ISSCR

VIENNA

ELUCIDATING PRINCIPLES OF DEVELOPMENT WITH STEM CELLS

4-6 DECEMBER 2023
VIENNA, AUSTRIA

PROGRAM BOOK

Welcome

Dear Colleagues,

On behalf of the International Society for Stem Cell Research (ISSCR), we are delighted to welcome you to the Vienna International Symposium, “Elucidating Principles of Development with Stem Cells” here in the historic imperial Palace, the “Hofburg.”

The potential of stem cells to self-organize and form three-dimensional models of organs (organoids) and embryos (stem cell-based embryo models) in a dish has revolutionized science and medicine. This discovery shows that simplified systems can recapitulate complex processes, and motivates us to mechanistically investigate biological processes that cannot be studied in animal models, thereby facilitating the exploration of design principles in biology. These stem cell-based models have raised immense hopes in various areas, from modeling development and disease to performing personalized drug and genetic screens.

Building on a unique dedicated research community focusing on stem cell-based models in Vienna, our conference program was designed to not only feature the latest advances in models of organs and organisms, but also to bring together experts across areas in developmental biology to tackle the biggest challenges in unlocking the key biological concepts that govern embryogenesis. We focus on six principles of development: genetic programs, genome evolution, tissue mechanics, morphogen gradients, self-organization, and timing of development. By harnessing the latest in stem cell technologies, including organoids and embryo models, and combining them with breakthroughs in single cell genomics, imaging, and computational techniques, we hope to better understand the mechanisms of development, and, ultimately, regeneration.

We invite you to explore the full program, from invited talks and innovation showcases to poster presentations, and to forge new collaborations here that will help unlock the unsolved mysteries of development. Engage with exhibitors to discover new technologies and platforms that can accelerate your research. Connect with friends and colleagues from around the world, network and build new relationships that will serve you throughout your career and discover the vibrant Vienna Christmas Market with friends old and new in picturesque Vienna. We hope you enjoy the conference.

Sincerely,

Vienna Program Organizing Committee:

Program Chair - Nicolas Rivron, PhD, *Institute of Molecular Biotechnology (IMBA), Austria*

Paola Arlotta, PhD, *Harvard University, USA*

Jürgen Knoblich, PhD, *Institute of Molecular Biotechnology (IMBA), Austria*

Kate McDole, PhD, *MRC Laboratory of Molecular Biology, UK*

ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

ABOUT THE ISSCR

The mission of the International Society for Stem Cell Research (ISSCR) is to promote excellence in stem cell science and applications to human health. The ISSCR is the largest society in the world dedicated to the advancement of responsible stem cell research – a field that strives to advance scientific understanding, treatments, and cures that better human health. We foster junior scientists, give voice and visibility to scientific advancement, and encourage a positive global environment for future discovery and treatment. Our promise is to help the field of stem cell research reach its potential.

Contact Us

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OFFICIAL MEETING JOURNAL

STEM CELL REPORTS

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ISSCR 2024

THE FUTURE STARTS HERE

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10-13 JULY 2024

PLEASE JOIN US FOR OUR 2024 ANNUAL MEETING AT THE CONGRESS CENTER HAMBURG

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Meeting Information

All times in Central European Time (Vienna, Austria)

ONSITE BADGE PICK UP

Pick-up your name badge in the designated areas below during posted hours. Name badges are required for admission to all sessions, social events, meals/breaks, and the Exhibit & Poster Hall. Badges may be picked up during the following times:

Satellite Pre-Event *Badge Pick-Up ONLY*	Le Méridien Vienna Lobby
Sunday, 3 December	*4:00 PM – 6:00 PM*
Registration Desk Hours	Hofburg Vienna Antekammer Mezzanine Level
Monday, 4 December	*8:00 AM – 5:00 PM*
Tuesday, 5 December	*8:00 AM – 5:00 PM*
Wednesday, 6 December	*8:00 AM – 3:30 PM*

ISSCR MEETING SITE

Log in to the Meeting Site using the ISSCR credentials you used to register. If you have trouble logging in, first try resetting your password. If the problem persists, please direct questions to isscrdigital@isscr.org.

Livestream will not be available for this event.
As a registrant, you will have access to on demand content after the event.

SMOKING

Smoking or the use of e-cigarettes is prohibited at the Hofburg Vienna.

LOST AND FOUND

Please bring found items to the ISSCR Registration Desk during posted hours. If you lost an item, stop by during registration hours for assistance.

PARKING

Motorists can park their vehicles at nearby car parks (for a fee). The following car parks are located within walking distance to the Hofburg Vienna. Attendees are responsible for their own parking fees.

Car park **Museumsquartier**	5 min walk
Car park **Opernringhof**	5 min walk
Car park **Robert-Stolz-Platz**	5 min walk

POSTER INFORMATION

Each poster is presented during a 45-minute session in the Foyer spaces outside Zeremoniensaal, the main session room. **Poster presenters must adhere to the scheduled date and time of their poster display and presentation.**

Monday, 4 December – Poster Sessions I & II

Poster Session I	**Poster Session II**
Poster Set-up: 3:30 PM – 3:45 PM	Poster Set-up: 4:30 PM – 4:45 PM
Poster Presentation: 3:45 PM – 4:30 PM	Poster Presentation: 4:45 PM – 5:30 PM
Poster Take-down: 4:30 PM	Poster Take-down: 5:30 PM

Tuesday, 5 December – Poster Session III

Poster Session III
Poster Set-up:
3:00 PM – 3:15 PM

Poster Presentation:
3:15 PM – 4:00 PM

Poster Take-down:
4:00 PM

Poster presenters are responsible for removing their posters upon completion of their presentation. Any posters that are not removed at the end of the session will be discarded.

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BY
SCIENTISTS.

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FOR STEM CELL RESEARCH

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Your support of ***Stem Cell Reports*** enhances the ISSCR's global mission and outreach to promote, educate and advocate for stem cell research and its application.

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ISSCR Meeting Policies

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These policies comprise the Code of Conduct for all ISSCR meetings and events and apply to all attendees, speakers, exhibitors, sponsors, staff, contractors, volunteers, media, and guests.

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Abstract content may not be announced, publicized, or distributed before the presentation date and time in any way including in media stories, blogs, and social media. ISSCR does permit promotion of general topics, speakers, and presentation days and times. This embargo policy applies to all formats of abstract publication—including abstracts in electronic or print version of Meeting Program Books/Poster Abstract Books, online via the Program Planner and Poster Abstract PDFs, the society's website(s), and other publications.

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MaxWell Biosystems is a technology leader providing instrumentation and solutions to boost scientific research and development in neurosciences, stem cell and tissue engineering, ophthalmology, and other fields involving electrogenic cells. The company engineered advanced high-density microelectrode arrays (HD-MEAs) as the core of easy-to-use platforms, MaxOne (single-well) and MaxTwo (multi-well), that equip scientists to record electrical signals of neurons in in-vitro 2D and 3D models. MaxWell Biosystems' HD-MEA technology allows to capture neuronal activity across multiple scales, from sub-cellular to single cells, up to full networks in unprecedented detail. Ultimately, MaxWell Biosystems' platforms facilitate the understanding of neurological diseases, enhance the efficiency of cell-based assays for toxicity and safety pharmacology, and accelerate drug discovery.

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Program Schedule

Monday, 4 December

Monday

4 December 2023

All times in Central European Time (Vienna, Austria)

9:00 AM – 10:05 AM — OPENING REMARKS AND GENETIC PROGRAMS I

ROOM: Zeremoniensaal

Chair: **Nicolas Rivron**, *Institute of Molecular Biotechnology (IMBA), Austria*

9:00 AM – 9:02 AM
Welcome Remarks
Keith Alm, *International Society for Stem Cell Research (ISSCR)*

9:02 AM – 9:05 AM
Opening Remarks
Nicolas Rivron, *Institute of Molecular Biology (IMBA), Austria*

9:05 AM – 9:35 AM
Denis Duboule, *Ecole Polytechnique Fédérale (EPFL), Switzerland*
USING STEMBRYOS TO STUDY BASIC DEVELOPMENTAL PRINCIPLES

9:35 AM – 10:05 AM
Paola Arlotta, *Harvard University, USA*
BRAIN CHIMEROIDS AS AVATARS TO STUDY HUMAN INTERINDIVIDUAL VARIATION

10:05 AM – 10:35 AM — BREAK

ROOM: Foyer

10:35 AM – 11:50 AM — GENETIC PROGRAMS II

ROOM: Zeremoniensaal

Chair: **Martin Leeb**, *Max Perutz Labs, Austria*

10:35 AM – 11:05 AM
Barbara Treutlein, *ETH Zürich, Switzerland*
RECONSTRUCTING BRAIN DEVELOPMENT AND REGENERATION WITH SINGLE-CELL TECHNOLOGIES

11:05 AM – 11:20 AM
Andre Dias, *Universitat Pompeu Fabra, Spain*
USING GASTRULOIDS TO ELUCIDATE THE ROLE OF SIGNALLING DURING MAMMALIAN GASTRULATION

11:20 AM – 11:50 AM
Maria Rostovskaya, *PhD, Babraham Institute, UK*
MOLECULAR TIMETABLE OF DIFFERENTIATION IN HUMAN PLURIPOTENT STEM CELLS

Program Schedule

Monday, 4 December

11:50 AM – 1:30 PM LUNCH BREAK

ROOM: Foyer

**Lunches may be brought downstairs to enjoy during the Innovation Showcase*

12:15 PM – 1:15 PM INNOVATION SHOWCASE

ROOM: Schatzkammersaal

12:15 PM – 1:15 PM

Presented by MaxWell Biosystems
Laura D'Ignazio, PhD, *MaxWell Biosystems, Switzerland*
Nina Dirkx, *Applied and Translational Neurogenomics Group - VIB Center for Molecular Neurology, VIB, Belgium*
Luca Guglielmi, PhD, *Lancaster Lab, MRC Laboratory of Molecular Biology (LMB), UK*
EXPLORING CUTTING-EDGE FUNCTIONAL CHARACTERIZATION OF IPSC-DERIVED 2D AND 3D IN VITRO MODELS IN MOLECULAR BIOSCIENCE

1:30 PM – 3:30 PM GENOME EVOLUTION IN DEVELOPMENT

ROOM: Zeremoniensaal

Chair: **Sarah Bowling**, *Harvard University, USA*

1:30 PM – 2:00 PM

Joanna Wysocka, *Stanford University School of Medicine, USA*
DNA-GUIDED TRANSCRIPTION FACTOR COOPERATIVITY SHAPES EVOLUTION AND VARIATION OF HUMAN FACES

2:00 PM – 2:30 PM

Madeline Lancaster, *MRC Laboratory of Molecular Biology, UK*
EXPLORING CELL IDENTITY IN BRAIN ORGANOIDS

2:30 PM – 2:45 PM

Ali Elagoz, *KU Leuven, Belgium*
CEPHALOPOD MOLECULAR APPROACH OF EVOLVING THE LARGEST INVERTEBRATE NERVOUS SYSTEM

2:45 PM – 3:00 PM

Thomas Vierbuchen, *Memorial Sloan Kettering Cancer Center, USA*
MAPPING THE IMPACT OF CIS-REGULATORY VARIATION ON MOUSE CORTICAL DEVELOPMENT ACROSS EVOLUTIONARY TIMESCALES

3:00 PM – 3:30 PM

Nicolas Rivron, *Institute of Molecular Biotechnology (IMBA), Austria*
MODELING THE HUMAN-SPECIFIC ASPECTS OF BLASTOCYST DEVELOPMENT AND IMPLANTATION WITH BLASTOIDS

3:30 PM – 5:30 PM WELCOME RECEPTION AND POSTER SESSION I & II

ROOM: Foyer

3:45 PM – 4:30 PM

Poster Session I

4:45 PM – 5:30 PM

Poster Session II

5:45 PM

Christmas Market Walk (optional)
Meet outside the Hofburg by 5:45 PM

Program Schedule

Tuesday, 5 December

Tuesday
5 December 2023

9:00 AM – 10:15 AM — MORPHOGEN GRADIENTS I

ROOM: Zeremoniensaal

Chair: **Katharina Sonnen**, *Hubrecht Institute, Netherlands*

9:00 AM – 9:30 AM

Jeremy Green, *King's College London, UK*
MORPHOGEN COCKTAILS AND MECHANICAL SYMMETRY-BREAKING: TWO NOT-QUITE-CLASSICAL PATTERNING MECHANISMS

9:30 AM – 9:45 AM

Jonathan Chubb, *University College London, UK*
ACTIVE DROPLET BEHAVIOUR OF CELL GROUPS DRIVES PATTERN FORMATION IN RESPONSE TO A SELF-GENERATED GRADIENT

9:45 AM – 10:15 AM

Anna Kicheva, *Institute of Science and Technology (ISTA), Austria*
INTERPRETATION OF MORPHOGEN SIGNALING IN THE DEVELOPING SPINAL CORD

10:15 AM – 10:45 AM — BREAK

ROOM: Foyer

10:45 AM – 11:45 AM — MORPHOGEN GRADIENTS II

ROOM: Zeremoniensaal

Chair: **Anna Kicheva**, *Institute of Science and Technology (ISTA), Austria*

10:45 AM – 11:15 AM

Sasha Mendjan, *Institute of Molecular Biotechnology (IMBA), Austria*
CARDIOIDS UNRAVEL HUMAN HEART DEVELOPMENT AND DISEASE

11:15 AM – 11:45 AM

Sharad Ramanathan, *Harvard University, USA*
ROLE OF MECHANICS IN THE PATTTERNING OF THE EARLY NEUROEPITHELIUM

11:45 AM – 1:15 PM — LUNCH BREAK

ROOM: Foyer

**Lunches may be brought downstairs to enjoy during the Innovation Showcase*

12:00 PM – 12:30 PM — INNOVATION SHOWCASES

ROOM: Schatzkammersaal

12:00 PM – 12:15 PM

Presented by Cytosurge AG
Tamás Gerecsei, PhD, *Cytosurge AG, Switzerland*
CYTOPLASMIC LIVE-CELL BIOPSIES FOR THE TEMPORAL PROFILING OF SINGLE-CELLS

12:15 PM – 12:30 PM

Presented by 3Brain AG
Antonella Di Bello, PhD, *3Brain AG, Switzerland*
REVOLUTIONIZING ORGANOID ANALYSIS: UNVEILING THE POWER OF 3D MICROCHIP TECHNOLOGY FOR ENHANCED LIFE SCIENCES INSIGHTS

Program Schedule

Tuesday, 5 December

1:15 PM – 3:00 PM TISSUE MECHANICS I

ROOM: Zeremoniensaal

Chair: **Diana Pinheiro**, *Research Institute of Molecular Pathology (IMP), Austria*

1:15 PM – 1:45 PM

Kate McDole, *MRC Laboratory of Molecular Biology, UK*
ILLUMINATING MECHANISMS OF MAMMALIAN MORPHOGENESIS USING ADAPTIVE LIGHT-SHEET MICROSCOPY

1:45 AM – 2:15 PM

Sebastian Streichan, *University of California Santa Barbara, USA*
DESIGN PRINCIPLES OF FATE, FORM, FORCES, AND FUNCTiON: FROM EMBRYOGENESIS TO SYNTHETIC MORPHOGENES

2:15 PM – 2:30 PM

Akanksha Jain, *ETH-Zurich, Switzerland*
MORPHODYNAMICS OF HUMAN EARLY BRAIN ORGANOID DEVELOPMENT

2:30 PM – 3:00 PM

Thomas Lecuit, *Institut de Biologie du Développement de Marseille (IBDM), France*
ENCODING SHAPE WITH GENETICS, MECHANICS AND GEOMETRY

3:00 PM – 4:00 PM BREAK AND POSTER SESSION III

ROOM: Foyer

3:15 PM – 4:00 PM

Poster Session III

4:00 PM – 5:00 PM TISSUE MECHANICS II

ROOM: Zeremoniensaal

Chair: **Madeline Lancaster**, *MRC Laboratory of Molecular Biology, UK*

4:00 PM – 4:30 PM

Edouard Hannezo, *Institute of Science and Technology (IST), Austria*
TOPOLOGY OF THE EARLY MAMMALIAN EARLY EMBRYO

4:30 PM – 5:00 PM

Ewa Paluch, *University of Cambridge, UK*
CROSS-TALK BETWEEN CELL MECHANICS, CELL SHARE AND CELL FATE

5:00 PM – 5:30 PM

End of Day with Exhibitors

Wednesday
6 December 2023

9:00 AM – 10:15 AM TISSUE SELF-ORGANIZATION I

ROOM: Zeremoniensaal

Chair: **Jürgen Knoblich**, *Institute of Molecular Biotechnology (IMBA), Austria*

9:00 AM – 9:30 AM
Francois Schweisguth, *Institute Pasteur, France*
SELF-ORGANIZED PATTERNING IN DROSOPHILA

9:30 AM – 9:45 AM
Noa Novershtern, *Weizmann Institute, Israel*
A HUMAN POST-IMPLANTATION EMBRYO MODEL DERIVED EX-UTERO SOLELY FROM GENETICALLY UNMODIFIED NAÏVE PLURIPOTENT CELLS

9:45 AM – 10:15 AM
Elly Tanaka, *Institute of Molecular Pathology, Austria*
CELL-FATE DECISIONS UNDERLYING FLOORPLATE SELF-ORGANIZATION IN ORGANOIDS

10:15 AM – 10:45 AM BREAK

ROOM: Foyer

10:45 AM – 11:45 AM TISSUE SELF-ORGANIZATION II

ROOM: Zeremoniensaal

Chair: **Paola Arlotta**, *Harvard University, USA*

10:45 AM – 11:15 AM
Aryeh Warmflash, *Rice University, USA*
SELF-ORGANIZING STEM CELL SYSTEMS TO STUDY SIGNALING DYNAMICS IN HUMAN GASTRULATION

11:15 AM – 11:45 AM
Jürgen Knoblich, *Institute of Molecular Biotechnology (IMBA), Austria*
ANALYZING FATE SPECIFICATION AND NEURAL NETWORK ARCHITECTURE BY TRANS-SYNAPTIC LABELING USING BARCODED RABIES VIRUS

Program Schedule

Wednesday, 6 December

11:45 AM – 1:15 PM LUNCH BREAK

ROOM: Foyer

**Lunches may be brought downstairs to enjoy during the Innovation Showcase*

12:00 PM – 1:00 PM INNOVATION SHOWCASE

ROOM: Schatzkammersaal

12:00 PM – 1:00 PM

Presented by the European Research Council (ERC)
Mariam Benjdia, PhD, *European Research Council Executive Agency, Belgium*
Katharina Sonnen, PhD, *Hubrecht Institute, Netherlands*
INFORMATION SESSION ON THE ERC PROGRAMME

1:15 PM – 2:30 PM TIMING OF DEVELOPMENT I

ROOM: Zeremoniensaal

Chair: **Sharad Ramanathan**, *Harvard University, USA*

1:15 PM – 1:45 PM

Alexander Aulehla, *European Molecular Biology Laboratory (EMBL), Germany*
METABOLIC CONTROL OF DEVELOPMENTAL TIMING

1:45 AM – 2:00 PM

Tomonori Nakamura, *Kyoto University, Japan*
ELUCIDATING THE IN VITRO PGC DIFFERENTIATION PATHWAY IN PRIMATES

2:00 PM – 2:30 PM

Cantas Alev, *Kyoto University, Japan*
TOWARDS RECONSTITUTING AXIAL DEVELOPMENT IN VITRO

2:30 PM – 3:00 PM BREAK

ROOM: Foyer

3:00 PM – 5:00 PM TIMING OF DEVELOPMENT II & CLOSING SESSION

ROOM: Zeremoniensaal

Chair: **Kate McDole**, *MRC Laboratory of Molecular Biology, UK*

3:00 PM – 3:30 PM

Katharina Sonnen, *Hubrecht Institute, Netherlands*
SIGNALING DYNAMICS IN THE CONTROL OF EMBRYONIC DEVELOPMENT AND TISSUE HOMEOSTASIS

3:30 PM – 4:00 PM

Alexander van Oudenaarden, *Hubrecht Institute-KNAW & University Medical Center, Netherlands*
NOVEL SEQUENCING TOOLS TO EXPLORE TRANSLATION IN INDIVIDUAL CELLS

4:00 PM – 4:30 PM

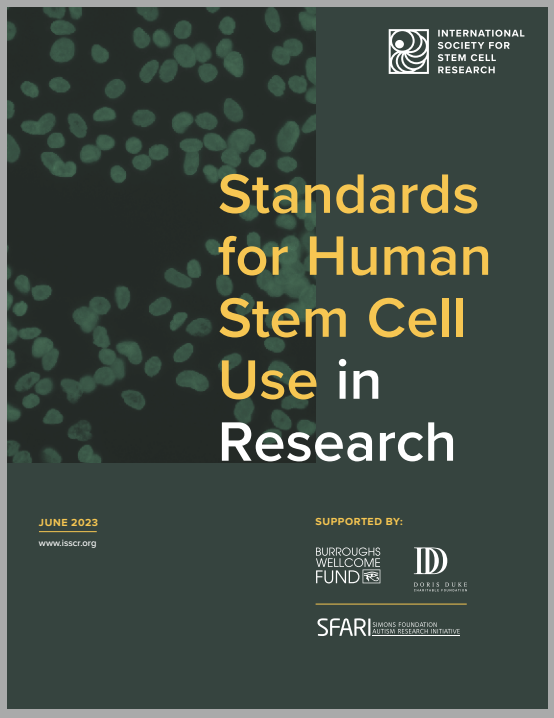
Sarah Bowling, *Harvard University, USA*
HIGH RESOLUTION MAPPING OF CELL LINEAGES DURING MAMMALIAN EMBRYOGENESIS

4:30 PM – 5:00 PM

Alexander Schier, *Biozentrum, University of Basel, Switzerland*
PATTERN FORMATION AND CELL FATE SPECIFICATION DURING VERTEBRATE EMBRYOGENESIS

ISSCR

Standards for Human Stem Cell Use in Research



Quality standards and core principles for the laboratory culture and characterization of both adult and pluripotent human stem cells and in vitro model systems using them.

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All exhibitor tables will be in the Foyer

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MaxWell Biosystems is a technology leader engineering advanced CMOS (complementary metaloxide-semiconductor)-based high-density microelectrode arrays (HD-MEAs) which are at the core of our easy-to-use systems. The technology allows integrating circuitry with thousands of electrodes per square millimeter on the same chip at high spatial resolution. MaxOne (single-well) and MaxTwo (multi-well), equip scientists to record electrical signals of neurons in in-vitro 2D and 3D models. MaxWell Biosystems' HD-MEA technology allows the capture of neuronal activity across multiple scales, from sub-cellular to single cells, up to full networks in unprecedented detail. Ultimately, MaxWell Biosystems' HD-MEA platforms facilitate the understanding of neurological diseases, enhance the efficiency of cell-based assays for toxicity and safety pharmacology, and accelerate drug discovery.

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CYTOSURGE AG

Sägereistrasse 25
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www.cytosurge.com

Cytosurge provides innovative solutions to shift boundaries in single-cell profiling and gene engineering based on the FluidFM® technology.

The FluidFM OMNIUM platform with our biopsy solution, enables semi-automatic collection of cytoplasmic extractions (biopsies) from single cells in such a gentle manner that the cells survive. Biopsies represent a snapshot of a cell's transcriptional state at a specific point in time and can be used for downstream, temporal transcriptome readouts of single cells along their lifepath.

