-like structures called cardioids. Cardioid complexity can be controlled by signaling that instructs morphogenesis and the separation of cardiomyocyte and endothelial layers. In vitro derived epicardium recapitulates important steps of cardiac development as epicardial cells spread over the myocardial layer and subsequently migrate inward. These cells express further differentiation markers and seem to interact with cardiac endothelial cells. As cardiogenesis involves highly coordinated and complex spatial and temporal interactions of all three cardiac lineages, we are aiming to use this platform to mechanistically dissect self-organization and lineage interactions during human heart development and disease.

3D self-organized microvascular network on a chip model formed by hiPSC-derived endothelial cells and pericytes

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The rapidly developing microfluidic chip technologies employ hiPSC-derived cells for personalized drug testing, disease modeling, and developmental studies. hiPSC-derived cells were successfully used to receive a 3D self-organized perfusable microvascular network on the chip [1]. The microvascular network formation however still required the presence of primary perivascular cells, such as pericytes isolated from human brain. The use of primary cells makes the personalized disease modeling challenging for most blood vessel pathologies, as the crosstalk between vascular endothelial cells and pericytes plays an essential role in the regulation of the blood vessel growth, and stability, as well as physical properties of the microvasculature, such as diameter and tightness. In this study we aim to receive the 3D self-organized microvascular network on the microfluidic chip, using endothelial cells and pericytes both derived from hiPSCs. As a cell source, we used endothelial cells and pericytes differentiated from the same keratinocyte-derived hiPSCs [2]. The differentiated cells were embedded in the fibrin gel and loaded to the commercially available 3D cell culture microfluidic chips from AIM Biotech. hiPSC-derived endothelial cells, loaded in different concentrations (90.000 and 120.000 cells/chip) were not able to form microvasculature. The addition of hiPSCs-derived pericytes induced the formation of a 3D microvascular network. We also found that the cell number and ratio play an important role in the microvasculature formation: the optimal microvasculature formation was achieved by using 90.000 endothelial cells and 30.000 pericytes. In summary, we developed a protocol that allows receiving the 3D self-organized microvascular network on a microfluidic chip, from hiPSC-derived endothelial cells and pericytes. Moreover, our results demonstrate that hiPSC-derived pericytes can efficiently support the microvasculature formation, and therefore fulfill their main function. The described hiPSC-derived 3D microvasculature on the chip can be used to model vasculopathies and cardiovascular disorders as well as for personalized drug testing and translational medicine approaches. 1. Campisi, M.; Shin, Y.; Osaki, T.; Hajal, C.; Chiono, V.; Kamm, R.D. 3D self-organized microvascular model of the human blood-brain barrier with endothelial cells, pericytes and astrocytes. Biomaterials 2018, 180, 117–129, doi:10.1016/j.biomaterials.2018.07.014. 2. Pars, S.; Achberger, K.; Kleger, A.; Liebau, S.; Pashkovskaia, N. Generation of Functional Vascular Endothelial Cells and Pericytes from Keratinocyte Derived Human Induced Pluripotent Stem Cells. Cells 2021, 10, 74, doi:10.3390/cells10010074.

A long-lived population of stem cells under neutral competition shapes the clonal composition of cerebral organoids

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Cerebral organoids have recently been established as a model system for studying the development of the human brain and of neuro-developmental diseases such as microcephaly and autism. During organoid development, roughly 30,000 ancestral hPSCs divide and differentiate to form a complex tissue that replicates many aspects of the human cortex, and which in particular comprises the expected types of differentiated cells. But while the individual differentiation trajectories that produce these cell types are well understood, many questions about the overall dynamics of this process remain open. To tackle these open questions, we introduce whole-organoid lineagetracing through genetic barcoding followed by high-throughput sequencing to determine the contribution of each ancestral hPSC to the final organoid. We demonstrate that our lineagetracing protocol is sensitive enough to detect single-cell lineages even in organoids comprising millions of cells, and observed that lineage size distributions are highly non-uniform and in fact follow a truncated Zipfian law. To explain these highly non-uniform Zipfian lineage size distributions, we introduce the stochastic SAN model and show that this model explains observed data. We show that this model is a useful as a null model that reflects wildtype behaviour in genetic perturbation screes. Through studying the conditions under which the observed Zipfian law emerges from the SAN model, we conclude that cerebral organoids likely contain a long-lived population of roughly 10,000 symmetrically dividing cells and that this population is the main driver of organoid growth.

Comparison of fracture healing and limb regeneration in aged axolotls to murine fracture healing.

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While the axolotls ability to completely regenerate amputated limbs is well known and studied, the bone healing capacity remains poorly understood. One reason might be the lack of standardized fracture fixation in axolotl. Here, we present for the first time a surgical technique that allows to stabilize the femur of the axolotl with a murine fracture fixator plate during osteotomy. We compared the plate-fixated group to a non-stabilized osteotomy and amputated limb groups. The healing outcome was evaluated 3 weeks, 3, 6 and 9 months upon osteotomy by microcomputer tomography (micro-CT) and histology as well as immunohistology examinations. In conclusion, micro-CT and histological analysis showed efficient restoration of the bone length and structure upon amputation. Remarkably, the bridging of a non-critical gap seems to be more difficult for axolotl than regrowing the complete distal limb, which was completed in the same time frame. In comparison to the non-fixated femur, plate-fixated bone healing upon osteotomy regains bone integrity more efficiently. This may be explained by necessity to produce larger callus in non-fixated femur to compensate for the bone fragments misalignment. In all three groups, we observed accumulation of PRRX-expressing mesenchymal progenitors during the early phase of fracture and amputated limb healing, coinciding with increased proliferation. PRRX1-positive cell population partially overlapped with LepR-expressing population. Immunohistology analysis revealed the presence of SOX9-positive cartilage progenitor cells suggesting the endochondral ossification as the mechanism of bone fracture healing in axolotl. The here demonstrated surgery with a standardized fixation technique allows the controlled bone healing experiments in axolotl, ensuring the comparability to fracture healing studies in mammals (mice).

