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Programme

Wednesday, 23rd March 2022

13:00 Welcome and introduction

Keynote lecture 1
Chair: Noelia Urban
13:15 James Briscoe (The Francis Crick Institute) About Time: The Dynamics of Neural Tube Development
14:15 coffee break & group picture

Session 1 - morphogenesis and signaling dynamics
Chair: Anna Kicheva
14:45 Katharina Sonnen (Hubrecht Institute) Signaling dynamics in the control of embryonic development and tissue homeostasis
15:15 Diana Pinheiro (IST Austria) Morphogen gradient orchestrates pattern-preserving morphogenesis via motility-driven (un)jamming
15:45 Yasuhiro Takashima (Kyoto University CiRA) Modeling in vitro embryonic development using naive human pluripotent stem cells
16:05 break

Session 2 - synthetic embryology / self-organization
Chair: Vincent Pasque
16:35 Leonardo Morsut (University of Southern California) Learning to Program Tissue Development with Artificial Genetic Circuits
17:05 David Oriola Santandreu (EMBL Barcelona) Towards a mechanochemical basis of symmetry breaking in a mammalian embryo-like system
17:35 Naomi Moris (The Francis Crick Institute) In vitro 3D gastruloid models of mouse and human development
17:55 coffee break

18:25 Harunobu Kagawa (IMBA Vienna) Four features of human blastoids that model human blastocysts
18:45 Siddarth Dey (UC Santa Barbara) Integrated single-cell sequencing reveals principles of epigenetic regulation of human gastrulation and germ cell development in a 3D gastruloid model

19:15 Symposium dinner
Thursday, March 24th

Session 3 - mechanobiology & cell fate transition
Chair: Ulrich Technau
09:00 Kevin Chalut (University of Cambridge) Mechanobiology of cell fate transitions
09:30 Marta Shahbazi (MRC Laboratory of Molecular Biology) Coordinating cell fate decisions and tissue shape changes during mammalian development
10:00 Charisios Tsaiiris (FMI Basel) Mechanical tissue stretching drives Wnt organizer establishment in Hydra
10:20 coffee break
10:50 Cornelia Schwayer (FMI Basel) Symmetry breaking in intestinal organoid formation
11:10 Alexandre Mayran (EPFL Lausanne) Cell-specific coordination of epithelial to mesenchymal transition potentiates gastruloids self-organization
11:30 coffee break

Session 4 - development & disease
Chairs: Sofia Grade & Frank Edenhofer
12:00 Federico Mauri (Boehringer-Ingelheim) Switching to the dark side: role of NR2F2 in regulating tumor malignant progression, stemness and maintenance.
12:30 Mercedes Paredes (UCSF) Late to the Game: Human Cortical Interneuron Development
13:00 Marcella Birtele (University of Southern California) The autism-associated gene SYN-GAP1 modulates human cortical neurogenesis
13:20 lunch
14:00 Meet-the-editor
14:20 Poster session 1
16:20 Agnete Kirkeby (University of Copenhagen) Repairing and modelling the human brain with stem cells
16:50 Oliver Harschnitz (Human Technopole) Human stem cell models to study host-virus interactions in the central nervous system
17:10 Aleksandra Savina (Institut Cochin) Single-cell transcriptomics reveals age-resistant maintenance of cell identities, stem cell compartments in naked-mole rats
17:30 Simona Lodato (Humanitas University) 3D Human Cortical Organoids to investigate early cortical activity and developmental and epileptic encephalopathy
18:00 end of talks – free evening for attendees
Friday, March 25th

Session 5 - metabolic control of stem cells
Chair: Stephanie Ellis
09:00 Nina Cabezas-Wallscheid (MPI of Immunobiology and Epigenetics) Regulation of dormant hematopoietic stem cells
09:30 Marlen Knobloch (University of Lausanne) How lipid droplet availability affects neural stem cell behaviour
10:00 Noelia Urban (IMBA Vienna) A full, dynamic view of neural stem cell quiescence
10:20 coffee break

Session 6 - cell identity and cell fate decision
Chair: Graziano Martello
10:50 Sven Falk (FAU Erlangen-Nürnberg) Molecular control of cellular identity acquisition
11:20 Merrit Romeike (Max Perutz Labs) Stem cell specific interferon stimulated gene expression is regulated by the formative pluripotency network through IRF1
11:40 Tom Wyatt (Wellcome-MRC Cambridge Stem Cell Institute) Patterning from the bottom up: hPSC patterning via spatially controlled stimulation from the basal side
12:00 coffee break
12:30 Moritz Mall (DKFZ Heidelberg) Transcriptional safeguarding mechanisms enable development and prevent disease
13:00 Antoine Zalc (Cochin Institute) Murine cranial neural crest cells reawaken pluripotency programs
13:20 Irene Talon (KU Leuven-University of Leuven) Polycomb repressive complex 2 restricts human trophoblast induction
13:40 lunch
14:40 Poster session 2

Keynote lecture 2
Chair: Christa Bücker
16:10 Kathrin Plath Dosage compensation of the X chromosome: An epigenetic phenomenon that teaches us how functional nuclear compartments form and how pluripotent cell states differ
17:10 closing remarks
17:25 networking with snacks & drinks
Keynote lecture 1

**About Time: The Dynamics of Neural Tube Development**

James Briscoe  
The Francis Crick Institute

The embryonic development of the vertebrate neural tube is a dynamic process coordinated by intercellular signaling that directs a gene regulatory network to assign cell fate. At the same time tissue growth and differentiation alters the arrangement and number of cells, contributing to the elaboration of pattern. Together these mechanisms determine the pattern, pace, precision, and proportion of the forming neural tube. Thus, accurate development of the neural tube and the specification of neuronal subtype identity relies on the interplay of cellular and molecular processes.

Keynote lecture 2

**Dosage compensation of the X chromosome: An epigenetic phenomenon that teaches us how functional nuclear compartments form and how pluripotent cell states differ**

Kathrin Plath  
David Geffen School of Medicine, UCLA, Los Angeles, USA

X chromosome dosage compensation represents an epigenetic phenomenon where coordinated regulation of a whole chromosome is required to compensate the imbalance of X-linked gene dosage between the sexes. We study this process in placental mammals, where X chromosome dosage compensation occurs through X chromosome inactivation (XCI), which results in the formation and maintenance of the silent nuclear compartment of the inactive X-chromosome (Xi). XCI is an essential developmental process in which roughly a thousand genes are silenced by the non-coding RNA Xist and therefore offers the unique opportunity to understand mechanistically how RNA molecules can establish a distinct nuclear compartment. The female-specific mosaicism resulting from random inactivation of one of the two X-chromosomes impacts human health as it affects the outcome of X-linked diseases. Our recent advances in studying the complex interplay among Xist RNA, interacting proteins, chromatin and transcription will be presented. The talk will also address how a distinct X chromosome dosage compensation process is employed in early human development and our efforts towards developing unique treatments for X-linked diseases.
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Session 1 - morphogenesis and signaling dynamics

Signaling dynamics in the control of embryonic development and tissue homeostasis

Katharina Sonnen
Hubrecht Institute

How information is transmitted between cells to govern development and tissue homeostasis in time and space remains a central question in biology. In particular, the role of signaling dynamics in this control is still largely unknown. While dynamic signaling has been found in various multicellular model systems, its function and mechanism has mainly been studied in cultures of single cells. We employ a combination of ex vivo and in vitro culture systems with high-resolution imaging of dynamic signaling reporters and microfluidics-based perturbation of signaling dynamics to dissect the function of such dynamics both during embryonic development and adult tissue homeostasis. Here, I will discuss our latest findings on the role of signaling dynamics in the segmenting mouse embryo and homeostasis of the adult small intestine.

Morphogen gradient orchestrates pattern-preserving morphogenesis via motility-driven (un)jamming

Diana Pinheiro
IST Austria

Embryo development requires both biochemical signalling generating patterns of cell fates and active mechanical forces driving tissue shape changes. Yet, how these fundamental processes are coordinated in space and time, and, especially, how tissue patterning is preserved despite the complex cellular flows occurring during morphogenesis, remains poorly understood. Here, we show that a Nodal/TGF-β morphogen gradient orchestrates pattern-preserving mesendoderm internalization movements during zebrafish gastrulation by triggering a motility-driven (un)jamming transition. We find that graded Nodal signalling, in addition to its well-established role in mesendoderm patterning, mechanically subdivides the tissue into a small fraction of highly protrusive leader cells able to locally unjam and thus autonomously internalize, and less protrusive followers, which remain jammed and need to be pulled inwards by the leaders. Using quantitative modelling and genetic perturbations, we further show that this binary mechanical switch, when combined with Nodal-dependent preferential adhesion coupling leaders to followers, is critical for triggering collective and orderly mesendoderm internalization, thus preserving tissue patterning. This provides a simple, yet quantitative, framework for how a morphogen-encoded (un)jamming transition can bidirectionally couple tissue mechanics with patterning during complex three-dimensional morphogenesis.

Modeling in vitro embryonic development using naive human pluripotent stem cells

Yasuhiro Takashima
Kyoto University CiRA

Most of our knowledge about peri-implantation development is based on mouse models. However, recent single-cell RNA sequencing (scRNA-seq) analyses of pre-implantation embryos have revealed differences between humans and rodents. Differences in the peri-implantation stage are obvious even morphologically when comparing mouse and human embryos. For example, in humans, epiblast and primitive endoderm (hypoblast) make a bilaminar disc, but in mouse, one sees a cylindrical structure. In addition, human embryos at this stage contain the amnion and extraembryonic mesenchyme, which do not exist in rodents before gastrulation. Therefore, human models are ideal for understanding human development although ethical limitations restrict access to human uteri during peri-implantation.

To overcome these issues and achieve our objective, our group has established a series of methods and human cells that correspond to human peri-implantation embryos. The blastocyst contains three cell types, epiblast, hypoblast, and trophectoderm. We successfully induced trophectoderm(*1) from naive human pluripotent stem cells (PSCs) (*2), which share features with pre-implantation embryos.

In this presentation, I will present in vitro lineage specifications for human extraembryonic lineages from naive human PSCs and in vitro development during peri-implantation.

(*1) Io et al. Cell Stem Cell 2021; DOI: 10.1016/j.stem.2021.03.013
(*2) Takashima et al. Cell 2014; DOI: 10.1371/journal.pgen.1009587
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Learning to Program Tissue Development with Artificial Genetic Circuits
Leonardo Morsut
University of Southern California
Dept. of Stem Cell Biology and Regenerative Medicine, Keck School of Medicine
Dept. of Biomedical Engineering, Viterbi School of Engineering
An ultimate goal of synthetic development is the generation of functional tissue assemblies. A key question in this area is: can we design artificial gene circuits that program the development of user-defined, multicellular structures and functions, even beyond those achieved with naturally-evolved genomes? An answer to this question would be broadly enabling as it would expand the landscape of possible functional structures that can be currently built from cells. Here I will present advancement in this area, including development of synthetic cell-cell communication pathways, implementation of synthetic development trajectories in mammalian cells for patterning and morphogenesis of spheroids, synthetic pathways for functional differentiation into skeletal muscle cells, development of computational pipelines for rational design of genetic networks for morphogenesis. We hope our work will inspire next generation of genetic engineers to continue this ambitious line of research.

Towards a mechanochemical basis of symmetry breaking in a mammalian embryo-like system
David Oriola Santandreu
EMBL Barcelona
How animals establish the body plan is a fundamental problem in biology. Molecular cues polarize the early embryo, generating an orthogonal coordinate system for subsequent regional patterning. In vertebrates, extra-embryonic tissues play a major role in body axis formation by sending instructive signals. However, in some systems such as mammalian blastocysts, radial symmetry breaking is thought to occur spontaneously within a group of cells. Recently, the in vitro reconstitution of 3D tissues has provided an ideal framework to study embryonic development in the absence of exogenous signals. One such example are 3D gastruloids, embryonic stem cell aggregates that recapitulate the axial organization of post-implantation embryos. Here we focus on the first symmetry breaking event that establishes anteroposterior polarity in 3D mouse gastruloids. By means of mechanical measurements, single-cell sequencing, FACS sorting and mathematical modelling, we show that the mesodermal transcription factor Brachyury (Bra/T) controls tissue rheology and the patterning proportions of mouse ESC aggregates through a mechanochemical coupling between cell signalling and cell sorting. Additionally, mathematical modelling and experiments suggest that Fgf signalling is key to control Bra/T proportions in the 3D aggregates. Finally, we discuss on how the initial pluripotency state of the cells might affect the symmetry breaking dynamics of 3D gastruloids.

In vitro 3D gastruloid models of mouse and human development
Naomi Moris
The Francis Crick Institute - Stem Cell & Human Development Laboratory
Human development at peri-gastrulation stages has been challenging to study experimentally, due to ethical and technical limitations on the use of human embryos in research. While much has been revealed about this stage of development using model organisms, such as the mouse, it is becoming clear that species-specific differences can lead to altered dynamics and mechanisms during development. Recently, we described a human Pluripotent Stem Cell (hPSC)-based model, using 3D aggregates of cells under defined conditions to generate human ‘gastruloids’ (Moris et al., 2020). Like their mouse counterparts, human gastruloids spontaneously break symmetry, polarise their gene expression and undergo morphological elongation with derivative cell types from all 3 germ layers organised in a spatiotemporally reproducible manner. Importantly, we observed transcriptional signatures of coordinated somitogenesis along the anteroposterior axis of the elongated gastruloids, suggesting similarity to a 19-22-day-old human embryo. We are now using these human gastruloids to explore the impact of genetic and environmental perturbations on the coordination of development, with a goal towards establishing new, patient-relevant disease models for conditions including congenital abnormalities.
Four features of human blastoids that model human blastocysts
Harunobu Kagawa
Institute of Molecular Biotechnology Austria

The human blastocyst comprises an inner cell compartment, that gives rise to the fetus and to the yolk sac, surrounded by an epithelial layer of trophoblast cells (trophectoderm, TE) that fulfills the crucial functions of mediating the implantation into the uterus and of forming the placenta. Of note, preimplantation development is prone to failure thus forms a major roadblock to establish successful pregnancy. Also, only 40% of the fertilized egg reaches a blastocyst stage with quality standards during IVF procedures. Despite clinical significance, the mechanisms underlying human blastocyst development remains largely unknown. This is because human embryos are available in limited numbers for the research and are extremely difficult to experimentally manipulate. Thus, the human embryo model that faithfully recapitulates the morphology, cell types, and function will allow us to study early human embryonic development. We have now established a robust and efficient model of human blastocyst from naive human pluripotent stem cells (hPSCs) by triple inhibition of the Hippo, TGF-β, and ERK pathways. I will introduce the four key features of these models that strongly suggests that they faithfully recapitulate several aspects of human blastocyst development.

Integrated single-cell sequencing reveals principles of epigenetic regulation of human gastrulation and germ cell development in a 3D gastruloid model
Siddarth Dey
UC Santa Barbara

The emergence of different cell types and the role of the epigenome in regulating transcription is a key yet understudied event during human gastrulation. Investigating these questions remain infeasible due to the lack of availability of embryos at these stages of development. Further, human gastrulation is marked by dynamic changes in cell states that are difficult to isolate at high purity, thereby making it challenging to map how epigenetic reprogramming impacts gene expression and cellular phenotypes. To overcome these limitations, we describe scMAT-seq, a high-throughput one-pot single-cell multiomics technology to simultaneously quantify DNA methylation, DNA accessibility and the transcriptome from the same cell. Compared to recently developed multiomics single-cell sequencing technologies, scMAT-seq uses a series of barcoding and simultaneous reaction steps to make all three measurements from the same cell without requiring physical separation of the nucleic acids prior to amplification, enabling high-throughput processing of thousands of single cells per day with minimal loss of starting material. Applying scMAT-seq to 3D human gastruloids, we characterized the epigenetic landscape of major cell types corresponding to the germ layers and primordial germ cell-like cells (hPGCLC). As the identity of the progenitors that give rise to human PGCLCs remain unclear, we used this system to discover that the progenitors emerge from epiblast cells and show transient characteristics of both amniotic- and mesoderm-like cells, before getting specified towards hPGCLCs. Finally, as cells differentiate along different lineages during gastrulation, we surprisingly find that while changes in DNA accessibility are tightly correlated to both upregulated and downregulated genes, reorganization of gene body DNA methylation is strongly related to only downregulated genes, with genes that switch on displaying a lineage trajectory-dependent correlation with DNA methylation. Collectively, these results demonstrate that scMAT-seq is a high-throughput and sensitive approach to elucidate epigenetic regulation of gene expression in complex systems such as human gastrulation that are marked by rapidly transitioning cell states.
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Mechanobiology of cell fate transitions
Kevin Chalut
University of Cambridge

The role of mechanics in cell fate choice has been largely overlooked; however, mechanics plays a significant role in getting the right cells to the right place at the right time in development. My lab is investigating the interplay between mechanics and signaling in cell fate decisions, both in stem cells and the embryo. I will present our work showing how cell surface mechanics influences early embryonic spatial patterning and fate transitions in the mouse embryo. I will further discuss a hypothetical feedback loop between mechanics and signaling that has significant impact on cellular plasticity both in development and stem cells.