In addition to extraction, the platform can also inject and pick cells providing a precision platform for single cell profiling.

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Speaker Abstracts

MONDAY, 4 DECEMBER

9:00 AM – 10:05 AM OPENING REMARKS AND GENETIC PROGRAMS I

9:05 AM – 9:35 AM

USING STEMBRYOS TO STUDY BASIC DEVELOPMENTAL PRINCIPLES

Duboule, Denis

Ecole Polytechnique Fédérale, Lausanne (EPFL), Switzerland

During vertebrate development, clustered Hox genes are activated in a precise time-sequence, leading to patterns necessary to properly establish the body plan. The mechanism underlying this phenomenon (the Hox timer) has remained elusive ever since its initial observation in 1989, due to the difficulty to approach it using early gastrulating mouse embryos. I will discuss our recent results using stembryos produced out of ES cells ('gastruloids') as an alternative approach to address this question and will show that the temporal dynamic of the system may rely upon the use of series of CTCF sites as successive boundary elements. While this mechanism can secure the deployment of Hox gene transcription and hence the proper establishment of axial structures within any given vertebrate species, it also offers some evolutionary flexibility, for minimal modifications in the number, position or affinity of these sites would translate into heterochronic transcription.

Keywords: Stembryos, Hox genes, transcriptional regulation

9:35 AM – 10:05 AM

BRAIN CHIMEROIDS AS AVATARS TO STUDY HUMAN INTERINDIVIDUAL VARIATION

Arlotta, Paola[1,2], Noelia Antón Bolaños*[1,2], Irene Faravelli*[1,2], Tyler Faits[1,2,3], Sophia Andreadis[1], Rahel Kestli[1,2], Xian Adiconis[2,3], Ralda Nehme[2], Steven A. McCarroll[2], Joshua Z. Levin[2,3]

[1]Department of Stem Cell & Regenerative Biology, Harvard University, Cambridge, MA, USA. [2]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, MA, USA. [3]Klarman Cell Observatory, Broad Institute of MIT and Harvard, Cambridge, MA, USA

**These authors contributed equally.*

Much remains to be understood regarding how genetic and environmental factors affect the human developing brain to ultimately cause disease. Emerging evidence indicates that similar disease risk factors are associated with distinct phenotypic outcomes depending on genomic context; such that different individuals show heterogeneous responses to negative stimuli. I will discuss progress in developing human brain organoids that are capable of reproducible, multi-donor development of the forebrain (we have named such organoids: "Chimeroids"). Chimeroids maintaining equal donor composition, as well as cell-type composition, over prolonged culture, even when the system is challenged by combining lines with different growth rates. We have leveraged this novel organoid system to investigate neurotoxic traits across multiple individual genomic context, identifying heterogeneity in the severity of neuropsychiatric conditions linked to exposure to stressors. Chimeroids have great potential to serve as a scalable system for screening drug responses and neurotoxicity in a personalized manner, across different human genetic backgrounds.

Keywords: neurotoxicity, chimeroids, neuropsychiatric, neurotoxicity, neurobiology

10:35 AM – 11:50 AM
GENETIC PROGRAMS II

10:35 AM – 11:05 AM

RECONSTRUCTING BRAIN DEVELOPMENT AND REGENERATION WITH SINGLE-CELL TECHNOLOGIES

Treutlein, Barbara

ETH Zürich, Switzerland

The brain is a highly complex and fascinating organ and we are interested in understanding how cellular heterogeneity emerges during brain development and how brain cells can regenerate upon injury. We are tackling these questions by applying and further developing integrative, multi-modal single-cell technologies. In the first part of my talk, I will present our work on human pluripotent stem cell (PSC) derived organoids that model human brain development in vitro. We generated a data set of paired single-cell transcriptome and accessible chromatin profiling over a dense time course of human brain organoid development, which we utilized to infer a gene regulatory network of human brain organoid development. We then used pooled genetic perturbation with single-cell transcriptome readout to assess transcription factor requirement for cell fate and state regulation in organoid and identified an important role of GLI3 during human telencephalon dorso-ventral patterning. Further, we have developed single-cell methodologies to directly track developmental lineages in brain organoids and could identify clonality of brain organoid regions as well as a temporal window of regional fate specification. Finally, we are working towards engineering diverse human neuronal populations in vitro using morphogen screening approaches. In the second part of my talk, I will present our work on understanding the organization and regeneration of the telencephalon in the axolotl salamander using single-cell genomics. We first generated a single-cell multiomic atlas of the axolotl telencephalon, identified evolutionary conservation of neuronal cell types and reconstructed trajectories of post-embryonic neurogenesis. We then showed that upon major injury, all neuronal cell types reemerge through regenerative neurogenesis and neuronal projections to other brain regions are re-established. Finally, we identified a regeneration specific state of neural progenitor cells that is characterized by expression of wound healing genes. Together, our work highlights the power of single-cell technologies to understand the gene regulatory logic underlying brain development and regeneration.

Keywords: human brain organoids, single cell genomics, brain development, brain regeneration, CRISPR/Cas9 gene perturbation screen, fate specification, single cell multiomics

11:05 AM – 11:20 AM

USING GASTRULOIDS TO ELUCIDATE THE ROLE OF SIGNALLING DURING MAMMALIAN GASTRULATION

Dias, André[1], Torregrosa, Gabriel[1], Pascual Mas, Pau[1], Robertson, Gaëlle[1], Balayo, Tina[1], Alemany, Anna[2] and Martinez Arias, Alfonso[1]

[1]Systems Bioengineering, MELIS, Universitat Pompeu Fabra, Barcelona, Spain, [2]Anatomy and Embryology, Leiden University Medical Center, Utrecht, Netherlands

Wnt/b-catenin and Nodal signalling have long been implicated in mammalian gastrulation, for primitive streak (PS) formation and mesoderm and endoderm specification. However, their role remains unclear as mouse mutants for its main effectors do not form a PS and die early during gastrulation. In addition, their molecular interaction has not been studied due to severe pleiotropic effects during embryonic development. To circumvent these issues, we used a non-integrated stem cell-based model system – 'gastruloids' – and devised protocols that allowed the independent study of these signalling during mammalian gastrulation. With such protocols, we were able to generate anterior mesoderm, endoderm and notochord, and spinal cord and paraxial mesoderm-like tissues in a Nodal and Wnt-dependent manner, respectively. As these Nodal and Wnt-engineered gastruloids mimic cellular and molecular events of the anterior and posterior domains of the PS, they reveal the existence of two distinct developmental modules controlling cell fate specification during mammalian gastrulation. Interestingly, through gain and loss of function experiments we concluded that besides the known cooperativity between Nodal and Wnt, there is also an antagonist interaction between the two signalling/developmental modules. In addition, and contrary to the current dogma, we found that the main role of Wnt/b-catenin during gastrulation is not to control the development of embryonic mesoderm but rather to activate the developmental module responsible for axial elongation (i.e. the 'posterior PS'). On this, we observed that Wnt signalling can control the pace of gastrulation in a concentration-dependent manner. Overall, our results point to the existence of a high degree of compartmentalization in the mammalian gastrula, with distinct modules regulating the formation of the different body parts. The role of Nodal and Wnt/b-catenin seems to be that of coordinating the activation of such modules through the activity of specific genetic programs (e.g. those involving Eomesodermin and Brachyury, respectively).

Funding Source: The authors' work is funded by an ERC Advanced Grant (834580) to AMA and an EMBO Postdoctoral Fellowship (ALTF 948-2022) to AD.
Keywords: Mammalian gastrulation, Wnt and Nodal signalling, Gastruloids

11:20 AM – 11:50 AM

MOLECULAR TIMETABLE OF DIFFERENTIATION IN HUMAN PLURIPOTENT STEM CELLS

Rostovskaya, Maria[1], Coussement, Loius[2], Ciarchi, Matteo[3], Della Rosa, Monica[4], Argelaguet, Ricard[5], Rulands, Steffen[6], Spivakov, Mikhail[4], Rugg-Gunn, Peter[1], Reik, Wolf[5]

[1]Epigenetics, Babraham Institute, Cambridge, UK, [2]Biobix, Ghent University, Ghent, Belgium, [3]Max-Plank Institute for the Physics of Complex Systems, Dresden, Germany, [4]MRC London Institute of Medical Sciences, London, UK, [5]Altos Labs Cambridge Institute of Science, Cambridge, UK, [6]Ludwig-Maximilians Universitaet Muenchen, Munich, Germany

Pluripotency is the ability of single cells to differentiate to any cell type of the body. In human, the pluripotent epiblast emerges 6 days after fertilisation; however, cell specification occurs later, with different lineages sequentially segregating from the epiblast in a defined order over the next 2 weeks. How this precise timing and order of lineage specification is controlled, remained unknown. Human pluripotent stem cells (hPSCs) are the in vitro counterparts of embryonic epiblast. Previously, we established an hPSC-based model of the entire 2 weekslong window of epiblast development, that closely follows its transcriptional dynamics and timing in embryos, also called pluripotent state transition. To understand the molecular control of its timing, we simultaneously probed transcriptome and chromatin accessibility in single cells. We found that the transition is a step-wise rather than a gradual process. As the transition occurs under constant conditions, this step-wise switch is not extrinsicallyinduced, but an intrinsic cell decision. Therefore, the pluripotent state transition is a self-guided linear endogenous genetic programme. We reconstructed the dynamic gene regulatory network and interpreted it as a TF cascade, ensuring directionality, timing and intrinsic decisions of the epiblast development ("transcriptional clock") Mathematical model closely reproduced the gene expression dynamics and allowed to predict key TFs of this genetic programme. We discovered that during the pluripotent state transition hPSCs step-wise change the abilities to respond to differentiation signals inducing definitive endoderm and neuroectoderm. Not only the emergence of these abilities recapitulated the order in which the respective lineages emerge in the embryo, it also correlated with the step-wise transcriptional switches during the pluripotent state transition, indicating their potential functional connection. Epigenetic profiling of hPSCs during the transition showed that the emergence of differentiation competence was associated with epigenetic priming of developmental enhancers. Therefore, we propose a model of transcriptional clock that establish a timetable of lineage segregation from the epiblast through epigenetic remodelling of cis-regulatory elements.

Keywords: Human pluripotent stem cells, Gene regulatory network and Epigenetic priming

12:15 PM – 1:15 PM
INNOVATION SHOWCASE

ROOM: Schatzkammersaal

12:15 PM – 1:15 PM

EXPLORING CUTTING-EDGE FUNCTIONAL CHARACTERIZATION OF IPSC-DERIVED 2D AND 3D IN VITRO MODELS IN MOLECULAR BIOSCIENCE

Laura D'Ignazio, PhD
MaxWell Biosystems, Switzerland

Nina Dirkx
Applied and Translational Neurogenomics Group - VIB Center for Molecular Neurology, VIB, Belgium

Luca Guglielmi, PhD
Lancaster Lab, MRC Laboratory of Molecular Biology (LMB), United Kingdom

Two-dimensional (2D) and three-dimensional (3D) cell cultures are instrumental tools in stem cell research, aiming to recreate in vivo conditions to varying degrees. When derived from human induced pluripotent stem cells (iPSCs), these models offer a promising alternative to animal models. Factors such as cell shape, differentiation, proliferation, drug sensitivity, and responses to stimuli influence the choice between 2D and 3D methods, depending on the specific scientific question and application.

Recognizing the equal value of both 2D and 3D in vitro cellular models in advancing stem cell research, we invited two distinguished speakers to shed light on novel applications and methodologies for the functional characterization of pluripotent stem cell-based models. Scientific presentations will be followed by an engaging Q&A session and a panel discussion to delve into the current perspectives on this critical and relevant topic.

**Lunches may be brought into the session room to enjoy during the Innovation Showcase*

1:30 PM – 3:30 PM
GENOME EVOLUTION IN DEVELOPMENT

1:30 PM – 2:00 PM

DNA-GUIDED TRANSCRIPTION FACTOR COOPERATIVITY SHAPES EVOLUTION AND VARIATION OF HUMAN FACES

Wysocka, Joanna

Stanford University School of Medicine, Stanford, CA, USA

Our laboratory uses facial embryonic stem/progenitor cells, called Cranial Neural Crest Cells (CNCCs), as a paradigm to study how genetic information harbored by cis-regulatory elements is decoded into a diversity of functions, behaviors and morphologies. We have established pluripotent stem cell differentiation models that recapitulate human and chimpanzee CNCC formation in vitro and utilized them – in combination with human facial genetics and in vivo modeling – to understand regulatory mechanisms by which CNCCs produce craniofacial forms that characterize us both as humans and as individuals. Transcription factors binding at cis-regulatory elements play central roles in determining cell type and positional identities in the developing face whereas genetically-encoded changes in their dosage are associated with both normal-range and disease-associated variation in facial morphology. I will discuss our latest work on how transcription factor dosage mediates phenotypic specificity and how lineage and positional identities are integrated through DNA-guided cooperativity between bHLH and homeodomain transcription factors at a long DNA sequence motif termed Coordinator, discovered in our evo-devo studies of enhancer divergence between human and chimp.

Keywords: neural crest, evolution, transcription factors, gene regulation

2:00 PM – 2:30 PM

EXPLORING CELL IDENTITY IN BRAIN ORGANOIDS

Lancaster, Madeline

MRC Laboratory of Molecular Biology, UK

The human brain sets us apart as a species, yet how it develops and functions differently to that of other mammals is still largely unclear. This also makes it difficult to understand how disorders of the brain such as neurodevelopmental defects and neurological disorders arise, and therefore how to treat them. In an effort to better understand the events that give rise to the complex human brain, we use a model system in a dish called cerebral organoids, or brain organoids. These 3D tissues are generated from pluripotent stem cells through neural differentiation and a supportive 3D microenvironment to generate organoids with the same tissue architecture as the early human fetal brain. Such organoids are allowing us to tackle questions previously impossible with more traditional approaches. Indeed, our recent findings provide insight into how the human brain becomes so large, and how external stimuli can influence brain development. Organoids can also be generated from patient-derived cells and are therefore providing new insight into how human conditions arise and even uncovering new drugs that can be used to treat these conditions. But the use of various starting cell lines brings with it certain issues related to reproducibility, as cell line variability can be a major hurdle for organoids and in vitro models in general. We have begun exploring why certain cell lines succeed at generating brain organoids and others do not, which has revealed a specific epigenetic footprint associated with suboptimal performing cell lines that can be reversed using a cocktail of small molecules. Not only can this technique open the door to more reliable disease modelling and therapeutics discovery, it is also revealing insight into the earliest stages of pluripotent stem cell fate determination.

Keywords: Organoids, brain development, stem cells

2:30 PM – 2:45 PM

CEPHALOPOD MOLECULAR APPROACH OF EVOLVING THE LARGEST INVERTEBRATE NERVOUS SYSTEM

Elagoz, Ali M., Deryckere, Astrid, Styfhals, Ruth, Aparicio, Daniela, Janssen, Dries, Maccuro, Sofia, Sannen, Benjamin, Van Dyck, Marie and Seuntjens, Eve

Department of Biology, KU Leuven, Belgium

The cephalopod nervous system is comparable to the nervous system of a small mammal in terms of neuronal number and richness in the behavioural repertoire it controls. Nevertheless, the last common ancestor between cephalopods and mammals was a worm-like marine organism that existed approximately 600 million years ago. Studying cephalopods presents an opportunity to understand the genetic drivers of neural development that evolved convergently with vertebrates. Octopuses have a centralized nervous system with circumesophageal brains and axial nerve cords passing through the center of each arm. Their nervous system consists of 500 million neurons. Approximately one-third of these neurons are found in the brain, and the rest is located mainly in the arms. While the morphological characterization of the octopus nervous system has been substantially carried out, molecular mechanisms driving the neurogenesis remain unclear. Our recent findings showed that there is a neurogenic zone located outside the brain adjacent to the eyes in lateral lips. We also discovered that newly born neurons display long-distance migration into the centralized brain, reminiscent of vertebrate neurogenesis. Our present study elaborates on the molecular characterization of octopus neurogenesis. We have identified another important pool of progenitors located at the basolateral epithelium layer in the arms. Live imaging experiments suggest these progenitor cells also display migration, strengthening the idea that migration is fundamental for large nervous system development. We have further investigated the spatiotemporal expression of intrinsic and extrinsic factors essential for neurogenesis and gliogenesis with in situ Hybridization Chain Reaction. Finally, using small molecules to impede the evolutionarily conserved signaling pathways, we unravel its functional implication in the morphogenesis of the octopus nervous system.

Funding Source: FWO
Keywords: Neuro-evo-devo, Neurogenesis, signaling pathways

2:45 PM – 3:00 PM

MAPPING THE IMPACT OF CIS-REGULATORY VARIATION ON MOUSE CORTICAL DEVELOPMENT ACROSS EVOLUTIONARY TIMESCALES

Vierbuchen, Thomas[1], Medina-Cano, Daniel[1], Islam, Mohammed[1], Petrova, Veronika[2], Yang, Marty[3], Balic, Zerina[1], Stadtfeld, Matthias[4] and Wong, Emily[2]

[1]Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA,[2]Victor Chang Cardiac Research Center, UNSW Sydney, Sydney, Australia, [3]Data Science and Biotechnology, Gladstone Institute, UCSF, San Francisco, CA, USA, [4]Department of Medicine, Weill Cornell Medical College, New York, NY, USA

Natural selection has shaped the gene regulatory networks that control neuronal cell fate specification and terminal differentiation, generating diversity in cell type composition, neural circuit formation, function, and behavior across mammals. However, it remains difficult to parse the specific genetic changes that contribute to this phenotypic diversity and to define the developmental mechanisms underpinning these differences at the molecular and cellular level. Here, we sought to use highly genetically and phenotypically distinct inbred mouse strains to examine how genetic changes contribute to differences in gene regulation during neocortex development. We generated and extensively characterized induced pluripotent stem cells (iPSCs) from F1 hybrid crosses between standard laboratory mice (C57Bl/6J) and four wild-derived inbred mouse strains (PWK/PhJ, MOLF/Ei, CAST/Ei, SPRET/Ei) from distinct sub-species that span ~1.5 million years of evolutionary divergence. These wild-derived inbred strains are known to exhibit extensive differences in gene expression, cell type composition, behavior, and susceptibility to neurological disorders, but the genetic changes that contribute to these differences remain almost completely unknown. To model brain development in these F1 hybrids, we developed a rapid and reproducible protocol to generate neocortical organoids from mouse epiblast stem cells. Mouse cortical organoids develop with kinetics that mirror the embryonic cortex in vivo, sequentially generating cortical neurons, astrocytes, and oligodendrocytes over ~10 days. We generated cortical organoids from each of these F1 hybrid backgrounds and mapped gene expression across developing cortical cell types using single cell RNA-seq. We took advantage of the high frequency of sequence

polymorphisms to map scRNA-seq reads to each allele, allowing us to identify genes whose expression has diverged as a result of cis-regulatory changes. These data will provide insight into how changes in transcriptional regulation contribute to differences in cortical development and establish new cellular resources and experimental methods for studying the impact of cis-regulatory variation on brain development and evolution.

Funding Source: NIH RO1 (R01-NS126921-01)
Keywords: Organoids, Genomics, Evolution

3:00 PM – 3:30 PM

MODELING THE HUMAN-SPECIFIC ASPECTS OF BLASTOCYST DEVELOPMENT AND IMPLANTATION WITH BLASTOIDS

Rivron, Nicolas

Institute of Molecular Biotechnology (IMBA), Austria

The blastocyst is the early mammalian organism before implantation in the uterus. We have promoted the self-organization of stem cells into models of mouse and human blastocysts, which we have named blastoids. Blastoids are morphologically and transcriptionally similar to the blastocyst and contain analogs of all three cell types that would eventually develop into the complete organism (embryonic and extraembryonic). Because blastoids are complete and model the preimplantation stage, they can be introduced into the uterus (mouse model) or combined in vitro with uterine cells (human model) to recapitulate aspects of the normally hidden implantation processes. Unlike blastocysts, blastoids come in large numbers and facilitate a more systematic modulation and analysis of development. As such, they represent both a scientific and ethical alternative to the use of embryos for research. Using this approach, we are investigating the genome evolution underlying species-specific aspects of blastocyst development and implantation, with the long-term goal of understanding the evolutionary basis of suboptimal human pregnancy (50% of fertilized eggs never develop). This knowledge could help solve the global health problems of family planning and developmental origin of health and disease.

Keywords: blastocyst, blastoid, human, implantation

TUESDAY, 5 DECEMBER

9:00 AM – 10:15 AM
MORPHOGEN GRADIENTS I

9:00 AM – 9:30 AM

MORPHOGEN COCKTAILS AND MECHANICAL SYMMETRY-BREAKING: TWO NOT-QUITE-CLASSICAL PATTERNING MECHANISMS

Green, Jeremy

King's College London, UK

Turing's Reaction-Diffusion (RD) is now well established as fundamental principle in developmental patterning. In a number of biological contexts we know the action and identities of morphogen molecules that fulfil the original activator-inhibitor theories to a remarkable degree. Using the patterning of rugae – periodic transverse ridges in the oral palate epithelium – as a model system, we found that, although FGF and Shh proteins can take the classical roles of Activator and Inhibitor, there are in fact more morphogens at work: there are at least five morphogen players in this ensemble, including Wnt, BMP and a second (mesenchymal) FGF. We used both perturbation by inhibitors and analysis of stripe formation dynamics to define a small set of network structures that could explain the robust periodic patterns that we see. Although appealing as a paradigm for RD-based stripe formation, the rugae system is a one-dimensional pattern that cannot explain, for example, the self-organisation and symmetry-breaking seen in gastruloids. Gastruloids, which model formation of the primary body axis of vertebrate embryos, turn themselves from spheres into rods, breaking symmetry to organise cell rearrangements known as convergent extension (CE). We have explored the possibility that a completely different, non-RD, self-organisation principle for symmetry-breaking by CE could exist, namely polarity-propagating mechanical feedback. Using in silico modelling, we show that extremely simple rules for mechanical interactions are sufficient to organise convergent extension. Thus, although Turing himself chose to consider only chemical morphogenesis, it may be that mechanical self-organisation may also have a role to play.

Keywords: Symmetry-breaking, self-organisation, gastruloids

9:30 AM – 9:45 AM

ACTIVE DROPLET BEHAVIOUR OF CELL GROUPS DRIVES PATTERN FORMATION IN RESPONSE TO A SELF-GENERATED GRADIENT

Chubb, Jonathan

University College London, UK

Gradients of extracellular signals organise cell behaviour in tissues. Although we have good models for how gradients organise the behaviour of a sheet of cells, it is not clear how cells react to gradients when the cell population is undergoing 3D morphogenesis, in which cell-cell interactions and cell-signal interactions undergo extensive emergent behaviour. Dictyostelium cells follow gradients of their nutritional source to feed and maintain their undifferentiated state. Using light sheet imaging to simultaneously monitor signalling, single cell and population dynamics, we show that these cells migrate towards nutritional gradients in swarms. As the swarm advances, it deposits clumps of cells at the rear, triggering their differentiation. Clump deposition is explained by a model in which the cell swarms behave as active droplets, with cell proliferation and signal gradient remodelling opposing surface tension to promote droplet shedding. The model predicts vortex motion of the cells within the droplet emerging from the local transfer of propulsion forces, which was validated by 3D tracking of single cells in the swarms. These vortices result from a transition in cell motion from advective to diffusive transport across the height of the swarm. Our data show how the emergent dynamics of cell groups impose specific cell states: the outcome for individual cells- to differentiate or not- is imposed by the stochastic positioning of the cell in the vortex as the droplet splits.