IPSC-derived pericytes for the alleviation of the muscle phenotype in myotonic dystrophy type 1

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Introduction: We are developing a stem cell-based treatment to alleviate the muscular phenotype of myotonic dystrophy type 1 (DM1). DM1 is caused by a (CTG)n-repeat expansion in the 3' untranslated region of the DMPK gene, which forms toxic RNA that remains retained in the nucleus. Furthermore, it sequesters proteins, such as MBNL1, leading to dysfunctional transcription, splicing and RNA export resulting in muscular atrophy, myotonia and progressive muscle wasting. We successfully isolated muscle progenitor cells, pericytes, from skeletal muscle of DM1 patients and healthy individuals. These primary pericytes have a high proliferative potential, yet their capacity to for in vitro expansion is limited to 20-25 passages. To ensure an unlimited source of cells, we generated pericyte-derived iPSCs (PC-iPSCs). Subsequently, the pathogenic trinucleotide repeat will be excised from these cells via CRISPR/Cas9-mediated gene editing to create isogenic lines. Corrected pericyte-derived iPSCs can then be differentiated to PC-iPSC-derived pericyte-like cells (PiPs), which have shown to maintain their restorative and myogenic potential. Methods: Skeletal muscle biopsies were taken from quadriceps muscle from DM1 patients and healthy individuals to be cultured for pericyte proliferation and isolation. Cells were sorted for ALP+/CD31- expression by flow cytometry, expanded in vitro, and transfected with non-integrating episomal vectors containing the Yamanaka factors. Repeat length was determined by small-pool PCR. Following published protocols, PC-iPSC were differentiated towards PiPs by media composition changes over 21 days. Characterization of PiPs was done via quantitative PCR and immunocytochemistry. Results and Conclusions: We were able to generate iPSCs from pericytes of DM1 patients and healthy controls. Quantitative PCR (qPCR) and immunocytochemistry proved that generated PC-iPSCs have pluripotency characteristics. Furthermore, we are optimizing the differentiation of these PC-iPSCs into PiPs. First results show that these PiPs have the same characteristics as pericytes. Finally, the PC-iPSCs will be used in CRISPR/Cas9-mediated gene editing via RNPs to remove and repair the expanded (CTG)n repeat mutation. Grant support: This work was funded by the Prinses Beatrix Spierfonds (grant numbers W.OP19-03 and W.OR18-06).

Round, reliable cell spheroids for reproducible cell-based assays

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3D in vitro model systems becoming more and more relevant in cancer and stem cell research as well as in pharmaceutical applications is due to their potential to resemble natural microenvironment and human pharmacokinetics. Unnatural 2D cell cultures restricted to cell-cell contacts in one dimension and cells reveal an altered cell behavior influencing e.g. drug response. 3D cell spheroids – dense cell aggregates – allow cell-cell contacts in all dimensions for more precise validation of cell-based assays. For this purpose, we developed a chemically defined and biologically inert coating solution which can be easily applied which prevents nonspecific binding of proteins and cells. In this environment cells start to aggregate and form cell spheroids by connecting with each other. We benchmarked our new technology using different cell lines such as hepatocytes showing a reproducible, rapid generation of round spheroids within 24 h outperforming current competitors. This new technology allows the development of spheroid-based model systems within a remarkable short time for pharmaceutical as well as medical research.

RAPID GENERATION OF FUNCTIONAL SENSORY NEURONS FROM HUMAN IPSC BY OVER-EXPRESSION OF NGN1, BRN3A and ISLET1

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Specialized peripheral sensory neurons are crucial for somatosensation and conscious representation of the outside world. Proprioceptors sense body position and movement, mechanoreceptors sense touch and pressure and nociceptors sense temperature, heat and pain. Disruption of proper nociceptor function is associated with a number of clinical syndromes including chronic pain, which affects one in five people in Europe. The vast majority of these patients report that their medical treatment does not meet their expectations. Human iPSC-derived sensory neurons provide perspectives for assessing potential therapies directly in disease-relevant cells, and recent progress in transcription factor-based cell programming may further promote this approach. We engineered iPSCs to express a combination of two (NGN1, BRN3A; 'NB') or three transcription factors (NGN1, BRN3A, ISLET1; 'NBI') from a safe harbor locus in an inducible manner. Upon 7 days of transgene induction both combinations yielded sensory neurons expressing key markers such as PRPH, TRPV1 and NAV1.7. However, NBI induced a stronger upregulation of PRPH already at day 2 of differentiation and enhanced expression of the nociceptor-specific gene TRPV1. Furthermore, NBI robustly induced pure neuronal cultures independent of the iPSC seeding density. Exposure to pain specific stimuli αβ-ATP (P2X3) and Capsaicin (TRPV1) significantly increased neuronal firing rate, validating nociceptor- specific functionality in these neurons. Finally we employed iPSCs from a chronic pain patient suffering from inherited erythromelalgia (IEM) for disease modeling. IEM is characterized by extreme burning pain and is attributed to gain-of-function mutations in the SCN9A gene coding for the peripheral sodium channel NAV1.7. Patient-derived NBI neurons showed an increased firing rate in multi-electrode array assays recapitulating changes in cellular functionality that could be associated with the sensation of chronic pain in affected individuals. In conclusion we here present a new forward programming approach for the fast and efficient generation of pure human sensory neurons suitable for disease modeling.

Identification of miRNAs regulated by E2F transcription factors in human embryonic stem cells

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Human embryonic or induced human pluripotent stem cells (hESCs and hiPSCs) have an unusual cell cycle structure which consists of a short G1 phase and absence of the G1/S checkpoint regulation. MicroRNAs (miRNAs) are small non-coding RNAs that play an important role in many key processes. E2F transcription factors (E2Fs) regulate G1/S transition. G1 duration contributes to hPSCs fate determination and miRNAs play a key role in achieving this cell cycle regulation. In hPSCs, the role of RB/E2F complexes remains uncertain and their expression profiles during cell cycle progression have not been fully studied yet. Due to this, the aim of this work was to explore if E2Fs mRNAs are constitutively or periodically expressed in hPSCs and to identify miRNAs that are regulated by E2Fs in hESCs. First, by RT-qPCR analysis, we observed high mRNA expression levels of the canonical E2Fs in hPSCs compared to somatic cells (human fibroblasts). At the same time, to determine if E2Fs are periodically or constitutively expressed we synchronized hPSCs in G1/S with PD0332991 (30h 5 µM), and in G2/M with Nocodazole (24h 100 ng/ml). RT-qPCR analysis of synchronized cells revealed a periodic gene expression profile of E2Fs transcripts. Then, we treated H9 hESCs line with the general inhibitor of E2Fs (pan-E2F inhibitor) HLM006474. Concentration and incubation time used for HLM006474 treatment was fine-tuned by studying the cell viability and cell cycle profile of hESCs-treated cells determined by XTT assay and BrdU-7AAD flow cytometry analysis. A 20µM HLM006474 concentration and 24 hours treatment was chosen for further experiments as it induced an increase in G1 cell population in H9 hESCs without affecting cell death rate. Next, we performed a RNA-seq analysis of small RNAs of H9 hESCs treated or not with HLM006474 inhibitor. From 52 miRNAs differentially expressed we selected 20 miRNAs candidates, some of which were already related with E2Fs family and others whose relationship with these factors or with hESCs-cell cycle has not yet been reported. Finally, upon validation of the expression levels of these candidates by RT-qPCR with specific stem loop primers, we concluded that miR-19a-3p, miR-19b-3p, miR-4454, miR-1260a, miR-1260b, miR-454-3p and miR-301a-3p would be transcriptionally regulated by canonical E2Fs.