Coordinating cell fate decisions and tissue shape changes during mammalian development
Marta Shahbazi
MRC Laboratory of Molecular Biology, Cambridge, UK

Embryo development entails the generation of diverse cellular identities and tissues morphologies. Cells need to take the right decision, at the right location, at the right time, and this decision needs to be coordinated with concurrent changes in tissue organisation. The mechanisms that ensure the tight coordination between cell fate decisions and tissue shape changes remain poorly explored. To address this question, my group focuses on the development of the mammalian embryo at the time of implantation into the uterus, a developmental stage that involves a global transcriptional and morphological transformation. We have recently developed a new 3D culture platform that maintains mouse pluripotent cells as self-renewing organised epithelial structures. Under these conditions, cells closely recapitulate the organization and transcriptional profile of the embryonic epiblast tissue of early post-implantation mouse embryos. Moreover, we have uncovered a functional relation between epithelial architecture and pluripotency maintenance. Our work establishes these 3D epiblast stem cells as a tractable model of the post-implantation embryo. We are currently applying this system to deconstruct the complexity of mammalian development.

Mechanical tissue stretching drives Wnt organizer establishment in Hydra
Charisios Tsiairis
Friedrich Miescher Institute for Biomedical Research

Hydra is a simple animal whose body resembles a hollow tube organized along a single oral/aboral axis. An organizer located in the hypostome, the oral end, patterns the axis. Hydra’s mythical regeneration potential depends on the ability of a tissue fragment, or even a clump of cells, to spontaneously establish an organizer de novo. Such regenerating pieces of Hydra tissue fold in hollow spheroids that undergo osmotically driven inflation and deflation cycles during the period that an organizer emerges. We have found that the mechanical stretching of the tissue that accompanies the inflation stage of the oscillations is necessary for the proper regeneration of the tissue. The levels of tissue stretching are transformed into corresponding levels of HyWnt3 expression, a gene that is a marker and effector of the organizer identity. Indeed, we observe that overexpression of HyWnt3 is sufficient for the regeneration of the tissue in the absence of mechanical stimulation. Given the localized nature of the organizer we examine with light-sheet microscopy the heterogeneities of mechanical properties in the tissue that are leading to biased, localized expression of HyWnt3. Patterning of epithelial lumen via mechanical signals is a widespread phenomenon, while the connection of Wnt signaling with mechanical stimuli seems to have very deep roots in evolution.

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Session 4 - development & disease

Symmetry breaking in intestinal organoid formation

Cornelia Schwayer
Friedrich Miescher Institute for Biomedical Research

Symmetry breaking is fundamental during initial cell fate determination in early development and regeneration and is a process during which a homogeneous system adopts asymmetry. In a self-organizing system, cells sense and integrate cell-intrinsic cues that depend on the cell’s internal state and cell-extrinsic cues, which enable the emergence of patterning and the establishment of complex structures. To better understand the mechanism of symmetry breaking, we study the formation of self-organizing intestinal organoids. In a uniform growth-promoting environment, only a subset of intestinal stem cells differentiates into secretory cells, which is a necessary step for later crypt-villus axis formation. We have previously shown that cell-cell heterogeneous activity of the mechano-sensor Yap1 is necessary but not sufficient to trigger Dll1/Notch lateral inhibition resulting in the emergence of the first secretory cell. Therefore, the underlying mechanism of symmetry breaking remains largely open. To tackle this question at the transcriptional and phenotypic level, we combine single-cell RNA sequencing, high-throughput and multiplexed imaging with the mechanistic insights provided by live tracking through light sheet imaging, perturbation experiments and biophysical modeling.

We show that growth factor signaling (EGF/ERK) as well as metabolic reprogramming are involved in the self-organization and symmetry breaking of intestinal organoids. We will discuss how the cell’s metabolic and mechanical state integrate extrinsic cues such as growth factors and extracellular matrix cues to guide the decision-making process at the single cell level. This tripartite framework of investigating biochemical, metabolic and mechanical axes, will allow us to decipher how extrinsic cues are integrated in a cell state-dependent manner resulting in symmetry breaking during intestinal regeneration and providing details for the emergence of tissue patterning.

Cell-specific coordination of epithelial to mesenchymal transition potentiates gastruloids self-organization

Alexandre Mayran
Ecole Polytechnique Federal de Lausanne

Mouse embryonic stem cells (ESC) can be stimulated to undergo gastrulation-like events to form gastruloids, structures that mimic post-implantation embryos. They can be easily generated in high numbers and are thus a promising model permitting large scale mechanistic studies of early embryogenesis. Gastruloids undergo cell fate diversification, break symmetry, and self-organize along a single antero-posterior axis. Here, we sought to investigate the mechanisms at play during gastruloid’s self-organization and its interplay with cell fate specification. First, we identified a critical window of time in gastruloid development that limits self-organization competence. This period is associated to an epithelial to mesenchymal transition (EMT). Using a combination of single cell transcriptomic and imaging of fixed gastruloids at a high temporal resolution, we dissected the different steps of EMT and cell fate acquisition in gastruloids. We found that EMT completion, but not its initiation is regulated in a cell-specific manner. Then, we generated a serie of ESC lines to genetically dissect the EMT process and its function during gastruloid development. Interestingly, we found that not all EMT steps are required for self-organization. However, repression of E-Cadherin is crucial for proper gastruloid organization, and in its absence gastruloid frequently elongate along multiple axes. Finally, we found that interfering with the EMT also had a strong impact to gastruloid cellular composition where the proportion of cell identities as well as the timing of differentiation of the two major gastruloid lineages is severely affected. In summary, we propose that the interplay between cell fate acquisition and EMT is critical for gastruloid self-organization.

Switching to the dark side: role of NR2F2 in regulating tumor malignant progression, stemness and maintenance.

Federico Mauri
Boehringer-Ingelheim

During the transition from benign tumors to malignant carcinoma, tumor cells need to repress differentiation and acquire invasive features, but the mechanisms regulating this process remain poorly understood. Using transcriptional profiling of cancer stem cells from benign tumors and malignant skin squamous cell carcinoma (SCC), we identified the nuclear receptor NR2F2 as uniquely expressed in malignant SCC. Using genetic gain- and loss-of-function in vivo, we show that NR2F2 is essential for promoting the malignant tumor state by controlling tumor stemness and maintenance in mouse and human SCC. We demonstrate that NR2F2 promotes tumor cell proliferation, epithelial-mesenchymal transition and invasive features, while repressing tumor differentiation and immune cell infiltration by regulating a common transcriptional program in mouse and human SCC. Altogether, we identify NR2F2 as a key regulator of malignant cancer stem cells functions that promotes tumor renewal and restricts differentiation to sustain malignant tumor state.
Late to the Game: Human Cortical Interneuron Development

Mercedes Paredes
UCSF

Creating a functional cerebral cortex requires a series of complex and well-coordinated developmental steps. These steps have evolved across species coinciding with the emergence of cortical gyrification and more complex behaviors. The presence of diverse progenitor cells, a protracted timeline for neuronal migration and maturation, and diverse neuronal types are developmental features that have emerged in the gyrated cortex. These properties could explain how gyrencephalic brains, such as in the human, has expanded in size and complexity. We discuss the molecular properties and organization of neural stem cells and migratory young neurons that persist in the infant brain yet disappear during infancy. Neural progenitor cells in the human medial ganglionic eminence, the birthplace of interneurons. This region harbors aggregates, or nests, of cells expressing early neuronal markers that continue to divide and produce GABAergic interneurons. This MGE-specific arrangement of neuroblasts in the human brain is present until birth, supporting expanded neurogenesis for inhibitory neurons. We also discuss how inhibitory neurons persist as extensive migratory streams of neurons targeting higher-order cortical regions in the frontal and temporal lobes. Using gyrencephalic models of postnatal cortical development, we outline the pathways and molecular composition of “late” migratory neurons. We propose protracted neuronal development as a mechanism for increased complexity and cognitive flexibility in larger, gyrated brains.

The autism-associated gene SYNGAP1 modulates human cortical neurogenesis

Marcella Birtele
University of Southern California

Advances in human genomics have dramatically accelerated our understanding of the genetics of neurodevelopmental disorders (NDDs), including autism spectrum disorders (ASD). De novo mutations in a variety of synaptic protein genes have been reported in individuals with ASD, making it a prototypical synaptopathy. However, it is important to characterize the role that classically defined synaptic protein genes have at different developmental stages with longitudinal modeling in a human cellular context. Here we utilize a human induced pluripotent stem cell-derived cortical organoid model to dissect the role of SYNGAP1, a Synaptic GTPase Activating Protein, which is amongst the highest confidence risk factors for ASD. For the first time, we reveal the expression of SYNGAP1 in human radial glia progenitors (hRGPs). We show that SYNGAP1 is highly expressed within the apical domain of hRGPs lining the wall of the developing ventricular zone. In a cortical organoid model of SYNGAP1 haploinsufficiency, we show a dysregulation in the filamentous actin dynamics of the hRGP. Disruption of cytoskeleton dynamics impairs the scaffolding and the division plane of the hRGPs ultimately resulting in disruption of cortical plate lamination and in accelerated cortical neurogenesis. Overall, our work reframes our understanding of the impairments in neural circuit function observed in SYNGAP1 patients by connecting it not only with the well-known alteration in synaptic transmission, but also with early developmental defects. Finally, this discovery highlights the importance of unraveling the stage specific function of genes associated with NDD to uncover new avenues for therapeutic interventions.

Repairing and modelling the human brain with stem cells

Agnete Kirkeby
University of Copenhagen

Agnete Kirkeby is an Associate Professor and group leader at the Department of Neuroscience at University of Copenhagen (Denmark) and at the Wallenberg Center for Molecular Medicine at Lund University (Sweden). Agnete and her group has unique expertise in using human pluripotent stem cells for production of subtype-specific human neurons, and the group has developed various protocols for accurate patterning of neural cells of different regional fates. In particular, the work of Dr. Kirkeby focuses on producing human neurons for regenerative therapy and disease modelling, i.e. dopaminergic neurons, hypothalamic neurons, telencephalic interneurons, basal forebrain cholinergic neurons etc. Part of this work also involves studying early brain development using advanced microfluidic culturing techniques to model the developing neural tube of humans with stem cells. A longstanding collaboration between Dr. Kirkeby and Prof. Malin Parmar at Lund University has led to the development of a human embryonic stem cell-derived dopamine cell product (STEM-PD) for treatment of Parkinson’s Disease (PD). This product has been manufactured under GMP and undergone preclinical safety and efficacy testing as required by regulatory authorities, and is expected to enter a first-in-human trial in early 2022 in collaboration with Prof. Roger Barker at Cambridge University and with Novo Nordisk as supporting industrial partner.
Human stem cell models to study host-virus interactions in the central nervous system

Oliver Harschnitz  
Human Technopole

Advancements in human pluripotent stem cell (hPSC) technology offer a unique opportunity for the neuroimmunology field to study host-virus interactions directly in disease-relevant cells of the human central nervous system (CNS). Neurotropic viruses target distinct CNS cell types often leading to severe neurological damage. With the unique flexibility and scalability of hPSC technology, it is now possible to examine the molecular mechanisms underlying acute infection and latency, determine which CNS subpopulations are specifically infected, study temporal aspects of viral susceptibility, and perform high-throughput chemical or genetic screens for viral restriction factors. The most common form of viral encephalitis in humans is herpes simplex encephalitis (HSE), caused by primary infections with herpes simplex virus type 1 (HSV-1). Inborn errors of cell-intrinsic immunity in the CNS can account for HSE, with defective TLR3 (Toll-like receptor 3) and DBR1 (RNA lariat debranching enzyme)-mediated immunity underlying forebrain and brainstem HSE, respectively. We have developed and applied hPSC-models to study the cellular and molecular mechanisms underlying HSE, showing that hPSC-derived cortical neurons rely on TLR-3-dependent cell-intrinsic anti-HSV-1 immunity, which is lacking in hPSC-derived trigeminal neurons. This closely mimics the in vivo setting, where trigeminal neurons are the site of viral entry and latency. To identify host antiviral factors for HSV-1 in human microglia, we performed pooled CRISPR/Cas9 screens in hPSC-derived microglia. Remarkably, and in contrast to cortical neurons, we found that TLR3-IFN (interferon) signalling is redundant in microglia for anti-HSV-1 immunity. Interestingly, we identified DBR1 to be a microglia cell-intrinsic antiviral factor against HSV-1. We report that DBR1 functions as an essential antiviral host factor in human microglia, as DBR1-mutated patient iPSC-derived and CRISPR/Cas9-engineered isogenic DBR1-mutant microglia are susceptible to HSV-1 infection. Based on our studies performed in highly defined hPSC-models, we show that CNS cells rely on cell-type-specific cell-intrinsic antiviral signalling pathways.

Single-cell transcriptomics reveals age-resistant maintenance of cell identities, stem cell compartments in naked-mole

Aleksandra Savina  
Institut Cochin

Skin represents an informative and convenient organ for the analysis of the aging process. Naked mole-rats (NMR) are subterranean rodents remarkable for their longevity, with unexplained resistance to aging. We performed extensive in situ analysis and single-cell RNA-sequencing comparing young and older animals skin and showed that NMR exhibited a striking stability of skin compartments and cell types, which remained stable over time without aging-associated changes. Remarkably, the number of stem cells was constant throughout aging. We also found three classical cellular states defining a unique keratinocyte differentiation trajectory that were not altered after pseudo-temporal reconstruction. Epidermal gene expression did not change with aging. Langerhans cell clusters were conserved and only a higher basal stem cell expression of Igfbp3 was found in older animals. In accordance, NMR skin healing closure was strictly similar in both age groups. Altogether, these results indicate that NMR skin is characterized by peculiar genetic and cellular features, different from those previously demonstrated for mice and humans. The remarkable stability of the aging NMR skin transcriptome likely reflects unaltered homeostasis and resilience.

3D Human Cortical Organoids to investigate early cortical activity and developmental and epileptic encephalopathy

Simona Lodato  
Humanitas University

The human cerebral cortex is characterized by an extraordinary complexity of neuronal and non-neuronal cell types wired together for the execution of high-order cognitive functions. Alterations, during fetal development as well as after birth, in the assembly of cortical circuits can lead to aberrant neuronal activity, shared sign of neurodevelopmental disorders. Developmental and Epileptic Encephalopathy (DEE), a heterogeneous group of devastating epilepsy disorders with a strong genetic component, constitute the most precocious syndromes that can affect infants as early as in the womb. De novo mutations in the hyperpolarization-activated cyclic nucleotide gated channels (HCN1, HCN2) are associated with severe and untreatable DEE forms. Both genetic and non-genetic components have been linked to DEE; however the precise causative mechanisms remain elusive. Here, we aim at decoupling the effect of aberrant activity per se from the patient-specific genetic makeup to uncover novel DEE mechanisms. By exploiting a highly reproducible human cortical organoids (hCOs) system, on which acute seizure-like currents are induced, we model infantile/pediatric epilepsy in vitro. In parallel, we generated hCOs from HCN1-DEE patient specific iPSC lines to study HCN1 specific variants. We aim at mapping, at the single-cell level - the epigenetic and transcriptional landscapes of both hCOs models: this will allow to deciphering the epigenetic fingerprints produced by exacerbate activity in distinct cortical neuron types along their trajectory and dissect the specific HCN1 effects on cortical assembly. Ultimately, the integrated analysis will identify specific molecular fingerprints downstream of aberrant activity per se and HCN1-DEE, and provide an invaluable resource for new drug targets for infantile epilepsy.
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Session 5 - metabolic control of stem cells

Regulation of dormant hematopoietic stem cells
Nina Cabezas-Wallscheid
MPI of Immunobiology and Epigenetics

Bone marrow (BM) hematopoietic stem cells (HSCs) are vital for the maintenance of hematopoiesis. In inbred mice housed in gnotobiotic facilities, the top of the hematopoietic hierarchy is occupied by dormant HSCs, which reversibly exit quiescence during stress. Whether HSC dormancy exists in human remains unclear. We show by single-cell RNA-seq analysis a continuous landscape of human BM HSCs displaying varying degrees of dormancy. We identified the orphan receptor GPRC5C as marker enabling enrichment of dormant human HSCs. Beyond its role as a marker, GPRC5C is also essential for HSC function as demonstrated by genetic loss- and gain-of-function analyses. Through structural modeling and biochemical assays, we uncovered that hyaluronic acid, a BM extracellular matrix component, preserves dormant HSCs through GPRC5C. Our work shows the existence and relevance of dormancy in human HSCs and identifies the hyaluronic acid-GPRC5C signaling axis as an essential component controlling the HSC state.

How lipid droplet availability affects neural stem cell behaviour
Marlen Knobloch
University of Lausanne - Department of Biomedical Sciences

Neural stem/progenitor cells (NSPCs) generate new neurons throughout adulthood, however, the underlying regulatory processes are still not fully understood. Lipid metabolism plays an important role in NSPC regulation: Build-up of lipids is crucial for NSPC proliferation, whereas the break-down of lipids has been shown to regulate NSPC quiescence. Despite their central role for cellular lipid metabolism, the role of lipid droplets (LDs), the lipid storing organelles, in NSPCs remains still underexplored. In this presentation I will discuss our recent findings on how LD availability affects NSPC behaviour and will present a new model to study LD dynamics in living mammalian cells and tissues.