Funding Source: Wellcome
Keywords: Morphogen, Imaging, Droplet

9:45 AM – 10:15 AM

INTERPRETATION OF MORPHOGEN SIGNALING IN THE DEVELOPING SPINAL CORD

Kicheva, Anna

Institute of Science and Technology (ISTA), Klosterneuburg, Austria

During spinal cord development, an elaborate dorsoventral pattern of molecularly distinct neural progenitor domains forms in response to opposing morphogen gradients. We are interested in understanding how the dynamics of morphogen signaling is controlled and how it influences the downstream pattern. To address these questions, we developed an in vitro system to generate cell types of the dorsal neural tube by directed differentiation of mouse embryonic stem cells. Our data reveal unexpected temporal dynamics of BMP signaling. Using a combination of perturbation experiments and mathematical modeling, we show how this signaling dynamics is regulated and interpreted by cells.

Keywords: pattern formation, morphogens, neural tube

10:45 AM – 11:45 AM MORPHOGEN GRADIENTS II

10:45 AM – 11:15 AM

CARDIOIDS UNRAVEL HUMAN HEART DEVELOPMENT AND DISEASE

Mendjan, Sasha

Institute of Molecular Biotechnology (IMBA), Vienna, Austria

The number one cause of human fetal death are defects in heart development. Because the human embryonic heart is inaccessible, and the impacts of mutations, drugs, and environmental factors on the specialized functions of different heart compartments are not captured by in vitro models, determining the underlying causes is difficult. Here, we established a human cardioid platform that recapitulates the development of all major embryonic heart compartments, including right and left ventricles, atria, outflow tract, and atrioventricular canal. By leveraging 2D and 3D differentiation, we efficiently generated progenitor subsets with distinct first, anterior, and posterior second heart field identities. This advance enabled the reproducible generation of cardioids with compartment-specific in vivo-like gene expression profiles, morphologies, and functions. We used this platform to unravel the ontogeny of signal and contraction propagation between interacting heart chambers and dissect how mutations, teratogens, and drugs cause compartment-specific defects in the developing human heart.

Keywords: cardioids, cardiac organoids, human heart development, cardiogenesis, hPSCs

11:15 AM – 11:45 AM

ROLE OF MECHANICS IN THE PATTERNING OF THE EARLY NEUROEPITHELIUM

Ramanathan, Sharad

Harvard University, USA

The patterning of the early neuroepithelium of the forebrain leads to the generation of multiple different progenitor cell types that give rise to the diversity of neuronal and glial cells of the cortex. Here we will study the early patterning of this human neuroepithelium through single-cell sequencing analysis of fetal tissue and in vitro models. These studies suggest that an early mechanical instability that drives some cells to expand and others to contract is a critical step in generating an early lineage decision leading to distinct neuronal and glial progenitors. Using a combination of an in vitro system, imaging, and optogenetics, we demonstrate a critical role for YAP and NOTCH signaling, as well as an underlying gene regulatory network in the lineage decision.

Funding Source: NIH
Keywords: early human development, cortical development, in vitro system

12:00 PM – 12:30 PM
INNOVATION SHOWCASES

ROOM: Schatzkammersaal

12:00 PM – 12:15 PM
CYTOPLASMIC LIVE-CELL BIOPSIES FOR THE TEMPORAL PROFILING OF SINGLE-CELLS
Presented by Cytosurge AG

Tamás Gerecsei, PhD

Cytosurge AG, Switzerland

Fluidic force microscopy or FluidFM is a biophysical technique for conducting single-cell biopsies. This innovative approach enables the extraction of a part of the cytoplasm from individual living cells while preserving their viability. These cytoplasmic biopsies can be used for subsequent highly-sensitive, low-input RNA-seq analysis to characterize single-cells multiple times throughout their lifetime. Moreover, the FluidFM Nanosyringes extend their utility by facilitating the targeted introduction of various molecular components into cells, including RNA, DNA, proteins and even molecular complexes such as CRISPR/Cas9 RNPs.This functionality streamlines the transfection processes for plasmids, and transcription factors, as well as enabling entire cell line engineering workflows. By exploring the capabilities of FluidFM in this seminar, we seek to uncover its potential implications for advancing the comprehension of intricate cellular processes, thus fostering new dimensions in cellular analysis and molecular investigation.

**Lunches may be brought into the session room to enjoy during the Innovation Showcase*

12:15 PM – 12:30 PM
REVOLUTIONIZING ORGANOID ANALYSIS: UNVEILING THE POWER OF 3D MICROCHIP TECHNOLOGY FOR ENHANCED LIFE SCIENCES INSIGHTS
Presented by 3Brain AG

Antonella Di Bello, PhD

3Brain AG, Switzerland

Human-derived iPCS model systems like brain organoids offer to recapitulate the complexity and functionality of human tissues. Microchip-based technologies that can directly access the functional activity of 3D neuronal assemblies hold promise to be the next gold-standard technique for non-invasive, kinetic, or long term measurements of physiological and pathological phenotypes. However, many current approaches struggle to fulfil accuracy, precision, sensitivity, reproducibility and throughput requirements. For 3D model systems specifically, current microchips have limited capability to record physiologically relevant cells and endpoints, causing a bottleneck in a wider adoption and research utility. In this innovation showcase, 3Brain will present their CMOS-powered cell-electronic biointerface solutions for high-content screening, cell-based assays and drug discovery. The showcase will highlight several advantages and capabilities of our first-in-class Accura-3D microchip, using thousands of electrodes mounted on ultrathin microneedles to gain unprecedented access to the inner layers of structured tissue. Accura-3D allows for non-destructive, label-free, real-time and parallel recording of thousands of neurons and provides a detailed granular description of the complex functional activity expressed by iPSC-derived brain organoids. By increasing data quality and reducing experimental variability, Accura-3D makes a technological leap toward mass adoption of brain organoids for studying neurodegenerative disease or drug discovery pipelines.

**Lunches may be brought into the session room to enjoy during the Innovation Showcase*

1:15 PM – 3:00 PM
Tissue Mechanics I

1:15 PM – 1:45 PM
ILLUMINATING MECHANISMS OF MAMMALIAN MORPHOGENESIS USING ADAPTIVE LIGHT-SHEET MICROSCOPY

McDole, Kate

MRC Laboratory of Molecular Biology, UK

Organ systems are complex, three-dimensional structures built for highly specialized tasks, yet they arise from a relatively simple, uniform population of cells. Despite this initial simplicity, our knowledge of how early organ systems develop and the role that physical forces play in sculpting these complex tissue structures is extremely limited. We use the mouse embryo to investigate the fundamental problem of symmetry breaking in development – how a uniform layer of cells can be mechanically sculpted into a complex 3D structure that makes a functional organ. As mammalian embryos are highly sensitive to environmental and culture conditions,

and in addition are extremely photosensitive, visualizing their development has been notoriously difficult. We have developed an advanced light-sheet microscope to gently and comprehensively image mouse embryo development at single-cell resolution over a course of days. With this system and a suite of computational tools, we can track individual cells and analyse patterns of divisions, as well as build dynamic cell fate maps over the course of two-days of development from gastrulation to early organogenesis. This allows us to describe the morphogenesis of complex three-dimensional structures such as the formation of the early heart, the neural tube, node and notochord, and the formation of the primitive streak.

Keywords: mouse embryo, development, light-sheet microscopy, morphogenesis

1:45 PM – 2:15 PM

DESIGN PRINCIPLES OF FATE, FORM, FORCES, AND FUNCTION: FROM EMBRYOGENESIS TO SYNTHETIC MORPHOGENESIS

Streichan, Sebastian J.

University of California Santa Barbara, USA

Organ architecture is often composed of multiple concentric tissue layers. Morphogenesis folds these organs into a specific shape that is required for proper function. Genetic signals that determine cell fate have been uncovered - yet the dynamic interplay of tissue layers giving rise to specific form remains elusive. We combine multi-layer analysis of cellular dynamics on evolving surfaces with physical modeling to obtain testable quantitative descriptions of how genetic patterning controls physics giving rise to shape. I will discuss two examples: (I) Quantitative analysis of visceral organogenesis in D. melanogaster reveals how a hox code in the mesoderm triggers a dynamic molecular mechanism to control physical processes in the adjacent endoderm layer. (II) A chip-based culture system enables self-organization of micro patterned stem cells into precise three-dimensional cell-fate patterns and form. This system recreates aspects of neural tube folding, and indicates basal interactions between non-neural and neural ectoderm are required for tube closure.

Funding Source: National Institutes of Health
Keywords: Synthetic Morphogenesis, Neural Tube Closure, In toto live imaging, quantitative biology

2:15 PM – 2:30 PM

MORPHODYNAMICS OF HUMAN EARLY BRAIN ORGANOID DEVELOPMENT

Jain, Akanksha[1], Gut, Gilles[1], Sanchís Calleja, Fatima[1], Okamoto, Ryoko[1], Streib, Simon[1], He, Zhisong[1], Zenk, Fides[1], Santel, Malgorzata[1], Seimiya, Makiko[1], Holtackers, Rene[1], Jansen, Sophie[1], Camp, J. Gray[2] and Treutlein, Barbara[1]

[1]D-BSSE, ETH-Zurich, Basel, Switzerland,[2]IHB, Roche, Basel, Switzerland

Brain organoids enable mechanistic study of human brain development and morphogenesis and provide opportunities to explore self-organization in unconstrained developmental systems. We have established long-term light sheet microscopy on unguided multi-mosaic neural organoids generated from fluorescently labeled human iPSCs, which enables tracking of tissue morphology, cell behaviors, and subcellular features over weeks of organoid development. We developed an analysis pipeline to demultiplex labels and segment cells in multi-mosaic brain organoids using deep learning to provide quantitative measurements of tissue and cellular dynamics, using Actin, Tubulin, plasma membrane, nuclei, and Lamin labels. Based on live imaging and single-cell transcriptome modalities, we find that lumenal expansion and cell morphotype transition within the developing neuroepithelium are associated with modulation of gene expression programs involving extracellular matrix (ECM) pathway regulators and mechanosensing. We show that an extrinsically provided matrix enhances neuroepithelium alignment, polarization and lumen expansion as well as telencephalon formation. Unguided organoids grown in the absence of an extrinsic matrix have altered tissue and lumen morphologies with increased neural crest and caudalized tissue identity. Finally, we show ECM induced morphological changes and patterning guidance is linked to modulations via the interactions between the HIPPO and the WNT signaling pathway, including spatially restricted induction of WNT pathway genes that mark the earliest distinction between telencephalic and non-telencephalic lineages. Altogether, our work provides a new inroad into studying human brain morphodynamics and supports a view that mechanosensing dynamics have a central role in constraining brain morphodynamics and regionalization.

Keywords: Brain organoid, Lightsheet microscopy, Morphodynamics

2:30 PM – 3:00 PM

ENCODING SHAPE WITH GENETICS, MECHANICS AND GEOMETRY

Lecuit, Thomas

The Marseille Developmental Biology Institute (IBDM), Switzerland

Abstract not available at time of printing.

4:00 PM – 5:00 PM
TISSUE MECHANICS II

4:00 PM – 4:30 PM

TOPOLOGY OF THE EARLY MAMMALIAN EARLY EMBRYO

Hannezo, Edouard

Institute of Science and Technology (IST), Austria

How living systems achieve precision in form and function despite their intrinsic stochasticity is a fundamental yet open question in biology. Here, we establish a morphomap of pre-implantation embryogenesis in mouse, rabbit and monkey embryos, which reveals that although blastomere divisions desynchronise passively, 8-cell embryos display robust 3D morphogenesis. Using topological analysis and genetic perturbations, we show that embryos progressively change their cellular connectivity to a preferred topology, which can be predicted by a physical model where actomyosin-contractility and noise facilitate topological transitions lowering surface energy. This favours embryo packing, promoting higher number of inner cells in the 16-cell embryo. Synchronised division reduces embryo packing and generates significantly more mis-allocated cells and less inner-cell-mass cells, suggesting that stochasticity in division timing contributes to robust patterning.

Keywords: mammalian embryo, mechanobiology, stochasticity

4:30 PM –5:00 PM

CROSS-TALK BETWEEN CELL MECHANICS, CELL SHAPE AND CELL FATE

Paluch, Ewa K.

University of Cambridge, UK

A precise control of cell morphology is key for cell physiology, and cell shape deregulation is at the heart of many pathological disorders. Furthermore, transitions in cellular fate and state are often associated with changes in cell shape, and strong evidence points to the existence of feedbacks between mechanics, morphology and fate decisions. Cell morphology is intrinsically controlled by mechanical forces acting on the cell surface, to understand shape it is thus essential to investigate the regulation of cellular mechanics. We investigate how cellular mechanical properties are regulated, how they drive cellular shape changes, and the cross-talk between mechanics and state in various cellular transitions in development and in stem cells. Taken together, using a combination of cell biology experiments, quantitative imaging, biophysical measurements, and modelling, we aim to understand the crosstalk between cell mechanics, morphology and fate.

Keywords: mechanobiology, morphogenesis, fate transitions, mechanosensing

WEDNESDAY, 6 DECEMBER

9:00 AM – 10:15 AM
TISSUE SELF-ORGANIZATION I

9:00 AM – 9:30 AM
SELF-ORGANISATION OF REPROGRAMMING CELLS INTO IBLASTOIDS

Schweisguth, Francois

Institute Pasteur, France

Self-organization can produce stereotyped patterns of cell fates when guided by positional cues. In my talk I will address how two distinct spatial patterns of cell fates dynamically emerge in Drosophila. I will first discuss how self-organization mediated by Notch contributes to the pattern of sensory organs at the body surface of adult flies. I will then describe how the pulsatile dynamics of a key fate-determining factor has led to a new model for the establishment of a crystal-like pattern in the fly eye.

Keywords: Pattern formation, lateral inhibition, oscillatory gene expression

9:30 AM – 9:45 AM
A HUMAN POST-IMPLANTATION EMBRYO MODEL DERIVED EX-UTERO SOLELY FROM GENETICALLY UNMODIFIED NAÏVE PLURIPOTENT CELLS

Novershtern, Noa[1], Oldak, Bernardo[1], Wildschutz, Emilie[1], Bondarenko, Vladyslav[1], Comar, Mehmet-Yunus[1], Zhao, Cheng[2], Aguilera-Castrejon, Alejandro[1], Tarazi, Shadi[1], Viukov, Sergey[1], Pham, Thi Xuan Ai[3], Ashouokhi, Shahd[1], Lokshtanov, Dmitry[1], Roncato, Francesco[1], Ariel, Eitan[1], Rose, Max[1], Livnat, Nir[1], Shani, Tom[1], Joubran, Carine[1], Pasque, Vincent[3], Petropoulos, Sophie[2], Lanner, Fredrik[2] and Hanna, Jacob[1]

[1]Department of Molecular Genetics, Weizmann Institute, Rehovot, Israel, [2]Department of Clinical Sciences, Karolinska Universitetssjukhuset, Stockholm, Sweden, [3]Department of Development and Regeneration, KU Leuven-University, Leuven, Belgium

Human post-implantation embryonic development is still an enigma in many aspects, due to clear ethical and technical limitations in the research of these stages. Recent progress was made on human gastruloids, in vitro cultured blastoids and other stem cell-based embryo models, however these typically do not contain both embryonic and extra-embryonic tissues, and lack adequate spatial organization. We and others have recently shown that mouse naïve pluripotent stem cells, giving rise to embryonic and extra-embryonic stem cells, are capable of self-assembling into post-gastrulation Stem cell-based Embryo Models (SEMs) with spatially organized morphogenesis, and eventually initiating organogenesis ex utero. Here, I will present our recent extension of these findings to human, while using genetically unmodified human naïve pluripotent stem cells, thus bypassing the need for ectopic expression of lineage promoting transgenes. Such human integrated SEMs recapitulate key hallmarks of post-implantation embryos up to 13-14 days post-fertilization, including epiblast (posterior and committed), primary and secondary yolk-sac, amnion, extra-embryonic mesoderm, trophoblast and PGCs. In this talk I will show a combination of structure staining, and single-cell RNA-seq analysis results, to prove each of these embryonic structures. To conclude, this new stem cell-based model may enable the experimental study of previously inaccessible windows of human early post-implantation up to initiation of gastrulation.

Keywords: embryo, model, synthetic

9:45 AM – 10:15 AM
CELL FATE DECISIONS UNDERLYING FLOORPLATE SELF-ORGANISATION IN ORGANOIDS

Tanaka, Elly

Institute of Molecular Pathology, Vienna, Austria

During vertebrate development the floorplate of the neural tube typically forms through an inductive signaling from the notochord. We found that in mouse and human organoids, a floorplate can self-organize in response to a global pulse of retinoic acid signaling through the generation of a scattered, heterogeneous distribution of FOXA2-expressing cells that undergo coalescence and competition to form the floorplate. Here I describe the molecular events leading to the induction of the heterogeneous state, the requirement of FOXA2-negative cells, and the subsequent spatial organization from this heterogeneous state.

Funding Source: The work was supported by funding from the Austrian Science Fund (FWF): F7803-B (Stem Cell Modulation) to E.M.T. and A.K, WWTF 10.47379/LS17037 and ERC AdG 742046 to E.M.T., Sir Henry Wellcome postdoctoral fellowship to H.T.S

Keywords: Floorplate, neural organoid, patterning, self-organisation

10:45 AM – 11:45 AM
TISSUE SELF-ORGANIZATION II

10:45 AM – 11:15 AM

SELF-ORGANIZING STEM CELL SYSTEMS TO STUDY SIGNALING DYNAMICS IN HUMAN GASTRULATION

Warmflash, Aryeh

Rice University, TX, USA

It is generally accepted that signaling molecules known as morphogens are a driving force during embryonic patterning. The mechanisms by which multiple dynamic morphogens specify the body axes of the embryo and determine cell fates has remained difficult to unravel, in part because of a lack of suitable systems for quantitative measurement and perturbation. This challenge is particularly acute for human development where access to embryos is extremely limited for both practical and ethical reasons. Our lab has developed systems in which early developmental patterns form in vitro starting from human embryonic stem cells. In these systems, a combination of geometric confinement and treatment with growth factors harnesses the intrinsic ability of stem cells to create patterns similar to those in the embryo. We use these systems together with live cell imaging to dissect how self-organized signaling activities underlie cell fate patterning. I will discuss the insights our work has provided into the signaling activities that trigger gastrulation and specify the embryonic germ layers.

Keywords: Gastrulation, self-organization, signaling dynamics, live-cell imaging, morphogens

11:15 AM – 11:45 AM

ANALYZING FATE SPECIFICATION AND NEURAL NETWORK ARCHITECTURE BY TRANS-SYNAPTIC LABELING USING BARCODED RABIES VIRUS

Knoblich, Jüergen

IMBA-Institute of Molecular Biotechnology, Vienna, Austria

Cerebral organoids derived from pluripotent human stem cells can recapitulate morphogenesis and cell fate specification in the fetal human brain. Our group uses stem cell derived 3D cultures to explore the mechanistic basis for neurodevelopmental disorders. We have modeled Tuberous sclerosis (TSC), a neurodevelopmental disorder caused by overactivation of the mTOR pathway and have identified a progenitor cell type responsible for tumor formation and brain pathologies in TSC patients. Our data suggest that these progenitor cells located in the caudal ganglionic eminence might be responsible for the generation of inhibitory interneurons that migrate into the cerebral cortex after birth in humans but not in mice. By analyzing neural network activity using extracellular recordings, we have identified characteristic phenotypes also seen in TSC patients during the interictal phase. To understand the modifications in neural network architecture underlying those changes, we developed “Connectomics by sequencing” a barcoded trans-synaptic labeling methodology that allows identification of synaptic connections in single-cell transcriptome experiments. Together, our results offer new insights into human brain development and the mechanistic defects that underlie neurodevelopmental disorders including Epilepsy or Autism.

Keywords: cerebral organoids, neurodevelopmental disorders, epilepy, autism, tuberous sclerosis, human brain development

12:00 PM – 1:00 PM
INNOVATION SHOWCASE

12:00 PM – 1:00 PM

INFORMATION SESSION ON THE ERC PROGRAMME

Presented by the European Research Council (ERC)

Presenters:

Mariam Benjdia, PhD

European Research Council Executive Agency, Belgium

Katharina Sonnen, PhD

Hubrecht Institute, Netherlands

The ERC, set up by the European Union in 2007, is an European funding organization for excellent frontier research. It funds creative researchers of any nationality and age, to run projects based across Europe, in any field of research.

The ERC offers 4 core grant schemes: Starting Grants, Consolidator Grants, Advanced Grants and Synergy Grants. With its additional Proof of Concept Grant scheme, the ERC helps grantees to explore the innovation potential of their ideas or research results.

The overall ERC budget from 2021 to 2027 is about €16 billion. To date, the ERC funded more than 12,000 projects and evaluated more than 100,000 research proposals.

During this session, the ERCEA representative will present the ERC’s funding schemes as well as provide answers to practical questions such as:

-How can the ERC support research careers?

-What are the main features of the ERC’s funding schemes?

-What are the selection criteria?
-How does the selection process take place?
-What are the changes foreseen from 2024?
The presentation will be followed by a Q&A session. In addition, ERC Grantee(s) will be present to share their experience(s) with the audience.

**Lunches may be brought into the session room to enjoy during the Innovation Showcase*

1:15 PM – 2:30 PM
TIMING OF DEVELOPMENT I

1:15 PM – 1:45 PM
METABOLIC CONTROL OF DEVELOPMENTAL TIMING

Aulehla, Alexander

EMBL Heidelberg, Germany

How environmental cues are linked to developmental programs and thereby impact the phenotypic outcome is a fundamental question that we aim to address. To this end, we focus on how metabolic activity, which responds dynamically to external environmental conditions, impacts cellular signaling and developmental timing. Our recent findings reveal a functional link between glycolytic flux, the period of collective signaling oscillation and the timing of mouse embryo segmentation. I will discuss our strategies to integrate physics-guided entrainment approaches to reveal the underlying principles.

Keywords: Timing, oscillators, metabolism

1:45 PM – 2:00 PM
ELUCIDATING THE IN VITRO PGC DIFFERENTIATION PATHWAY IN PRIMATES

Nakamura, Tomonori

The Hakubi Center/WPI-ASHBi, Kyoto University, Kyoto, Japan

Just after implantation, human embryo initiates critical events such as dynamic morphogenesis and three germ layer differentiation associated with gastrulation. However, ethical constraints have made it impossible to collect samples during this stage, leaving the developmental mechanisms unelucidated. Recent advances in techniques for culturing human and non-human primate embryos for a long period in vitro, as well as methods for differentiation or embryoid model from pluripotent stem cells (PSCs), are beginning to address post-implantation embryonic development. On the other hand, despite the essential need to validate these in vitro models through comparison with in vivo conditions, knowledge about the embryos of humans and non-human primates developing under physiological conditions in the uterus, especially at the molecular level, has been limited. We have previously utilized the cynomolgus macaque as a model organism to study the post-implantation embryonic development of primates, employing our original scRNA-seq method. And other research groups have also reported transcriptomic data for later stages in both human (E17-19) and cynomolgus macaque embryos (E20-29). However, these studies have primarily focused on the middle to late stage of gastrulation, which is around ten days after implantation. Therefore, a gap exists in the data covering the earlier stages. In this study, we used the comprehensive scRNA-seq method to obtain transcriptome data from embryos, ranging from early E15. We identified nearly all cell types, including small populations such as PGCs and Node cells. We also discovered authentic marker genes for all cell types, including those that can distinguish between Amnionic ectoderm and trophectoderm cells. Since these data sets can be applied to validate in vitro differentiation models, we obtained the PGCLC data sets from monkey ESCs and compared them with the in vivo dataset. We found that, in an undifferentiated state, the ESCs are homologous to epiblasts; however, as days pass, the differentiating cells migrate to amnionic ectoderm clusters and then differentiate into PGC fate, consistent with our previous findings that the monkey PGCs are derived from the top of the Amnion dome. This result suggested that the in vivo dataset is valuable for evaluation of in vitro models.