Impaired Differentiation: Understanding a Single Cell State Transition

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Development is characterized by distinct, coordinated cell state transitions. During each transition, an existing gene expression program is dismantled, and a new cellular identity has to be established.

We use the exit from naïve pluripotency as a highly accessible and controllable model system for a single cell state transition: mouse embryonic stem cells cultivated under defined conditions are homogenous naïve pluripotent and maintained by a well-established core gene regulatory network. Upon change of culture conditions, this network is rapidly dismantled, and cells irreversibly commit to differentiation into formative pluripotency, a less characterized cell state. Despite extensive screening for factors required for exiting naïve pluripotency, so far not a single factor has been identified which completely abrogates differentiation ability. Differentiation impaired mutants rather exhibit a phenotype which is mostly described as prolonged expression of pluripotency markers or clustering of bulk transcription profiles with naïve wildtype.

Single cell methods open up a window into understanding these aberrant cell states. We collected fine-tuned differentiation time courses of control and selected differentiation impaired mutants, targeting the major signalling pathways involved in this transition. We built a shared trajectory across these genotypes and could recapitulate a delay in cell state of mutants in comparison to time matched control cells. With this, we now address fundamental questions: are observed differences based on differences in cell state, or are they directly affected by the altered gene regulatory networks? How are these networks responding to the challenge of genetic manipulations? How is the remarkable robustness of this cell state transition ensured?

Taken together, our work will elucidate the mechanism of a single cell state transition and its intrinsic robustness.

Determination of the role of stem cell characteristics as key factors, allowing the production of wished molecules and changing the cellular differentiation direction

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Capability of non-lymphoid and non-myeloid cells to produce receptor glycoproteins and immunoglobulins was studied. In pre-incubation of low-differentiated non-malignant mammal embryonic cells in the presence of malignant antigens, malignant cells and viral particles, initial myeloid and lymphoid phenotypes were noted and further confirmed by established osteoclast-like cells (OCLCs), hybrid cells and activated cellular proliferation after freezing in the presence of cryo-protector Dimethylsulfoxide (DMSO), subsequent thawing and re-incubation. The OCLCs phenotype was confirmed by osteoblast-like cells (OBLCs), derived from de novo-incubated fibroblasts in cultural fluid from the received OCLCs, but also by the destroyed OBLCs monolayer in co-cultivation with OCLCs. Both OCLCs and OBLCs were very similar to the derived from normal

mammal adult mesenchymal stem cells (MSCs). The explanation was eventually activated fusion between separate cells (from the same or different types), and between cells and viral particles (probably causing nucleotide sequences exchange) on the influence of DMSO and drastic temperature changes. The possibility for production of antibodies/immunoglobulins was stronger for the mixed cultures of normal and malignant cells compared to each separate type, probably due reversed stem-like cellular phenotype. Antibody production was assessed also in non-lymphoid anatomic organs, probably because cells in various maturation and differentiation phases are available. On the other hand, however, because these antibodies are out of the so called „germinative centers", the control of their functions by low molecular mass molecules as for instance the complex glycosphingolipids – gangliosides, was proved. These phenomena could be explained with activation of respective genes or with changed protein functions. These data suggested the stem cell character importance about wished molecules production and changed cell differentiation direction. In confirmation was the successfully inserted additional oncogene in normal mouse embryonic stem cells (mESCs), transfected with recombinant viral vectors, but also with initial myeloid and lymphoid progenitors, derived from non-transfected and transfected mESCs sub-populations in the presence of appropriate factors, including of malignant and viral antigens. Both mESCs types proved preserved normal/non-malignant features and presented animal analogues of non-immortalized and immortalized by virus SV40 normal human embryonic trophoblasts.

Novel imprinted genes exemplify predominantly H3K27me3-dependent imprinting in mouse blastocysts

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Parent-of-origin specific gene expression depends on epigenetic marks (imprints), which are asymmetrically distributed between maternal and paternal mammalian genomes. Genomic imprints correspond to DNA methylation or to recently reported allele specific H3K27me3. However, current maps of the imprinting landscape in early embryos are likely incomplete. We therefore set out to functionally and physically map the parent-of-origin-specific gene expression landscape in mouse preimplantation embryos. Transcriptome profiling of blastocysts from genetically distinct reciprocal crosses revealed 71 novel uniparentally expressed genes (nBiX: novel blastocyst-imprinted expressed genes). We validated candidate genes in independent blastocysts and observed that all tested genes lose their imprinted status upon implantation. To identify the epigenetic mechanisms underlying the monoallelic expression of nBiXs, we performed micro-whole-genome bisulfite sequencing on uniparental blastocysts, uncovering 859 differentially methylated regions (DMRs). Only 16% of nBiXs were associated with a DMR, whereas most were associated with, and/or dependent on, parentally-biased H3K27me3, indicating a major role for Polycomb-mediated imprinting in blastocysts. Many known imprinted genes are located in clusters. We could assign nBiX genes to 5 existing clusters, and further identified 5 new imprinted gene clusters containing exclusively nBiX genes. Collectively, our data suggest a complex program of stage-specific imprinting involving different tiers of regulation.

Oral stem cells as a potential source for dopaminergic neurons

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Currently, stem cells provide a variety of research possibilities with great potential in medical therapeutics. Previous research has discovered that the transplantation of fetal midbrain tissue could improve neurological symptoms of Parkinson's disease. This discovery increased interest in cell-based therapy for the disease. A differentiation protocol that induces midbrain dopaminergic neurons from human induced pluripotent stem cells (iPS) is already established. Because the iPS cell system is expensive and elaborative, this study explores the potential of gingival mesenchymal stem/progenitor cells (G-MSCs) as an alternative cellular source for dopaminergic neurons. The hypothesis was that G-MSCs would offer a cellular source with great medical potential, which can be easily isolated from the patient's oral cavity via minimally invasive procedures for dopaminergic neurons formation.

The current study's results suggest G-MSCs as a promising cell source for developing neurons in vitro. We hope to further optimize the here suggested protocol to improve neuronal differentiation in order to make stem/progenitor cell-based therapy clinically applicable and further explore disease mechanisms of specific cell-types.