A full, dynamic view of neural stem cell quiescence
Noelia Urbán
Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter (VBC), Dr. Bohr-Gasse 3, 1030 Vienna, Austria.

The majority of adult neural stem cells (aNSCs) are in a distinct metabolic state of reversible cell cycle exit also known as quiescence. The rate of aNSC activation determines the number of new neurons generated and directly influences the long-term maintenance of neurogenesis. Despite its relevance, it is still unclear how aNSC quiescence is regulated. Many factors contribute to this, like aNSC heterogeneity, the lack of reliable quiescence markers, the complexity of the neurogenic niches or the intricacy of the transcriptional and posttranscriptional mechanisms involved. For instance, we have shown that the same signal -canonical WNT stimulation - can elicit different responses in active and quiescent aNSCs. In order to integrate this and other recent discoveries on the regulation of quiescence in aNSCs we need a new framework that goes beyond our existing concept of a linear shift from quiescence to activation. Instead, we must acknowledge the full complexity of aNSC states, which include not only activation but also differentiation and survival as behavioural outcomes. I propose a model where aNSCs dynamically transition through a cloud of highly interlinked cellular states driven by intrinsic and extrinsic cues. This new perspective enables us to incorporate current results into a coherent framework and aids the formulation of new testable hypothesis. Having a more complete view of aNSC transitions and embracing their complexity will bring us closer to understanding how aNSC activity and neurogenesis are controlled throughout life.
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Session 6 - cell identity and cell fate decision

Molecular control of cellular identity acquisition
Sven Falk
FAU Erlangen-Nürnberg

Proper acquisition of cellular identity is crucial for the functioning of any tissue. The human brain showcases how the ample variety of functions it preforms every second of our lives depends on a tremendous number and diversity of distinct cells. We use direct lineage reprogramming of adult human brain resident cells towards induced neurons as a heuristic approach to identify molecular frameworks crucial for the acquisition of cellular identity and test the arising concepts in human brain organoids to study their implication in natural neurogenesis.

Stem cell specific interferon stimulated gene expression is regulated by the formative pluripotency network through IRF1
Merrit Romeike
Max Perutz Labs

Stem cells intrinsically express a subset of genes which are normally associated with interferon stimulation, thus the innate immunity response. Expression of these interferon stimulated genes (ISGs) in stem cells is independent from external stimuli such as viral infection. Here we show that the interferon regulatory factor 1, Irf1, is directly controlled by the murine formative pluripotency gene regulatory network and therefore upregulated in the transition from naive to formative pluripotency. IRF1 in turn binds at regulatory regions of a conserved set of ISGs, and is required for their faithful expression in formative pluripotent cells. IRF1 also binds to an enhancer of the formative pluripotency transcription factor Oct6 and is partially required for upregulation of Oct6. Presence of IRF1 increases resistance against viral infection during differentiation from naive to formative pluripotency. Irf1 therefore acts as a link between the formative pluripotency network, the regulation of innate immunity genes and viral resistance in formative pluripotency.

Patterning from the bottom up: hPSC patterning via spatially controlled stimulation from the basal side
Tom Wyatt
Wellcome-MRC Cambridge Stem Cell Institute

Pluripotent cells of the human epiblast differentiate into a diversity of cell types during gastrulation. These decisions give rise to a spatially organised body plan and thus differentiation is necessarily linked to spatial patterning. We use human pluripotent stem cells (hPSCs) to model the epiblast in vitro and study patterning through developing bioengineered approaches that enable quantitative control of the environment. The morphogen BMP4 is crucial during gastrulation and it was recently discovered that BMP receptors are located baso-laterally in hPSC, as well as in the mouse epiblast, making those cells effectively insensitive to apically applied BMPs. We have thus developed a bioengineered system which stimulates hPSC monolayers on their basal side, whilst using microfluidics to quantitatively control the spatial profile of morphogen concentration. Using this system, we show that a parabolic shaped concentration gradient applied to the basal side is converted by hPSC to a classic Wolpertian ‘French flag’ pattern of 3 cell identities via a sigmoid-shaped signalling response which is stable in time. The high degree of control afforded by the system allows us to vary the shape of the profile and tissue independently, allowing us to establish that the patterning occurs via a true dose-response with respect to two independent concentration thresholds. In contrast, we find that a sharp (step-shaped) gradient, leads to altered patterning and the appearance of endodermal cell fates, as stimulated cells induce differentiation in their non-stimulated direct neighbours. Our approach thus precisely distinguishes between the roles of two fundamental patterning mechanisms, that of morphogen gradients and secondary inductions.
Transcriptional safeguarding mechanisms enable development and prevent disease

Moritz Mall
DKFZ Heidelberg

Cell identity and function requires both induction of desired genes and repression of unwanted programs. While master regulators that activate gene networks during development are well characterized, potential mechanisms that terminally repress alternative fates remain poorly understood. We recently found that the neuron-specific transcription repressor Myt1l enhances neuronal cell identity. Myt1l is expressed in virtually all neurons throughout life and its loss in postmitotic neurons impairs neuronal gene expression and function, suggesting a role in maintaining cell fate. Unlike known repressors such as REST that specifically silences neuronal genes in non-neuronal cells, Myt1l represses many non-neuronal programs in neurons, acting as a novel “many-but-one” repressor. Myt1l mutations occur in mental disorders, such as autism, suggesting that failure to repress non-neuronal genes could contribute to brain diseases. Here, we generate genetically-engineered stem-cell derived human neurons and mice to address this question. Using these preclinical models, we show that MYT1L deficiency caused upregulation of non-neuronal genes and is sufficient to induce autism-associated phenotypes ranging from altered gene expression and neurodevelopment to behavior phenotypes. Unexpectedly, we found that continuous loss of MYT1L affected synaptic gene expression and transmission and that acute application of approved drugs rescued the electrical phenotypes in postmitotic mouse and human neurons, providing a potential therapeutic avenue for patients with MYT1L syndrome. Our work showcases how stem cell technologies allow to study mental disorders in human neurons, and how combining mouse and human models offers unique opportunities to decipher novel disease-causing mechanisms and translational strategies. Moreover, silencing non-neuronal genes in neurons represents a novel mechanism that enables normal development and prevents brain disorders and raises the possibility that factors similar to Myt1l exist in other lineages. Identifying such factors could provide insight into fighting diseases associated with cell identity loss and to efficiently generate cells for regenerative medicine using reprogramming.

Murine cranial neural crest cells reawaken pluripotency programs

Antoine Zalc
Cochin Institute

Cell differentiation progresses via a continuous lineage restriction process where cell potential is reduced as the embryo develops. Pluripotent embryonic cells can beget all somatic cell types, but this capacity is rapidly restricted during the formation of the three germ layers, each giving rise to distinct cell types. Uniquely among vertebrates, a stem cell-like population arising in the embryo rostral part – called cranial neural crest cells (CNCC) – challenges this paradigm. CNCC have a much broader differentiation potential than their ectodermal lineage of origin as they not only give rise to ectoderm derivatives, such as neurons and glia, but also to cell types canonically associated with the mesoderm such as bone and cartilage of the face. We identified a CNCC precursors population that re-expresses Oct4 and associated pluripotency factors to reverse cell differentiation during development. We demonstrated this return into a higher pluripotency state is essential for subsequent formation of ectomesenchyme and proper craniofacial development. We showed open chromatin landscapes of Oct4+ CNCC precursors resemble those of pluripotent epiblast stem cells, with additional features suggestive of priming for mesenchymal programs. Altogether, our data suggest CNCC undergo a natural in vivo reprogramming event allowing them to climb uphill on Waddington’s epigenetic landscape and expand their developmental potential via a transient re-acquisition of molecular signatures of pluripotency.
Polycomb repressive complex 2 restricts human trophoblast induction

Irene Talon
KU Leuven-University of Leuven

During human development, the first cell fate specification event leads to the distinction between the extraembryonic trophoderm cells and the embryonic pluripotent epiblast. However, lineage commitment is not complete in the early blastocyst. This unrestricted lineage potential is retained in vitro in naïve human pluripotent stem cells (hPSCs), which can give rise to embryonic and extraembryonic lineages, and are able to form blastoids resembling human blastocysts. Yet, it is currently unknown which chromatin mechanisms enable the developmental plasticity present in naïve hPSCs. Here, we show that the Polycomb Repressive Complex 2 (PRC2) acts as a barrier to alternative cell fates in naïve hPSCs and in human blastoids. First, we detected the presence of PRC2-mediated H3K27me3 at the promoters of lineage regulators in naïve hPSCs, including trophoblast regulators, suggesting that PRC2 might oppose cell fate specification in naïve hPSCs. Next, we inhibited PRC2 during naïve to trophoblast conversion. We found that PRC2 inhibition (PRC2i) increased the number of GATA3-positive nuclei, the activation of trophoblast genes and accelerated the exit from naïve pluripotency during trophoblast fate induction. Single-cell RNA-seq analyses showed that an increased proportion of PRC2i-treated cells aligned with the human embryo trophoderm and trophoblast lineage compared to control, in line with increased trophoblast induction upon PRC2i. To further investigate a role for PRC2 in trophoblast specification and morphogenesis, we used human blastoids. PRC2i increased the proportion of TROP2 and GATA3-positive trophoblast-like cells during blastoid formation, and decreased the ratio of epiblast-like cells. Additionally, we measured blastocyst-like cavity formation in blastoids, whose expansion is critical for embryonic uterine implantation, and observed that blastoid cavities appeared earlier upon PRC2i, supporting an acceleration of trophoblast specification and epithelial morphogenesis. Thus, our results show that naïve hPSCs are not epigenetically unrestricted, and instead possess PRC2 as a chromatin barrier to the trophoblast fate.
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Posters

Please note that although all posters will be displayed at the same time, the presentations will take place on different days. Presenters with even numbers will present in Session 1 on Thursday, March 24, and presenters with odd numbers will present on Day 2, Friday, March 25.

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**Poster number: 1**

**Anillin-mediated cortical actomyosin governs epidermal shape and growth**

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Epidermal development and homeostasis require tight coordination between cell shape dynamics, proliferation, and differentiation. Defects in the balance between these fundamental processes can be detected in common skin diseases such as cancer and psoriasis. However, the underlying mechanisms are poorly understood, particularly in physiologically relevant mammalian systems. Here, we identify the actin-binding protein Anillin (encoded by Anln) as a regulator of epidermal shape and growth. In utero depletion of Anln transcripts in the mouse embryos gave rise to hyper-proliferation and hindered tissue morphogenesis. We further show that ANLN localized to the cell cortex, and cortical actomyosin levels and activity were upregulated without its activity. Using pharmacological reagents, we demonstrated that these cortical defects were not computable with cell adhesion and normal epidermal architecture. Together, these observations demonstrate that Anin is a major regulator of epidermal development and provide novel insights into the regulation of cortical actomyosin and its importance in tissue shape and growth.

**Poster number: 2**

**CRISPR/Cas9 mediated gene editing of human iPSC to track haematopoietic development**

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Background: Our group recently reported enhanced erythroid differentiation from human iPSC by closer mimicry of physiological cell contacts in a three dimensional network, termed „haematopoietic cell forming complex“ (HCFC). From this HCFC, CD43+ hematopoietic cells (purity >95 %) were continuously released into the culture supernatant and could be collected repeatedly for further erythroid differentiation. Characterization of the HCFC in more detail is currently limited by accompanying destruction of the complex, hindering further haematopoietic cell fate tracking. To overcome this limitation, we developed a CD43 fluorescence reporter hiPSC line (CD43R-iPSC) that allows live-cell imaging of haematopoietic cells as they emerge in a spatiotemporal manner from the HCFC.

Methods and Results: We have successfully performed gene knock-in of a CD43 promoter region tagged with GFP using CRISPR/Cas9-mediated homology-directed repair (HDR) mechanisms in the AAVS1 safe harbor locus. Adeno-associated viral vectors (AAV) were used for efficient DNA donor delivery into hiPSCs. The CRISPR/Cas9 reagents were firstly successfully tested in the K562 cell line. Following HDR-mediated donor integration, clones with on-target seamless DNA integration were isolated, which was confirmed by Sanger sequencing and in-out PCR. To confirm the functionality of the CD43 fluorescent reporter, haematopoietic and erythroid differentiation of the CD43R-iPSC was induced. GFP-expression of CD43+ hematopoietic cells inside the HCFC was monitored by fluorescence microscopy and live cell imaging. Haematopoietic nature of released GFP+ cells was confirmed by flow cytometry (CD43, CD34, CD45) and colony formation assay. Differentiation and expansion kinetics during further erythroid maturation did not differ between CD43R-iPSCs and unmanipulated iPSC clones.

Conclusion: The established CD43 fluorescent reporter system allows to track hematopoietic development from hiPSCs within three-dimensional structures like organoids. Therefore, the system represents an attractive tool to investigate the hematopoietic development from hiPSCs. The established system can be used for different hiPSC lines or other primary cells.
Poster number: 3

STRAIGHT-IN: A platform for high-throughput targeting of large DNA payloads in human pluripotent stem cells
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Inserting large DNA payloads (>10 kb) into specific genomic sites of mammalian cells remains challenging. Applications ranging from synthetic biology to evaluating the pathogenicity of disease-associated variants for precision medicine initiatives would greatly benefit from tools that facilitate this process. We have merged the strengths of different classes of site-specific recombinases and combined these with CRISPR/Cas9-mediated homologous recombination to develop a platform for stringent site-specific replacement of genomic fragments >50 kb in size in human induced pluripotent stem cells (hiPSCs). This platform, termed STRAIGHT-IN (Serine and Tyrosine Recombinase Assisted Integration of Genes for High-Throughput INvestigation), enables a genomic locus to be repeatedly modified not only rapidly but also efficiently. We demonstrate the versatility of STRAIGHT-IN by (i) inserting various combinations of fluorescent reporters into hiPSCs to assess excitation-contraction coupling cascade in derivative cardiomyocytes, and; (ii) simultaneously targeting multiple variants associated with an inherited cardiac arrhythmic disorder into a pool of hiPSCs. Furthermore, we confirmed the expected electrophysiological phenotype was observed for one of the variants introduced. In summary, STRAIGHT-IN offers a precise approach to generate panels of hiPSC lines containing either multiple combinations of transgenes or potential disease variants in the same cell line and genomic context both efficiently and cost-effectively.

Poster number: 4

Analysis of Leukemia Stem Cells in Acute Myeloid Leukemia to Predict Clinical Response to Venetoclax-Based Therapy
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Acute Myeloid Leukemia (AML) is the most common adult leukemia, with a high frequency of relapse. Treatment with Hypomethylating agents (HMA) such as 5-Azacytidine (AZA) in combination with the BCL-2 inhibitor Venetoclax (VEN) has recently become standard therapy for patients unsuitable for intensive standard chemotherapy. Additionally, AZA/VEN-therapy is currently under investigation to replace standard chemotherapy in high risk patients but the criteria are still not well defined. Therefore, studies linking clinical response with molecular parameters are essential to select the most suitable therapy choice.

Rare leukemia stem cells (LSCs) are a likely cause for refractory disease and hence the most relevant subpopulation to investigate therapy response. Using a flow cytometry panel which combines myeloid differentiation markers CD64 and CD11b and the recently described LSC marker GPR56, we identified three distinct sub-populations present in all investigated AML cases (N=95) irrespective of the underlying mutations: GPR56+ LSC-, GPR56- immature- and CD64+CD11b+ mature-population. Ex-vivo AZA/VEN treatment showed high resistance of Mature-population owing to overexpression of anti-apoptotic proteins MCL1 and BCL2A1. However, transplantation into immune-deficient mice and transcriptomic analysis of 19 diagnosis samples revealed that GPR56+ subpopulation contains functional LSCs, display an enrichment for LSC signatures and aberrant BCL2 expression. The dependence on BCL2 for survival was consolidated by apoptotic characterization using novel single-cell BH3 profiling. The daily analysis of GPR56+ LSCs abundance in AML patients upon induction of AZA/VEN therapy revealed that in responsive patients, LSCs are the main target of AZA/VEN therapy and clearance of LSCs is essential for therapy success. Importantly, the response of other subpopulations did not follow treatment outcome. Lastly, we characterized the expression of LSCs from 38 de-novo patients with known treatment outcomes and established a novel flow cytometry-based response score which predicts response to AZA/VEN and identifies patients most suitable for AZA/VEN as frontline and salvage therapy.
Poster number: 5

Single-cell transcriptomics identifies conserved regulators of neurosecretory lineages in embryos and adults
Julia Steger, Alison G. Cole, Andreas Denner, Tatiana Lebedeva, Grigory Genikhovich, Alexander Ries, Robert Reischl, Mark Lassnig, and Ulrich Technau
University of Vienna

Bilaterian neurons secrete neuromediators to transmit signals, and some cells of neural origin also produce hormones. However, the relation of neurons to other secretory cell types is not obvious. Here we use developmental single cell RNA-sequencing validated by transgenics to demonstrate that neurons, stinging cells, and gland cells arise from a common multipotent progenitor population in the sea anemone Nematostella vectensis - a member of the bilaterian sister group Cnidaria. We identify the conserved transcription factor gene SoxC as a key upstream regulator of all neurosecretory lineages, and demonstrate that SoxC knockdown eliminates both the neuronal and the secretory cell types. While in vertebrates and many other bilaterians neurogenesis is largely restricted to early developmental stages, we show that in the sea anemone differentiation of neurosecretory cells is maintained throughout all life stages, and follows the same molecular trajectories from embryo to adulthood, ensuring lifelong homeostasis of neurosecretory cell lineages.