Funding Source: This research was supported by AMED under Grant Number 23gm6310013h0004
Keywords: Primate, scRNA-seq, implantation development

2:00 PM – 2:30 PM
TOWARDS RECONSTITUTING AXIAL DEVELOPMENT IN VITRO

Alev, Cantas

Kyoto University, Kyoto, Japan

Early embryonic events including somitogenesis, during which the metameric body plan of vertebrates is laid out, have been extensively studied using model organisms such as mouse, chick or zebrafish, but remain largely elusive and poorly understood when it comes to human and other primates. Using induced pluripotent stem cell (iPSC)-derived presomitic mesoderm (PSM), we previously succeeded to quantify oscillatory activity of the segmentation clock, a molecular oscillator believed to control somite formation. Interestingly, these in vitro models of the segmentation clock did not show any sign of

segmentation despite the presence of oscillatory activity of clock genes such as HES7. Extending on these earlier findings we then asked whether we could recapitulate not only the clock but also the actual process of segmentation and epithelial somite formation in vitro. Utilizing again pluripotent stem cells as starting material we established a 3D in vitro model of human somitogenesis, which exhibited periodic formation of properly patterned epithelial somites in synchrony with the segmentation clock. Our selforganizing 'axioloids' shared further morphological and molecular features of the human embryo and emerging vertebrate embryonic axis including presence of opposing morphogen gradients. We also demonstrated a critical role of Retinoic Acid (RA) signalling in the stabilisation of segments, suggesting synergy of RA and ECM in the formation and epithelialisation of somites during human somitogenesis. We are currently applying our axioloid model for the study of axial development & disease in human and other species.

Keywords: axioloid, axial development, somitogenesis, segmentation clock, in vitro embryo model

3:00 PM – 5:00 PM
TIMING OF DEVELOPMENT II & CLOSING SESSION

3:00 PM – 3:30 PM

SIGNALING DYNAMICS IN THE CONTROL OF EMBRYONIC DEVELOPMENT AND TISSUE HOMEOSTASIS

Sonnen, Katharina

Hubrecht Institute, Netherlands

Cells communicate with each other via dynamic signalling pathways to govern development and tissue homeostasis. For instance, segmentation of vertebrate embryos is coordinated by oscillating signaling pathways. To enable the investigation of such dynamics, we established a microfluidic system to entrain endogenous signaling oscillations to external pulses of pathway modulator. Combined with real-time imaging of signaling reporters this enables the functional dissection of complex dynamic signaling networks in a multicellular context. Here, I will present our findings on the control of mouse segmentation by multiple interacting oscillators and how we have adapted such methods to study signalling dynamics in the context of the small intestine.

Keywords: Signalling dynamics, oscillations, embryonic development, tissue homeostasis, somitogenesis, small intestine

3:30 PM – 4:00 PM

NOVEL SEQUENCING TOOLS TO EXPLORE TRANSLATION IN INDIVIDUAL CELLS

van Oudenaarden, Alexander

Hubrecht Institute-KNAW and University Medical Center, Netherlands

In recent years novel single-cell sequencing methods have allowed an in-depth analysis of the diversity of cell types and states in a wide range of organisms. Due to the continuous optimization of experimental and computational methods by many research groups, it is now possible to sequence the transcriptomes of thousands to millions of individual cells. Albeit an exciting development, transcription only covers the first step in the central dogma. The second step, the process of translation, is currently much harder to explore in single cells. Despite recent progress in detecting proteins by mass spectrometry with single-cell resolution, it remains a major challenge to measure translation in individual cells. Building upon existing ribosome profiling protocols our laboratory recently increased the sensitivity of these assays allowing ribosome profiling in single cells. Here I will present our ongoing efforts to determine translation efficiency in single cells and to correlate translation efficiency to tRNA levels and tRNA modifications.

Keywords: single-cell sequencing, translation

4:00 PM – 4:30 PM

HIGH RESOLUTION MAPPING OF CELL LINEAGES DURING MAMMALIAN EMBRYOGENESIS

Bowling, Sarah

Harvard University, USA

Understanding the routes through which a single cell populates the adult organism is one of the most fundamental yet elusive areas of biology. In recent years, immense progress has been made in cataloguing cell identity during mouse development through single-cell RNA sequencing, though these data alone do not shed light on the ancestry and fate choices taken by cells. To address this, we have recently developed and published a new mouse model, named CARLIN, that uses CRISPR-mediated cellular barcoding to trace thousands of cells in vivo with unique, transcribed tags in an inducible manner. Using this system, we have mapped the clonal and anatomical origins of the hematopoietic system. Our data demonstrate the existence of diverse embryonic origins for both fetal and long-lived blood cells and shed light on the drivers of hematopoietic heterogeneity in adult tissues. Furthermore, we have generated single-cell RNA

sequencing libraries of whole barcoded early mouse embryos, allowing us to create a blueprint of lineage decisions taken by cells during gastrulation. Our work sheds light on long-standing questions in developmental biology and can be used to understand the cell-of-origin of pediatric diseases, and to bring insight into very basic biological questions concerning cell fate commitment.

Funding Source: NIH-NHLBI, Wellcome Trust, EMBO
Keywords: Hematopoiesis, cellular barcoding, gastrulation

4:30 PM – 5:00 PM

PATTERN FORMATION AND CELL FATE SPECIFICATION DURING VERTEBRATE EMBRYOGENESIS

Schier, Alexander

Biozentrum, University of Basel, Switzerland

How do embryos generate the correct cell types at the right places and right times? This classic question can now be revisited through revolutionary advances in imaging, genomics and computation. I will discuss our recent efforts in spatial transcriptomics, single-cell sequencing and gene regulatory network reconstruction to determine how zebrafish embryos are patterned and specify cell fates.

Keywords: embryo, zebrafish, Nodal

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Poster Abstracts

MONDAY, 4 DECEMBER

All times in Central European Time (Vienna, Austria)

Poster Session I
3:45 PM – 4:30 PM

TOPIC: GENETIC PROGRAMS

101

6P24 SNP ASSOCIATED WITH CORONARY ARTERY DISEASE ALTERS TALE FAMILY COMPLEX TRANSCRIPTION BINDING AND LINKS TO CIS-GENE EXPRESSION IN ARTERIAL ENDOTHELIAL CELLS

Tay, Kai Yi

Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

Cardiovascular diseases are the primary global cause of mortality, with coronary artery disease (CAD) being responsible for most cardiovascular-related deaths. A single nucleotide polymorphism (SNP) rs6903956 located on chromosome 6p24.1 within the non-coding region of ADTRP was initially identified as a susceptibility locus for CAD in a large Chinese population cohort through a genome-wide association study (GWAS). However, the exact mechanism by which rs6903956 influences CAD risk remains elusive. Through a comprehensive population meta-analysis, we found an association between rs6903956 and endothelial function. Utilizing in silico data mining and affinity purification mass spectrometry with arterial endothelial cell nuclear extracts, we discovered that the TALE family transcription factor complex exhibits differential binding affinity to the rs6903956 risk allele versus the non-risk allele. Using CRISPR base editing, we converted the risk allele A to the non-risk allele G in CAD patient-derived induced pluripotent stem cells. Remarkably, CAD arterial endothelial cells with homozygous GG genotype showed a significant decrease in the expression of cis-genes compared to their isogenic AA counterparts. These cis-genes, located proximally to rs6903956, are implicated in vascular effects in endothelial cells (ECs) and are acknowledged GWAS loci for CAD. Interestingly, the TALE family complex is noted to predominantly bind to promoters of perturbed cis-genes. Our findings establish a connection between intronic SNPs in ADTRP, the TALE family transcription factor complex, and altered cis-gene expression levels in arterial endothelial cells, shedding light on potential mechanisms underlying CAD risk.

Funding Source: We acknowledge funding sources, the National Research Foundation, SG (Project 370062002), MOE Academic Research Fund Tier 1 (2018-T1-001-030), Human Frontier Science Program (RGY0069/2019), and Nanyang Assistant Professorship.
Keywords: Induced pluripotent stem cells (iPSCs), Arterial Endothelial Cells, CRISPR genome editing

102

BRD9-CONTAINING NON-CANONICAL BAF COMPLEX IS A BARRIER TO SOMATIC CELL REPROGRAMMING

Onder, Tamer T.

School of Medicine, Koç University, Istanbul, Turkey

Epigenetic reprogramming to pluripotency requires extensive remodeling of chromatin landscapes to silence existing cell-type-specific genes and activate pluripotency genes. ATP-dependent chromatin remodeling complexes are important regulators of chromatin structure and gene expression; however, the role of recently identified Bromodomain-containing protein 9 (BRD9) and the associated non-canonical BRG1-associated factors (ncBAF) complex in reprogramming remains unknown. Here, we show that genetic or chemical inhibition of BRD9, as well as ncBAF complex subunit GLTSCR1, but not the closely related BRD7, increase human somatic cell reprogramming efficiency and can replace KLF4 and c-MYC. We find that BRD9 is dispensable for human induced pluripotent stem cells under primed but not under naive conditions. Mechanistically, BRD9 inhibition downregulates fibroblast-related genes and decreases chromatin accessibility at somatic enhancers. BRD9 maintains the expression of transcriptional regulators MN1 and ZBTB38, both of which impede reprogramming. Collectively, these results establish BRD9 as an important safeguarding factor for somatic cell identity whose inhibition lowers chromatin-based barriers to reprogramming.

Funding Source: This work was supported by TUBITAK project 219Z209 and by a Newton Advanced Fellowship (Royal Society)
Keywords: Chemical Reprogramming, Chromatin remodeling, iPSC

Poster Abstracts

103

DECODING THE EPIGENETIC DETERMINANTS OF HUMAN SKELETAL DEVELOPMENT USING AN IN VIVO ORGANOID SYSTEM

Hidalgo Gil, David[1], Garcia Garcia, Alejandro[2], Grigoryan, Ani[3], Zacharaki, Dimitra[2], Bourgine, Paul[2]

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Endochondral ossification (EO) is the essential developmental pathway governing the formation and repair of the skeletal system. This process is orchestrated by mesenchymal stem/stromal cells (MSCs) predominantly derived from the mesoderm layer. Following condensation and proliferation, MSCs form a transient cartilage tissue progressively vascularized and remodeled into a fully mature bone organ. A fraction of MSCs persist in the mature organ, regulating the hematopoietic activity and bone homeostasis. Animal models have allowed identifying some of the key regulators of the EO process, including IHH/WNT initiating chondrogenesis. Histone Deacetylases (HDACs) are also pivotal, since HDACS 4 and 5 knockouts produce embryos with defective skeletal development due to compromised EO. Nevertheless, the precise epigenetic landscape and transcriptional drivers of EO remain cryptic, and no models offered gathering information in a human-specific context. Progress in tissue engineering and development biology led to the recapitulation of the EO process using human bone marrow-derived MSCs. By priming cells in vitro, human cartilage tissue can be obtained. Following subcutaneous implantation in immunocompromised mice, the human tissue spontaneously recapitulates the latest stages of the EO process, establishing a fully mature human bone organ/ossicle with persisting MSCs. We here propose to harness this process and combine it with IF and single cell multi-omics analysis to unveil the human transcriptional and epigenetic drivers of EO. Our data confirmed the formation of mature human ossicles within 4-6 weeks in vivo. By collecting implants at early timepoints (Day 3), IF indicates early priming and tissue self-organization. By performing single nuclei RNA and ATAC sequencing, we generated the first multiome dataset of the human EO pathway. Our project is expected to shed light on the key drivers of the EO process and the differentiation of MSCs. This could be exploited in regenerative context, whereby cell differentiation remains unstandardized since donor and tissue dependent. Last, our study exemplifies the potential of harnessing human tissue engineering protocols to decode the developmental/repair processes in a human specific setting.

Funding Source: The project was supported by the Knut and Alice Wallenberg Foundation, the Medical Faculty at Lund University, Region Skåne (to PB), the European Research Council (ERC) (Starting grant hOssicle #948588 to PB)
Keywords: Endochondral ossification, Epigenetics, Human Skeletal development

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ESTABLISHING A MYOCARDIAL INFARCTION MODEL IN EPICARDIOIDS USING NITROREDUCTASE FOR CELL ABLATION

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Myocardial infarction (MI) is the leading causes of mortality around the world, with 1.8 million deaths in Europe alone. Following MI, the regenerative capacity of the adult human heart after is limited, particularly after significant cardiomyocyte loss. Neonatal mice studies suggest age-related changes in regenerative capacity and highlight the epicardium's role in this process. However, little is known about the role of the epicardium in the regeneration of the human heart. Cardiac organoids, specifically epicardioids, closely mimic the human heart's structures and functions, making them a valuable tool for studying human heart development and regeneration. Combining epicardioids with genetic cell ablation techniques represents an innovative in vitro model of human heart regeneration after myocardial infarction. In this study, we established a system to induce specific cell death of cardiomyocytes in 2D and 3D settings, which involves transgenic expression of a bacterial nitroreductase (NTR) in cells, making them susceptible to prodrugs like metronidazole (MTZ). Using lentivirus, hiPSC-derived 2D cardiomyocytes were transduced to express NTR under the control of the cardiac-specific promoter, cTnT. Following MTZ treatment, we observed the selective and concentration-dependent ablation of cardiomyocytes expressing NTR. To mimic the application of the NTR system in an organoid setting, we generated cardiomyocyte spheroids by mixing NTR-transduced and non-transduced CMs creating a mosaic structure suitable for partial CM ablation. Compared to 2D, higher MTZ concentrations were needed to efficiently ablate CMs in a 3D setting. Furthermore, CMs located in the outer layer of the spheroids showed a more efficient ablation compared to those in the core. In conclusion, this study demonstrates the potential of combining epicardioids with the NTR-system to create a novel in vitro model for investigating myocardial infarction. Our findings highlight the versatility of this approach in selectively ablating cardiomyocytes and provide insights into dose -dependent MTZ effects in both 2D and 3D settings.

Keywords: cell ablation, heart regeneration, organoids

Poster Abstracts

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GAIN OF 1Q CONFERS AN MDM4-DRIVEN GROWTH ADVANTAGE TO UNDIFFERENTIATED AND DIFFERENTIATING HESC WHILE ALTERING THEIR DIFFERENTIATION CAPACITY

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Human pluripotent stem cells have become a promising research tool for transplantation and disease modeling due to their unique ability to differentiate to any cell type of the adult human body. In vitro propagation can induce certain chromosomal abnormalities in stem cells, which confers them with growth advantage and take-over of the culture. Gains of 1q chromosome are highly recurrent in human pluripotent stem cells. Currently, the effect of 1q abnormality on lineage specification is not yet understood. In this work, we show that gains of 1q impact the differentiation capacity to derivates of the three germ layers, leading to miss-specification to cranial placode and non-neural ectoderm during neuroectoderm differentiation and by lower expression of lineage specific markers in 64% of hepatoblast markers and 63% of cardiac progenitor markers. Competition assays show that the cells retain their selective advantage during differentiation. The population of cells with a 1q duplication increased from 11% to 45% during neuroectoderm differentiation, from 11,6% to 60,9% during hepatoblast differentiation and 10,8% to 33,5% during cardiac progenitor differentiation. Selective advantage is mediated by a higher expression of MDM4, a gene located in the common region of gain. MDM4 drives the winner phenotype of 1q cells in both the undifferentiated and differentiating state by reducing the cells' sensitivity to DNA-damage through decreased p53-mediated apoptosis. Finally, we find that cell density in culture plays a key role in promoting the competitive advantage of the cells by increasing DNA damage. When cells reach density above 265.224 cells/cm2, 1q cells with high DNA damage load keep proliferating while wild-type cells undergo apoptosis. Our work reveals that the selective advantage of 1q gain is retained during differentiation, showing that mosaic culture at the beginning of the differentiation can result in heterogeneous end population of poorly specified cells.

Funding Source: The Research Foundation – Flanders (FWO)

Keywords: Human pluripotent stem cells, 1q chromosomal abnormality, Growth advantage during differentiation

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IDENTIFICATION OF A PREDICTIVE BIOMARKER FOR THE ANGIOGENIC POTENTIAL OF HUMAN MESENCHYMAL STEM CELLS BY SINGLE CELL-TRANSCRIPTOME ANALYSIS

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Human mesenchymal stem cells (MSCs) have already been utilized as a source of cell therapy for various diseases, and the use of MSCs for clinical applications will increase in the future. However, the heterogeneity of MSC-based products and the lack of robust predictive biomarkers linked to therapeutic efficacy limit the possibility of developing an efficient and effective manufacturing process for MSC-based products. In this study, we aimed to identify biomarkers to predict the efficacy of angiogenic therapy using bone marrow-derived MSCs (BM-MSCs). To assess angiogenic potency, we measured the secretion of vascular endothelial growth factor (VEGF), a well-known angiogenic factor, in 11 donor-derived BM-MSC lines under in vitro ischemic culture conditions. Significant variations were observed in VEGF secretion ability among the 11 lines. Next, by clarifying the heterogeneity of BM-MSCs using single-cell RNA-sequencing (scRNA-seq) analysis, we identified the functional cell subpopulation that contributes to the VEGF-producing ability under ischemic conditions. Gene Ontology analysis suggested that energy production pathways were strongly activated in the identified cell subpopulation. In addition, leucine-rich repeat-containing 75A (LRRC75A) was more highly expressed in this cell subpopulation than in the other subsets. Knockdown of LRRC75A using small interfering RNA resulted in significant inhibition of VEGF secretion in ischemic BM-MSCs, indicating that LRRC75A regulates VEGF secretion under ischemic conditions. Conversely, the proportion of LRRC75A-expressing cells in this particular cell

subpopulation was notably diminished in MSCs derived from the umbilical cord and placenta, neither of which induced VEGF expression under ischemic conditions. Therefore, LRRC75A may be an efficient biomarker for predicting the angiogenic potential of BM-MSCs. Together, our findings indicate that scRNA-seq provides a useful tool for identifying cell subsets and biomarkers associated with a particular cellular function in heterogeneous MSCs.

Funding Source: This work was supported by the Japan Agency for Medical Research and Development and by grants from the Kanagawa Prefectural Government.
Keywords: human mesenchymal stem cells, angiogenesis, single cell-transcriptome analysis

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MODELING GLIOMA INITIATION USING PRIMARY HUMAN NEURAL STEM AND PROGENITOR CELLS

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Glioblastoma multiforme (GBM) is a deadly brain cancer notable for its significant intratumoral heterogeneity, which is believed to drive therapy resistance. GBM has been observed to mimic a neural stem cell hierarchy reminiscent of normal brain development. However, it is still unclear how cell-of-origin and specific driver mutations shape intratumoral heterogeneity. Here, we develop a model of glioma initiation using neural stem and progenitor cells (NSPCs) purified from fetal human brain tissue. We previously described a method to prospectively isolate and culture tripotent neural stem cells (NSCs), bipotent glial progenitor cells (GPCs), and unipotent oligodendrocyte precursor cells (OPCs). We transduced these isogenic lines with dominant-negative TP53-R175H and NF1 knockdown, a commonly-used genetic model of GBM in mice. These reprogrammed lines robustly engrafted when transplanted into the brains of immunodeficient mice, and showed significant expansion over time. Engrafted cells were reextracted from the mouse brain for single cell RNA sequencing (scRNA-seq), in order to quantify how the cell-of-origin modulates the cellular subtypes found in the resulting tumor. Our platform is highly adaptable and allows for modular and systematic interrogation of how cell-of-origin and specific sets of driver mutations shape the tumor landscape.

Keywords: Glioblastoma, Neural stem cell, Cancer stem cell

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MODELLING THE BARDET-BIEDL SYNDROME CILIOPATHY USING HUMAN RETINAL ORGANOID AND CELL MODELS

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Bardet Biedl Syndrome (BBS) is a rare autosomal-recessive ciliopathy that results from mutations in a set of highly-conserved BBS genes. BBS patients experience retinal degeneration caused by a progressive loss of rods and cone photoreceptors, which is commonly diagnosed during early childhood. However, studying the retinal defects associated with BBS presents several significant challenges. These challenges include the limited availability of relevant tissue samples, the inability to study the human retina on a cellular and molecular level, the lack of a patient-specific drug testing platform, and the limitations of animal models in reproducing human pathophysiology. Addressing these challenges is crucial for advancing our understanding of BBS-related retinal degeneration and developing effective treatments for this condition. Here we aimed to study genes BBS2 and BBS6, as they account for most of the mutations present in BBS with a severe retinal phenotype. We edited BBS2/6 using CRISPR/Cas9 technology to introduce knockout mutations underlying the BBS phenotype into two fully characterized hiPS cell lines from healthy individuals. We have differentiated these cells carrying BBS mutations into retinal organoids to define the photoreceptor phenotype and retinal pigmented epithelial cells to characterize the ciliary defects caused by the BBS genes mutations. Our findings undeniably substantiate the retinal organoid model as an invaluable developmental biology tool for the elucidation of BBS-related retinal anomalies, unveiling the potential for insights within the developmental biology discipline as well as regenerative medicine.

Keywords: Organoids, hiPS, BBS

Poster Abstracts

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NEUROECTODERM SPECIFICATION REQUIRES TET3 CATALYTIC AND NONCATALYTIC FUNCTIONS TO ACTIVATE ECTODERMAL AND SILENCE MESODERMAL PROGRAMS RESPECTIVELY

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The ten-eleven translocation family of proteins (TET1/2/3) are epigenetic enzymes that regulate gene expression by promoting DNA demethylation (i.e. catalytic activity) and partnering with regulatory proteins (i.e. non-catalytic functions). Unlike Tet1 and Tet2, Tet3 is not expressed in embryonic stem cells (ESCs) but is induced upon ESC differentiation. However, the significance of its dual roles in lineage specification is less defined. By generating Tet3 catalytic mutant (Tet3m/m) and knockout (Tet3–/–) mouse ESCs and differentiating them to neuroectoderm (NE), we have identified distinct catalytic dependent and independent roles of TET3 in NE specification. We found that the catalytic activity of TET3 is important for activation of neural genes while its non-catalytic functions are involved in suppressing mesodermal programs. Interestingly, the vast majority of differentially methylated regions (DMRs) in Tet3m/m and Tet3–/– NE cells were hypomethylated. The hypo-DMRs were associated to aberrantly upregulated genes while the hyper-DMRs were linked to downregulated neural genes. We found the maintenance methyltransferase Dnmt1 as a direct target of TET3, which is downregulated in TET3 deficient NE cells and may contribute to the increased DNA hypomethylation. Our findings establish that the catalytic dependent and independent roles of TET3 have distinct contributions to NE specification with implications in development.

Funding Source: Supported by the Paul S. Frenette Scholar Awards Program of the Ruth L. and David S. Gottesman Institute for Stem Cell Research and Regenerative Medicine and Training Program in Cellular & Molecular Biology and Genetics (T32 GM007491).