Modeling Drug-Induced Cardiotoxicity Utilizing Hpsc-Derived Cardiomyocytes: Towards Protective Therapy For The Heart

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Advances in the development of anti-cancer agents have made the treatment of several cancers more effective, but unfortunately have also increased the risk for cardiotoxic side effects. Cardiotoxicity is one of the main adverse effects of cancer therapy and has primarily been assessed in different in vivo animal models. However, animal models fail to recapitulate human physiology because of species differences and therefore are not predictive for drug-induced cardiotoxicity in humans. Human pluripotent stem cell-derived cardiomyocytes (hPSC-cardiomyocytes) provide a reliable source of human cardiomyocytes and have already proven valuable for cardiotoxicity studies. We utilized high throughput time-lapse imaging of cardiomyocytes differentiated from an NKX2.5eGFP-α-actininmRuby2 hPSC cardiac reporter for assessment of cell viability and sarcomeric disarray after treatment with Doxorubicin, one of the most effective anti-cancer drugs. Using bioinformatics, we created binary masks of the GFP area to quantify the survival after exposure to Doxorubicin. As expected, after 5 days only 20% of cardiomyocytes survived treatment with 1 uM Doxorubicin when compared to the DMSO control group. Findings from the high throughput imaging were confirmed by staining of live marker calcein. We also created binary masks of the α-actininmRuby2 signal to quantify disassembly of the sarcomeres and observed that less than 25% of sarcomeres are still present after 5 days. We evaluated the cardiotoxic effect of Doxorubicin on a functional level by evaluating calcium transients in cardiomyocyte monolayers using the calcium dye Fluo4 and in-house developed software. We identified increased contraction frequency (2 vs 1 Hz) with reduced time to peak (0.1 vs 0.2 s) and decay time (0.4 vs 0.5 s) in hPSC-cardiomyocytes treated for 24 h with 1, 5 or 10, but not 0.1 uM Doxorubicin. To more closely resemble the human heart compared to simple 2D monolayers, we established an advanced platform for creating 3D engineered heart tissues, so called EHTs. In this medium throughput platform 36 EHTs can be prepared per experiment with 3 technical replicates per condition. Robust formation of contractile EHTs allowed us to measure the effect of different concentrations of Doxorubicin on contractile force, a clinical relevant output. We quantified absolute force of contraction every 12 h and observed total loss of contraction force 72 h after treatment with 5 or 10 uM Doxorubicin. Similar to many novel drugs, the underlying mechanisms of cardiotoxicity from Doxorubicin are not fully understood. Despite DNA damage, apoptosis and oxidative stress, calcium accumulation has been one of the proposed mechanisms. We thus analyzed, expression of DNA damage marker pH2AX, apoptotic marker Annexin 5, cell rox and calcium accumulation after treatment with increasing concentrations of Doxorubicin. Most interestingly, we discovered that 1 uM of Doxorubicin is enough to induce eviction of cardiac transcription factor Nkx2.5 from the nucleus 24 h after exposure to the drug, a possible novel mechanism of cardiotoxicity. In summary, these hPSC-based assays provide a versatile screening platform for assessment of cardiotoxicity. In our next steps, we will analyze different Doxorubicin analogues, which may ultimately lead to a better and safer treatment of patients.

Activity-dependent Gene Modulation in Glia-derived Induced Neurons

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Generation of neurons through overexpression of fate-determining transcription factors is widely used to study neurodevelopmental mechanisms such as cell fate differentiation, to model neurological diseases or develop therapeutic approaches to neurodegenerative conditions. Cortical astrocytes can be reprogrammed into glutamatergic forebrain neurons through overexpression of the basic-helix-loop-helix (bHLH) transcription factor Neurog2. Over a period of 2-6 weeks in vitro, the reprogramming process stably induces properties such as a distinctly complex neuronal morphology, expression of neuron-specific proteins and the gain of two principal functional properties of neurons, namely action potential firing and establishment of functional synaptic connections. Albeit characterization of such iNs has been focusing on their electrophysiological phenotype, marker expression or transcriptomic analysis, little is known regarding whether and to which extent iNs undergo lasting transcriptional and functional changes in response to changing network activity patterns. Here, we have established an in vitro model for assessing iN plasticity by co-culturing murine postnatal astroglia one day after initiation of Neurog2-induced reprogramming, together with pre-existing cortical neurons that have already established a robustly active network. We show that iNs mature and integrate into the cortical network with functionally active synaptic connections being established between iNs and cortical neurons. We show that iNs participate in coordinated patterns of network activity and express several activity-regulated genes, which are crucial for different forms of homeostatic plasticity. Following iN integration, we performed a two days long pharmacological inhibition of network activity with subsequent single-nucleus RNA-sequencing. Preliminary analysis reveals that iNs and cortical neurons differ in their level of maturation, with iNs occupying an intermediate position between astrocytes and cortical neurons. In spite of the presence of distinct clusters within each neuronal population, mapping single-nucleus profiles onto existing single-cell CNS datasets points to a dorsal forebrain neuronal identity of iNs. Ongoing analysis is focusing on identifying differentially expressed genes upon activity modulation in iNs and cortical neurons.

Investigation of different cell sources and their influence on endothelial cell network quality

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Introduction: One of the leading problems of modern-day medicine is the shortage of tissue and organ donors. The field of tissue engineering aims to solve this problem through generation of bioartificial organs and tissues. For achieving clinically relevant dimensions the establishment of a perfusable vascularized system is mandatory. Stability of such network is dependent on the presence of supporting cells, named pericytes (PCs). Various cell sources have the ability to act as PCs, but the absence of single cell-specific markers, the biological variability, and also study-dependent marker criteria hinder the accuracy of gathered data for PC-like cells. We aim to investigate different cell sources on a functional and molecular level for their ability to act as PCs.

Methods and Results: Three different cell types were investigated for PC function by employing a 3D hydrogel constructs. For the generation of these constructs GFP labelled human umbilical vein endothelial cells (GFP-HUVECs) and respective cell types were mixed with a hydrogel containing rat tail collagen. The mixture was cast onto decellularized porcine small intestine submucosa (SIS) mounted in a metal frame serving as a carrier matrix. The formation of a GFP-HUVEC network and the stability of the network was analysed.

In this setting, human adipose tissue-derived stroma cells (hASCs) and human bone marrow mesenchymal cells (hBM-MSCs) were supporting the formation of an endothelial cell (EC) network which was stable for at least 14 days. On the contrary, constructs containing human foreskin fibroblast (HFFs) showed weaker stability of the EC network than hASCs and hBM-MSCs based constructs.

Additional experiments showed that direct contact between supporting cells and ECs was needed for network stability. Therefore, membrane bound and intercellular proteins were investigated to understand the underlying mechanism of communication between EC and supporting cells. A ready-to-use array enabled the screen of more than 300 membrane bound proteins. The screen was conducted for two independent isolations of hASCs, one isolation of hBM-MSCs, and one isolation of HFF. For most of the analysed membrane-bound proteins a negative and a positive fraction was detected within tested cell source. By eliminating proteins that HFFs have in common with hASCs and hBM-MSCs, the number of possible surface markers of interest, was narrowed down. Analysis revealed around 30 membrane proteins as candidates for further analysis including LAP, EphB4, AN2 and syndecan.