Poster number: 6

The specialist in regeneration – the Axolotl – a suitable model to study bone healing?
Research Institute of Molecular Pathology (IMP)

While the axolotl’s ability to completely regenerate amputated limbs is well known and studied, the mechanism of axolotl bone fracture healing remains poorly understood. One reason might be the lack of a standardized fracture fixation in axolotl. We present a surgical technique to stabilize the osteotomized axolotl femur with a fixator plate and compare it to a non-stabilized osteotomy and to limb amputation. The healing outcome was evaluated 3 weeks, 6 and 9 months post-surgery by microcomputer tomography, histology and immunohistochemistry.

Plate-fixated femurs regained bone integrity more efficiently in comparison to the non-fixated osteotomized bone, where larger callus formed, possibly to compensate for the bone fragment misalignment. The healing of a non-critical osteotomy in axolotl was incomplete after 9 months, while amputated limbs efficiently restored bone length and structure. In axolotl amputated limbs, plate-fixated and non-fixated fractures, we observed accumulation of PCNA+ proliferating cells at 3 weeks post-injury similar to mouse. Additionally, as in mouse, SOX9-expressing cells appeared in the early phase of fracture healing and amputated limb regeneration in axolotl, preceding cartilage formation. This implicates endochondral ossification to be the probable mechanism of bone healing in axolotls.

Poster number: 7

Direct conversion of human fibroblasts into induced neural stem cells as a model for studying neural ageing and regeneration
Spathopoulos, Angeliki - University of Innsbruck, Innsbruck, Austria

Mammalian tissue regeneration, particularly in the central nervous system, is limited. Comparative analysis with regeneration in fish and axolotl indicates evolutionary conserved transcriptional programs, that are not well understood in the human brain. Cellular reprogramming of human somatic cells such as adult dermal fibroblasts (ADFs) into induced pluripotent stem cells (iPSCs) provides new opportunities to analyze regeneration trajectories. During reprogramming cells get epigenetically rejuvenated, whereas direct conversion into induced neurons (iNs) circumvents the rejuvenation and maintains the majority of epigenetic ageing marks. The novel approach of the direct conversion of ADFs into proliferating induced neural stem cells (iNSCs) is offering for the first time a system for studying neuronal regeneration. However, the molecular mechanism underlying this rejuvenation process has been poorly investigated. In this study, we aim to assess the reprogramming trajectories during the conversion of ADFs into iNSCs. For this purpose, we employed a lentiviral cell barcoding method, CellTagging, to label cells with barcodes at multiple stages during direct conversion. Subsequently, we collected multiple samples along the iNSC conversion, in order to analyze the transcriptome and epigenome on a single-cell level by multi-omics. Initial bioinformatic analysis of the end point of conversion reveals the generation of a highly homogeneous iNSC population, enriched for NSC-specific markers, like Nestin and PAX6, and the absence of the pluripotency markers OCT4, cMYC and KLF4. Analysis of markers for the anterior/posterior and ventral/dorsal axis of the neural tube revealed a hindbrain, dorsal cellular identity. In the next steps of our study, we will analyze the multi-omics data prior to and during the conversion process, we will explore the key genes affecting the conversion efficiency and we will perform high resolution lineage analysis. Ultimately, this study will contribute to gain comprehensive insight into neural rejuvenation as a proxy to understand ageing and regeneration.
**Poster number: 8**

**Identification of Novel Light Regulated microRNAs in Human Retina Using Retinal Organoid Model.**

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MicroRNA molecules (miRNAs) represent a class of small non-coding RNAs that act as post-transcriptional gene regulators. The expression of miRNAs occurs in a tissue-specific manner and in this context the retinal miRNAs expression has unique profiles in the developing and adult retina. These retinal-specific miRNAs regulate many cellular functions including the adaptation of the retina to different light intensities, rapid turnover of the phototransduction cascade, circadian rhythms, and maintenance of cellular homeostasis. Although retinal-specific miRNA species have been already identified in mice, it is challenging to study these miRNAs in human, as there is a lack of human retinal tissues that are available for experimental procedures.

Here we aimed to study light-regulated miRNAs in the human retina using a retinal organoid model. We show that retinal organoids, derived from human pluripotent stem cells, exhibit cellular heterogeneity and composition that mimics the retina in vivo. In order to identify light-regulated miRNAs, we photo-stimulated retinal organoids and performed miRNA next-generation sequencing. We identified three miRNA clusters to be significantly up-regulated and ten clusters to be down-regulated upon photostimulation. Detailed analysis of miRNA expression revealed a rapid turnover of these light-regulated miRNA clusters. Taken together our data indicate an interesting and unique miRNA expression pattern in photo-stimulated retinal organoids. Interestingly, most of these miRNAs have not been previously associated with the retina and/or light adaptation and their role is unknown.

This study was supported by the Czech Science Foundation (GA21-08182S) the Grant Agency of Masaryk University (GAMU) - MU-Ni/G/1391/2018.

**Poster number: 9**

**CTCF-driven insulation at the HoxB locus using gastruloids at a model system**

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The HoxB cluster present a unique structure among Hox genes family. The central Hoxb9 and posterior Hoxb13 are disconnected by a large genetic desert, presenting an interesting pattern of CTCF binding sites with the same orientation. During mice development, delay in Hoxb13 posterior activation is mandatory for a proper elongation. Here, we hypothesized that this DNA segment is important to unsure the insulation of Hoxb13. In mice, deletion of this region leads to premature Hoxb13 expression and a reduction of the antero-posterior axis length. Using gastruloids as an in vitro model, we decided to dissect the insulation mechanism of Hoxb13 and more precisely, the qualitative and quantitative function(s) of the CTCF sites present in this interval. We performed multiple genetic rearrangements within the boundary sequence between Hoxb9 and Hoxb13 using CRISPR-cas9. First, we deleted the intergenic region and observed a gain in Hoxb13 expression, corroborating its importance for Hoxb13 insulation. Then, we introduced a small CTCF-cassette containing six CTCF binding sites within the deleted configuration. Interestingly, the CTCF cassette was sufficient to re-establish insulation of Hoxb13 despite the small distance between Hoxb13 and Hoxb9. Finally, to challenge the robustness of the CTCF-cassette boundary, we deleted 5 out of the 6 introduced CTCF binding sites. Interestingly, a low Hoxb13 expression was detected, indicating that one CTCF is able to insulate Hoxb13, although the boundary was weaker than with the full CTCF cassette. We are currently investigating the chromatin conformation in these mutant cell lines in order to understand the mechanism of Hoxb13 repression and the consequences on DNA-DNA interactions. On the other hand, we are also assessing the importance of these CTCF binding sites orientation on Hoxb13 insulation.
Cardioids unravel mechanisms of compartment-specific cardiac defects
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3Heart beat.bio, Vienna, Austria

The number one cause of fetal death are defects in heart development. Most of these cardiac deficiencies are due to unknown factors; these deaths could be caused by genetic mutations, environmental influences, or a combination of several factors. Determining the underlying causes in vivo faces many challenges, including the complexity and inaccessibility of the human fetal heart and the impossibility of drug testing during pregnancy. Here we test how genetic (knockout of ISL1, TBX5, and FOXF1) and environmental factors (retinols, thalidomide, and plastic residues) affect cardiac development using cardioids representing all major compartments of the human heart. Using this platform, we could dissect how genetic and environmental factors specifically impact different regions of the developing heart, allowing us to unravel the underlying mechanisms of cardiac defects.

Modeling all major compartments of the developing heart using cardioids
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Congenital cardiac defects are the most common birth defects affecting about ~1% of newborns. These defects are often chamber-specific, but it is challenging to discern the underlying molecular mechanisms in vivo. We established 3D human in vitro protocols representing all major compartments of the heart, including the right ventricle, atria, outflow tract and the atrioventricular canal. By using in vivo-like signaling, we specified the lineage-specific cardiac progenitors with the capacity to differentiate into cavity-forming cardioid subtypes. These cardioids have the compartment-specific in vivo-like gene expression profile, morphology, and functionality. Thus, the cardioid platform of all major compartments represents a powerful tool to understand the complexity of heart development, its malformations and study the interactions between cardiac compartments.

Uncoupling Human Chimerism from Developmental Stage
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Contrary to human Primed Pluripotent Stem Cells (hPSC), human Expended Pluripotent Stem Cells (hEPSC) have the ability to form chimera in pre-implantation embryos and contribute to both embryonic and extraembryonic lineages. This observation led to the hypothesis that hEPSC might represent a totipotent-like state. However, hEPSC have yet to be properly benchmarked in order to determine precisely their state and fate. Here we provide an extensive characterization of the hEPSC. Despite having chimeric abilities, hEPSC closely resemble hPSC on the transcriptomic, metabolic and epigenetic levels. Thus, we propose that human chimerism ability could be uncoupled from developmental stage. This new finding could help to better understand interspecies chimerism mechanisms.
Telomere length in human pluripotent stem cells
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Human pluripotent stem cells (hPSC), whether human induced pluripotent stem cells (hiPSC) or human embryonic stem cells (hESC), are a powerful tool in research and hold great potential for clinical applications. One of their basic properties is active telomerase, which leads to telomere length maintenance or even lengthening in low passages (Zeng Sicong et al., 2014).

Short telomeres can lead to early senescence in cells differentiated from hPSC. Therefore we used Southern blot analysis of terminal restriction fragments (TRFs) to test if the telomere length changes during reprogramming and culture of hiPSC in low passages. Contrary to previously published work (Yehezkel Shiran et al., 2011), we did not find any trend within the tested hiPSC lines.

We next tested the possible impact of culture conditions on telomere length. We compared the telomere length of hPSC lines cultured for 15 passages in feeder-dependent and feeder-free systems. We also tested the effect of hypoxic/normoxic culture conditions. We did not prove any influence of culture conditions on the telomere length.

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Gastruloids as a model to study complex developmental gene regulation – case study of Mesp1 and Mesp2
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Gastruloid are an ES-cell based model of embryos mimicking early mammalian development. They undergo symmetry breaking, diversification of cellular identities and establishment of body axes. They are easily grown and engineerable, opening the door to large-scale experiments that would not be easily attainable in vivo. Here, we demonstrate the usefulness of gastruloids to study the regulation of a dynamic genetic locus. The Mesp1/2 locus is composed of two genes (Mesp1 and Mesp2) required for proper mesoderm specification.

We validated that gastruloids recapitulate Mesp1/2 dynamic embryonic expression. We could observe that Mesp1 and Mesp2 are expressed during two waves; an early Mesp1-only expression followed by a second wave comprising both Mesp1 and Mesp2. Therefore, we propose to use the Mesp1/2 locus as a case study to understand the mechanisms at play during the tight establishment of gene expression patterns.

First, we developed a long-term enhancer reporter assay to test each enhancers’ activity using live imaging on gastruloids. We identified putative Mesp1/2 regulatory elements using a time resolved ATAC-seq time course of gastruloids. These were then analyzed using this new enhancer reporter assay to dissect the dynamic spatio-temporal Mesp1/2 enhancer usage.

In parallel, to assess the contribution of each regulatory element present in the Mesp locus, we generated single deletions of each element. We’ll use these deletions to study the function of each element on Mesp1/2 gene expression in time and in space.

Overall, we hope to convince that gastruloids are a useful tool to investigate regulatory landscapes. By applying convergent approaches, we hope to obtain a comprehensive view of the mechanisms at play during Mesp1/2 dynamic expression. Moreover, the enhancer reporter assay developed for this project, can be scaled up to allow high-throughput studies of enhancer activity.
Human pluripotent stem cells (hPSCs) are intrinsically able to self-organize into cerebral organoids that mimic features of developing human brain tissue. These three-dimensional structures provide a unique opportunity to generate cytoarchitecture and cell-cell interactions reminiscent of human brain complexity in a dish. However, current in vitro brain organoid methodologies often result in intra-organoid variability, limiting their use in recapitulating later developmental stages as well as in disease modeling and drug discovery. In addition, cell stress and hypoxia resulting from long-term culture lead to incomplete maturation and cell death within the inner core. Here, we used a recombinant silk microfiber network as a scaffold to drive hPSCs to self-arrange into engineered cerebral organoids. Silk scaffolding promoted neuroectoderm formation and reduced heterogeneity of cellular organization within individual organoids. Bulk and single cell transcriptomics confirmed that silk cerebral organoids display more homogeneous and functionally mature neuronal properties than organoids grown in the absence of silk scaffold. Furthermore, oxygen sensing analysis showed that silk scaffolds create more favorable growth and differentiation conditions by facilitating the delivery of oxygen and nutrients. The silk scaffolding strategy appears to reduce intra-organoid variability and enhances functional maturation during spontaneous self-patterning in human brain organoid differentiation.

Modelling human brain ageing in vitro is a major challenge aimed at discovering mechanisms involved in physiological and pathological conditions, such as neurodegenerative disorders. Recent studies in neurons derived from human induced pluripotent stem cells (iPSCs) have shown that cells overexpressing Progerin, a protein associated with the Hutchinson-Gilford progeria syndrome, show diverse hallmarks of ageing. However, the ability to induce ageing in complex 3D organoid models remains to be investigated. In this study, we have generated iPSCs that overexpress Progerin in a doxycycline (dox)-inducible manner and used them to establish a human model of age-induced cerebral organoids. We show that GFP-T2A-Progerin transgenic iPSCs can give rise to brain organoids that look morphologically homogeneous. Upon administration of dox, d60 and d90 organoids show a widespread expression of GFP in up to 90% of the total cells, as judged by flow cytometry, whereas vehicle-treated cells remained GFP-negative. Interestingly, GFP expression is even detectable after long-term culture indicative of resistance toward transgene silencing. Immunostaining validated the expression of Progerin in the majority of GFP-positive cells compared to the controls. Moreover, Progerin expression gives rise to various well-established hallmarks of ageing. We detected via immunostaining a strong reduction of heterochromatin markers, such as H3K9me3 and HP1γ. Moreover, at the level of DNA damage, we observed a marked increase in dsDNA breaks (γH2Ax) and oxidative damage as shown by 8oxoG staining. Notably, γH2Ax signal is highly abundant in the neural precursor regions, whereas 8oxoG appears more evident in the mature neuronal regions of the organoids.

We plan to comprehensively characterize the ageing phenotype of our age-induced brain model to understand the impact of Progerin-mediated ageing on mitochondrial stress, DNA methylation status, and transcriptome. Finally, the Progerin system will be employed in patient-specific iPSCs to study the contribution of ageing to the pathophysiology of Parkinson’s disease.
Live imaging reveals cerebellar neural stem cell dynamics and the role of VNUT in lineage progression

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Poster number: 17

Little is known about the intrinsic specification of postnatal cerebellar neural stem cells (NSCs) and to what extent they depend on information from their local niche. Here we have used an adapted cell preparation of isolated postnatal NSCs and live imaging to demonstrate that cerebellar progenitors maintain their neurogenic nature by displaying hallmarks of NSCs. Furthermore, by employing this preparation, all the cell types produced postnatally in the cerebellum, in similar relative proportions to those observed in vivo, can be monitored. The fact that neurogenesis occurs in such organized manner in the absence of signals from the local environment, suggests that cerebellar lineage progression is to an important extent governed by cell-intrinsic or pre-programmed events. Finally, we took advantage of the absence of the niche to assay the influence of the vesicular nucleotide transporter inhibition, which dramatically reduced the number of NSCs in vitro by promoting their progression towards neurogenesis.

Regulation of E-cadherin-mediated contacts via cortical F-actin flows

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The regulation of intercellular adhesion is a key process in the development and physiology of multicellular organisms. At a molecular scale, regulation of cadherin-mediated cell-cell adhesion entails the reorganization of cadherins and the associated actomyosin cortex locally at the contacts. This reorganization in many systems manifests itself in clustering of cadherins at the contact rim, where they function to stabilize the contact. However, how this stereotypical clustering is achieved remains unexplored due to technical limitations in high-resolution live imaging of cell-cell contacts. Here, we engineered supported lipid bilayers functionalized with E-cadherin ectodomains as a cell surface model, and showed that centrifugal flows of F-actin and E-cadherin clusters contribute to the enrichment of these molecules at the contact rim of cells adhering to the bilayers. The cortical flows arise due to a gradient of Myosin-II activity that peaks at the contact rim, carrying E-cadherin clusters along and dilating the F-actin network. The Myosin-II gradient forms due to the depletion of Myosin-II at the contact center during contact formation, which again is regulated by E-cadherin engaged in trans binding over the contact, reducing RhoA GTPase activity in the center. Consistent with this, we found that manipulation of Myosin-II activity affects the translocation and accumulation of F-actin and E-cadherin at the contact rim, and associated dilation of the F-actin network at the contact disc. Thus, using a biomimetic assay, we identify contractility-driven cortical flows as a novel mechanosensitive mechanism regulating E-cadherin and F-actin reorganization at cell-cell contacts.