Keywords: ESC differentiation, DNA methylation, Tet enzymes

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NUCLEAR SHUTTLING OF FOXO TRANSCRIPTION FACTORS INSTRUCTS EARLY EMBRYONIC CELL FATE TRANSITIONS

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Nuclear shuttling of FoxO transcription factors instructs the transition to formative pluripotency NUCLEAR SHUTTLING OF FOXO TRANSCRIPTION FACTORS INSTRUCTS EARLY EMBRYONIC CELL FATE TRANSITIONS Naïve pluripotency is sustained by a self-reinforcing gene regulatory network (GRN) composed of core and naïve pluripotency specific transcription factors (TFs). Upon exit from the naïve state, ES cells enter differentiation by transitioning through formative, post-implantation like pluripotency. This cell fate transition is instructed by a set of crucial signalling pathways, but how exactly changes in pathway-activities are translated into decommissioning the naïve and the initiation of the formative GRN is still largely unknown. One of the key input signals into pluripotency-progression is funnelled through the kinase Akt. Accordingly, multiple components up- and downstream of Akt are top hits in genetic screens for factors required for the exit from naïve pluripotency. Here we show that a reduction of Akt-activity by the tumor supressor PTEN is required for proper differentiation by controlling the activity of FoxO-family TFs. FoxO TFs are well-known as regulators of longevity and metabolism, but their involvement in cell fate transition is far less studied. We find that in naïve ESCs phosphorylated AKT acts as gatekeeper to stop FoxO TFs from entering the nucleus. Reduction of Akt-activity by PTEN at the onset of differentiation allows shuttling of FoxO TFs into the nucleus, where they enforce the transition from naïve to formative pluripotency by binding and activating formative pluripotency specific enhancers. At the same time, enhancers of crucial naive specific TFs, such as Klf2, Nanog and Esrrb, are bound by FoxO TFs at the onset of differentiation. We speculate that this indicates a dual role for FoxO TFs in decommissioning the old naïve, while establishing the new formative gene expression programme. Highlighting the pivotal role of Foxo TFs in the exit from pluripotency, they are both required and sufficient for the exit from naïve pluripotency. Our work identifies a central role for FoxO TFs in instructing early embryonic cell fate transitions and provides a mechanistic explanation for the role of Akt signalling during differentiation.

Keywords: pluripotency, Akt and FoxO signaling, cell fate transition

Poster Abstracts

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THE ESSENTIALOME OF IMPRINTED AND REGULATORY GENES DURING NEURAL DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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Essential gene networks for the maintenance of pluripotency and early germ layers differentiation have been recently identified by us by high-throughput functional genomics studies utilizing CRISPR/Cas9 technology. To this end, we generated a loss-of-function library in haploid human pluripotent stem cells (hPSCs) by targeting 18,000 protein coding genes with over 180,000 sgRNAs. Using this library, we identified essential genes for the normal growth and survival of hPSCs and their differentiation into the ectoderm, mesoderm, and endoderm. Mapping the essentialome of neurons can assist uncovering new therapeutic targets and develop models for neurodevelopmental disorders. To this aim, we utilized the genome-wide loss-of-function library which we differentiated into neurons. We show that essential genes and pathways for neurogenesis are enriched for secreted and membrane proteins, and that a large group of neurological conditions, including neurodegenerative disorders, manifest early neuronal differentiation phenotypes. Furthermore, the essential transcription factors are enriched with HOX genes demonstrating synergistic regulation and surprising non-redundant functions between HOXA6 and HOXB6 paralogs. Moreover, we establish the essentialome of imprinted genes during neurogenesis, demonstrating that maternally expressed genes are non-essential in pluripotent cells and their differentiated germ layers, yet several imprinted genes are essential for neuronal development. These include Beckwith-Wiedemann syndrome and Angelman syndrome related genes, for which we suggest a novel regulatory pathway. Overall, our work identifies essential pathways for neuronal differentiation and stage-specific phenotypes of neurological disorders. Nature Cell Biology (2018) Cell Stem Cell (2020) Nature Communications (under revision)

Keywords: Genome-wide screening, Human pluripotent stem cells, Neural differentiation

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THE ROLE OF BAF COMPLEXES IN THE MAINTENANCE OF HUMAN INTESTINAL EPITHELIAL HOMEOSTASIS

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Intestinal homeostasis is ensured by a constantly renewing epithelium consisting of fast-cycling intestinal stem cells and their short-lived differentiated progeny. Importantly, cellular differentiation in the intestine is maintained by the concerted interplay between cell type-specific transcription factors, niche signals, and the dynamic open chromatin landscape of mot intestinal cells. Current knowledge on the regulation of homeostatic chromatin accessibility and its dynamic changes during cellular differentiation in the intestine is limited. BRG1/BRM associated factor (BAF) complexes are multisubunit chromatin remodelers that exist in distinct biochemical forms. They play a fundamental role in diverse developmental processes and regulate cellular differentiation in multiple adult tissues. Limited evidence from previous genetic studies in mice suggests, that regulation of chromatin accessibility by BAF complexes is crucial for proper intestinal differentiation. Additionally, loss-of-function mutations in genes encoding various BAF complex subunits are frequent in colorectal cancer (CRC). Drawing from these insights, in this project we aim at deciphering cell type-specific BAF complex-mediated chromatin regulatory mechanism and related gene expression patterns in the intestine and define their contribution to intestinal homeostasis as well as to CRC. To investigate BAF complex function in the intestinal epithelium, we have generated human induced pluripotent stem cell -derived intestinal organoids that closely recapitulate cellular heterogeneity and gene expression profile of the human intestinal epithelium. Using pharmacological and genetic perturbations of distinct BAF complexes, we show that BAF complexes are required for homeostatic gene expression and that inhibition of BAF complex function alters the differentiation, growth, and cellular composition of the intestinal organoids. These findings unveil a previously unexplored mechanism governing the regulation of the chromatin landscape during intestinal cellular differentiation. Furthermore, they suggest a possible mechanism by which mutations in the BAF complexes might play a role in the development of CRC. Ultimately, these insights may facilitate the development of more effective therapies for CRC.

Keywords: Chromatin regulation, Intestinal epithelium, Cellular differentiation

TOPIC: GENOME EVOLUTION IN DEVELOPMENT

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DEVELOPMENTAL EXPRESSION DYNAMICS OF STEM CELL MARKERS AND HYPOXIA-INDUCIBLE FACTORS IN FORMING DENTAL PULP

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Dental pulp stem cells (DPSCs) are capable of regenerating a tissue and have a high capacity for differentiation, thus provide promising source for treatment strategies. In general, stem cell niches are characterised by a low partial oxygen pressure. This physiological hypoxia helps stem cells to maintain their major characteristics such as multipotency or ability to differentiate. So far, the majority of DPSCs research has been performed in vitro. The knowledge in in vivo developmental context is limited. Based on in vitro data, a set of positive and negative DPSCs markers has been established, including a key trio of MSC markers (Nt5e, Thy1, Eng). To follow developmental expression dynamics, postnatal stages of the dental pulp formation were investigated in the first mouse mandibular molar. The expression of positive and negative DPSCs markers along with hypoxia signalling pathway genes was examined by customised PCR Arrays. Among the positive markers, Vcam1, Fgf2, Nes were identified as increasing and Cd44, Cd59b, Mcam, Alcam as decreasing between perinatal vs. postnatal stages. Within the panel of negative DPSC markers, Cd14, Itgb2, Ptprc displayed increased and Cd24a decreased levels at later stages of pulp formation. The key trio of markers, Nt5e did not show any significant expression difference, Thy1 displayed a strong decrease between P0 and P7 while Eng increased between these stages. Hypoxia signalling pathway genes showed decreased trend in the expression during the development of dental pulp. The downregulating trend was identified e.g. in genes that encode glycolytic enzymes and glucose transporters. This trend suggests the shift from glycolysis to more energetically efficient oxidative phosphorylation which corresponds to known metabolic flexibility of MSCs during their transition to more differentiated phenotype during development. This in vivo insight reflects the natural environment which is of critical importance in the case of stem cell maintenance. Additionally, it provides findings to the general knowledge about hypoxic state during formation of dental pulp in vivo.

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Keywords: Dental pulp, In vivo development, Stem cell markers

TOPIC: MORPHOGEN GRADIENTS

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LIGHT-INDUCIBLE PATTERNING OF ORGANOIDS: UNRAVELING THE ROLE OF WNT3A IN HUMAN HIPPOCAMPAL DEVELOPMENT

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Brain organoids, three-dimensional in vitro models derived from stem cells, have emerged as a valuable tool for studying brain development and neurodevelopmental disorders. One of the critical aspects of brain organoid research is the ability to replicate specific brain regions accurately. Morphogens orchestrate the patterning of the brain in a spatial and temporal manner during embryonic development, determining cell types and brain regions. These signaling molecules can be used in vitro to generate brain organoids of distinct regions, such as the hippocampus. The hippocampus is a pivotal brain region critically involved in learning, memory, and cognitive functions. It is severely affected in diseases such as Alzheimer's and linked to neurodevelopmental disorders, including autism spectrum disorder, and epilepsy. Wnt morphogens, particularly Wnt3a, contribute to the formation of the hippocampus. Wnt3a signaling is known to influence the differentiation of neural stem cells into excitatory pyramidal neurons and inhibitory interneurons, thereby shaping the intricate neural circuitry of the hippocampus. Here, we utilize a light-inducible gene expression system that allows defined spatial activation of Wnt3a in induced pluripotent stem cells and organoids. By activating Wnt3a in embryoid bodies, we aim to direct stem cells to differentiate towards hippocampal-like structures. Wnt pathway key effector beta-catenin exhibits upregulation in induced pluripotent stem cells and organoids upon light induction of Wnt3a. Prox1, known for its expression in the dentate gyrus of the hippocampus during embryonic

development, is also detected in light-switched cells of organoids at day 30. This novel approach will provide insights into the molecular mechanisms governing Wnt3a driven human hippocampal development and offers a promising platform to study neurological disorders.

Keywords: Wnt3a, light-inducible patterning, brain organoids - hippocampus

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SECRETED PROTEIN ACIDIC AND RICH IN CYSTEINE PROMOTES EPITHELIA TO MESENCHYMAL TRANSITION AND CARDIOMYOGENESIS IN MURINE CARDIAC STEM CELLS

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Mammalian hearts contain cardiac stem cells throughout life; however, these cells could not be harnessed to repair damaged myocardium in vivo so far. Assuming physiological relevance of these cells which had evolved and had been maintained during evolution, we seek after their function using murine cardiac stem cell lines as an in vitro model system. Here we address the hypothesis that cardiac stem cells may fulfil a gland-like function in the adult heart and focus on factors secreted by cardiac stem cells. Cardiac stem cells increasingly express and secrete Secreted Protein Acidic and Rich in Cysteine (SPARC) during differentiation to cardiac cells in the absence of Leukemia Inhibitory Factor secreting niche cells. Autocrine and paracrine SPARC promotes cardiomyogenesis in a dose dependent manner including but not limited to the promotion of epithelial to mesenchymal cell transition of cardiac stem cells. Demonstration that secretion of SPARC from cardiac stem cells promotes cardiomyogenesis opens up the possibility that a physiological function of cardiac stem cells in the adult and ageing heart might be the gland-like secretion of factors which modulate age related and adverse environmental influences and thereby contribute to homeostasis in the heart throughout life.

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Keywords: Cardiac stem cells secrete SPARC, SPARC promotes cardiomyogenesis, SPARC promotes EMT

TOPIC: TIMING OF DEVELOPMENT

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BENCHMARKING HAIR-BEARING HUMAN PSC-DERIVED SKIN ORGANOIDS WITH HUMAN PRENATAL SKIN USING SINGLE-CELL AND SPATIAL GENOMICS

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Recent advances in stem cell research have enabled generation of hair-bearing skin organoids from human pluripotent stem cells (hPSC) comprising embryonic (hESC) and induced pluripotent stem cells (hiPSC). This presents a unique opportunity to gain insights into the intricate cellular and molecular processes governing human skin development. However, our understanding of how closely the hPSC-derived skin organoids mimic the complex processes and dynamic tempo of in vivo skin development remains incomplete. Here, using advanced single-cell genomic technologies, we have benchmarked the hESC- and hiPSC-derived skin organoid development over a five-month culture period (n=84) with first and second trimester human prenatal skin (n=15). Our results suggest that skin organoids faithfully recapitulate the processes of in vivo human skin development and its appendages such as de novo hair follicle formation. In addition, using spatial transcriptomics paired with histological observations, we have, for the first time, characterised the spatial organisation of the skin and hair follicle cells in the mature five-month-old skin organoids (n=3). Overall, our findings validate the prenatal hair-bearing skin organoid as a faithful model to derive human hair follicles and other skin appendages in vitro, opening new avenues for regenerative medicine and disease modelling including congenital skin disorders and hair loss.

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Keywords: hPSC-derived hair-bearing skin organoids, Prenatal skin development, Single-cell and spatial genomics

Poster Abstracts

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DEVELOPMENT OF AN EX VIVO ORGAN PERFUSION SYSTEM FOR THE MOUSE UTERUS: A NOVEL APPROACH TO STUDYING EMBRYONIC IMPLANTATION AND DEVELOPMENT

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Studying mammalian embryonic developmental events poses significant challenges due to the inaccessible nature of embryos. Existing imaging methods provide only snapshots and require uterus removal, which can potentially impede growth. Embryo culture ex-utero has proven to be difficult and negates fetal-placenta-uterus interactions. To overcome these limitations, we aimed to develop an ex vivo organ perfusion (EVOP) system for culturing the mouse uterus. This system would provide easier access to the embryos for imaging and real-time longitudinal data collection while maintaining a physiologic environment for growth. This system offers the potential for studying blastoid implantations and disease modeling. For this, the uterus is isolated, and the aorta is cannulated and connected to a bioreactor previously developed by the Rogers Lab. Hormones, various culture media, and nutrient supplements are being tested to determine the ideal culture medium for mouse uterus growth. We assess the performance of the ex vivo cultured uterus by measuring oxygen consumption using oxygen sensors and analyzing metabolic activity through clinical chemistry of the culture media. Hematoxylin and eosin (H&E), Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay, and immunohistochemistry (IHC) staining with Cleaved-Caspase 3 are used to evaluate cell death, determine organ viability, and assess culture media compatibility. Results show that utilizing the infrarenal as a cannulation point leads to successful perfusion of the uterus and ovaries, and cells are viable after a 24-hour culture period. The uterus EVOP culture will enable observation of development in real-time and help us understand fundamental questions of developmental biology.

Keywords: Ex-vivo, Development, Uterus

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DIVERGENCES IN BIOELECTRIC STATE AND CALCIUM SIGNAL CORRELATE WITH EARLY GERM LAYER DIFFERENTIATION OF EMBRYONIC STEM CELLS

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Resting membrane potential (Vmem) and calcium signaling are spatiotemporally-specific, as a function of developmental stage and location. Changes in Vmem and increases in intracellular calcium ([Ca2+]i) regulate proliferation and differentiation of embryonic cells, with prior evidence suggesting calcium signaling is downstream of Vmem. However, it remains unclear whether changes in the bioelectric state and calcium signaling of precursor cells are connected with their differentiation trajectory. In this study, we measured Vmem and [Ca2+]i signals during differentiation of pluripotent embryonic stem cells (ESCs) towards neuroectoderm (NE) and mesendoderm (MZ) cells. We hypothesized that Vmem and calcium signals diverge from ESCs prior to acquisition of NE and MZ-like states. We differentiated mouse ESCs in monolayer towards NE- or MZ-like states for 4 days and measured cells every 24 hours using DiBAC4(3) (for Vmem) and OGB1 (for [Ca2+]i). NE and MZ differentiation cultures expressed germ layer transcription factors, Sox1 and T, respectively. Cultures featured morphologies characteristic of early NE and MZ-like states, as well as distinct regions with flattened or dense, three-dimensional ('cluster') morphologies. NE-directed cells were hyperpolarized, with lower [Ca2+]i relative to MZ-directed cells prior to germ layer marker acquisition. Colonies with clustered morphologies maintained relatively depolarized Vmem and higher calcium signals, closer to those of ESCs despite their distinct cluster appearance. Within NE cultures, Sox1+ cells were hyperpolarized and had lower [Ca2+]i relative to Otx2+ (epiblast-like) cells. T+ cells are depolarized within MZ-like cell populations, with spatial variation in [Ca2+]i correlating with T expression. Our results demonstrate that divergences in Vmem and [Ca2+]i occur prior to germ layer marker protein expression, suggesting that a cell's bioelectric state may be connected with differentiation trajectory from ES cells. Identification of regional differences in both Vmem and [Ca2+]i suggests that even within in-vitro systems that lack the complexity of in vivo microenvironments, stem cells maintain regulated resting membrane potentials and calcium signals during early germ layer differentiation.

Funding Source: Supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany´s Excellence Strategy – EXC-2068 – 390729961 and a Regent's Junior Faculty Fellowship (AD).
Keywords: germ layer differentiation, bioelectricity, calcium signaling

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INCREASED APOPTOTIC CELL DEATH AND AUTOPHAGY ALTERATION IN RIBOFLAVIN TRANSPORTER DEFICIENCY

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Riboflavin Transporter Deficiency (RTD) is a rare, neurological disorder characterized by hearing loss and sensory ataxia associated with spinal motor neuron (MN) degeneration. The disease is caused by loss of function mutations in SLC52A2 or SLC52A3 genes, respectively encoding riboflavin transporters hRFT2 and hRFT3. As RF is the precursor of the coenzymes FMN and FAD, their abnormally low levels result in defective functionality of flavoproteins, which are involved in cellular bioenergetics and cell survival processes. As this disorder lacks dependable in vivo models, we took advantage of iPSC technology to recapitulate human neuronal features of RTD. More specifically we perform combined confocal and immunoblotting analyses aimed at characterizing the pathomechanisms associated to RTD. Patient-specific iPSCs and iPSC-derived MNs have been analysed by Focused Ion Beam/Scanning Electron Microscopy (FIB/SEM) that demonstrates mitochondrial ultrastructural alterations, involving shape, number, and intracellular distribution of organelles. Increased apoptosis was observed in RTD cells, confirmed by the presence of vesicles and blebs budding from the cell surface of RTD cells and by activated caspase-3 immunofluorescence, WB and TUNEL assays. Consistent with these results and due to the high cell mortality associated with RTD, we investigated survival mechanisms, such as autophagy, an essential process for cell viability that ensures an effective turnover of impaired cytoplasmic components and organelles. Immunoblotting experiments and confocal analyses demonstrate an abnormal activation of autophagic process associated with alteration of the lysosomal compartment. Overall, our work contributes to the knowledge on the multiple cellular features associated to RTD phenotype, supporting a central role played by mitochondrial apoptosis in its pathogenesis, thus suggesting potential targets for future therapies.

Keywords: iPSC, Apoptosis and autophagy, Neurodegenerative disease

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MACROPHAGE DERIVED PDGF SIGNALING GOVERNS HSD11B2-MEDIATED DENTAL PULP STEM CELL AGING

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Teeth undergo ongoing restorative procedures throughout their lifetimes, relying on the dental pulp stem cells (DPSCs) and their niches to act as a healing reservoir in times of tooth injury to sustain dental health. The restorative capabilities decline with ageing, culminating in DPSC fibrosis and functional deterioration. However, the underlying molecular dynamics and functional attributes of DPSCs in the context of ageing remain largely unexplored. Using single cell RNA sequencing, we delineated the heterogenous cell populations present in the dental pulp of mouse incisors across different developmental stages. Our analysis revealed that the Lypd1+ cells in the apical region of the pulp, a specific subset of DPSCs, undergo substantial changes as aging progresses which marked by a heightened expression of Hsd11b2, an enzyme pivotal in transforming the active hormone corticosterone into its inactive form. This process ensures the selectivity of the mineralocorticoid receptor (MR), highlighting a potentially significant pathway in the aging process of DPSCs. In vivo pharmacological inhibition of Hsd11b2 effectively mitigates the extent of DPSC fibrosis observed in the aging pulp, thus underpinning the pivotal role Hsd11b2 plays in pulp aging. Metabolomics assessments have uncovered that signals pertaining to the synthesis and discharge of aldosterone are distinctly amplified in the aging dental pulp. This is in conjunction with an increased expression of NR3C2 which encodes the mineralocorticoid receptor. This suggests that the escalated presence of Hsd11b2 in ageing DPSCs exacerbates pulpal fibrosis by diminishing corticosterone's engagement with the MR, facilitating the enhanced affinity of aldosterone for its receptor. To delve deeper into the potential cellular interactions within the dental pulp that might be instigating DPSC fibrosis, we implemented CellChat analysis. This revealed PDGF signaling originating from macrophages and targeting Lypd1+ DPSCs through the mediation of the Pdgfb/Pdgfrb/p38 axis, fostering pulp fibrosis. Blocking p38 phosphorylation led to a reduction in Hsd11b2 expression, reversing the pulp fibrosis. Our research suggesting that targeting Hsd11b2 and the upstream signals could pave a promising pathway for the treatment of pulp problem associated with ageing.

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Keywords: Dental Pulp Stem Cells, Immunomodulation, Pulpal Fibrosis

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RNAI MANIPULATION OF HIPPO PATHWAY REVEALS POTENTIAL TARGETS FOR STEM CELL PROLIFERATION STUDIES IN TRIBOLIUM CASTANEUM MIDGUT

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The Hippo pathway is a critical regulator of cell proliferation and tissue homeostasis in various organisms. In this study, we investigated the role of the Hippo pathway in the proliferation of stem cells in the midgut of Tribolium castaneum, a widely used model organism in developmental biology research. Hippo pathway components, including hippo, salvador, warts, and yorkie, were targeted via RNAi. Using immunostaining and confocal microscopy, we found that knockdown of salvador, a critical mediator of the Hippo pathway, resulted in the complete absence of stem cell clusters in the midguts of T. castaneum. To gain deeper insights into the molecular mechanisms underlying this absence, we plan to compare cell proliferation and apoptosis in the developing midgut of normal and knockdown beetles. This study provides novel insights into the role of the Hippo pathway in regulating stem cell clusters in the midgut of T.castaneum. Our findings highlight the crucial function of Salvador in midgut stem cell maintenance and suggest there could be an unknown molecular mechanism at play, besides its function as a key regulator within the T. castaneum Hippo pathway. The results will also contribute to our understanding of molecular mechanisms governing stem cell proliferation in the midgut and pave the way for future studies aimed at elucidating specific functions of downstream targets. The manipulation of the Hippo pathway using RNAi provides a powerful tool for functional analysis in T.castaneum, that may have implications for regenerative medicine.