Furthermore, immunohistochemistry was used to observe intracellular proteins in supporting cells. So far, ACTA2 and transgelin have been shown to be expressed in hACSs and hBM-MSCs, but are absent in HFFs in respective constructs.

Conclusion and Further Plans:

hASCs and hBM-MSCs are a reliable source of PC-like cells supporting EC network formation and stability. Heterogeneity of surface markers within the cell population indicates the existence of subpopulations. ACTA2 and transgelin were only expressed in hACSs and hBM-MSCs and not on HFFs in the constructs suggesting their importance for EC network stability.

Upcoming investigation of around 100 angiogenesis-related genes on RNA level and gene silencing starting with ACTA2 lentiviral based sh-RNA, will bring further insight to ECs and PCs communication.

Developing Novel Methods to Protect Endogenous Tenocytes from Inflammation using Equine Embryonic Stem Cells

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Tendon injuries occur commonly in human and equine athletes, and poor tendon regeneration leads to the formation of biomechanically inferior scar tissue which is prone to re-injury. The comparable nature of certain equine and human tendons essential for efficient high-speed locomotion makes the horse an attractive large animal model for the study of human injury. Despite the prolonged recuperation periods, increased retirement rate, and substantial welfare burden associated with tendinopathy, these injuries remain poorly understood. This has motivated the development of model systems which further investigate tendinopathy and allow the advancement of regenerative strategies which aim to restore the functionality of tendon tissue post-injury. Equine embryonic stem cells (ESCs) have been derived successfully and can be differentiated into tenocytes in vitro, making them the ideal model to improve our understanding of tendinopathy. In vivo, equine ESCs differentiate into tenocytes following their injection into injured equine tendon and survive at high and consistent levels (>60%) with no detrimental effects detected, but the long-term consequences are unknown. Modern molecular techniques have identified a strong inflammatory component to tendinopathy onset and progression, with upregulation of cytokines including IFN-γ, TNFα and IL-1β. Previously, IL-1β was demonstrated to exhibit adverse effects on the functional ability of adult tenocytes. Nevertheless, these consequences could be rescued by using exogenous IL-1 receptor antagonist protein (IL1Ra). However, in vivo, other inflammatory cytokines are present, and we evidence IL1Ra administration alone fails to have any beneficial effects on adult tenocytes stimulated with multiple cytokines simultaneously. Our aim is to identity novel methods to protect adult tenocytes from the negative consequences of inflammatory cytokines on matrix remodelling and gene expression. We demonstrate that in combination, IFN-γ, TNFα and IL-1β have even greater adverse effects on adult tenocytes, increasing matrix metalloproteinase and tenascin-C expression, and decreasing the expression of scleraxis and cartilage oligomeric matrix protein. When cultured in 3-dimentional collagen gels, adult tenocytes exposed to IL-1β, TNFα and/or IFN-γ have a reduced contraction ability, attributed to diminished matrix reorganisation. In contrast, equine ESC-derived tenocytes exposed to IL-1β exhibit no detrimental effects on tendon gene or matrix metalloproteinase expression. Additionally, following exposure to combinations of IL-1β, TNFα and IFN-γ, equine ESC-derived tenocytes generate tendon-like constructs in vitro indistinguishable from controls. To determine the mechanisms behind ESC-derived tenocytes inflammatory protection, we identified the inflammatory signalling pathways activated during tendinopathy. The proinflammatory transcription factor nuclear factor NF-κB regulates the expression of over 500 genes involved in inflammation and immunoregulation. Using immunocytochemistry, we demonstrate that the NF-κB pathway is activated in adult tenocytes within one hour of stimulation with combinations of IFN-γ, TNFα and IL-1β. In contrast, NF-κB was not shown to be activated by inflammatory stimulation in equine ESC-derived tenocytes. Successfully identifying important inflammatory signalling pathways using in vitro models will enable the future development of novel pharmaceutical interventions which protect endogenous tenocytes from the adverse consequences of inflammation during tendinopathy. In turn, this will result in major economic and welfare benefits to both equine and human athletes.

Reprogramming and rejuvenation trajectories of the direct conversion of human fibroblasts into induced neural stem cells

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The reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) provided new opportunities for disease modeling and drug discovery. However, during reprogramming the cells are epigenetically rejuvenated, therefore, iPSC-based models are not optimal for studying age-related mechanisms and pathologies. This challenge can be bypassed through the direct conversion of differentiated cells directly from one cell type to another. Direct conversion of adult dermal fibroblasts (ADFs) into induced neurons (iNs) circumvents the rejuvenation, which allows deciphering mechanisms of cellular ageing. The rejuvenation associated with conversion into proliferating induced neural stem cells (iNSCs) has been poorly investigated. In this study, we aim to study the reprogramming trajectories during the direct conversion of ADFs into iNSCs, by overexpressing the OSKM transcriptional factors and subsequently inducing the neural fate by culturing the cells in a chemically defined medium, containing small molecule inhibitors modulating the WNT and TGFβ pathways. Furthermore, we will compare the epigenomic profiles between iNSCs, iNs and iPSC-derived NSCs when converted from ADFs of various age groups. For this purpose, we will employ the CellTagging barcoding methodology to label the cells with 8bp random barcodes at day 0, day 7, day 13 and day 30 during the direct conversion process and collect samples for analyzing the transcriptome and epigenome on a single-cell level. In order to adapt the CellTagging protocol to the direct conversion of human iNSCs, we performed a series of pilot experiments for defining the lentiviral barcoding efficiencies by transducing different cell densities of foreskin fibroblasts, ADFs and iNSCs with various viral concentrations, We transduced foreskin fibroblasts or ADFs with 0.5x, 1x, 2.5x and 5x viral concentrations. In the case of iNSCs, which were considered to be more fragile upon infection, we expanded

the viral concentration conditions by testing a range of 0.05x – 5x. The infection efficiency was validated by fluorescent microscopy of the GFP+ successfully transduced cells and was quantified by flow cytometry analysis over a period of 10 weeks post-infection. This analysis revealed that foreskin fibroblasts or ADFs were optimally infected with a viral concentration of 2.5x resulting in 98% transduction, while in the case of iNSCs the optimal viral concentration was 2x at 200,000 cell number, leading to 95% successfully transduced cells. Moreover, we concluded that polybrene does not appear to increase the percentage of the GFP+ cells and in the case of the iNSCs leads to massive cell death and stress. In the next steps of our study, we will perform the sequencing of the barcode library and generate a "whitelist" that will be used for the filtering of the CellTags in our final analysis. Finally, we will proceed with the direct conversion experiments, followed by single-cell transcriptomics, high-resolution lineage analysis, and DNA methylation analysis. This study will help to exploit reprogramming technologies to gain further insight into neural ageing and regeneration pathways.