Dissecting direct neuronal reprogramming as a tool to identify novel key players in human neurogenesis

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For many years advances in the study of human CNS development and function have been hindered by the inaccessibility of human tissue and hence the molecular basis of neuronal subtype specification and formation in mammals largely stems from studies in rodent models. Albeit similar, the human brain differs in many aspects from that of rodents. Direct lineage reprogramming of terminally differentiated somatic cells into induced neuronal cells (iNSs) opened up the possibility to shed light on the molecular mechanisms driving human neurogenesis. This technique allows to generate potentially clinically relevant cells in the anatomical site of interest without resetting the age of the patient-derived starting cells. Among the somatic cells amenable for iN reprogramming, pericytes, naturally residing within the human brain, represent an ideal candidate. Indeed, it has been demonstrated that, via retroviral over-expression of Ascl1 and Sox2 (AS) in pericytes derived from the adult human cerebral cortex, it is possible to obtain actively firing neurons [Karow et al., 2012; Karow et al., 2018]. However, the key molecular nodes that orchestrate this reprogramming trajectory have not been fully explored yet. Here, we use pericyte-to-iN conversion as a tool to identify genes potentially involved in human neuron formation by performing high resolution molecular analysis of the conversion process on a single cell level. Deconstructing the reprogramming process by simultaneous profiling of the transcriptional and the chromatin accessibility changes in the very same cells, allows both to formulate hypotheses on the molecular nature of reprogramming barriers and target cell subtype-specification. Thus, we speculate that molecular key nodes responsible for acquiring neuronal identity during direct lineage reprogramming are also key in developmental neurogenesis. Ultimately our aim is to investigate whether and to what extent such forced neurogenesis recapitulate the naturally occurring one and extract the key essence of becoming a human neuron.
**Poster number: 20**

**Daam plays opposing roles in the canonical and non-canonical Wnt signaling pathways regulating intestinal stem cells**

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The small intestine is among the fastest self-renewing tissues in adult mammals. This rapid turnover is fueled by intestinal stem cells residing in the intestinal crypt, capable of generating all the differentiated cells that populate the intestinal epithelium to maintain homeostatic function. Among the several signaling pathways governing the balance between stem cell renewal and differentiation, Wnt signaling plays a pivotal role, and dysregulation of this pathway leads to cancer formation. Several feedback mechanisms have evolved to tightly control Wnt signaling, especially at the cell surface as exemplified by the transmembrane E3 ligases Rnf43/Znrf3.

Using biochemical and molecular analysis on cell cultures we show that the diaphanous-related formin (DRF) Daam1 interacts with Rnf43, and this complex is required to attenuate Wnt/beta-catenin signaling at the receptor level. Accordingly, Daam1/2 knock-out allows R-spondin-independent growth in mouse intestinal organoids, similar to Rnf43/Znrf3 knock-out. Furthermore, RNA-seq on organoids as well as genetic analysis in vivo suggest that Daam is also required for secretory lineage specification through the non-canonical Wnt, also known as Planar Cell Polarity (PCP) pathway. In conclusion, we show that Daam1/2 play opposing roles on Wnt/beta-catenin and PCP pathways via Rnf43, a function required for homeostatic maintenance of the murine small intestine.

**Poster number: 21**

**Mapping early human neurogenesis in an in vitro model of human brain development using Single Cell RNASeq**

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Understanding the intermediate steps in human neural subtype specification is crucial for producing accurate patterning of stem cells into specific lineages of neurons for regenerative therapy and disease modelling.

Here, we study early human neural subtype specification by implementing a microfluidic, gradient-based in vitro stem cell culture model of brain development called MiSTR. This model is based on a morphogenetic gradient of increasing WNT signalling, inducing regionalisation of human embryonic stem cells into a rostro-caudally organised neural tissue, providing >80% reproducibility. Single-cell transcriptomics revealed that rostro-caudal fate was established already at 48 hours of differentiation, prior to specification of neural identity. Furthermore, the gene expression profile of rostro-caudal MiSTR organisation was consistent with the gene expression patterns of the early rostro-caudal neural plate in mouse embryos, including the successful modelling of the midbrain-hindbrain boundary. By implementing large scale single cell RNAseq on the cells from MiSTR tissue at different time points and regions, we aim to produce a comprehensive spatio-temporal map of human neural subtype specification, spanning human neural lineages from the forebrain to the hindbrain. For this, we have sequenced approximately 250K cells and are building a web-based neural subtype development atlas depicting gene expression and neural lineage trajectories. MiSTR thereby represents a useful in vitro model of human neurodevelopment to deconstruct and systematically analyse gene regulatory networks responsible for neural regionalisation and patterning.

Furthermore, MiSTR development atlas will prove to be an instrumental resource for scientific community helping in identification and understanding expression dynamics of key markers. This, in turn can be used for development of improved stem cell therapies against various neurodevelopment and neurodegenerative diseases.
Barrett’s esophagus (BE) is categorized, based on morphological appearance, into different stages, which correlate with the risk of developing esophageal adenocarcinoma. More advanced stages are more likely to acquire chromosomal instabilities (CINs) but stage-specific markers remain elusive. Here, we performed single-cell DNA-sequencing experiments (scDNAseq) with fresh BE biopsies. Dysplastic BE cells frequently contained CIN regions, and these CIN cells carried mutations corresponding to the COSMIC mutational signature SBS17, which were not present in biopsy-matched chromosomal stable (CS) cells or patient-matched non-diseased control cells. CS cells were predominantly found in non-dysplastic BE biopsies. The single-base substitution (SBS) signatures of all CS BE cells analyzed were indistinguishable from those of non-diseased esophageal or gastric cells. Single-cell RNA-sequencing (scRNAseq) experiments with BE biopsies identified two sets of marker genes, which facilitate the distinction between columnar BE epithelium and non-dysplastic/dysplastic stages. Moreover, histological validation confirmed a correlation between increased CLDN2 expression and the presence of dysplastic BE stages. Our scDNAseq and scRNAseq datasets, which are a useful resource for the community, provided novel insight into the mutational landscape and gene expression pattern at different stages of BE development.

The kinase mTOR (mammalian target of rapamycin) integrates growth factor-dependent stimuli, amino acid availability, and cellular energy levels to coordinate cell growth and proliferation. The immunosuppressant and anticarcinogenic drug rapamycin works by inducing inhibitory protein complexes with the kinase mTOR, an important regulator of growth and proliferation. The obligatory accessory partner of rapamycin is believed to be FK506-binding protein 12 (FKBP12).

Hedgehog (HH) signalling is important for embryonic patterning and stem cell differentiation. The G-protein coupled receptor Smoothened (SMO) is the key HH signal transducer modulating both transcription-dependent and independent responses. We show that SMO protects naive mouse ESCs from dissociation-induced cell death. We exploited this SMO dependency to perform a genetic screen in haploid ESCs where we identify the Golgi proteins TMED2 and TMED10 as factors for SMO regulation. Super-resolution microscopy shows that SMO is normally retained in the ER and Golgi compartments and we demonstrate that TMED2 binds to SMO preventing localization to the plasma membrane. Mutation of TMED2 allows SMO accumulation to the plasma membrane, recapitulating early events after HH stimulation. We demonstrate the physiologic relevance of this interaction in neural differentiation, where TMED2 functions to repress HH signal strength. Identification of TMED2 as a binder and upstream regulator of SMO opens the way for unravelling the events in the ER-Golgi leading to HH signalling activation.
Poster number: 25

**Modeling Langerhans Cell Histiocytosis using induced Pluripotent Stem Cells**

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Langerhans Cell Histiocytosis (LCH) is a rare myeloid neoplasm of the pediatric age with an accumulation of CD1a+/CD207+ cells in various organs. High-risk patients can develop a life-threatening form of the disease with hepatosplenomegaly, fever and pancytopenia and have poorer prognosis. This cancer is characterized by alterations in the MAPK pathway with BRAFV600E being the most common mutation. Due to its rarity and lack of cell lines no proper in vitro model of LCH exists. We are establishing a LCH model starting from induced Pluripotent Stem Cells (iPSCs) to answer fundamental questions regarding the pathogenesis of LCH and better understand how to improve current treatments. Our results indicate that iPSCs can be differentiated into CD1a+/CD207+ Langerhans Cells via a CD14+ Monocyte precursor. Furthermore, treatment with JAG2, a NOTCH ligand induces iPSC-derived monocytes to express surface markers and acquire a transcriptomic profile similar to LCH tumor cells from lesions. We have then engineered iPSCs to express BRAFV600E upon tamoxifen treatment and shown that upregulation of this oncogene gives rise to a larger fraction of CD14+ cells during differentiation. We have also reprogrammed PBMCs from a LCH patient into iPSCs and generated isogenic pairs of BRAFV600E/WT and BRAFV600E/WI clones that will allow us to study the role of this oncogene within its endogenous regulatory network. Overall, our results show that LCH-like cells and their physiological counterparts can be generated starting from iPSCs and that clones of pluripotent cells expressing BRAFV600E will be instrumental in creating an in vitro model for LCH.

Poster number: 26

**Innovative, animal free biomaterial for induced stem cell cultivation**

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Induced pluripotent stem cells (iPSCs) have a high potential in the field of regenerative medicine and as model system to discover organ physiology, genetic diseases or as drug testing system. Nevertheless, current standard for iPSCs cultivation, to maintain their pluripotency, is the usage of animal-derived matrices (e.g. Matrigel). These matrices have significant disadvantages limiting their applications such as batch to batch variants, cross-contamination risk as well as animal- dependence. For this purpose, we developed a new, animal-free polymer-peptide based matrix to maintain and multiply iPSCs. The new polymer was tagged with a bio-instructive peptide tag, which consists of different cell binding motifs and adsorbs to almost any cell culture consumable without any chemical reaction. Via Fluorescent staining we could confirm consistent expression of stemness markers on iPSCs after 20 passages on our synthetic coating, comparable to cells cultured of the animal-based standard. In conclusion, with our new fully synthetic culture system we have the possibility to overcome the drawbacks of the animal-based standard method of iPSC culture and allows the application in regenerative medicine and development of reliable model systems to replace insufficient animal-based models.

Poster number: 27

**Simultaneous single cell profiling of open chromatin and gene expression in B cell lymphoma highlights tumor-specific regulatory**

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Characterizing tumors by simultaneous transcriptomic and the epigenomic profiling of the same cells at single cell resolution can enable direct reconstruction of cell type-specific regulatory gene networks without relying on assumptions. This approach can significantly enhance the understanding of tumor biology. Here, we characterized single nuclei derived from 14,000 cells of a human diffuse small B-cell lymphoma sample by simultaneous measurement of gene expression and chromatin accessibility using the Chromium Single Cell Multiome ATAC + Gene Expression assay. Leveraging a microfluidic platform, we generated paired barcoded gene expression and ATAC-seq libraries, linking transcriptomic and open chromatin information at the single cell level.

In B cell lymphoma, we used known markers of malignancy to segregate tumor B cells from normal B cells. We constructed regulatory networks in both cell states using the observed transcription factor motif enrichment in open chromatin regions combined with gene expression from the same cells. Comparing regulatory networks in healthy vs. tumor B cells highlighted the presence of unique regulatory networks in tumor B cells. Notably, the PAX5 tumor regulator was observed only in tumor B cells, and its impacts on upregulation of many downstream genes was characterized. This regulatory network analysis was enabled through the joint profiling of open chromatin and gene expression at the single cell level. Our data demonstrate that simultaneous profiling of both epigenomic and transcriptomic data is a powerful tool for characterizing cells in cancer state and in extracting functional information from tumor samples.
Polarity inversion reorganises the stem cell compartment of the trophoblast lineage
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The mouse embryo undergoes drastic morphological changes during the implantation stages, transforming from a hollow-shaped blastocyst into a tube-like egg cylinder. The blastocyst cavity defines two trophectodermal compartments – mural trophectoderm, surrounding the blastocoel and polar trophectoderm, in direct contact with the inner cell mass. The implantation of the blastocyst is initiated by the mural trophectoderm that during this process exhibits terminal differentiation. In contrast, the polar trophectoderm preserves its stem cell properties and forms the extraembryonic ectoderm, which later gives rise to the placenta. Previously, it was suggested that the extraembryonic ectoderm is formed through folding of the polar trophectoderm. Instead, we found that the tissue-scale architecture of the trophoblast lineage is reorganized via inversion of the epithelial polarity axis. Our findings show the developmental significance of polarity inversion and provide a new model for the trophoblast morphogenesis during the implantation stages.

The role of the SWI/SNF chromatin remodeling complex in trophoblast identity
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Placenta provides an interface for the exchange of nutrients, gases and metabolites and thus proper placenta development is essential for both maternal and fetal health. Defective placentation is an underlying factor of many pregnancy complications such as preeclampsia, fetal growth restriction or miscarriage. The structure of the human placenta is characterized by multipotent self-renewing cytotrophoblasts (CT) that give rise to invasive extravillous trophoblasts (EVT) or multinuclear syncytiotrophoblasts (ST). The transcriptional and epigenetic machinery mediating the differentiation events of human CT is a novel and to a certain extent unexplored field. Using human trophoblast stem cells (hTSC) we aim to investigate the role of SWI/SNF complex in CT-ST transition. ATP-dependent SWI/SNF chromatin remodelling complex modifies chromatin accessibility and thus mediates transcriptional activation or repression. SWI/SNF is well-characterized in multiple biological systems, for example, it was discovered to be essential for self-renewal and differentiation of human embryonic stem cells. The function and composition of SWI/SNF have not been studied in placental development and disease. To gain a greater understanding of the problematics we depleted multiple components of the SWI/SNF complex and interestingly, double knockdown (dKD) or inhibition of ATPases BRG1 and BRM resulted in severe ST differentiation defect. Upon forskolin-mediated ST differentiation, the dKD/inhibited hTSC failed to express ST markers while maintaining CT markers. We aim to uncover transcriptional and epigenetic regulation of BRM/BRG1-bound genes necessary for the CT-ST transition and improve the understanding of placenta developmental events and work towards a safe pregnancy for all women.

Mechanistic dissection of primary ciliogenesis during embryonic development using a 3D in vitro model system
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The primary cilium is an antenna-like organelle protruding from the surface of mammalian cells and serves as a communication hub between the cell surface and internal stimuli. In development, its correct assembly and function is essential to prevent malformations of the embryo. It has previously been shown that cilia first arise on epiblast cells of embryos shortly after implantation at the time of cavitation (e5.5). How the primary cilium is established de novo in development is an open question. Common model systems to study ciliogenesis in development show severe limitations since embryos need to be extracted from the uterus at the day of gestation e5.5, which only allows to capture a status quo. To overcome this problem, my PhD project aims to establish a 3D in vitro model suitable to study the assembly and role of this fascinating organelle in the early steps of mouse embryonic development.
Recent studies of gastric tissues under homeostasis and oncogenesis have shed light on the signaling pathways required for gastric tissue maintenance and how aberration in some of these pathways leads to gastric cancer formation. Although it has been shown that the Wnt pathway is important in gastric epithelial maintenance, the identity and source of the specific Wnt ligands important in this process remained unknown. Furthermore, the process by which early gastric cancer acquires Wnt independence from the niche environment has not been previously described. Using gastric organoid technology, we identified that the mesenchymal compartment secretes Wnt2b and Wnt17b to maintain the gastric epithelium in homeostasis. Through genetic studies and single-cell multiomics analysis, we additionally discovered that the acquisition of a MAPK pathway activation is closely related to the epithelial secretion of Wnt17b. We further found that the second method of epithelial Wnt acquisition is through the genetic amplification of Wnt2, a mutation commonly detected in human gastric cancer. Together, our results reveal that the normal gastric epithelial turnover relies on mesenchymal Wnt ligands while gastric epithelial transformation often involves the onset of Wnt secretory phenotype, a potential target for therapeutics.
Poster number: 34

An imaging approach to identify cellular origins of species-specific differentiation timing
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Mammalian species typically undergo similar developmental stages from early embryonic development to childhood and maturity but they do so at different time scales. Recent studies show that species-specific time scales of development are recapitulated by in vitro differentiating pluripotent stem cells (PSCs), making them an ideal model to investigate how timing is determined on the cellular level. Here, we use PSCs of mouse, human and cynomolgus monkey to elucidate cell-intrinsic mechanisms of differentiation timing during neural progenitor differentiation.

To compare neural differentiation speeds and cell cycle durations between the species on the single-cell level, we make use of a time-lapse imaging approach. So far, we measured cell cycle durations of mouse and human PSCs in pluripotency and upon perturbation. One candidate factor that could affect differentiation timing is the mTOR pathway that links cell growth and proliferation to metabolism. Inhibition of mTOR signalling strongly elongated the cell cycle in human PSCs while the effect was much weaker in mouse PSCs, indicating higher sensitivity towards mTOR inhibition in human cells.