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Keywords: RNAi, Stem Cells, Genomics

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SINGLE-CELL TRANSCRIPTOMIC ANALYSIS UNRAVELS THE DISTINCT CONTRIBUTION OF SFRP2+ DPSCS TO TOOTH GROWTH IN BOTH MICE AND HUMANS

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Unlike human teeth, mouse incisors grow continuously throughout their lifespan, suggesting a sustained activation of a stem cell population that promotes proliferation and differentiation to maintain tissue homeostasis. Recent advances in single-cell technology have shed light on the diversity and complexity of dental pulp stem cells (DPSCs) present within teeth. Leveraging publicly available datasets along with our exclusive single-cell transcriptomic data of dental pulp cell, sourced from developed human molars, human apical papilla (representative of pulp cells from a developing root), mouse molars, and mouse incisor, we have clarified tissue similarities and differences, helping uncover mechanisms guiding diverse stem cell functions in distinct environments. Initially, we engaged in a comparative analysis of human and mouse molar pulp tissues, identifying analogous cell types albeit in varying proportions. Our CellChat analysis revealed pronounced MIF (Macrophage Migration Inhibitory Factor) signaling, originating from pulp cells and directing towards monocytes and T cells in both human and mouse molars, showcasing a regulatory network with substantial similarities in both tissues. Strikingly, we discerned a unique subset of Sfrp2+ fibroblasts, exclusively present in mouse incisors and human apical papillae — the models associated with persistent growth capabilities. Further scrutiny through cell trajectory and RNA velocity analyses affirmed the stem cell attributes of Sfrp2+ fibroblasts. These cells have the potential to differentiate into all other fibroblast subsets, thereby facilitating tissue homeostasis and nurturing root development, notably with reference to the Sfrp2+ component in human apical papilla. To delve deeper into the molecular mechanisms guiding Sfrp2+ stem cell fate, we analyzed related transcription factors, identifying Twist1 as a pivotal transcription factor interacting with the Sfrp2+ promoter. Remarkably, the regulation of Twist1 expression levels is intricately managed through the MAPKs pathway, which in turn inhibits WNT signaling in Sfrp2+ fibroblasts, a crucial process to preserve their stem cell identity. Our research brings to light a hitherto undiscovered subset of DPSCs instrumental in tooth growth and evolution in both humans and mice.

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Keywords: Stem Cell Heterogeneity, Dental Pulp Mesenchymal Stem Cells, Tooth Regeneration

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UNRAVELLING MECHANISMS OF NEUROEPITHELIAL TO RADIAL GLIAL TRANSITION USING NOVEL ORGANOID DAMID TECHNOLOGIES

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Timely transitions in stem cell identity and competence during brain development underpin the capacity of neural stem cells (NSCs) to produce a vast array of neuronal and glial cell-types. One such transition during the earliest stages of cerebral cortex development is the conversion of symmetrically dividing neuroepithelial cells to neurogenic radial glia. The timing of this transition determines brain size in humans and great apes and deviations in this timing may underlie neurodevelopmental abnormalities in brain growth. Studying these early events in human neurodevelopment has proven challenging due to the lack of early-stage human fetal tissue. Brain organoids derived from human pluripotent stem cells provide a tractable system for this purpose. The neuroepithelial to radial glial transition in human cerebral organoids is characterised by broad transcriptional changes, orchestrated at least in part by the transcription factor ZEB2. How ZEB2 regulates this transition, and how ZEB2 expression is controlled in a timely manner, are two outstanding questions. Here, we combined novel organoid DamID methodologies and chromatin conformation capture to investigate the role of ZEB2 in the neuroepithelial to radial glial transition. Our aim is to construct a comprehensive network of transcriptional and epigenetic regulation that provides broad insights into the mechanisms underlying the regulation of brain size.

Keywords: Neurodevelopment, Epigenetics, DamID

TOPIC: TISSUE MECHANICS

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DEVELOPMENT OF A 3D KIDNEY-ON-A-CHIP MODEL USING IPSC-DERIVED PROXIMAL TUBULE CELLS

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The development of physiologically relevant kidney models is of high interest for pharmaceutical companies as drug-induced nephrotoxicity accounts for a high percentage of drug development failures in pre-clinical and clinical stages. Mimicking the embryonic development of proximal tubule (PT) cells in vitro has the potential to generate cells, which show high expression of transporter proteins. These transporter proteins are responsible for xenobiotic clearance and make this type of cell especially susceptible to drug-induced cell damage. Further, the culture device might improve the physiological relevance. In contrast to standard 2D cell culture, 3D organ-on-a-chip models allow tubular architecture, physiological fluid flow and interaction with extracellular matrix (ECM), other tissue-specific cell types and vasculature. This mimics the native microenvironment and may improve the clinical translation of knowledge about new drugs. In this study we differentiate human induced pluripotent stem cells (iPSC) into proximal tubular-like cells (PTL) within 14 days1. These cells are characterized in 2D, as well as in static transwells, and in 3D tubes under flow using the OrganoPlate® system from Mimetas. To optimize the 3D culture conditions, several seeding densities, maturation times, and ECMs or chip coatings are evaluated in the 3D system. PT-specific protein expression and cell polarization is validated by immunohistochemistry and high content imaging. Cell maturation and barrier integrity is analyzed with transepithelial electrical resistance (TEER) and fluorescent barrier permeability assays. Additionally, functional screening assays with these cells show active transport e.g. mediated by P-glycoprotein (ABCB1), Megalin (LRP2), and other organic anion and cation transporters. To allow the comparison with similar models in the field, all results of iPSC-derived PTL are compared to a standard human proximal tubular cell line, which is commonly used for 3D models and toxicity assessments. As a further advancement of this model system, the co-culture of PTL with primary or iPSC-derived endothelial cells is under examination in all formats.

Keywords: iPSC, kidney, MPS

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DISTINCT DYNAMICS OF MUSCLE STEM CELL SUBTYPES ASSOCIATED WITH THE HYPERPLASIA DECLINE IN TROUT

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Post-larval growth in salmonids is originally characterized by intense muscle hyperplasia. This process results from the specific proliferation and differentiation of muscle stem cells, also known as satellite cells (SCs). While the evidence for the existence of a diversity of SC subtypes are clear in mammals, similar data on fish are still lacking. We aimed to elucidate the cellular and molecular dynamics of SCs during the decline of muscle hyperplasia in trout. First, we examined the kinetic of hyperplasia decline by evaluating the fiber size distribution in the white muscles of trout weighing from 10 g to 2 kg. Our analysis revealed a continuous increase in mean diameter of fibers associated with a decrease of small fibers (< 30 µm) strictly observed between 10 g and 1 kg. This suggests that hyperplasia in muscle decreases very early and ceases around 500 g - 1 kg. We then determined the evolution of SCs numbers in relation to this decline. In situ quantification of pax7+ cells indicated a weight-related decrease, reaching a minimum plateau at 500 g. Finally, we studied the SCs heterogeneity and its evolution during hyperplasia decline, using a scRNAseq method on whole mononucleated cells extracted from trout muscle. Sixteen cell clusters were identified. Eight subtypes belonging to myogenic lineage were observed from quiescent SCs to myocytes. While a lower proportion of the myogenic precursors most involved in the differentiation program is observed in the absence of hyperplastic growth, we interestingly showed that the proportion of the most primitive cells increases with weight gain. These shifts directly impact on SC heterogeneity and genetic networks, yielding specific transcriptomic signatures within myogenic cell subtypes. Additionally, we distinguished mesenchymal cells (including fibroblasts and fibro-adipogenic progenitors) which display shifts in proportions and molecular signatures with muscle growth, suggesting a potential involvement of the SC niche. Our results show the loss of generation of new committed myogenic precursors associated with the hyperplasia arrest, although the most primitive SCs are preserved, suggesting an orientation of the latter towards symmetrical division modalities. This study provides key insights into the fundamental biology of muscle growth in fish.

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Keywords: Muscle Stem Cells, Single Cell RNAseq, Fish muscle growth

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MORPHOLOGICAL COMPARISON OF MOUSE AND HUMAN PRE-IMPLANTATION EMBRYOS

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Preimplantation development is a self-organising process, that is largely conserved between humans and mice. In both species, the blastocyst-stage embryo consists of a liquid filled cyst formed by extraembryonic Trophectoderm (TE) cells, comprising an inner cell mass (ICM) that subsequently forms the Epiblast (EPI) and the extraembryonic Primitive Endoderm (PrE). Despite an overall morphological similarity, here we show that the inner cells of the mouse embryo are more spread out while in human the inner compartment is rounder and it shares a smaller interface with the TE. This species-specific difference is also recapitulated by the human blastoid, a stem-cell based model of the human blastocyst. Here, we aimed to dissect the underlying mechanisms that are responsible for the species-specific morphology and their potential consequences in embryogenesis. While the mouse embryo has more inner cells and is significantly smaller than the human, our data suggests that the species-specific shapes are neither affected by the number of cells or the size of the blastocoel cavity. Furthermore, morphological analysis showed that the second event of lineage segregation, separating the EPI from the PrE, does not reshape the inner compartment. Looking for mechanisms leading to species-specific shapes, we have identified differences in the expression patterns of force transmitting proteins, which can potentially translate into distinct levels of membrane tension. To understand whether such differences can affect the tissues geometries, we are currently studying membrane tension using the mechanosensitive probe FLIPPER-TR, together with Fluorescence lifetime imaging microscopy. Species-specific mechanisms driving different shapes and geometries of this inner tissue and of its interaction with the TE might contribute to a divergence exemplified, a few days later, by a mouse cup- or human disc-shape morphology of the embryo.

Keywords: Species comparison Tissue mechanics, Tissue mechanics, Fluorescence lifetime imaging

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EXOSOMES DERIVED FROM HUMAN DENTAL PULP STEM CELLS ARE EFFECTIVE BIOMATERIALS FOR ANTI-INFLAMMATION AND ODONTOGENESIS

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Human dental pulp stem cells (hDPSCs) have received special attention in tissue engineering strategies for its certain advantages, including that they present less ethical problems, low immunogenicity, and high plasticity than other mesenchymal stem cells. Exosomes are nano-sized vesicles containing genetic information of cells. Although much evidence has shown the potential of exosomes for use in regenerative medicine, the role of hDPSC-derived exosomes (hDPSC-Exos) has rarely been reported. We tried to evaluate the therapeutic potential of hDPSC-Exos for anti-inflammation and odontogenic differentiation in LPS treated hDPCs and rat model of LPS-induced pulpitis. hDPSCs were obtained from healthy supernumerary teeth. Exosomes were isolated by Exoquick-TC reagent following the manufacturer's procedures. The effect of hDPSC-Exos on anti-inflammation and the mineralization of hDPCs were investigated by real-time PCR, RNA-seq, Western Blot, ALP staining and Alkaline phosphatase assay. For evaluation of effects of hDPSC-Exos in LPS-induced pulpitis model of rat, a gelatin film with exosomes absorbed was placed in the pulp cavity after inducing pulpitis by LPS. After one month, HE staining was performed with paraffin block tissue obtained from dental samples of rats. We characterized exosomes derived from hDPSCs. It showed that hDPSC-Exos induced decreased inflammation-related markers and increased expression of odontogenesis-related genes in inflammatory hDPCs. In Gene Set Enrichment Analysis (GSEA), it was found that TNF-α signaling via NF-κB and hypoxia-related pathway were down-regulated and angiogenesis-related pathway was up-regulated in the exosome treated hDPCs. In the LPS-induced pulpitis model of rats, hDPSC-Exos-treated group showed significantly less inflammatory cell infiltration into the pulp tissue and increased odontogenesis compared to the control group. Taken together, these results showed that hDPSC-Exos have anti-inflammatory and regenerative therapeutic effects in LPS-induced pulpitis. hDPSC-Exos could be utilized as a cell free therapeutic tool for various diseases.

Keywords: Exosomes, human dental pulp stem cells, pulpitis

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THE ROLE OF TOLL-LIKE RECEPTOR 5 SIGNALING ON NEUROGENESIS IN MOUSE EMBRYONIC STEM CELLS AND ADULT HIPPOCAMPAL NEURAL STEM CELLS

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Toll-like receptors (TLRs) make a crucial contribution to the innate immune response. TLR5 was expressed in SOX2- or DCX-positive cells in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) where adult neurogenesis occurs. TLR5 inhibited the proliferation of adult hippocampal neural stem cells (NSCs) by regulating the cell cycle and facilitated the neural differentiation from the adult hippocampal NSCs via JNK pathway. Also, TLR5 deficiency impaired fear memory performance in mice. Our data suggest that TLR5 is a crucial modulator of neurogenesis from adult hippocampal NSCs in mice and represents a new therapeutic target in neurological disorders related to cognitive function.

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Keywords: neural stem cell, neurogenesus, TLR5

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TOPIC: TISSUE SELF-ORGANIZATION

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A MULTI-LINEAGE EMBRYONIC MOUSE LIVER CULTURE RECAPITULATES TISSUE DEVELOPMENT AND FUNCTION

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A major challenge in modern biology is to establish in vitro systems where multiple cell types can recapitulate the multi-cellular structural complexity of tissue architecture in vivo. Traditionally, in vitro culture systems that support a high cell diversity do it at the expense of tissue organization. Here, we developed a system based on systematic high-content screening of developmental stages, extracellular matrix and culture conditions of dissociated liver cells to leverage their self-organization potential and reproduce features of the tissue architecture in vitro. We termed this system Screenable in vitro Embryonic Liver For tissue Organization studies (SELF-O). We benchmarked SELF-O to the tissue by combining quantitative fixed and live-cell imaging with single-cell RNA sequencing analyses. SELF-O recapitulates the development of over twenty liver cell populations, self-organizing reproducible ordered structures comprising hepatocyte cords that drain bile into functionally connected bile ducts. Hepatocyte cords interact with the sinusoidal network through hepatic stellate cells, liver pericytes associated with resident liver macrophages called Kupffer cells. Remarkably, this technology supports liver functions, including haematopoiesis, metabolism, and bile flow, enabling the systematic interrogation of liver development in health and diseases.

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Keywords: Self-organization, Liver, Development

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CRYPTOCHROME 2 REPRESENTS A CRITICAL COMPONENT IN HUMAN RETINAL ORGANOID DIFFERENTIATION AND FUNCTION

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Cryptochrome 2 (CRY2) is a highly conserved protein found in a wide range of organisms, from plants to animals, including humans. It plays a crucial role as a blue light-sensitive photoreceptor in the regulation of circadian rhythms, providing organisms with the ability to perceive and respond to changes in light-dark cycles. Additionally, mutations in the CRY2 gene have been associated with sleep disorders and mood disturbances in humans, emphasizing its clinical relevance. In recent years, CRY2 has emerged as a multifunctional protein with roles beyond circadian clock regulation, including development and differentiation. However, little is known about the function of CRY2 in the human retina which represents a key component for the synchronization of the circadian rhythms in the human body. Here we used the human retinal organoid model to investigate the role of CRY2 in the human retina closely. We show that CRY2 is predominantly localized to ganglion and photoreceptor cell layers. Using the CRISPR/Cas9 approach we generated CRY2 knock-out hiPSCs and assessed the effect of CRY2 deficiency on retinal organoid differentiation and the cell composition. Our data sets indicate that CRY2 deficiency leads to impaired differentiation leading to low numbers of correctly structuralized organoids or organoids with altered cell composition. RNA sequencing revealed alterations in FGF signaling in CRY2-deficient hiPSCs, potentially responsible for impaired differentiation of hiPSCs and downregulated retinal-specific genes in CRY2-deficient embryoid bodies, thus suggesting a novel link between circadian regulation and FGF signaling. These findings expand our understanding of CRY2's significance in the human retina and the differentiation of hiPSCs. The observed alterations in retinal-specific genes and FGF signaling in CRY2-deficient cells highlight the association between circadian regulation and broader developmental processes.

Keywords: Cryptochrome 2, human retinal organoid, FGF signaling

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EPICARDIOIDS: A NOVEL TOOL TO STUDY HUMAN HEART DEVELOPMENT AND DISEASE

Zengerle, Sophie[1], Ellinger, Tobias[2], Monge Mora, Luis Felipe[2], Meier, Anna[2], Zawada, Dorota[2], Goedel, Alexander[2], Laugwitz, Karl-Ludwig[2], Dorn, Tatjana[2], Moretti, Alessandra[2]

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Our knowledge of congenital heart diseases remains limited due to the lack of suitable models. As the heart forms within the first weeks of embryonic development, human native tissue samples are largely inaccessible. Moreover, as cardiac development varies significantly between species, better models to study the complexities of human heart development are required. Here, we generated hiPSC-derived heart organoids showing self-organization of the epicardium and myocardium, which we call 'epicardioids'. The epicardial layer gives rise to epicardial-derived cell lineages (e.g., fibroblasts and smooth muscle cells) via epithelial-to-mesenchymal transition and promotes the formation of compact and trabecular-like myocardial layers that display molecular and functional patterning typical of the ventricular wall. Time course single-cell RNA sequencing revealed that epicardioids are formed through the specification of first heart field progenitors, a subset of which correspond to HAND1+ MAB21L2+ juxta-cardiac field cells that have recently been described in mouse development as multipotent progenitors of the myocardium and epicardium. Epicardioids are well suited to study the cell-cell interactions of various cell populations in a 3D environment. In a proof-of-concept study, the utility of epicardioids for disease modeling was illustrated by the hypertrophic and fibrotic response to endothelin-1 (ET1) treatment (stress-induced acquired hypertrophy) and by recapitulating features of the congenital cardiomyopathy associated with Noonan Syndrome using patient-iPSCs. In Noonan epicardioids, we observed hyperproliferation of cardiomyocytes and no increase in cardiomyocyte size or expression of hypertrophic genes seen in the ET1-disease model. Notably, we also detected early formation of fibrotic clusters within the myocardium layer, which were characterized by increased fibrotic gene expression and secretion of fibrotic proteins. This suggests that fibroblasts may play an important role in Noonan disease on-set and progression. Our epicardioids provide a powerful platform for investigating the role of various cell populations in heart development and disease. Also they can be used as a suitable human model for the development of potential regenerative therapies and drug discovery.

Keywords: Epicardioid, Disease modelling, cardiac development

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GENERATING HUMAN BLASTOIDS FROM SINGLE NAÏVE PSCS IN 3D BIOREACTOR CULTURE

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Blastoid is a useful model of the blastocyst derived from naïve pluripotent stem cells to study early human development. Human blastocyst is formed about five days after fertilization with a fluid-filled cavity and contains different lineages including the epiblast, trophectoderm, and primitive endoderm. Current methods can make blastoids within 4 days starting with cell aggregates, while natural embryos develop from a single cell. Here, we use a stirred tank bioreactor to form blastoids in seven days starting from single cells, which provides a convenient strategy for large-scale production of blastoids. More importantly, the bioreactor method allows strict environment control in the process. The bioreactor-blastoids have a diameter of about 150um-300um and comprise around 200 cells, which are comparable to the human blastocyst. We compared our bioreactor blastoids with static culture blastoids and human blastocyst using scRNA-seq. We also showed that our bioreactor blastoid platform could be useful for drug screens. Thus, we propose that the bioreactor-blastoid can be used to study human implantation and early development in a faithful and ethical manner.

Keywords: blastoid, human early development, naive stem cell

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LOSS-OF-FUNCTION MUTATION OF KIF3B CAN CAUSE A DEFECTIVE BILIARY DEVELOPMENT IN BILIARY ATRESIA: EVIDENCE FROM IPSC-DERIVED BILIARY ORGANOID

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Biliary Atresia (BA) is a poorly understood devastating fibro-obliterative biliary disease of newborns. Limited access to primary biliary tissue, difficulties in culturing primary biliary cells (cholangiocytes) and inadequate animal disease model have led to a slow advancement in unravelling the patho-mechanisms, diagnosis and treatment for BA. Human iPSC-derived biliary organoids provide us an unprecedented cellular model to study BA. We have conducted whole exome sequencing on 85 BA

trios, identified deleterious loss of function (LOF) mutations in cilia-related genes including KIF3B in 31.5% non-syndromic BA patients. KIF3B encodes Kinesin-like protein KIF3B that is a subunit of the anterograde intraflagellar transport (IFT) motor protein kinesin-II in cholangiocyte cilia. Functional analyses demonstrated absence of cilia in the BA livers with KIF3B mutation and knockdown of KIF3B in human fibroblasts resulted in reduced number of cilia. Additionally, CRISPR/Cas9-engineered zebrafish knockouts of KIF3B displayed reduced biliary flow. In this study, we generated KIF3B+/- & KIF3B-/- human iPSC cells and differentiated them into biliary organoids to investigate the impacts of the KIF3B LOF mutation in biliary development in BA. Single-cell-RNA-seq analysis and immuno-staining showed that KIF3B+/- and KIF3B-/- iPSCs are less capable in the differentiation of hepatoblast and cholangiocyte progenitors (CPs). Individual cell AUC revealed down-regulation of Wnt, Notch and TGF-beta pathway activity, while cell-cell interaction analysis showed a defective cell-cell interaction mediated by TGAV and ITGB8 (integrin αvβ8) in the KIF3B+/- and KIF3B- /- CPs. Furthermore, KIF3B+/- & KIF3B-/- biliary organoids were few, tiny and with abnormal or no cilia. Bulk-RNA-seq and immunostaining analysis of biliary organoids revealed a shift from cholangiocyte to hepatocyte differentiation in KIF3B+/- & KIF3B- /- biliary organoids. Taken together, our data indicate that KIF3B plays a key role in cholangiocyte differentiation, which demonstrates that the human iPSC-derived biliary organoid is a valuable disease model for patho-mechanistic study of BA.

Funding Source: The study is supported by the Theme-Based Research Scheme (T12-712/21-R) and HMRF 09201836. Research Grant Council. Hone Kong Special Administrative Region Government.
Keywords: Biliary Atresia, KIF3B, iPSC-derived biliary organoids

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DECIPHERING MICROENVIRONMENT CUES TO MAINTAIN HUMAN NOTOCHORDAL CELLS PHENOTYPE AND TO DRIVE THEIR MATURATION TOWARD NUCLEUS PULPOSUS CELLS

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Low back pain prevalence is increasing with population aging, but so far, no treatment is available. The central part of healthy adult intervertebral discs, the nucleus pulposus, is composed of nucleopulpocytes (NPC) embedded in a highly hydrated matrix. Genetic studies in mice showed that NPC derive from the embryonic notochord. Notochordal cells (NC) have been shown to maintain disc homeostasis by secreting factors supporting matrix synthesis and NPC proliferation. Human iPSC-derived notochordal cells (NLC) have been proposed as cell source for regenerative medicine, but they often lack maturation to exert their expected rejuvenating effects. Essential cues driving the transition from immature NC to mature vacuolated cells, and to terminally differentiated NPC remain to be elucidated. HiPSC are differentiated into mesendoderm progenitors (MEPC) upon WNT activation and then commitment toward NLC is triggered by NOTO mRNA transfection. First, we investigated maturation in 3D aggregates in suspension or in encapsulation of single cells in hyaluronic acid-based hydrogel. In parallel, we studied terminal differentiation in aggregates in a medium supplemented with the growth factors TGF-β1 and GDF-5. Our results showed that MEPC aggregation in micromass, in NLC differentiation media, triggers a higher level of expression of the NC markers TBXT and NOTO, but is not sufficient to induce maturation. We identified that covalent hydrogel grafted with RGD peptide (fibronectin-derived) performs better than viscoelastic hydrogel to maintain NLC phenotype, and the formation of vacuole-like structures indicated the beginning of maturation process. We showed that media supplementation with TGF-β1 and GDF-5 promoted further differentiation of MEPC micromass and the secretion of a glycosaminoglycan rich matrix, suggesting a shift toward NPC phenotype. The lastest constructs indicated the coexistence of both cell types, NLC and NPC, suggesting support for NC maturation and terminal differentiation. Our work identified critical cues to preserve NLC phenotype, to guide their maturation toward a secretory phenotype and their differentiation to NPC. These cues will be helpful to propose a maturation model of NC to be used for studying diseased disc tissue and to facilitate its regeneration.