Investigation of m6A modified lncRNAs for glioma prognosis

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Introduction Gliomas are one of the most prevalent type of brain tumors. Even with optimal treatment, the prognosis of the most malignant form of glioma - glioblastoma (GBM) patients are dismal with an overall survival less than 15 months after diagnosis [1]. An unfavorable outcome of GBM patients, tumor relapse and lethality likely arise from glioma stem cell (GSC) subpopulation, which are highly tumorigenic and resistant to therapy [2]. Due to high mortality, it is necessary to identify molecular mechanisms underlying tumor progression and to find potential targets for therapy. There is emerging evidence that modifications in long non-coding RNA (lncRNA) are functionally significant and plays an important role in biological processes and diseases, including cancer [3–5]. Therapeutically controlling epitranscriptomic modification levels in cancer stem cells could be critical for maintaining its growth, self-renewal, and tumor development. Therefore, the aim of this pilot study was to evaluate the most m6A modified long non-coding RNA molecules in glioma stem cells as a potential molecular markers for glioma prognosis. Methods Glioblastoma stem-like cell line (NCH421k) and glioblastoma (U87 MG) cells were used for the experiments. RNA was extracted from cell cultures, polyA enriched and prepared for sequencing with direct RNA sequencing. LncRNA m6A epi-modifications were detected by direct RNA sequencing on MinION device (Oxford Nanopore) and analyzed with "MasterOfPores" and "Nanocompore" pipelines. The expression of TMEM99 and RNF216P1 in different grade glioma tumors were evaluated using data from TCGA and GTEx databases via the Xena and Gepia2 webtools. Results In total, 25 unique lncRNA genes and 77 lncRNA transcripts were detected. Out of which, TMEM99 and RNF216P1 lncRNAs were found to be the most differentially m6A modified between glioblastoma stem-like and differentiated glioblastoma cells. These lncRNAs had at least 75% differences of m6A modifications in 8 and 4 different sites (TMEM99 and RNF216P1, respectively) comparing U87 and NCH421k cells. The contrasting TMEM99 motifs were found in 1st and 3rd exons and RNF216P1 motifs in 3rd and 6th exons. In addition, TMEM99 expression was 126 times higher in U87 as compared to NCH421k cells (22.8 vs 0.18 (TPM)), whereas RNF216P1 expression was similar in both U87 and NCH421k cells (7.6 vs 9.5 (TPM)). These results were in line with the TCGA and GTEx database analysis; TMEM99 and RNF216P1 genes were overexpressed in higher grade glioma tumors and associated with poor overall survival prognosis of glioma patients, suggesting their importance in tumorigenesis process. Conclusion The research proposes that m6A epitranscriptomic modification of TMEM99 and RNF216P1 could be an important factor for evaluating glioma cell stemness. However, these results are only preliminary and should be validated in a wider glioma patients' cohort. Nevertheless, this pilot study indicates the potential of m6A modifications in evaluating similarly expressed lncRNAs and their importance to glioma characteristics.

The Connection Between Deregulated Glioma Stem Cells Genes and GBM Subtype

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Background. Glioblastomas (GBM) are the most common, aggressive, and lethal malignant brain tumor in adults. GBM Patients' median overall survival after the diagnosis does not exceeds 15 months even administering optimized therapy [PMID:27157931]. Even though histological characteristics of tumors appear to be very similar, the molecular landscape of GBMs evidently indicates very high inter-and intra-tumor heterogeneity. The heterogeneity of the GBMs is still indicated as one of the main challenges in the treatment of the disease, therefore molecular GBM classification is applied to classify GBM into smaller and more homogeneous groups. The pivotal GBM classifier proposed by Verhaak et al., 2010 [PMID:20129251] identifies four GBM subtypes, nevertheless, the impact of tumor-initiating and therapy-resistant cells - glioma stem cells (GSC) signature for GBM classification is poorly analyzed up to date. The aim of the study was to evaluate if the level of specific GSCs genes are associated with GBM subtype and patient survival. Materials and methods. RNA-seq analysis was performed on MinION Oxford Nanopore sequencing system and analyzed with "MasterOfPores" pipeline on GenomeDK server. Human glioblastoma derived GBM stem-like cells NCH421K and human glioblastoma cell line U87MG cultivated on standard conditions were applied for sequencing analysis. RNA from cell pellets was purified using TRIzol, Dynabeads mRNA purification system (#61012) was used for polyA enrichment and direct RNA sequencing (#SQK-RNA002) kit was used to prepare 3'-polyA RNA for sequencing. Affymetrix HG-U133a data of GBM expression (n=539) available at TCGA coordination center was used to evaluate GSCs specific genes expression in GBM subtypes. Orange Data Mining machine learning and data visualization soft was used for data analysis. Results. Nanopore-seq analysis identified 38891 transcripts, which were mutual in GCSc and U87MG cells. In total 860 of the most deregulated transcripts between NCH421K and U87MG cells were selected for the study. Univariate linear regression analysis was applied to narrow down gene list to 30 the most informative genes, which were used for the analysis. The higher expression of selected genes were characteristic in mesenchymal and partially classical GBM subtypes, while lower levels were observed in proneural subtype. Current finding meets the provision that NCH421K cells are described as mesenchymal GCS [PMID:25806680], therefore high mRNA expression of the same genes are characteristic in mesenchymal GBM subtype specimens and NCH421K. After hierarchical clustering patients were divided into 3 expression groups and patients in high-level group showed worse overall survival compared to other groups (LogRank p

A self-generated Toddler gradient directs mesodermal cell migration during zebrafish gastrulation

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Directed cell migration is one of the underlying mechanisms that coordinate essential tissue arrangements during embryogenesis. The majority of these arrangements occur during gastrulation, during which complex global cell movements form the three germ layers. In gastrulating zebrafish embryos, mesoderm is specified at the margin and then undergoes directed migration to the animal pole. However, the molecular mechanisms underlying mesoderm guidance have remained a mystery. Traditionally, directed cell migration is guided by a chemokine gradient that arises from a localized source. In contrast to this idea, we discovered that mesoderm migration is guided by a self-generated gradient of the small protein Toddler/Apela/ELABELA, and that gradient formation is coordinated by its own receptor, the Apelin receptor (Aplnr), which is expressed in the responding mesodermal cells. Our studies show that Toddler acts as a chemoattractant for Aplnr-expressing cells, yet rescue of mesoderm migration defects in toddler mutants is independent of the site of Toddler expression. To reconcile these contradicting results, we combined computational modeling and experimental approaches to show that (i) the location of the sink, not the source, determines direction of mesoderm migration, (ii) a collective of Aplnr-expressing cells, but not an individual cell, can undergo directed migration in a uniform Toddler environment and (iii) Aplnr takes on a dual role by providing a sink for Toddler at the margin and sensing the self-generated gradient to drive mesoderm migration. Taken together, our work provides a compelling explanation for the long-standing question of how mesodermal cells are directed to the animal pole during zebrafish gastrulation. This model of a self-generated gradient, in which generation and reading of the guidance cue are mediated by a single receptor, provides a simple yet robust mechanism for mesodermal cells to steer their own directional migration.