Following up on this, we will analyse the effect of mTOR inhibition during differentiation and expand our observations to induced PSCs of cynomolgus monkey. We will also use fluorescent neural reporters to compare neural marker expression onset between the species upon mTOR inhibition. In the end, we want to uncover molecular and metabolic mechanism that enable us to manipulate timing during differentiation.

Poster number: 35

Characterization of human-specific regulators of neurodevelopment
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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. As a proof of principle, dCas9-KRAB hiPSCs were transduced with gRNAs targeting SOX2, OCT4, and ARHGAP11B, leading to an interruption of proliferation, and mesodermal differentiation.

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.
EARLY HUMAN FETAL NEURAL PRECURSORS WITH HIGH SELF-RENEWAL AND BROAD NEURAL DIFFERENTIATION CAPACITY

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Recently, we reported neural plate border stem cells (NPBSC) differentiating into central and peripheral lineages directly converted from human somatic cells. Although NPBSCs resemble cells of anterior hindbrain region in neurulation-stage embryos, it remains unclear to which extent physiological correlates exist. Therefore, we isolated early neurulation-stage stem cells from human fetal brain tissue (7-11 wpc) employing a defined medium which modulates signaling pathways (SHH, WNT, FGF) orchestrating neurodevelopment. We identified conditions enabling formation of highly proliferative, homogenous fetal neural precursor cells (fNPCs). fNPCs exhibit non-polarized morphology and an early neuroepithelial profile including expression of SOX1, PAX6, Nestin, SOX2 and ZO-1 as judged by immunofluorescence and qRT-PCR. Flow cytometry revealed CD133 and CXCR4 and PSA-NCAM-expressing cells. Notably, fNPCs can be monoclonally expanded >45 passages maintaining their primitive NPC phenotype and normal karyotype. Transcriptional profiling of fNPCs by scRNA seq revealed little variance among different biological replicates. Moreover, GO enrichment revealed genes related to neurodevelopment and neural tube formation. Interestingly, fNPCs express not only neuroepithelial (TJP1, HES1), but also radial glia (VIM, CDH) and NPB genes (SOX3, ZIC1). Further data suggest patterning towards ventral regional identity characterized by upregulated NKX6-1 and SFRP2 and downregulated NKX2.2. The differentiation analysis of fNPCs unraveled strong neurogenic potential. Moreover, the presence of astrocytes and oligodendrocytes following targeted differentiation and transplantation confirms lineage potential. Electrophysiology revealed spontaneous action potentials and immunofluorescence indicate GABAergic, glutamatergic and dopaminergic subtypes. Additionally, putative synapse formation was detected by immunostainings and ultrastructural examination. As shown for NPBSCs, the differentiation of fNPCs can be directed towards sensory neurons. In conclusion, our data suggests a thus far unknown primitive neuroepithelial precursor population with broad CNS and neural crest differentiation capacity. These fNPCs will be instrumental to elucidate early human neurodevelopmental mechanisms and represent a novel source for cell replacement and drug screening approaches.

Exploring the regulation of human neural stem cell quiescence in vitro

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Quiescence is crucial to maintain stem cell pools throughout life. In the adult brain, quiescent neural stem cells (NSCs) are present beside others in the dentate gyrus in the hippocampus. Upon activation, the stem cells start proliferating and produce new neurons, but adult NSCs (aNSCs) are not very efficient at generating new stem cells (self-renew) and the stem cell pool declines with age, causing a drop in neurogenesis over time. Studying the mechanisms that control quiescence is challenging in-vivo due to the complexity of the niche and the rarity of stem cells. NSCs can be derived from the embryonic and adult mouse brain at different ages, and differentiated from embryonic stem cells. These cells are maintained in-vitro and upon BMP4 addition, quiescence is induced. However, we lack a human model and accessing human material is difficult due to obvious reasons. I am establishing a novel human NSC quiescence in-vitro model by differentiating human pluripotent stem cells (hiPSCs) into granule neurons and maintain as hippocampal NSCs. In response to BMP4, cells drop proliferating and upregulate characteristic markers. Interestingly, marker associated with the transition to quiescence, are differently expressed in human NSCs, indicating that quiescence might be regulated differently in human NSCs.

With age, the remaining quiescent cells enter a deeper quiescence, making its harder to activate. To investigate changes over time, we will compare directly reprogrammed NSCs, which maintain certain ageing characteristics, with our NSCs derived from hiPSCs, corresponding to embryonic brain stages. With this, we can investigate the mechanisms involved and understand the effects of age on aNSC transitions between active and quiescent states.
Topological morphogenesis of neuroepithelial organoids
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Animal organs exhibit complex topologies involving cavities and tubular networks, which underlie their form and function. However, how topology emerges during organ morphogenesis remains elusive. Here, we combine tissue reconstitution and quantitative microscopy to show that trans and cis epithelial fusion govern tissue topology and shape. These two modes of topological transitions can be regulated in neuroepithelial organoids, leading to divergent topologies. The morphological space can be captured by a single control parameter which is analogous to the reduced Gaussian rigidity of an epithelial surface. Finally, we identify a pharmacologically accessible pathway that regulates the frequency of trans and cis fusion, and demonstrate the control of organoid topology and shape. The physical principles uncovered here provide fundamental insights into the self-organization of complex tissues.

Glycogen modulates naïve pluripotency in embryonic stem cells and pre-implantation embryo development
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Distinct phases of pluripotency, e.g., naïve and primed, capture embryonic potential of epiblast cells in pre- and post-implantation embryo. Both states of cells are capable of self-replication and differentiation, yet are differ from several biological characters, such as epigenetic, mitochondrial and metabolic features. Particularly, metabolic remodeling occurs in embryo in order to adapt dramatic changes of extracellular circumstances before-and-after the implantation. For instance, high glucose demands and lower level of glycolysis are distinct characters of naïve embryonic stem cells (ESCs) in pre-implantation embryo. Previously, we found that glycogen storage is an unique feature of naïve ESCs and inner cell mass of pre-implantation embryo, however, roles of the accumulated glycogen in naïve ESCs and embryos remained unclear. Here, we found that intracellular glycogen modulated naïve pluripotency by directly regulating Ampk activity followed by de novo fatty acid synthesis. Also, loss of glycogen in Glycogen synthase 1 (Gys1) knockout ESCs showed reduction of fatty acids with decreased acylation and secretion of Wnt proteins. Moreover, few glycogen ESCs induced decline of Bmp4 and Nodal expression which are necessary for trophectoderm development. These findings reveal that glycogen in naïve ESCs can act as a “signaling molecule” to directly regulates Ampk activity and signals embryo to further process development during implantation.

Sphingolipids Control Dermal Fibroblast Heterogeneity
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Human cells produce thousands of lipids that impact biological processes in ways we are only starting to characterize. The cellular composition in lipids changes during differentiation and also varies across individual cells of the same type. Yet, whether and how cell-to-cell differences in lipid composition affect cell phenotypes remain unknown. Here we have measured the lipidomes and transcriptomes of individual human dermal fibroblasts by coupling high-resolution mass spectrometry imaging to single-cell transcriptomics. We find that the cell-to-cell variation of specific lipid metabolic pathways contributes to the establishment of cell states involved in the organization of skin architecture. In fact, sphingolipid composition defines fibroblast subpopulations and sphingolipid metabolic rewiring drives cell state transitions. These data uncover a role for cell-to-cell lipid heterogeneity in the determination of cell states and reveal a new regulatory component to the self-organization of multicellular systems.
Poster number: 41

AktTORs in differentiation: the role of PTEN and TSC2 in coordinating ES cell exit from naïve pluripotency
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Mouse embryonic stem cells (mouse ES cells) are captured in the ground state of pluripotency. This cell state, also known as the naïve pluripotent state, is sustained by a self-reinforcing gene regulatory network (GRN) composed by core pluripotency transcription factors (TFs) and naïve-specific TFs. Upon appropriate instructions, ES cells exit the naïve pluripotent state and enter the differentiation process, transitioning through the recently described formative pluripotent state. This is accompanied by the dismantling of the naïve GRN, and the establishment of a formative-specific GRN. A balanced interplay of several signalling pathways regulates this cell state transition. Among those, an important role in supporting the self-renewing, naïve pluripotent state of ES cells is exerted by the PI3K/Akt pathway. Two negative regulators of this pathway, Pten and Tsc2, are indeed found among high confidence hits in genetic screens performed to identify factors driving ES cell differentiation. Although Pten and Tsc2 are already reported to be involved in the control of ES cell exit from naïve pluripotency, the molecular mechanisms underlying their differentiation promoting function remains to be addressed.

Combining the use of knock-out (KO) cell lines with small molecules inhibitors, we confirmed the requirement of Pten and Tsc2 for proper ES cell differentiation. Mechanistically, we observed that TSC2, as expected from textbook knowledge, controls ES cell differentiation via mTORC1 signalling. PTEN signal is mainly, but not completely, integrated through mTORC1. Several observations point to a parallel involvement of FoxO signalling downstream PTEN. Conversely, the GSK3 pathway seems to play only a minor role in integrating PTEN signalling. Our ultimate goal is the identification of common and specific downstream targets of the signalling cascades elicited by PTEN and TSC2. This will provide a better understanding of molecular events orchestrating the early differentiation process of ES cells.

Poster number: 42

Application of an inflammatory skin model with induce pluripotent stem cell-derived macrophages to study granuloma formation
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Introduction: Sarcoidosis is an inflammatory skin disease of unknown etiology, characterized by the aggregation of macrophages, lymphoid cells and fibroblasts. These granulomas represent the pathological hallmark of sarcoidosis, yet the process of granuloma formation is not known. Therefore, we aim to engineer an immunocompetent skin model reflecting the tissue microenvironment, allowing us to study the inflammatory niche occurring in vivo.

Results: In this study we focused on the potential use of induced pluripotent stem cells (iPSC) and their ability to develop into every other cell type. We established a protocol to successfully differentiate macrophages from healthy and patient-specific iPSC. In a two-step process, monolayer differentiation of monocytes is induced followed by their differentiation into macrophage subtypes using IL-4 or INF-γ and LPS. iPSC-derived macrophages (iPSDM) are compared to monocyte-derived macrophages (MoDM) from the same individuals. iPSDM and MoDM expressed the canonical markers CD45, CD14 and CD68, as well as macrophage subtype specific markers and the respective cytokines. Next, we incorporated MoDM into human skin equivalents to assess their behavior in a three-dimensional setting. We were able to localize the cells by immunofluorescence staining and fluorescent cell tagging allowed us to track their migratory behavior. Interestingly, patient-derived MoDM tend to migrate into the same direction similar to granuloma formation, whereas control cells were more evenly distributed within the matrix. To identify potential disease drivers, we performed single cell RNA sequencing and identified a switch in the metabolic program of MoDM from sarcoidosis patients. In the next steps, we aim to validate this data in iPSDM and study their interaction with other cell types in a 3D in vitro skin model. We combine primary cell culture, direct differentiation, bioprinting and the intrinsically driven self-assembly of cells to decipher cellular processes involved in inflammatory skin diseases.
Myotonic dystrophy type 1 (DM1) is an inherited multi-systemic disorder, originating from the expansion of an unstable CTG repeat in the 3’ UTR of the DMPK gene. Typically, a longer repeat correlates with more severe symptoms and an earlier age of onset. The disease pathogenesis is thought to be mainly caused by a deleterious gain-of-function effect of the expanded DMPK transcripts. DM1 is known for features like myotonia and muscle wasting, but it is also characterized by cognitive impairments such as executive dysfunction, visuospatial deficits and excessive daytime sleepiness. Unfortunately, knowledge about the underlying mechanisms that cause the cognitive defects is limited and everything so far is known from DM1 mouse models.

We successfully generated iPSC from fibroblasts from two patients with DM1 and differentiated these to glutamatergic excitatory neurons (iNeurons). First, we looked at DM1-specific hallmarks, such as RNA foci. Additionally, we looked at the electrophysiological activity of iNeurons generated from these two DM1 patients and a control, measured by multi-electrode array (MEA). The generated data is preliminary but in vitro DM1 neurons seem to be hyperexcitable which was also found in a DM1 mouse model. In the upcoming period we aim to characterize DM1 iNeurons to identify deficits of patient-derived neurons and to identify disrupted biological processes underlying the DM1-specifics neural defects. For this we will investigate neuronal morphology by immunocytochemistry and reconstruction with the Neurolucida 360 software, furthermore we will determine the repeat length by Bionano Sapphire, look at alternative spliced genes by RT-PCR and continue establishing an electrophysiological profile of DM1 neurons.

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Poster number: 43

Elucidation of the neuropathological defects in iPSC-derived iNeurons from patients with DM1
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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. Moreover, the NAGs follow a similar expression dynamic in vitro and in vivo during epiblast transition, suggesting in vivo relevance. Additionally, 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 fundamental role in the naïve state, we proposed to perform both CRISPR activation and interference-based screens. For this purpose, dCas9 fused to transcriptional effectors and a customized pooled gRNA library were delivered into the cell line reporter “Rex1-GFP”. This cell line loses GFP signal under cell differentiation, enabling the monitoring of the exit from naïve pluripotency in high resolution. Furthermore, in order to explore interactions with pluripotency-related pathways, the setup of these screens also included the individual addition of components known to maintain the naïve pluripotent state such as LIF, PD0325901 and CHIR99021. Thus, we expect to identify novel gene roles that can contribute to a better understanding of the underlying mechanisms involved in the naïve pluripotent state.

Poster number: 44

Systematic dissection of an extended naïve pluripotency gene regulatory network
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Two distinct modes of cis regulation control cell specification in response to Shh during spinal cord development
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Precise spatial and temporal control of gene expression is essential for development. In many developing tissues the pattern of gene expression is determined by secreted signals functioning in a graded manner over several cell diameters. Cis Regulatory Elements (CRES) interpret signalling inputs to direct appropriate gene expression. How this is accomplished remains poorly understood. The morphogen Sonic hedgehog (Shh) acts in a graded manner to direct neural progenitor specification in the ventral neural tube. Here, we uncover two distinct ways in which CRES translate graded Shh signaling into differential gene expression. For all but the most ventral neural progenitors a common set of CREs are used to control gene activity. These integrate cell type specific inputs to control expression of the associated genes. By contrast, in the most ventral progenitors, extensive chromatin remodelling is required for cell type specification. This is mediated by the pioneer factor Foxa2 engaging a distinct set of CRES, paralleling the pioneering role of Foxa2 in endoderm. Moreover, Foxa2 binds these neural sites in endoderm cells. Together the data identify distinct cis regulatory strategies for the interpretation of morphogen signaling and raise the possibility of an evolutionarily conserved regulatory strategy for Foxa2-mediated cell specification across tissues.
Intestinal stem and progenitor cells acquire cell-intrinsic inflammation during ageing

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During ageing, cell-intrinsic and extrinsic factors often act in concert, leading to stem cell exhaustion, declining tissue function, and organismal health. We study the ageing intestine, which displays a prime model to disentangle cell-intrinsic and extrinsic factors. The mouse intestinal epithelium forms a dynamic interface with its microenvironment, including immune cells and the microbiome, and receives many extrinsic signals affecting its homeostasis and tissue ageing. At a single-cell level, we found in vivo that aged intestinal stem cells (ISCs) and progenitors exhibit a chronic inflammation phenotype (inflammaging) that showed heterogeneity between individual cell types. In aged ISCs, which function as antigen-presenting cells in the intestine via MHC class II (Biton et al., 2018), we detected a strong upregulation of MHC class II expression, concurrent with alterations in T cell composition in the immune microenvironment. Current work aims to disentangle the dependency of these two ageing phenotypes.

To identify ageing phenotypes in ISCs and progenitors that are independent of extrinsic signals (such as immune cell signaling), we compared transcriptional profiles of young and aged cells from in vivo tissue and ex vivo cultured intestinal organoids. Intestinal organoids are cultured isolated from signals by the microenvironment and reveal cell-intrinsic ageing signatures. Surprisingly, intestinal organoids from aged mice still showed a similar inflammaging signature after weeks in culture. Mechanistically, we identified increased chromatin accessibility of inflammation-associated loci, suggesting the development of an epigenetic memory of inflammation. Our results reveal a cell-intrinsic, stable inflammation phenotype in aged ISCs and progenitor cells, which is in part independent from extrinsic signals.

Moreover, a potential epigenetic memory of inflammation raises the question of whether early in life inflammatory events in stem cells could prime later in life susceptibility to chronic inflammatory diseases.