Funding Source: Financial support from EU Horizon 2020, “iPSpine” and the French Society of Rheumatology, “Spherodisc”.
Keywords: 3D culture, Hyaluronic acid-based hydrogels, Notochordal cells

Poster Abstracts

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NEXT-GENERATION ELECTROPHYSIOLOGY FOR FUNCTIONAL CHARACTERIZATION OF HUMAN NEURAL ORGANOIDS

D'Ignazio, Laura, Gong, Wei, Guella, Elvira, De Gennaro, Martina, Li, Zhuoliang, Obien, Marie Engelene

MaxWell Biosystems, Zurich, Switzerland

The human brain's complexity presents challenges for direct optical observation and experimental manipulation. However, recent progress has made human induced pluripotent stem cell (hiPSC)-derived brain models indispensable tools in studying neurological disorders like epilepsy, Alzheimer's, and Parkinson's diseases. The ability to measure the electrical activity of a self-organizing in vitro cellular model in real-time, live and label-free, offers invaluable insights into the intricacies of its neuronal network. High-density microelectrode arrays (HD-MEAs) provide an unprecedented means of conducting non-invasive in vitro electrophysiological recordings. These arrays can be employed to gather data from various electrogenic samples, including iPSC-derived neurons, retina explants, brain slices, and neural organoids. Our study utilized MaxWell Biosystems' HD-MEA platform, featuring 26,400 electrodes per well, to capture extracellular action potentials in neural organoids at different scales, ranging from cell population networks to single-cell resolution and even subcellular levels, with high precision and minimal noise. This system allows for flexible electrode selection for recording and stimulation, enhancing data reproducibility and statistical power. We extracted critical metrics including firing rates, spike amplitudes, and network burst profiles in a parallelized manner, even detecting subtle neuronal signals. Furthermore, we conducted a comprehensive characterization of the axonal function and structure of hiPSC-derived neural organoids using the AxonTracking Assay. This tool automates the recording and analysis of action potential conduction along individual axonal arbors of multiple neurons simultaneously, enabling the measurement of action potential conduction velocity, latency, axonal length, and the number of axonal branches. MaxWell Biosystems' HD-MEA platforms, in combination with automatically generated plots and extracted metrics, offer a user-friendly and powerful approach for identifying and isolating functionally active areas within a 3D culture. This is applicable in both acute recordings and longitudinal studies, making it possible to conduct long-term in vitro disease modeling and compound testing with precision and efficiency.

Funding Source: This work is funded by the NEUREKA project, GA 863245, within the H2020 Framework Program of the European Commission. This work is funded by the HyVIS project, GA 964468, within the H2020 Framework Program of the European Commission.
Keywords: Electrophysiology, HD-MEA, Neural Organoids

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PROTEIN KINASE A AS A POTENTIAL PLAYER IN THE NEPHRON-TUBULE BALANCE IN IPSCS-DERIVED KIDNEY ORGANOIDS

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Chronic kidney disease affects 11–13% of the global population. The shortage of donors for kidney replacement therapy increases the pressure to find alternative solutions. One regenerative medicine alternative comprises the use of induced pluripotent stem cell (iPSC)–derived kidney organoids as a therapeutic engraftment to the dysfunctional kidney. However, several drawbacks need to be overcome before clinical translation, including the lack of maturation and the presence of non-renal cell populations such as cartilage. Protein kinase A (PKA) is known to play an important role in renal function. The aim of this work was to assess the impact of PKA activation or inhibition on the development of kidney organoids. We modified the culture protocol, first described by Takasato et al., by adding an activator (DbcAMP) or inhibitor (PKI) of PKA, alone or in combination with FGF9, which we previously found to reduce the amount of cartilage. We harvested the organoids at day 7+25 when their development reaches a plateau in the original protocol and evaluated the renal structures by immunostaining. We observed that activation of PKA with DbcAMP increased the number of nephrons (NPHS1) in the organoids, but induced the appearance of adipocyte-like structures. By comparison, the inactivation of PKA with PKI increased the number of tubules (LTL) but induced the appearance of cartilage. The concomitant treatment of kidney organoids with both FGF9 and DbcAMP led to a higher number of nephrons compared to the control along with their engulfment in the proximal tubules, with more nephrons than tubules. To improve the nephron–tubule balance in the organoids, we cultured them first in PKI and then in DbcAMP. With this sequential treatment, we observed a similar number of tubules and nephrons in the kidney organoids with engulfment of the nephrons in the proximal tubules and no off-target cartilage. These results demonstrate improved maturation and organization of kidney organoids cultured at the air–liquid interface and showed a potential role for PKA in the development of renal structures. This protocol could help produce a higher-quality kidney organoid that can be maintained longer in culture for further in vitro and in vivo work.

Funding Source: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101065328
Keywords: Nephron-tubule, PKA, Self-organization

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REVASCULARIZATION OF DECELLULARIZED MOUSE KIDNEYS USING HUMAN STEM CELLS TO STUDY RENAL VASCULAR DEVELOPMENT

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Chronic kidney disease affects over 950 million people globally, however treatment options remain limited. Consequently, there is a high demand for bioartificial kidneys which can be used to gain new insight into kidney development. The extracellular matrix (ECM) is critical in organogenesis, guiding spatial organization and the timing of differentiation. We have previously demonstrated that the same biological pathways that direct differentiation during organogenesis are maintained in the adult ECM and can direct the differentiation of cells during organ regeneration. We aim to take advantage of this by using adult acellular kidney ECM as a substrate, along with patient iPSCs to ultimately produce functioning kidneys. Using acellular mouse kidneys along with hiPSCs allows us to make mini humanized kidneys for drug and cell therapy development. My project assesses the role of a decellularized mouse kidney scaffold in providing human stem cells with critical site-specific cues for adherence, differentiation, and growth of the renal vasculature. Mouse kidneys are decellularized by perfusion with sodium dodecyl sulfate (SDS). Our data demonstrates that a low SDS concentration and flow rate achieve effective cell clearance while maintaining the ECM microarchitecture and glycosaminoglycan (GAG) levels. GAGs sequester site specific growth factors which guide cell differentiation, thus their retention post decellularization is crucial. For revascularization, human mesoderm cells are perfused into the scaffold via the renal artery. Previous work in our lab has found mesoderm cells derived from stem cells to be a good starting point for our purposes. The recellularized kidney is cultured in a bioreactor previously developed in the Rogers Lab. The recellularized tissue is analyzed to determine tissue health, proliferation, and maturation. Single cell RNA sequencing will be conducted to identify the endothelial populations present within the scaffold. This work will improve our understanding of the role of the ECM in tissue development. In the future this system can be adapted to study specific cell-cell interactions during development for example by recellularizing with the addition of podocytes to elucidate the endothelial-podocyte cross talk.

Funding Source: New Frontiers in Research Fund Canada Graduate Scholarship-Master's, Canadian Institutes of Health Research
Keywords: Kidney engineering, Decellularization, Recellularization

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THE IMPROVEMENT OF PARACRINE ACTIVITY OF MULTIPOTENT MESENCHYMAL STROMAL CELLS IN 3D CULTURE CONDITIONS

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Multipotent mesenchymal stromal cells (MSCs) are extensively used in cell therapy and regenerative medicine. MSCs can promote the repair and regeneration of injured tissue due to paracrine activity. However, the large-scale expansion of MSCs in vitro in a highly oxygenated 2D environment affects cell metabolic status, proliferation, differentiation, growth factor/cytokine secretion, and induces cell senescence. The microenvironment plays a significant role in the fate and behaviour of MSCs, which may subsequently influence their repair potential. Returning the MSCs into 3D culture conditions that mimic the natural microenvironment can restore cellular functions and improve their efficacy in therapeutic applications. Nevertheless, no comparative research has been reported to determine the optimal 3D culture conditions for enhancing the paracrine activity of MSCs. In this study, we evaluated the metabolic profile of human adipose tissue-derived MSC spheroids as well as compared the cell distribution, viability, and production of selected growth factors by MSCs cultured in different 3D culture conditions: a) ECM-free spheroid cultures, b) hydrogel cultures supported by ECM (collagen, plasma-based hydrogel, GeltrexTM), c) porous GeltrexTM - based scaffolds. Standard 2D monolayer culture conditions served as a control. We found that in most cases 3D culture conditions significantly increased the growth factor secretion by MSCs. The amount of produced growth factors varied in different 3D culture microenvironments, showing variations depending on the presence of extracellular matrix, its origin, or the structural composition of the 3D constructs. The most prominent growth factor secretion was detected in ECM-free 3D MSC spheroid cultures, which had the densest structural composition and significant changes in metabolic and metabolomic profile compared to monolayer cultures. Therefore, we believe in depth studying of the effects of microenvironment on the paracrine activity of cells may be the key to achieving successful and effective MSC-based therapies. All human tissue donors provided their written informed consent before any intervention. All studies involving human tissues or cells were approved by the Ethics Committee of the Institute of Experimental Medicine of the CAS, Prague.

Funding Source: The study was supported by Czech Science Foundation grant GAČR 22-31457S, and Charles University Grant Agency grant GAUK 390722.
Keywords: The multipotent mesenchymal stromal cells, 3D microenvironment, Paracrine activity

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UNCOVERING MECHANISMS OF ROSTROCAUDAL PATTERNING OF HUMAN SOMITES

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Recent studies have revealed multiple instances of developmental mechanisms that are different between humans and model organisms. We computationally identified genes involved in human somitogenesis that are not present in mice, suggesting human-specific changes in the segmentation program. However, the inaccessibility of human embryos to measure the dynamics of signaling, differentiation, and cell movements, and the challenge of performing penetrant genetic manipulations at scale has impeded a mechanistic understanding of human somitogenesis. Here we overcome these challenges by employing a robust organoid system that accurately recapitulates essential features of somitogenesis, including axial elongation, traveling segmentation clock waves, and the periodic and sequential segmentation of rostrocaudally compartmentalized somites. The rostral and caudal halves of each somite have cell types with distinct transcriptional signatures. Through analyzing single-cell RNA sequencing data, we identify two novel precursor populations responsible for generating caudal and rostral somite cells. Furthermore, leveraging endogenous fluorescence tagging, time-lapse imaging, and single-cell tracking, we determined that these early caudal and rostral cells are specified prior to spatial segregation within the prospective somite. Using an inducible CRISPRi system, we conduct perturbations targeting 46 differentially expressed genes encompassing transcriptional regulators, cell adhesion molecules, and signaling components within the prospective rostral and caudal somite cells. Our organoid model combined with comprehensive transcriptomic analysis, single-cell tracking, and high-throughput genetic perturbations unveils critical signaling mechanisms and transcriptional machinery governing somite fate specification and key molecules orchestrating the anteroposterior compartmentalization of human somitogenesis.

Funding Source: This work was supported by NIH (SR).
Keywords: Human Organoid, Somitogenesis, Differentiation Dynamics

MONDAY, 4 DECEMBER

All times in Central European Time (Vienna, Austria)

Poster Session II
4:45 PM – 5:30 PM

TOPIC: GENETIC PROGRAMS

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A MITOTIC PERSPECTIVE ON A GENETIC GENERALIZED EPILEPSY SYNDROME

Anand, Anuranjan[1], Sinha, Sanjib[2], Joshi, Shrilaxmi[1], Raju, Praveen[1], Chinthapatla, Sri Charani[1], Iyer, Vishwanathan[2], Choudhury, Debopriya[3]

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While subtle structural brain abnormalities have been observed in individuals with certain generalized epilepsies, cellular and developmental genetic aspects of these apparently weakly penetrant phenotypes are poorly elucidated. Through our studies on EFHC2 (EF-Hand Domain Containing 2), an X-linked gene associated with a range of brain-related phenotypes, among them, epilepsy, autism, and fear recognition in individuals with Turner's syndrome, we are examining the roles of EFHC2 behind brain development employing mammalian cultured cells, genetically engineered mice, and human embryonic stem cells. EFHC2 is related to EFHC1, a known epilepsy gene. It is predicted to be intolerant to complete loss of function among humans and escape X-inactivation. It is synthesized in the hippocampus, hypothalamus, cerebellum, and cortical regions in human and mouse brains. In BJNhem20, a human embryonic stem cell line, EFHC2 localizes to the nucleus and is expressed during trilineage differentiation indicating its broader biological functions. It is a microtubule interacting protein that localizes to centrosomes, spindle poles, and midbody. In mammalian cultured cells expressing EFHC2 mutations, significantly enhanced cell division defects consisting of monopolar spindles, multipolar spindles, and misaligned chromosomes are observed. Interestingly EFHC1 rescues these defects due to EFHC2 variants in cultured cells. Likewise, defects due to EFHC1 mutations are rescued by EFHC2, suggesting these two genes play redundant cell biological roles - an observation with implications for the

clinical manifestation of the disorders. Our future work plans to harness the potential of human embryonic stem cells to address questions regarding EFHC2's role in early embryonic and brain development and the disorders.

Funding Source: ICMR, New Delhi (BMS/Trans-Neuro/2014-3681); SERB, New Delhi (JCB/2020/000026); JNCASR, Bangalore (Intramural funds).
Keywords: Epilepsy, Microtubule-associated protein, Cell division

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DECODING REGULATORY CODES OF TROPHECTODERM SPECIFICATION AND IMPLANTATION

Pradhan, Saurabh Jagdish, Khoei, Heider, Slovakova, Jana, Novatchkova, Maria, Seong, Jinwoo, Scholte Op Reimer, Yvonne, Kagawa, Harunobu, Brennecke, Julius, Rivron, Nicolas

IMBA GMBH, Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter (VBC), Vienna, Austria

Implantation of the mammalian embryo into the uterus is a crucial process that establishes a connection between the competent blastocyst and maternal tissues necessary for nutrient exchange and waste removal during gestation. During implantation, the blastocyst first interacts with the uterine tissue and then later invades it. While the basic scheme of implantation is similar across mammalian species, variations in the mechanisms have evolved. Mouse embryos, for example, attach to the endometrium (the uterus lining) by the mural trophectoderm cells, the ones located away from the inner cell mass, whereas human blastocysts interact via the opposite pole, the polar trophectoderm. How these subpopulations of trophectoderm cells gained differential abilities in different species remains largely elusive. To delineate the underlying mechanisms encoded in mammalian genomes, we constructed trophectoderm-specific gene regulatory networks for both species. We performed single-cell multiome (scATAC+scRNA) analysis using a recently introduced human blastoids and a mouse Trophectoderm Stem Cells differentiation system. Our comparative analysis identified a number of transcription factors that may act in tandem. We hypothesise that evolution of genomic traits is central to controlling these key regulatory modules in a spatiotemporal manner. These differences in cell fate specification and mode of implantation highlight the importance of evolutionary adaptations that have allowed different mammals to successfully reproduce and thrive in their respective environments.

Keywords: Human Preimplantaion, Gene regulatory networks, Trophectoderm specification

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DEEP DYNAMICAL MODELLING OF DEVELOPMENTAL TRAJECTORIES WITH TEMPORAL TRANSCRIPTOMICS

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Developmental cell fate decisions are dynamic processes driven by the complex behaviour of gene regulatory networks. A challenge in studying these processes using single-cell genomics is that the data provides only a static snapshot with no detail of dynamics. Metabolic labelling and splicing can provide time-resolved information, but current methods have limitations. Here, we present experimental and computational methods that overcome these limitations to allow dynamical modelling of gene expression from single-cell data. We developed sci-FATE2, an optimised metabolic labelling method that substantially increases data quality, and profiled approximately 45,000 embryonic stem cells differentiating into multiple neural tube identities. To recover dynamics, we developed velvet, a deep learning framework that extends beyond instantaneous velocity estimation by modelling gene expression dynamics through a neural stochastic differential equation system within a variational autoencoder. Velvet outperforms current velocity tools across quantitative benchmarks, and predicts trajectory distributions that accurately recapitulate underlying dataset distributions while conserving known biology. Velvet trajectory distributions capture dynamical aspects such as decision boundaries between alternative fates and correlative gene regulatory structure. Using velvet to provide a dynamical description of in vitro neural patterning, we highlight a process of sequential decision making and fate-specific patterns of developmental signalling. Together, these experimental and computational methods recast single-cell analyses from descriptions of observed data distributions to models of the dynamics that generated them, providing a new framework for investigating developmental gene regulation and cell fate decisions.

Funding Source: This work was supported by core funding from Cancer Research UK (CC001051), UK MRC (CC001051), the Wellcome Trust (CC001051); EU Horizon 2020 research and innovation program grant 742138 and by the Wellcome Trust (220379/D/20/Z).
Keywords: single-cell transcriptomics, dynamical modelling, gene regulatory networks

Poster Abstracts

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ESTABLISHMENT OF MOUSE NEURAL STEM CELL IDENTITY, GLIOGENIC COMPETENCE, AND LINEAGE-SPECIFIC ENHANCER ACTIVATION REQUIRES TET-MEDIATED DNA DEMETHYLATION

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DNA methylation is extensively reconfigured during mammalian development, but the functional significance of DNA demethylation in lineage commitment remains poorly understood. Here we find that TET DNA dioxygenases, enzymes whose catalytic activities promote DNA demethylation, are essential for establishment of neural stem cell (NSC) identity and gliogenic potential. Using Tet1/2/3 triple knockout (TKO) mouse embryonic stem cells (ESC) in an ESC-to-NSC differentiation model, we have shown that loss of TET enzymes leads to formation of NSCs that do not properly induce expression of critical neurodevelopmental transcription factors (TF), such as Sox1, Pax6, and Otx1, and that have limited self-renewal. TKO NSCs could efficiently form neurons but were completely unable to differentiate into astrocytes and oligodendrocytes, demonstrating a selective loss of gliogenic competence as opposed to a categorical impairment of neural differentiation. Consistent with the glial differentiation block, the Nfi- and Olig-families of glial TFs were significantly downregulated in TKO NSCs. Mechanistically, we found that hundreds of NSC-specific enhancers in proximity to downregulated neural and glial TF genes were hypermethylated in TKO NSCs. Intriguingly, enhancer hypermethylation was associated with dramatic loss of histone H3K4me1 and H3K27ac marks, suggesting that TET enzymes are critical for proper commissioning and activation of neural enhancers. Our findings establish TET-mediated DNA demethylation of neural enhancers as an essential regulatory modality underlying the developmental establishment of NSC identity and selective acquisition of gliogenic competence.

Funding Source: NIH/NICHD F30HD107921, NIH 5T32GM007288

Keywords: neural stem cells, DNA methylation, TET enzymes

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GAIN OF 1Q RESULTS IN A GROWTH ADVANTAGE DURING HESC RETINAL PIGMENTED EPITHELIUM DIFFERENTIATION WHILE IMPAIRING CORRECT CELL MATURATION

Couvreu de Deckersberg, Edouard[1], Krivec, Nuša[2], Lei, Yingnan[2], Scheyltjens, Isabelle[3], Tsuiko, Olga[4], Regin, Marius[2], Sermon, Karen[2], Spits, Claudia[2]

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A safety concern in the clinical translation of hPSC is their susceptibility to genomic instability, reminiscent to that found in cancerous cells. In this study we investigate genetic mosaicism upon differentiation of hESC to retinal pigmented epithelium cells (RPE), a cell type broadly used in clinical trials. We aimed at establishing which copy number variants arise or enrich during differentiation and how they affect RPE gene expression. We performed scRNAseq of 5 RPE cultures obtained from genetically balanced hESC lines and scDNAseq of 3 of its undifferentiated source lines. The scDNA-seq showed that 2.5% of cells in all 3 hESC lines had complex karyotypes with multiple monosomies and trisomies, and between 5% and 12% of cells carried CNV >10Mb. Each line carried at least one of the CNV recurrently found in hPSC cultures as a low-grade mosaic: VUB02 carried 0.1% of cells with trisomy 12, VUB04 carried 3% of cells with a gain of 1q and 0.4% with an isochromosome 20 and VUB07 carried 2.2% of cells with a gain of 20q11.21. InferCNV analysis of the scRNAseq showed that the RPE cultures of VUB07 and VUB04 carried 2.7% and 42% of cells with a gain of 1q, respectively. The raise from 3% to 42% during the differentiation of VUB04 suggested that this variant confers a growth advantage to the cells during differentiation. The scRNAseq showed, next to the RPE, the cultures contained 2% of cells with an astrocyte-like transcriptomic signature and 0.6% of cells resembling Pigmented ciliary body cells. scGSVA showed that the 1q RPE cells had increased signatures of apoptosis and lower of DNA replication, a shared general characteristic of aneuploid cells. They also showed a decreased VEGF signalling, retinol metabolism and tight junction gene expression, all of which are essential functions of RPE. To confirm the ability of cells with a gain of 1q to take over RPE differentiation cultures, competition assays were performed between 2 additional 1q lines and their genetically balanced counterpart. We found that 1q cells, spiked in at < 1% at the start, represent >15% of the RPE cells at the end of differentiation. Bulk RNAseq of these additional lines confirmed the transcriptomic signatures suggestive of impairment of key RPE functions and incorrect cell maturation.

Funding Source: Fonds Wetenschappelijk Onderzoek (FWO)

Keywords: Copy number variants (CNV), RPE differentiation, Single cell and bulk RNA sequencing

Poster Abstracts

TOPIC: TIMING OF DEVELOPMENT

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EXPLORING THE RARE PROLIFERATIVE CARDIOMYOCYTE SUBSET

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The lack of proliferation in terminally differentiated cardiomyocytes (CMs) projected the heart as a post-mitotic organ. However, the advent of the 21st century met with reports of a small percentage of CMs turnover through adulthood, suggesting that homeostatic renewal of the myocardium relies on pre-existing CMs. This rare population(s) represents phenotypically more immature CMs, but their prospective identification still requires a definitive cell surface signature. We identified the immune checkpoint regulator CD24, a glycoprotein often expressed in tumour cells, undifferentiated cells of the epithelia, and hematopoietic cells, as able to discriminate CM subpopulations, of distinct differentiation stages, coexisting in embryonic life. While CMs expressing CD24 decreased during development to a minor fraction in the adult heart, we detected an increase when the adult myocardium was challenged by ischemic injury. In addition, a higher percentage of the CD24+ CMs associated with actively cycling cells at mid-gestation. This led us to explore further the dynamics of expression of this molecule, also known as “differentiation antigen”, in development, and to set conditions in vitro for mechanistic studies. In situ immunolocalization, in wild type and genetically modified mice, showed a differential spatial and temporal pattern of expression for Cd24a in the developing heart. While CD24+ CMs seem to be prevalent in the ventricular compact zone (where more proliferative CMs were detected) early in development, their frequency showed a sharp decline over time, coincident with coronary artery development. Flow cytometry analysis confirmed a higher frequency of CMs (Troponin+) in cycle (Ki67+) in the CD24+ subset. These results were also corroborated in HL-1 cells, a prototypical CM cell line that we are investigating as a surrogate in vitro system in mechanistic studies. We will also discuss our latest data, including results from ongoing RNA sequencing analysis performed on FACS sorted embryonic day (E)13.5 and E16.5 CMs. A first sketch of relevant transcriptional differences between CD24+ and CD24- CMs is anticipated.