Modelling Spastic Paraplegia and Psychomotor Retardation with or without Seizures (SPPRS) Using Patient-Derived Stem Cells

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Spastic Paraplegia and Psychomotor Retardation with or without Seizures (SPPRS) is a rare autosomal recessive neurodevelopmental syndrome caused by HACE1 deficiency. The most common features amongst affected individuals include hypotonia, intellectual disability, cerebral atrophy with enlarged ventricles, and hypoplastic corpus callosum. Additionally, HACE1 has been shown to be downregulated in certain tumours. Current models to study SPPRS are murine HACE1 knockout lines and patient-derived fibroblasts. In order to better understand the effect of this mutation on human development, we used induced pluripotent stem cells (iPSCs), small molecule-derived neural progenitor cells (smNPCs), and neurons derived from two affected individuals and a control line. Our aim is to recapitulate the findings from previous models and to identify novel disease features. The findings suggest that HACE1-deficient iPSCs and smNPCs express lower levels of pluripotency and neural progenitor markers, respectively. However, in patient-derived neurons, both neuronal and astrocytic markers were upregulated compared to the control. In all cell types, there were indications for dysregulation of cancer pathways in HACE1-deficient lines, as well as increased production of reactive oxygen species (ROS). The results imply that HACE1 is an important regulator for progenitor maintenance and the preservation of healthy status. Stem cells are an important tool to model neurodevelopmental disorders because they allow the study of neuronal cells and progenitors in a human context. Moreover, a better understanding of the role of HACE1 will shed a light into other disorders in which it is involved, such as tumour formation and neurodegenerative diseases.

Myoblast differentiation, fusion and oncogenesis are influenced by the syndecan-4-mediated activation of Rac1 GTPase

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Skeletal muscle is constantly renewed in response to injury, exercise, or muscle diseases. The ubiquitously expressed, transmembrane, heparan sulfate proteoglycan syndecan-4 (SDC4) influences cell-matrix adhesion, cytokinesis, cell migration by regulating the activity of Rac1 GTPase. The Rac1-mediated actin remodelling is required for myoblast fusion. Here we demonstrate that SDC4 knock-down increased the MyoD expression, differentiation index, fusion index, myotube size and length in C2C12 myoblasts. The Rac1 activity was required for the increased fusion of SDC4 silenced cells. Atomic force microscopy studies revealed that SDC4 silenced cells have reduced elasticity, and super-resolution direct stochastic optical reconstruction microscopy (dSTORM) images indicated nanoscale changes in the actin cytoskeletal architecture. The genomic analysis of human rhabdomyosarcoma samples showed SDC4 gene amplification in 28% of the Pax gene fusion negative tumors, and significantly higher expression of SDC4 mRNA. Our studies suggest that the gradually decreasing level of SDC4 during myoblast differentiation allows the activation of Rac1 leading to myoblast fusion, while the high SDC4 expression inhibits myogenesis and promotes oncogenesis. Our results contribute to the understanding of the role of SDC4 in skeletal muscle development, regeneration, and tumorigenesis. This research was supported by the National Research, Development and Innovation Office of Hungary [grant numbers: GINOP-2.3.2-15-2016-00040, EFOP-3.6.2-16-2017-00006 and NKFI FK 134684], and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

Development of a human in vitro model to study the effects of age on adult neural stem cell quiescence

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Adult neurogenesis is the generation of new neurons in the adult brain. In mice, neurogenesis occurs throughout life in certain areas of the brain including the dentate gyrus in the hippocampus. The new neurons integrate into the hippocampal circuitry, contributing to memory formation, spatial learning and behavior. These new neurons arise from adult neural stem cells (aNSCs), which are present in 2 states, the active and the quiescent state. The majority of the stem cells are in the quiescent state and transition to the active state to proliferate and produce new neurons. But aNSCs are not very efficient at generating new stem cells (self-renew) and thereferore, the stem cell pool declines with age. This causes a drop in neurogenesis with age that is exacerbated by the fact that the remaining quiescent cells enter a deeper quiescence, which makes them harder to activate. We know that the response of aNSCs to regulatory stimuli changes with age, but the underlying mechanisms behind this change are still unknown. NSCs can be derived from the embryonic and adult mouse brain at different ages, as well as differentiated from embryonic stem cells. We can maintain these cells in vitro and upon addition of BMP4, quiescence is induced. Using this system, we are exploring the effects of age on aNSC transitions between active and quiescent states. However, we are still lacking such in vitro system for studying of human NSC quiescence, mostly due to the limited accessibility of primary human material. For this, I am first establishing a novel human NSC quiescence model using human pluripotent stem cells that we can efficiently differentiate into hippocampal NSCs. In response to BMP4, these cells acquire quiescence, indicated by a drop in proliferation and upregulation of characteristic markers. In order to investigate the changes over time, we will compare directly reprogrammed NSCs, which maintain certain ageing characteristics, with our NSCs derived from human pluripotent stem cells, which correspond to embryoic brain stages. This system will allows us to investigate the mechanisms involved in the changing behaviour of aNSCs during ageing and directly compare the regulation of the quiescent state between mouse and humans.