Contribution of fetal stem cells to maternal wound healing in sickle cell disease

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Refractory chronic leg ulcers are a frequent complication of sickle cell disease (SCD) that induces disabilities and severely impairs life quality. As their treatment is particularly difficult, therapeutic innovation is urgently needed. We have shown that during pregnancy, fetal microchimeric cells (FMC) transferred to the mothers are recruited to maternal wounds and improve healing. Here, we sought to evaluate FMC healing capacities in both normal and delayed wound healing during post-partum. We found that healing was similarly improved in post-partum mice as in pregnant mice, through increased proliferation and angiogenesis. In a SCD mouse model (SAD) recapitulating SCD refractory ulcers, we showed an accelerated healing in post-partum SAD mice compared to virgin ones, associated with FMC recruitment to wounds and increased neovascularization. In a retrospective cohort of SCD patients, we demonstrated that ever parous SCD women had a decreased burden related to leg ulcers compared to nulliparous women. Taken together, these results indicate that healing capacities of FMC are maintained long after delivery and may be therapeutically targeted to promote wound healing in post-partum SCD patients.
Voltage-gated calcium channels (VGCCs) are involved in many physiological processes and are highly expressed in human cardiac, endocrine and brain tissues. In addition, increasing evidence emerges that VGCC are key modulators of early neurodevelopment. VGCC gain-of-function mutations, as observed in the Cav1.3 encoding CACNA1D gene, have been linked to a range of neurological pathologies, including Autism Spectrum Disorders (ASD). One such mutation affects the Cav1.3 L271 residue, which is highly conserved among VGCC pore-forming α1-subunits. Electrophysiological studies in tSA-201 cells overexpressing Cav1.3 L271H indicate that this mutation induces channel gain of function by lowering the voltage dependency of channel activation and inactivation, thereby permitting increased subthreshold inward Ca2+ currents. However, currently no functional studies are available on how this mutation affects early neurodevelopment or the physiology of disease-relevant human neurons.

Here, we describe the generation of an induced pluripotent stem cell (iPSC)-line, carrying the heterozygous Cav1.3 L271H mutation, through reprogramming of peripheral blood mononuclear cells (PBMC) obtained from a patient diagnosed with a severe neurodevelopmental disorder. By employing Sendai virus OSKM vectors, we have generated stable iPSC lines expressing pluripotency markers. The obtained Cav1.3-mutant lines can be differentiated into all three germ layers and show a normal karyotype. Additionally, a neural progenitor cell (NPC) line, expressing NPC markers SOX2, NESTIN and PAX6, has been generated. We demonstrate that these NPCs express Cav1.3, as confirmed by RT-qPCR, and that these cells can readily be used for in vitro differentiation into neurons typically associated with abnormal Cav1.3 activity, such as dopaminergic neurons.

We will present a comprehensive analysis including immunostainings, electrophysiological recordings and calcium imaging aimed to investigate how the Cav1.3 L271H mutation interferes with neural differentiation and neuronal function. Overall, this study will broaden our knowledge regarding the role of Cav1.3 channels during neurodevelopment and their pathogenic role in CACNA1D channelopathies, thereby paving the way for novel therapeutic strategies for affected individuals.

Poster number: 49

CCL2 dampens brain excitotoxic damage in post-partum mice by recruiting foetal microchimeric cells
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Improving tissue repair is a challenge, particularly for brain lesions. It was shown that brain stroke in parous mice is featured with neoangiogenesis partly involving fetal-derived endothelial cells. Our group previously reported the implication of CCL2/CCR2 signaling pathway in the trafficking of fetal progenitor populations to maternal cutaneous wounds. We therefore injected CCL2 in parous or virgin mice’s injured brains. Parous mice showed an increased fetal recruitment to cortical lesions along with improved repair mechanisms and reduced tissue damage. This study introduces a „natural stem cell therapy” based on the selective recruitment of fetal progenitors, found in all parous mammals, to repair maternal brain injury.
A systematic characterization of intrinsically formed microglia-like cells during retinal organoid differentiation

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Human induced pluripotent stem cell – derived brain organoids provide a unique opportunity to investigate the development, organization, and connectivity of neurons in a complex cellular environment. However, organoids usually lack mesoderm-derived microglia, the brain-resident immune cells which are both present in the early human embryonic brain and participate in neuronal circuit development. Here, we find that IBA1+ microglia-like cells intrinsically develop in unguided retinal organoid differentiation between week 3 and 4 in 2.5D culture. We immunostained the 2.5D culture for microglia-specific markers as PU.1, CX3CR1 and P2RY12 and confirmed their expression in IBA1+ cells. Interestingly, we found that microglia preferentially occupied floating and non-pigmented 3D-cystic compartments later in differentiation. We enriched for cystic structures using a low-dosed BMP4 application and performed mass spectrometry, thus defining the protein composition of microglia-containing compartments. We found that cystic compartments expressed both mesenchymal and epithelial markers with Vimentin and E-Cadherin among the most abundant. We validated the expression of both markers by immunostaining and showed cell layer specific expression in 3D cysts. Microglia mostly occupied the mesenchymal region. Interestingly, microglia-like cells started to express the border-associated macrophage marker CD163. The preferential localization of human microglia to a mesenchymal compartment and the border-associated signature provides insight into the behavior and migration of microglia. The model will ultimately allow detailed study of these enigmatic cells and how they enter and distribute within the human brain.

Stepwise activation of selective mRNA decay synchronises pluripotency progression and morphogenesis

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The temporal coordination of cell differentiation with morphogenesis is an essential but poorly understood feature of embryonic development. The peri-implantation period involves a rapid progression through rosette formation and lumenogenesis, coupled with rosette to formative pluripotency transition. Guided by machine learning, metabolic RNA sequencing, RBP interactome analyses we identify crosstalk between signalling, transcription and mRNA decay that ensures the coupling of cell differentiation and morphogenesis by clearing the pluripotency-associated mRNAs and restricting the time window of the rosette stage. Loss of WNT and gain of MEK signalling is both required for transcriptional induction and relocalisation of LIN28A, which only upon MEK-dependent phosphorylation induces selective decay of mRNAs while also creating a delayed negative feedback system that restricts the MEK response to the rosette. Strikingly, ablation or nuclear retention of LIN28A arrests cell fate at the rosette whereas morphological determination proceeds to lumenogenesis, leading to an unforeseen embryonic multiplication with impaired gastrulation. These findings show how interdependencies between signalling, TFs and post-transcriptional mechanisms implement the order and timing of coordinated morphogenetic and cell fate transitions in early development.
In organisms ranging from insects to songbirds, the adult brain is not simply a post-mitotic tissue, but subject to dramatic remodeling that includes formation of new neurons (neurogenesis). Such changes correlate with shifts in life cycle phases and periodic reproductive behavior, governed by the organism’s own timing mechanisms. Recently, also neuronal stem cells of the mammalian brain were found to exhibit activation on a day-night cycle. This suggests that internal timing might be a fundamental regulator of adult brain neurogenesis. The molecular mechanisms of such a regulation, however, remain enigmatic, in part due to the need for a model system that combines significant shifts in adult neurogenesis with experimental accessibility.

To bridge this knowledge gap, we are studying the marine worm Platynereis dumerilii, which possesses a highly plastic brain, and is also amenable to a range of analytical and functional techniques. We have characterized major fluctuations in brain proliferation across distinct stages of the adult life, and pioneered a single-cell approach compatible with the specific isolation and sequencing of proliferating cells at distinct stages of lineage commitment, as well as over a given period of time. Here we outline how these tools can be used to generate a multidimensional, time-resolved neuronal cell atlas and pinpoint when distinct populations of neurons are given rise to, in order to shed light at the adult neurogenesis and how this fundamental process is conditioned by internal timing mechanisms.

Minor Fatty Acid Profiles of Transitional Breastmilk: Metabolomic Analysis
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Human breast milk is one of the most important nutritional resources of infant growth. Its lipidomic profile of major fatty acid was well-documented. However, the detailed information of minor fatty acids or its derivatives is far more limited. For example, branched fatty acids, which originated from branched amino acids were frequently found in breastmilk. But the structures or quantitative characteristics are scarcely reported. As a preliminary analysis of lipidomes, analytical methods for such minor fatty acids were developed and the detailed profiles in transitional breastmilk were determined in this study. For the chemical analysis, branched fatty acids (iso- and anteiso-branched) were prepared by Wittig reaction and applied on GC-MS/GC-FID based fatty acid profiling. For rapid multi-sample analyses, efficient one-pot extraction and derivatization methods were developed. According to instrumental analysis, approximately 32 different fatty acids were observed, among which 10 minor fatty acids with branched (C15-C18 carbons) and odd-numbered fatty acids took 0.2-1% of total fatty acids. Statistical analysis of the quantitative results was performed with discriminant analysis. Possible relationship between donor’s physiological data and lipid profiles were explored. This work was supported by the National ResearchFoundation of Korea (NRF) grant funded by the Koreangovernment (MSIT) (No. 2020R1A2C1005082).

Tissue-Wide Genetic and Cellular Landscape Instructs the Execution of Sequential PRC2 Functions in Neural Stem Cell Lineage Prog
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The generation of a correctly-sized cerebral cortex with all-embracing neuronal and glial cell-type diversity critically depends on faithful radial glial progenitor (RGP) cell proliferation/differentiation programs. Temporal RGP lineage progression is regulated by Polycomb Repressive Complex 2 (PRC2) and loss of PRC2 activity results in severe neurogenesis defects and microcephaly. Yet, how PRC2-dependent gene expression precisely instructs RGP lineage progression on the single cell level is unknown.

Here we utilize Mosaic Analysis with Double Markers (MADM)-based single cell technology and demonstrate that PRC2 is not cell-autonomously required in neurogenic RGPS but rather orchestrates RGP proliferation programs at the global tissue-wide level. Conversely, cortical astrocyte production and maturation is cell-autonomously controlled by PRC2-dependent transcriptional regulation.

We thus reveal highly distinct and sequential PRC2 functions in RGP lineage progression that are dependent on complex interplays between intrinsic and tissue-wide mechanisms. In a broader context our results imply a critical role for the genetic and cellular niche environment in neural stem cell behavior.
Forward programming of human sensory neurons by overexpression of NGN1, BRN3A and ISLET1

Disruption of sensory neuron (SN) and particularly nociceptor function is a major cause of chronic pain and other clinical disorders. IPSC-derived SNs provide an exciting platform for modeling such disorders in vitro. However, conventional small molecule-based in vitro differentiation requires adaptation for each iPSC-line and is prone to batch-to-batch variations. With the aim to make this process more efficient and robust, we engineered iPSCs to express key transcription factors involved in the development of sensory neurons in an inducible manner. To that end, a doxycyclin-inducible expression cassette encoding NGN1, BRN3A and ISLET1 ('NBI') was introduced in the AAVS1 safe harbor locus. After 7 days of continuous transgene induction we observed robust formation of sensory neurons expressing PRPH, NTRK1, TRPV1, NAV1.7 and NAV1.8. Interestingly, overexpression of ‘NBI’ robustly induced pure neuronal cultures independent of the iPSC seeding density, while a combination of only NGN1 and BRN3A generated a larger proportion of glial cells. Membranome analyses revealed expression of nociceptor-associated proteins such as PKA-RIIβ, P2X3 and the TRPV1 interacting protein CHMP4B. Exposure to pain-specific stimuli αβ-ATP and Capsaicin resulted in increased neuronal firing rates, thus confirming functionality of P2X3 and TRPV1 receptors, respectively. Finally we employed iPSCs from two chronic pain patients 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 neurons showed an increased firing rate, as well as a higher number of bursts in multi-electrode array assays, recapitulating changes in cellular functionality that could be associated with the sensation of chronic pain in affected individuals. Taken together, we present a forward programming approach for the fast and efficient generation of pure and functional human sensory neurons suitable for disease modeling.

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

Myotonic dystrophy type 1 (DM1) is the most common form of adult muscle disease with a prevalence of 1:2500. It is a toxic RNA gain-of-function disorder caused by an expanded (CTG)n-repeat in the 3’ untranslated region of the DMPK gene. The toxic RNA is retained in the nucleus where it sequesters proteins, such as MBNL1, leading to dysfunctional transcription and aberrant splicing. Clinical symptoms involve muscular atrophy, myotonia and progressive muscle wasting with symptomatic treatment as the only option. However, our aim is to set-up a personalized treatment with the use of induced pluripotent stem cells to alleviate the muscular phenotype.

We successfully isolated muscular progenitor cells, pericytes, from quadriceps muscle biopsies of six DM1 patients and two healthy individuals. A main characteristic of pericytes is that they can be delivered into the muscle via systemic injection. However, they have a limited expandability in vitro and limited survival rate for clonal expansion. To circumvent these issues we generated pericyte-derived iPSCs (PC-iPSCs) by nucleofection with non-integrating episomal vectors containing the Yamanaka factors. Next, we excised the pathogenic trinucleotide repeat from these cells via CRISPR/Cas9-mediated gene editing by ribonuclear proteins (RNPs) with a dual cut, furthermore we now focus our work on replacing the disease-causing repeat by a healthy repeat via HDR. We determined an HDR efficiency of 1-14% with digital droplet PCR.

For therapeutic purposes, the corrected PC-iPSCs can be differentiated to pericyte-like cells (PiPs) by a published protocol. PC-iPSCs were differentiated into PiPs by media changes and cell density limitations. Currently, our PiPs show the same markers as primary pericytes, with no remaining pluripotency on immunocytochemistry and RT-qPCR. However, compared to primary pericytes the PiPs are smaller in size and are not able to spontaneously form muscle fibers in 2D. At the moment we are optimizing the differentiation of PC-iPSCs to PiPs and determining the in vivo characteristics of transplanted cells in immunodeficient mice.

Grant support: This work was funded by the Prinses Beatrix Spierfonds (grant numbers W.OP19-03 and W.OR18-06).
Adult neural stem cells (aNSCs) are the source of newly born neurons in the adult brain. Unlike developmental neural stem cells, most aNSCs are quiescent and seldom activate to generate progeny. The regulation of their activation and return to quiescence is crucial to determine the neurogenic output, but also the long-term maintenance of the aNSC pool and neurogenesis. Intermittent fasting (IF), known to extend life and healthspan, has been proposed to increase neurogenesis, and therefore holds a great potential as a strategy to improve cognitive abilities and promote a healthier aging. We used lineage tracing and label retention experiments to characterize the impact of IF on adult neurogenesis, focusing on aNSC behaviour. We observed that a short period of IF reduced the neuronal output and altered aNSC transitions between quiescence and activation. IF caused clear weight oscillations, where mice lost up to 10% of their weight upon fasting and recovered it with refeeding. Nonetheless, the total number of neuroblasts and of newly born neurons were unaffected after 3 months of IF, contradicting previous reports. Additionally, aNSC proliferation and maintenance were alike in IF and control mice. We ruled out that mouse strain, tamoxifen, or the time of refeeding introduced any confounding effects and found consistently that IF did not increase neurogenesis. Overall, this data suggests that aNSCs can sense and react to extrinsic stimuli such as diet, but that neurogenesis is more robust than previously thought and adapts to a changing systemic environment to maintain homeostasis.

Mesenchymal stem cells (MSCs) are primary candidates in tissue engineering and stem cell therapies due to their intriguing regenerative and immunomodulatory potential. Their ability to self-assemble into three-dimensional (3D) aggregates further improves some of their therapeutic properties, e.g., differentiation potential, secretion of cytokines, and homing capacity after administration. However, high hydrodynamic shear forces and the resulting mechanical stresses within commercially available dynamic cultivation systems can decrease their regenerative properties. Cells embedded within a polymer matrix, however, lack cell-to-cell interactions found in their physiological environment. Here, we present a “semi scaffold-free” approach to protect the cells from high shear forces by a physical barrier, but still allow formation of a 3D structure with in vivo-like cell-to-cell contacts. We highlight a relatively simple method to create core–shell capsules by inverse gelation. The capsules consist of an outer barrier made from sodium alginate, which allows for nutrient and waste diffusion and an inner compartment for direct cell-to-cell interactions. Next to capsule characterization, a harvesting procedure was established and viability and proliferation of human adipose-derived MSCs were investigated. In the future, this encapsulation and cultivation technique might be used for MSC-expansion in scalable dynamic bioreactor systems, facilitating downstream procedures, such as cell harvest and differentiation into mature tissue grafts. (parts published in MDPI Bioengineering, DOI: 10.3390/bioengineering9020066)
In salamanders, limb regeneration occurs regardless of the amputation site. However, distally amputated limbs grow slower than proximally amputated ones, resulting in an overall time of regeneration, independent of the tissue volume to be reformed. This suggests that cell proliferation/size could be adjusted with the plane of amputation. Interestingly, intercellular adhesion strength has shown to be higher between distal blastema cells than between proximal ones. We propose that such differential adhesion strength modulates the mechanical properties of the regenerating tissue, that ultimately leads to differential growth rate.

By amputating axolotl forelimbs at different levels, we measured growth rate and analysed cell cycle stages, observing that differences in proliferation readily occur during early regeneration steps, reaching its maximum values during later stages, i.e., after digit patterning.

To probe the tissue’s elastic properties in vivo, we used an emerging technique in the tissue mechanics field, Brillouin Confocal Microscopy (type of optical elastography combined with confocal microscopy) and our results indicate that tissue compressibility is lower during regeneration of distally amputated limbs when compared to proximally amputated ones, indicating higher rigidity. Accordingly, our indentation measurements ex vivo with the well-established Atomic Force Microscopy show that that distally amputated limbs are stiffer during different stages of regeneration as well.

We analysed several Yap/Taz transcriptional targets by qPCR. Our results are consistent with a regulation in gradient along the proximo-distal axis during regeneration. This data suggests that the mechanotransduction mechanism responsible for differential proliferation rates may be, at least in part, explained by the Hippo pathway.