Interplay of Oncogenic Fusions and Cellular Context in Sarcoma

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Our project tackles a fundamental question in cancer biology: how and why distinct genetic alterations promote cancer in one cellular context but not another. We focus on fusion oncogenes, which are cancer type-specific and tumorigenic only in certain cellular contexts. A major class of tumors driven by fusions are sarcomas. A systematic analysis of the molecular response of particular cellular contexts to different sarcoma-linked fusions will lead to mechanistic insights of carcinogenesis and to cell context-specific therapeutic vulnerabilities. We are using a human embryonic stem cell (hESC) line, which is capable of relocating a receptor-bound Cre recombinase (ERT2-Cre) into the nucleus upon Tamoxifen treatment. This flexible and versatile system is used to conditionally express different oncogenic fusion proteins during the course of developmental differentiation. We decided on a set of five different fusion proteins (EWS-FLI1, EWS-ATF1, EWS-WT1, EWS-NR4A3, FUS-DDIT3), all leading to individual types of sarcoma. With our platform, we are able to perform any given differentiation protocol linked to fusion protein induction at any given time point during development. In a first step, we are focusing on a high temporal resolution of fusion protein expression patterns within the mesenchymal lineage. We will use an unbiased embryoid body differentiation to capture a large variety of cell types within this trajectory. Along this 21 day differentiation protocol, we are going to induce fusion gene expression on ten different time points and analyze transcriptional profiles of single cells. Together with expression levels of the oncogenic fusion proteins, we will be able to identify specific cell types susceptible to oncogenic transformation. In a next step, we will compare certain cell state/fusion gene combinations to single cell sequencing data of individual sarcoma tumors, aiming to identify essential mutations, transcription factors or active regulatory elements, necessary for full oncogenic transformation. Finally, we will be able to draw a blueprint for individual oncogenic fusion proteins and the required cell state, expression timing and (epi)genetic landscape needed for robust and accurate in vitro and in vivo models. Beyond that, this information will be invaluable to describe detailed mechanistic and regulatory pathways of sarcoma development further enhancing targeted treatment options.

Genome-engineered human stem cells to study the pathophysiology of ASD related VGCC mutations.

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Voltage-gated calcium channels (VGCCs) are key-modulators of early neural development and play an import role in maintaining functionality of the adult human brain. Mutations within VGCCs are known to contribute to a wide range of neurodevelopmental and neuropsychiatric disorders, such as Schizophrenia, Epilepsy and Autism-Spectrum-Disorder (ASD). However, inaccessibility to human brain tissue represents a major roadblock when aiming to study the biomolecular mechanisms underlying these disorders. Therefore, in vitro approaches exploiting the differentiation potential of human induced pluripotent stem cells (iPSC) and human neural progenitor cell (NPC) lines are nowadays widely used to gain insights into molecular aspects of neurodevelopment and related disorders. The aim of this study is to decipher the molecular role of two de novo mutations in the pore-forming Cav1.3 VGCC α1-subunit encoding gene CACNA1D, which have previously been reported in two patients with a neurodevelopmental disease spectrum including ASD. Previous electrophysiological studies in tsA201 cells indicate that these mutations affect the gating properties of Cav1.3 channels permitting a gain-of-channel-function. However, up to date, no data is available on how these mutations affect disease-relevant human neurons. To investigate the pathophysiology of these mutations more thoroughly, mutated human iPSC and NPC lines will be generated through means of CRISPR/Cas9 technology. These cell lines will subsequently be subjected to in vitro differentiation protocols to obtain disease-relevant human cortical glutamatergic, midbrain dopaminergic and striatal GABAergic medium spiny neurons. Through immuncytochemical stainings, qPCR analysis, electrophysiological recordings and calcium imaging we will investigate how these mutations interfere with early neurodevelopment and what functional alterations they cause in the fully differentiated neurons. A central prerequisite for this study will be the analysis of the temporal gene expression pattern, not only of CACNA1D, but also of other VGCC subunit encoding genes, and their splice variants, throughout various stages of differentiation. This will broaden our knowledge regarding the role of VGCC subunits and splice variants during early neurodevelopment and will serve as a theoretical basis for the later disease modeling. Overall, these experiments will deepen our understanding of the molecular pathophysiology underlying ASD, as well as related neuropsychiatric disorders. Moreover, the generated cell lines may be used as drug screening platforms for pharmacological modulators in the future and by that build the basis for novel therapeutic approaches.

Optimized culture conditions to generate fully-formed 3D human retinal organoids to model inherited blinding diseases

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Inherited retinal diseases (IRD) are a major cause of blindness worldwide for which no therapies are currently available. Small and large animal models are available for IRD. Although these models have proven critical to understand the molecular bases of the corresponding IRD and to test potential therapies, they still pose important challenges due to species-specific differences with humans. Human three-dimensional (3D) organ and tissue cultures may provide unique, patient-specific models to study both disease mechanisms and the efficacy of novel therapies. We implemented our 3D retinal organoids differentiation protocol by culturing them in a bioreactor which improves aeration and nutrient distribution resulting in high photoreceptors yields with increased rhodopsin localization and outer segments formation with developing membranous discs. In addition, our Quant-seq analysis revealed that human retinal organoids closely mimic human fetal retina. These findings support retinal organoids as models to phenocopy human retinal diseases and as a reliable and renewable source of transplantable cells for personalized therapies.

Human Bone Marrow Adipose Tissue: Novel Stem Cell Niche Complexity and Perspective

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The development of bone marrow adipose tissue (BMAT) occurs throughout the lifespan as a repercussion of red-to-yellow conversion of bone marrow (BM), and independently of peripheral fat accumulation. While the cellular origin and physiology of BMAT is mostly studied in mouse models, in depth knowledge of human BMAT is still lacking. As an energy-rich hub of the stromal-reticular network, BMAT is recognized as an endocrine organ and a nutrient interface between diverse BM cellular compartments. Since adult BM is a main source of cells with multiple regenerative properties, understanding of the contribution of BMAT to stem cell phenotypes is a paramount aim of the fundamental investigations. Our current research on human BMAT brings a strategy for more accurate evaluation of BMAT cell phenotypes and metabolic activity. To achieve this, we isolate and compare BMAT and subcutaneous peripheral adipose tissue (PAT) form matched donors. Based on tissue digestion, further separation of BMAT and PAT into adipocyte-enriched as well as non-adipocytic cell fractions, allowing us to study the BMAT-associated cells. Ex vivo and in vitro analyses of BMAT-associated cells indicate increased presence of the cells with multiple differentiation potential (osteogenic, adipogenic and chondrogenic) and endothelial phenotype than in liquid phase of BM, while comparable to those in PAT. On the other side, BMAT, and particularly PAT, contain lower frequency of cells with hematopoietic potential. Interestingly, our findings suggest presence of undifferentiated cells within separated adipocyte-enriched fraction of BMAT, in the close proximity to mature adipocytes. Bioenergetic states and metabolic plasticity of BM, BMAT and PAT adipocytes as well as non-adipocytic cells, appeared to be distinct. Our further research will identify and characterize the BMAT precursor population at single-cell level, evaluating the complex interplay of their differentiation commitment, metabolic profile, mitochondrial biogenesis and epigenetic mark. Deconvolution of cellular phenotype that contributes to the integrity and heterogeneity within the BMAT niche will contribute to understanding of BMAT, not only as regulator, but as a peculiar stem cell niche. In addition, this might define novel therapeutic strategies to target BMAT-contributing cells, to combat undesirable BMAT expansion during aging and pathologic process. Keywords: bone marrow, adipose tissue, stem cells, metabolism, differentiation

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