Extracellular matrix biomechanical properties affect several aspects of cell behaviour, such as reprogramming and proliferation. Thus, our results contributing to better understanding the impact of tissue mechanics in regeneration, may have important implications for the design of biomaterials in the future.

Poster number: 60

Embryo-like stem cell aggregates reveal the potential of the primitive endoderm for morphogenesis and differentiation

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Interactions between the embryonic epiblast (Epi) and two extraembryonic lineages control morphogenesis and cell differentiation in the mammalian peri-implantation embryo. Current models posit that the extraembryonic primitive endoderm (PrE) arranges around the epiblast. Furthermore, differentiation of the PrE cells towards anterior visceral endoderm (AVE), a cell population that controls anterior-posterior patterning of the embryo, is thought to be locally restricted by signal emanating from the extraembryonic ectoderm. Whether the PrE has autonomous potential for morphogenesis and patterning is not known. Here we address this question using Epi-like and PrE-like cells differentiated from mouse embryonic stem cells. When cultured on low adhesion substrates, mixtures of these two cell types spontaneously form 3D aggregates that resemble part of the peri-implantation embryo and that we term bilayered embryo-like aggregates (BELAs). While pure cultures of Epi-like cells do not undergo spontaneous morphogenesis, PrE-like cells alone can form epithelial cysts that resemble the outer layer of BELAs. This reveals an hitherto unappreciated autonomous morphogenetic potential of PrE-like cells. To test whether PrE-like cells also had potential for autonomous patterning, we performed single cell RNA sequencing and in situ mRNA staining on BELAs and PrE cysts. This revealed the differentiation of a subset of PrE-like cells towards AVE in BELAs, but not in PrE cysts. AVE cells often formed single spatial clusters in individual BELAs, and their differentiation required Nodal signaling, recapitulating key aspects of AVE differentiation in the embryo. These findings challenge current models in which the extraembryonic ectoderm patterns the AVE, and instead open the possibility of autonomous, self-organized AVE differentiation in BELAs.
Self-organized pattern formation in the developing dorsal neural tube

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The vertebrate dorsal neural tube gives rise to a remarkable diversity of cell types; neural crest, roof plate and six domains of neural progenitor subtypes are established in precise spatiotemporal order. These progenitor types are specified in response to parallel gradients of BMP and Wnt signalling. However, it is unclear how these gradients are established and interpreted to control pattern formation. To address this question, we established a novel in vitro assay in which we direct mouse embryonic stem cells to differentiate, on micropatterns, into cell types of the dorsal neural tube. Our experiments revealed that in response to exogenous BMP, cells spontaneously self-organize into concentric domains of gene expression. Using time-resolved inhibition and rescue experiments, we further showed that pattern formation occurs in two sequential steps. This temporal sequence is controlled by the dynamics of endogenous BMP and Wnt signalling gradients in this system. By constructing a data-driven theoretical model of signalling and cell fate specification of dorsal progenitor cells, we further identified a minimal network that captures the observed signalling dynamics. We are currently testing key predictions of the model using pharmacological and genetic manipulations. Altogether, our findings suggest that pattern formation in the dorsal neural tube relies on an intrinsic timing mechanism encoded in the signalling dynamics to generate cell types in the correct spatiotemporal order.

Reassessing transcriptomic landscapes of stem-cell based embryo models

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Pluripotent mice-stem cell aggregates, when grown in a favourable medium, and supplanted with factors to initiate asymmetry, go on to elongate and form structures that mimic early embryonic development and are termed as ‘gastruloids’. Several groups worldwide have attempted to make these gastruloids using similar and different protocols. These groups use their own distinctive methods to annotate and report the cell types they get from their models. The publication of these models often includes single-cell RNA (scRNA) sequencing data, a new revolutionary technique that sequences the messenger RNA of individual cells in a tissue, giving us a readout of the genes about to be expressed in a cell.

The authors are attempting to scrutinize the single-cell RNA transcriptomic data of several published stem-cell-based embryo models, by employing several approaches to analyse the single-cell transcriptomic data. Initial results suggest that structures following the same protocols in different labs possess different transcriptomic signatures. The analysis also revealed that the ‘reference’ data used to annotate the cell types is problematic, as different references seem to annotate different identities to the same cell. The analysis reveals fundamental problems of scRNA analysis - lacking consensus on what transcriptomic signature belongs to which cell type.

Understanding the role of the cell cycle in the exit from naïve pluripotency

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Development is characterized through multiple cell fate transitions. The exit from naïve pluripotency is an ideal model system to study how cells change their identity during differentiation in vitro, since the mouse embryonic stem cells (mESC) start from a highly homogenous starting population. However, as soon as differentiation is initiated, some cells initiate differentiation earlier compared to others, thereby leading to a highly asynchronous exit from naïve pluripotency. Heterogeneous cell cycle profile of mESCs, stochasticity in gene expression or intercellular communication could be potential reasons for observing the rise in asynchrony. We explored here if the underlying cell cycle phase at the time of initiation of differentiation could explain different rates of cell fate transition. In spite of a few reports in human and mESCs showing the role of cell cycle in cell fate determination we demonstrate here that the cell cycle phase at the initiation of differentiation does not influence the timing of transition.
Image-based lineage tracing has allowed the interrogation of adult tissue turnover kinetics and lineage potential of different cell populations. Based on the multicolor reporter systems, several mosaic genetic systems have been developed. Previously, we reported Red2Onco, which ectopically express oncogenes together with the RFP, thereby allowing the dissection of expansion kinetics and neighboring effects of oncogenic clones. In the current study, we report Red2Flpe, a new mosaic knockout system with multicolor reporters for both mutant and wildtype cells. Red2Flpe shows efficient and specific recombination in the RFP+ clones both in vitro and in vivo. To facilitate new conditional knockout (cKO) mouse line generation, we have developed a Short Conditional intrON (SCON) technology that is suitable for one-step cKO allele generation via zygote injection. SCON is compatible with both Cre/loxP- and Flp/frt-based cKO recombination systems. SCON shows no signs of hypomorphism prior recombination, while knockout is efficiently induced upon recombination. Utilizing Red2Flpe and Sox2-SCOnRt, we investigated the functions of Sox2 in the adult esophagus in which Sox2 has been thought to be crucial for stem cell maintenance and tissue turnover. However, mosaic Sox2 knockout clones in a wildtype environment persist after >2 months of lineage tracing, suggesting a non-essential role of Sox2 in maintaining stem cell characteristics. Clone size of Sox2-KO cells are smaller compared to the wildtype clones in the same tissues, which indicates a lowered fitness upon Sox2 knockout. We performed single-cell RNA sequencing (scRNAseq) of sorted cells and found differences in proliferation characteristics of wildtype and Sox2-KO cells. We conclude that Sox2 is not an essential stemness marker, but a regulator of proliferation kinetics in basal cells. Overall, we have constructed a toolkit for in vivo mosaic knockout studies that is suitable for clonal tracing with internal controls and a pipeline for one step generation of cKO alleles.

During embryonic development, cellular and molecular interactions induce symmetry breaking that allows for self-organization, tissue patterning and correct organ formation. In vitro, neural tube (NT) organoids represent a unique model system to study symmetry breaking and self-organization under defined and controllable conditions. Starting from single mouse pluripotent stem cells, clonal 3D organoids form by coupling neuroectoderm differentiation with single lumenogenesis to structurally recapitulate the early NT in vivo. Remarkably, a globally applied pulse of retinoic acid (RA) results in formation of a localized floorplate and consequent ventral-dorsal pattern formation. Here, we investigate how this symmetry breaking and pattern self-organization occurs. We show that, following RA pulse, the floorplate marker FoxA2 is initially upregulated in a spatially scattered distribution. Live-imaging reveals that these scattered FoxA2+ cells form multiple pre-clusters, which interact with each other by a combination of physical sorting and long-range mutual inhibition, resulting in the formation of a single stabilized cluster that later secretes the ventral morphogen SHH. We identify the BMP signaling pathway as a key governor of long-range pre-cluster interaction, with FoxA2+ cells expressing BMP4 which acts as a diffusible inhibitor of Foxa2, and Noggin that protects FoxA2+ cells from BMP-driven downregulation. Therefore, we propose that Foxa2+ cluster self-organization is driven by a Turing-type reaction-diffusion signaling mechanism, in which Noggin and BMP4 control number and size of the Foxa2 domain. We created an analogous human NT organoid system, in which we observe a similar BMP-based mechanism governing self-organization. Intriguingly, the resolution of scattered FoxA2 into pre-clusters then mature floorplate takes around 3 times longer in human than mouse NT organoids, in keeping with each species’ embryonic pace, but their resultant pattern geometry is similar, as are their embryonic NT counterparts at this stage. This reveals a decoupling between temporal vs spatial scaling during self-organization in different species.
Establishment of human embryonic stem cells for stem-cell therapies
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Human embryonic stem cells are able to differentiate into every cell type in the human body making them a tremendous cell source for regenerative medicine.

However, the production of human embryonic stem cell lines for clinical use is challenging as clean rooms, highly-qualified personnel, standard operating procedures for both manufacture and quality control are required.

Here we present the derivation of clinical-grade hESC lines in cooperation with the Centre of assisted reproduction - University Hospital Brno which provided 6-day old blastocysts. Laminin 521 in combination with Nutristem, Human serum albumin, and E-Cadherin was used for the mechanical derivation. Human embryonic stem cells were cultured on laminin 521 in a Nutristem medium.

The derivation itself and subsequent culture are xeno-free, feeder-free, and fulfill standards of current good manufacturing practices. In-depth quality control provides essential information about the safety, pluripotency, and differentiation potential of lines. Presented clinical-grade hESC lines were established according to state-of-the-art technology that makes them excellent cell source for stem cell-based therapies.

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Molecular characterization of loss-of-function HACE1 mutations identified in individuals with a rare neurodevelopmental disorder
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Spastic Paraplegia and Psychomotor Retardation with or without Seizures (SPPRS) is an ultra-rare complex autosomal recessive neurodevelopmental disorder (NDD), typically shows an infantile-onset, starting with hypotonia at birth, followed by severely impaired global development, intellectual disability, and notable motor disability. Mutations in the HACE1 gene, which encodes for the HECT domain, and ankyrin repeat-containing E3 ubiquitin-protein ligase (HACE1) have been identified to be causative of SPPRS. Previous studies have implicated HACE1 as a tumor suppressor gene involved in multiple cancers. However, HACE1 is ubiquitously expressed in the brain and targets proteins for proteasomal degradation. The HACE1 pathology was never studied before in the human neuronal context, therefore, we intend to understand the cellular and molecular mechanisms leading to the abnormal synaptic plasticity using HACE1 patient-derived stem cells.

Here we molecularly and functionally characterized the loss-of-function HACE1 mutations known to be causative of SPPRS. We performed a detailed analysis into neurodevelopmental pathophysiology of SPPRS patients (compound heterozygous variant p.R748*), that was previously reported. Firstly, we studied the molecular mechanisms underlying the HACE1 mutation in patient-derived neuronal cells and rescue the HACE1 deficiency-induced pathologies using gene-editing. We differentiated healthy controls and patient-derived induced pluripotent stem cells (iPSCs) into mature neurons. A combination of molecular, biochemical, and high-throughput functional assays was used to study the extent of neurodevelopmental perturbations in patient-derived neurons. Most importantly, we recorded alterations in cortical excitability using IncuCyte live imaging (spontaneous) & intracellular calcium imaging (evoked). Lastly, we will rescue the disease phenotypes by correcting the deleterious HACE1 mutations using CRISPR-Cas9 prime editing. Altogether, the whole project helps to identify fundamental mechanisms causing the HACE1 phenotype and opens doors for investigating new therapeutic interventions for SPPRS patients. Besides, the novel genome editing technology has a great potential for NDDs that are currently suffering from a paucity of targeted therapeutics.
Characterization of human microglia colonizing developing retinal organoids
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Microglia are resident immune cells that infiltrate the embryonic central nervous system from the yolk sac. In rodent development, microglia impact neural organization and connectivity, yet, little is known if they have a similar role in the human embryonic environment due to the lack of a model system. Human induced pluripotent stem cells (hiPSC) provide a unique opportunity to generate brain models. One example of a spatially well-defined brain region is the retina. However, retinal organoids commonly lack microglia. Here, we are generating microglia-assembled retinal organoids (MAR) to characterize the occupation pattern of microglia in retinal organoids, which allows to study embryonic microglia-neuron interaction.

We have previously shown that we can differentiate hiPSC into microglia. To monitor microglia in retinal organoids, we generated a hiPSC line in which we integrated a transgene expressing tdTomato in the AAVS1 locus using CRISPR/Cas9. We confirmed that this hiPSC line allows the differentiation of tdTomato+ microglia expressing markers as IBA1, PU.1 and P2Y12. Next, we added these tdTomato+ cells to retinal organoids and they occupied OTX2 and Recoverin labeled retinal structures. We then performed a time course to follow up their integration pattern: Microglia maintain at least for up to ten weeks, and follow a localization pattern as previously described in vivo. First, they preferentially accumulate close to the ganglion cells, and later invade the developing synaptic layers. Now, we will use calcium imaging to explore how microglia presence impacts neuronal function.

Overall, MAR represent a model system to study microglia distribution and their interaction with neurons during human embryonic development.

HiPSC-derived three-dimensional cardiac microtissues to study the contribution of cardiomyocytes and cardiac fibroblasts in Arrh
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Arrhythmogenic cardiomyopathy (ACM) is a cardiac genetic disorder characterized by fibro-fatty replacement of the myocardium, arrhythmias and sudden cardiac death. The cellular origin and underlying molecular mechanisms underlying disease progression remain poorly defined due to the lack of appropriate models able to capture the disease phenotype.

The aim of our work is to generate a complex in vitro three dimensional (3D) multicellular microtissue model (MT) in order to evaluate the contribution of CMs and non-CM population in ACM pathogenesis.

We generated CMs and cardiac fibroblasts (CFs) from ACM hiPSCs of a patient carrying the pathogenic heterozygous c.2013delC PKP2 mutation, and its isogenic control line correcting the mutation using CRISPR/Cas9. We produced 3D cardiac MTs composed of 5000 cells in a defined ratio of 70% CMs, 15% CFs and 15% cardiac endothelial cells. We generated four groups of MTs combining CTRL CMs with either CTRL or ACM CFs, or ACM CMs with CTRL or ACM hiPSC-CFs.

Bulk RNAseq and Gene Ontology analysis on the differentially expressed genes between MT groups showed the enrichment for pathways related to protein localization to membrane, sarcomere organization and extracellular matrix when comparing MTs including mutated cells to MTs with all CTRL cell types. Immunofluorescence analysis revealed distinct localization patterns of connexin 43 throughout the MTs among the four different groups. We characterized the electrical properties of MTs by stimulating them at increasing pacing frequencies. Inclusion of only ACM CFs or ACM CMs affected the ability of MTs to respond to high stimulation frequencies (≥ 2Hz), but the presence of both ACM CMs and CFs showed an additive effect in causing arrhythmic behaviour under high-frequency.

Our findings provide evidence of non-myocyte contributions to ACM pathogenesis and demonstrate the utility of our tricellular MT in modelling multicellular cardiac diseases, enabling investigation of diseases non-autonomous to CMs.
Human PGCLC differentiation is influenced by hiPSC tissue-of-origin.
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Primordial germ cells (PGCs) are the founding population of all germ cells that can give rise to either sperm cells or eggs in mammals. Human induced pluripotent stem cells (hiPSCs) can be differentiated to generate primordial germ cell like cells (PGCLCs) in vitro, which enables tremendous opportunities such as modelling germ cell development in vitro, or generation of human gametes that may be used as treatment of infertility.

Current protocols for the generation of hPGCLC from hiPSCs suffer from vast variation depending on the hiPSC lines used. For example, male hPSC lines were reported to differentiate more efficiently to hPGCLCs than female hiPSC lines. X chromosome inactivation (XCI) is an important epigenetic event in female cells, that ensures dosage compensation of the X chromosome in male (XY) and female (XX) cells. However, female hPSCs are notorious for erasing XCI-marks such as the accumulation of XIST or histone modification H3K27me3 on the inactivated X chromosome (Xi). The reactivation of the Xi prior to meiotic recombination is also a crucial step in female germ cell development.

Here, we characterized the XCI states of 7 female hiPSC lines reprogrammed either from kidney epithelium (urine) or skin fibroblasts. Subsequently, we assessed their differentiation efficiency to hPGCLCs together with three male hiPSC lines. We observed that the XCI states have no significant effect on the differentiation efficiency of hPGCLCs; however, urine-derived hiPSCs seem to have higher differentiation efficiencies compared to skin-derived hiPSCs. To further investigate this difference, we performed transcriptomic analysis on both urine- and skin-derived hiPSCs cultured under similar pluripotent conditions and determined their differential gene expressions and enriched pathways.

Our results contribute to a better understanding of the impact that different characteristics of hiPSCs (sex, tissue of origin, XCI state) play in obtaining a highly efficient hPGCLC differentiation protocol.
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