Funding Source: This work is funded by national funds through FCT – Fundação para a Ciência e Tecnologia, I.P. in the scope of the project [2022.09110.PTDC].
Keywords: CD24, Cardiomyocyte renewal, Heart development

TOPIC: GENETIC PROGRAMS

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MAPPING NEURONAL NETWORKS IN HUMAN STEM CELL-DERIVED CEREBRAL ORGANOIDS WITH SINGLE-CELL TRANSCRIPTIONAL RESOLUTION

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The human brain is immensely complex with billions of interconnected neurons that are organized into finely tuned networks. This, in turn, gives rise to precisely coordinated dynamics that are vulnerable to disease as single mutations can disrupt connectivity throughout the brain. To understand neuronal connectivity and determine how networks are disrupted in disease, one first needs a comprehensive map of interconnected neurons. However, developing a connectomic map while simultaneously revealing disease relevant mechanisms is challenging. This is especially true in the human context due to the brain’s complexity and its unique disease pathology. Unfortunately, in vivo models often don’t recapitulate human specific processes and standard methods used to map neuronal networks are either too low-throughput or low-resolution to reveal the biological mechanisms that control their structure or perturbation. To overcome these challenges, we have developed a ‘connectomics-by-sequencing’ approach that utilizes barcoded retrograde rabies viral transsynaptic labeling in combination with single-cell RNA sequencing to map thousands of neuronal networks while simultaneously recovering the transcriptional information from the neurons that comprise them. Here, I demonstrate our progress on applying this technique to human stem cell-derived cerebral organoids which mimic, to a degree, the structure of the human cortex and allow us to investigate disease pathology in a tractable in vitro system. To date we have mapped thousands of networks from individual organoids, revealed the cells that comprise them, and are working to understand the gene expression patterns that define their composition. Additionally, we have begun collaborating with other members of the Knoblich group to determine how neuronal networks are perturbed in Tuberous Sclerosis Complex, an mTORopathy manifesting in 90% of patients as intractable epilepsy. Our goal is to describe the neuronal networks that exist within human cerebral organoids across multiple genetic backgrounds, define the transcriptional principles that determine their connectivity, and reveal how transcriptional dysfunction in disease results in aberrant connectivity and ultimately pathology.

Keywords: Cerebral Organoid, Connectomics, Epilepsy

Poster Abstracts

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MOLECULAR AND PHARMACOLOGICAL INVESTIGATION OF α7 NICOTINIC ACETYLCHOLINE RECEPTORS IN HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED DENTATE GYRUS GRANULE CELLS

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Nicotinic acetylcholine receptors (nAChRs) are present throughout the mammalian CNS and involved in a wide range of presynaptic and postsynaptic neuronal activities by mediating endogenous cholinergic transmission. nAchRs are pentametric structures consisting of six α and three β subunits. The α7 nAchRs are homopentametrical structures containing five α7 subunits. α7 nAchRs are particularly interesting due to their selective central nervous system localization and unique physiological and pharmacological properties. Intensive pharmacological research is focused on this receptor population as a potential target for developing procognitive agents. α7 nAChRs are also present in human induced pluripotent stem cell (hiPSC)-derived dentate gyrus granule cells, an in vitro model of hippocampal neurogenesis. Therefore this model system can be used to study the properties and function of α7 nAChRs in human neurons. We generated dentate gyrus granule cells from induced pluripotent stem cells following the protocol of Yu et al (2014) and validated the obtained cells by MAP2 and Prox1 staining and functional assays. We demonstrate the expression and localization of α7 nAChR on neuronal cells by qPCR and immunofluorescence staining. Ca-imaging was used to trace intracellular Ca transients in cells after treating neuronal cultures with choline (selective agonist), PNU-120596 (allosteric modulator), and methyllaconitine (MLA, α7 nAChR antagonist). We successfully generated MAP2 Prox1 positive dentate gyrus granule cells from induced pluripotent stem cells and demonstrated the presence of α7 nAChR by qPCR and immunostaining. Functional activity of cells were verified using Ca-imaging and showing that cells responded both to choline and PNU-120596 treatment by increasing calcium transients, but this response could be abolished by adding MLA.

Funding Source: This study is funded by the National Brain Research Program (NAP) of Hungary (Grant NAP-B KTIA_NAP_13-2014-0011 to JR and Grant NAP 2017-1.2.1-NKP-2017-00002 to AÁ).
Keywords: human iPSC, hippocampus, alpha7 nAChR

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NUCLEAR RECEPTOR FACTOR MODULATES INTRINSIC DEVELOPMENT PROGRAM CONSERVED ACROSS BLASTOIDS AND BLASTOCYSTS

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Embryonic stem cells possess the remarkable ability to self-organize into blastocyst-like structures upon induction. These synthetic embryo models serve as invaluable platforms for studying embryogenesis and therapeutic developments. Nevertheless, the specific intrinsic regulators that govern this potential for blastoid formation remain unknown. Here we demonstrate a novel intrinsic program that plays a crucial role in both blastoids and blastocysts across multiple species. We first establish metrics for grading the resemblance of blastoids to mouse blastocysts, and identified the differential activation of gene regulons involved in lineage specification among various blastoid grades. Notably, abrogation of nuclear receptor factor (Nrf) drastically reduces blastoid formation. Nrf activation alone is sufficient to rewire conventional ESC into a distinct pluripotency state, enabling them to form blastoids with enhanced implantation capacity in the uterus and contribute to both embryonic and extraembryonic lineages in vivo. Through integrative multi-omics analyses, we uncover the broad regulatory role of Nrf in the transcriptome, chromatin accessibility and epigenome, targeting genes associated with embryonic lineage and the transposable element SINE B1. The Nrf-centred intrinsic program governs and drives the development of both blastoids and early embryos.

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Keywords: Blastoid, Expanded pluripotency, Multiomics

Poster Abstracts

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TET PROTEINS CONTROL EARLY-STAGE STEM CELL DIFFERENTIATION VIA H3K27ME3, NOT DNA DEMETHYLATION

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TET proteins are instrumental in facilitating DNA demethylation. To investigate the significance of TET proteins in early ESC differentiation, we conducted mapping experiments on ESCs, during exit from pluripotency-state, and at early differentiation-phase. Before differentiation, minimal disparities surfaced between wild-type and TET-TKO cells. However, analysis of active enhancers, DNA methylation, and single-cell RNA-seq maps illuminated a distinct narrative during differentiation. Notably, TET-TKO cells exhibited a marked deficiency in activating the differentiation process towards extraembryonic endoderm (XEN). Intriguingly, this impairment did not stem from escalated DNA methylation levels; rather, it was intricately linked to diminished H3K27me3 levels during the pluripotency-stage. Remarkably, we achieved the restoration of TET-deficiency-associated effects through co-culturing TET-TKO ESCs with their wild-type counterparts. This co-culturing strategy successfully reinstated XEN differentiation, despite the absence of TET proteins during the differentiation phase. This intriguing set of observations leads us to propound that, during the nascent stages of differentiation; the catalytic role of TET-mediated DNA demethylation holds marginal significance. Instead, the crux of TET proteins' regulatory prowess lies in the establishment of the ESC ground state, intricately facilitated by their deposition of H3K27me3. This paradigm-shifting insight invites a novel perspective on the multifaceted functions of TET proteins in orchestrating cellular differentiation.

Keywords: DNA methylation, Early differentiation, Sequential ChIP-bisulfite-seq

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THE ROLE OF BAF CHROMATIN REMODELING COMPLEXES IN HUMAN NEURODEVELOPMENT AND DISEASE

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The polymorphic ATP-dependent BRG1/BRM associated factor (BAF) chromatin remodeling complexes play a pivotal role in maintaining cellular homeostasis. Their main function consists of sliding and ejecting nucleosomes to regulate DNA accessibility, especially at gene regulatory regions, and thus control gene expression. Their dysfunction owing to genetic aberrations is associated with various human pathologies, including cancer and developmental diseases. In particular, mutations in several subunits of the BAF complexes are linked to neurodevelopmental disorders such as Coffin-Siris-Syndrome (CSS), Nicolaides-Baraitser-Syndrome (NCBRS), and autism spectrum disorder (ASD). Notably, haploinsufficiency of ARID1B is one of the most frequent causes of CSS and Intellectual Disability. However, the cellular and molecular mechanisms that drive these faulty neurodevelopmental processes are largely unknown. To investigate the role of BAF complexes during neurodevelopment and to unravel how BAF complex perturbations contribute to neurodevelopmental disorders, we utilize differentiation of human induced pluripotent stem cells (hiPSCs) into cerebral organoids as a model system. Their differentiation recapitulates early human embryonic neurodevelopment at both the cellular and molecular levels. Inhibiting the chromatin remodelling activity of BAF complexes at different time-points during organoid differentiation revealed substantial developmental stage-specific changes in gene expression, chromatin organization and organoid morphology. These results suggest that BAF complexes have distinct functions at different stages of neurodevelopment that affect further developmental processes. In the future, these studies will be extended to investigate how these processes are affected in neurodevelopmental disorders caused by mutations in genes encoding BAF subunits.

Keywords: BAF complex, neurodevelopment, cerebral organoid

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ZMYND11 MUTATION IMPACT ON SCHIZOPHRENIA IPSC-DERIVED NEURONS

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Our research group specializes in modeling neurodevelopmental psychiatric disorders in vitro using induced pluripotent stem cells (iPSCs). Among the conditions under investigation, schizophrenia stands out due to its characteristic deviations in brain development and synaptic activity. In a prior study, we successfully reprogrammed somatic cells from a schizophrenic patient bearing a potentially pathogenic de novo mutation in the ZMYND-11 gene into iPSCs. Subsequently, these iPSCs were differentiated into PROX-1 positive hippocampal dentate gyrus granule neurons, as existing knowledge points toward the hippocampus playing a significant role in schizophrenia pathogenesis. Our study aims to explore whether the ZMYND-11 mutation induces alterations in neuronal differentiation, resulting in differential gene expression between the patient-derived cell line and isogenic controls. Utilizing CRISPR-based genome editing techniques, we rectified the de novo mutation in the patient-derived cell line and introduced the same mutation into a genetically unrelated healthy control cell line. We then differentiated these cells into PROX-1 positive dentate gyrus granule cells through an in vitro protocol. RNA samples were collected from the patient-derived cell line and isogenic controls at two distinct stages during the differentiation process: neural progenitor cells and mature hippocampal neurons. Comparative analysis of gene expression was performed between the mutant cell lines and wild-type cells in both genetic backgrounds. At the neural progenitor stage, we observed overexpression of genes linked to neural differentiation and concurrent underexpression of genes associated with glial function in the mutant lines. In the mature neural stage, there was noticeable overexpression of genes related to neural function. Further investigation unveiled differentially expressed genes participating in synaptic transmission, particularly within the glutamatergic synapse. Our findings lend support to the notion that the de novo mutation in the ZMYND-11 gene, specific to this schizophrenic patient, exerts influence on nervous system development and synaptic function. This mutation may have contributed to the pathogenesis of schizophrenia in this particular case.

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Keywords: schizophrenia, CRISPR, ZMYND11

TOPIC: GENOME EVOLUTION IN DEVELOPMENT

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HUMAN PLURIPOTENT STEM CELL-DERIVED NEUROEPITHELIAL CELLS DEVELOP INTO AN ORGANIZER FOR OPTIC TECTUM FORMATION IN THE CHICKEN TELENCEPHALON

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Human brain development critically depends on the formation of secondary organizers, which determine specification of neural territories along the antero-posterior axis. Studies on human secondary organizers

have proven to be difficult due to technical limitations and ethical concerns. Here, we used interspecies transplantations to demonstrate that neuroepithelial cells (NECs) derived from human pluripotent stem cells (hPSCs) acquire properties of the isthmic organizer (IsO) when transplanted into the HH11 chicken telencephalon, driving formation of a fully laminated optic tectum. hPSCs-derived NECs initially do not express the whole range of characteristic IsO molecules, but develop these features after transplantation and interaction with chicken host tissue. We show that engineered human NECs act as dominant IsOs, overriding local signals in the chicken telencephalon to form a mesencephalic architecture, the optic tectum. The generation of IsOs from hPSCs offers new opportunities to study the pathogenesis of brain defects caused by malfunctions of the IsO.

Keywords: neuroepithelial cells, human pluripotent stem cells, organizer, Chicken optic tectum

TOPIC: MORPHOGEN GRADIENTS

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FINE-TUNING MECHANO-CHEMICAL SIGNALLING TO DRIVE DISTINCT MORPHOGENESIS IN DIFFERENT SEGMENTS OF THE EMBRYONIC CHICK GUT

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Uncovering the cellular processes that define and maintain organ shapes has been a long-standing objective in developmental biology. This is exemplified in the context of vertebrate gut morphogenesis, where a combination of biochemical signals and overarching mechanical forces play pivotal roles in shaping the gut into its mature form (Savin et al., Shyer et al., Nerurkar et al., Huycke et al., Gill et al.). Beginning as a simple tube, the vertebrate gut develops into three distinct compartments (foregut, midgut, and hindgut), each ultimately adopting unique morphological attributes finely tuned to fulfil specialized functions. During the early stages of chick gut development, the Sonic Hedgehog (Shh) and Bone Morphogenetic Protein (BMP2/4) signalling systems regulate the patterning of muscle tissue along the gut's radial axis. An intriguing question arises: how does this shared biochemical patterning mechanism (input) result in distinct morphological outcomes (output) within different gut regions? In this study, we employ a combination of quantitative microscopy and mathematical modelling to provide compelling evidence elucidating how both biochemical signals and variations in growth processes contribute to the observed differential muscle patterns along the gut axis.

Keywords: Vertebrate gut morphogenesis, Morphogen signalling, Smooth Muscle

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OPPOSING ROLES OF DORSAL MORPHOGENS IN THE REGULATION OF NEURAL TUBE GROWTH

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Morphogen signalling molecules orchestrate tissue development by controlling the specification of cell identities as well as the rates of cell proliferation and cell loss. An excellent example of this is the dorsal neural tube. BMP and Wnt signalling gradients in this organ control both the specification and cell cycle progression of neural crest and dorsal interneurons. However, the exact mechanisms by which Wnt and BMP signalling are interpreted to achieve the correct number of cells of each type are poorly understood. We developed a quantitative in vitro culture system in which we direct mouse embryonic stem cells to differentiate into dorsal spinal neural progenitor cells on micropatterns. This system allows us to precisely manipulate the dynamics of morphogen signalling and assess the cellular response. We are combining this approach with quantitative microscopy to measure changes in signalling, as well as progenitor number and proliferation rates in different conditions. Preliminary data suggests opposing roles for BMP and Wnt in the regulation of proliferation. Inhibition of BMP signalling or addition of exogenous Wnt3a results in increased proliferation and expansion of the neural progenitor population.

Keywords: morphogens, growth, neural tube

Poster Abstracts

TOPIC: TIMING OF DEVELOPMENT

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A SYSTEMS VIEW OF CELLULAR STATE HETEROGENEITY IN HUMAN PLURIPOTENT STEM CELLS

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Early human embryogenesis relies on cellular fate transitions of pluripotent stem cells orchestrated by various signalling pathways and spatio-temporal dynamics. While progress has been made in understanding the gene regulatory programs underlying these processes, the molecular determinants governing cellular state heterogeneity of pluripotent stem cells at the single-cell level remain incompletely quantified and understood. Here, we investigate the functional and phenotypic variability of induced pluripotent stem cells (iPSCs) upon small-molecule-based drug perturbations using a combination of high-content microscopy, computer vision, and high-throughput molecular profiling. The quantification of our imaging data, facilitated by a novel self-supervised deep learning approach, has unveiled scale-crossing morphological heterogeneity across diverse drug mode-of-actions. We then profiled a selected set of drug perturbations using multiplexed transcriptomics and high-throughput proteomics, uncovering condition-specific expression dynamics of key proteins and transcripts involved in early differentiation. Intriguingly, our multimodal data suggests that, in response to perturbation, iPSCs navigate a delicate equilibrium between committing to specific cellular fates and adapting to the perturbed conditions. Overall, our study provides valuable insights into the intricate molecular landscape underlying scale-crossing phenotypic heterogeneity in a cellular system characterised by maximal developmental plasticity.

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Keywords: Multi-Omics, Single-Cell High-Content Screen, Deep Learning

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CD32 ALLOWS CAPTURING BLOOD CELL EMERGENCE IN SLOW MOTION DURING HUMAN EMBRYONIC DEVELOPMENT

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During early development, blood cells in the embryo proper emerge from a subset of specialized endothelial cells, named hemogenic endothelial cells (HECs), via a process known as endothelial-to-hematopoietic transition, which is driven by time-specific Notch signaling activation. HECs represent an elusive cell population as they are rare and transient, rapidly generating blood cells, and specific markers are lacking. Therefore, it remains unclear how and when the hematopoietic fate is specified and how blood cell emergence is molecularly regulated in the human embryo. Notably, thorough characterization of this process is essential to guide the generation of therapeutic blood products in vitro from human pluripotent stem cells (hPSCs). To identify specific human HEC markers, we performed transcriptomic analysis of 28-32-day human embryos, a developmental stage characterized by active hematopoiesis. We observed that the expression of FCGR2B, encoding for the Fc receptor CD32, is highly enriched in the ACE+CD34+ endothelial cell population that contains HECs. Functional ex vivo analyses confirmed that multilineage hematopoietic potential is highly enriched in CD32+ endothelial cells isolated from human embryos. In addition, clonal analysis revealed that 90% of CD32+ hPSC-derived endothelial cells are bona fide HECs. We leveraged this specificity to study how HECs commit to the blood fate. Remarkably, our analyses indicated that HECs progress through different states, culminating with the one identified by CD32 expression. Indeed, CD32+ HECs no longer require Notch to generate hematopoietic progeny and fully commit to hematopoiesis even before the expression of hematopoietic markers. These findings provide a precise method for isolating HECs primed to the blood fate from human embryos and hPSC cultures, thus allowing the efficient generation of hematopoietic cells in vitro.

Keywords: Hemogenic endothelial cells, human pluripotent stem cells, embryonic hematopoiesis

Poster Abstracts

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DEVELOPMENTAL POTENCY OF HUMAN ES CELL-DERIVED MESENCHYMAL STEM CELLS REVEALED IN MOUSE EMBRYOS FOLLOWING BLASTOCYST INJECTION

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Mesenchymal stem cells (MSCs) are present in almost all the tissues in the body critical for their homeostasis and regeneration. However, the stemness of MSCs has been under debate as it is mainly an in vitro observation and lacking exclusive markers for endogenous MSCs makes it difficult to study the development and multipotency of MSCs in vivo, especially for human MSCs. To address this hurdle, we injected GFP-tagged human embryonic stem cell (hESC)- derived MSCs (EMSCs) into mouse blastocysts. Although assumed developmentally incompatible, EMSCs survived well and penetrated both the inner cell mass and trophectoderm whereas few hESCs did so. This correlates to the higher anti-apoptotic capability of MSCs than hESCs. Injected EMSCs contributed to murine skeletal, dermal, and even extraembryonic tissues in E16 chimera, and partially rescued skeletal defects in Sox9+/- mouse fetuses. Thus, this study provides the first evidence for the stemness and developmental capability of human MSCs through chimerization with the mouse blastocyst. It may serve as a model for studying human mesenchymal and skeletal development and a platform for human skeletal organogenesis for clinical applications.

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Keywords: Human mesenchymal stem cells, Chimera, mouse embryo

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EFFICIENT GENERATION OF OLIGODENDROCYTES FROM IPSC-DERIVED NEURAL PRECURSOR CELLS

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Oligodendrocyte loss in the central nervous system causes demyelinating diseases such as multiple sclerosis and several leukodystrophies. Yet effective pharmaceutical drugs which fundamentally cure those diseases are unavailable and thus cell replacement therapy is considered to be an alternative strategy. Oligodendrocyte progenitor cells (OPCs) which are a cellular source of oligodendrocytes have been generated from induced pluripotent stem cells (iPSCs). However, this process is shown to be time-consuming and exhibits a limited efficiency. Here, we have developed an efficient method by which over 80% OPCs can be generated from iPSC-derived neural precursor cells (NPCs) within 45 days. We first generate neural progenitor cells (NPCs) from human iPSCs through dual SMAD/GSK3 inhibition. These NPCs express a number of NPC makers including SOX1, SOX2, PAX6, and NESTIN and can differentiate into OLIG2+ and NKX2.2+ cells within 4 days. These cells are then mechanically dissociated into small clumps and cultured them in the ultra-low-attachment plate to allow forming spheroids till day 22. The spheroids are then plated into Poly-L-ornithine/ Laminin coated-dishes and further cultured in OPC medium till day 45. During this period, OPCs which are positive for PDGFRα, SOX10, NG2, and O4 are migrated out from the edges of spheroids and highly proliferative. Isolated PDGFRα+/SOX10+/O4+ OPCs by cell sorting can be terminally differentiated to myelin basic protein–positive (MBP+) oligodendrocytes. The MBP+ oligodendrocytes are capable of tightly ensheathing axons of neurons and nanofibers. Overall, the method presented herein enables OPC generation in a very rapid and efficient manner. The iPSC-derived OPCs can be utilized for preclinical and clinical trials to investigate therapeutic potential of demyelination diseases.

Keywords: iPSC-derived neural precursor cell, Oligodendrocyte progenitor cell, Oligodendrocyte

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INVESTIGATING EVOLUTIONARY DIFFERENCES BETWEEN HUMAN AND NON-HUMAN PRIMATE BRAIN DEVELOPMENT USING FOREBRAIN ORGANOID MODELS

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There are human-specific molecular and cellular processes underlying brain development that are not shared even with our closest Hominidae relatives like chimpanzees. Forebrain organoids (FBOs) derived from human pluripotent stem cells (hPSCs), referred to as hFBOs, provide access to early structures of human cortical brain development, including outer subventricular-like zones (oSVZs), cavities reminiscent of ventricles, and organization like that found in the intermediate zone (IZ) and preplate, which are not represented in two-dimensional neural rosettes or in the developing cortex of rodents. Using protocols developed by Dr. Guo-li Ming's lab, we have generated FBOs from a diverse range of primate species made up of two human (embryonic and induced PSC lines) and four non-human primate (NHP) PSC lines, including apes (bonobo and chimpanzee), old world monkeys (rhesus macaque), and new world, lissencephalic monkeys (common marmoset). Immunohistochemical analysis (IHC) of FBOs derived from all six PSC lines demonstrate neural (observed via labelling of GFAP+, GABA+, and NeuN+ cells) and forebrain lineages with SVZs and oSVZs. SVZs are comprised of SOX2+, radially organized neural progenitor cells (NPCs) followed by an oSVZ layer of TBR2+ intermediate progenitor cells. Additionally, FBOs express forebrain markers Ctip2 (layers V and VI) and Satb2 (upper layer neuronal marker) and demonstrate electrophysiological activity measured by multi-electrode arrays. Preliminary bulk RNA sequencing, qPCR, and IHC comparing hFBOs to those derived from the induced PSCs (iPSCs) of bonobos, rhesus macaques, and marmosets suggests that NHP FBOs experience astrogenesis earlier in development. NHP FBOs appear to have increased GATA3 expression at days 50 and 100 compared to hFBOs which could indicate earlier serotonergic and glutamatergic neuronal subtype maturation and specification with a related decrease in glycolysis gene transcription. The primate-specific, outer radial glia gene TMEM14B also appears to be upregulated in rhesus macaques at days 50 and 100 of development and could coincide with earlier cortical thickening and gyrification in macaques. This work provides evidence for distinct trajectories of cortical brain development between humans and several species of NHPs.

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Keywords: Neurodevelopment, Forebrain organoids, Nonhuman primate

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METABOLIC SHIFTS IN GLUTAMINE-DERIVED NITROGEN CONTRIBUTES TO THE ACQUISTION OF THE EARLIEST CELL FATES

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Shifts in metabolite levels and fluxes help determine whether human pluripotent stem cells (hPSCs) self-renew or undergo differentiation. Using a nutrient-balanced media approach to directly differentiate hPSCs into mesoderm, endoderm, or ectoderm lineages, we previously identified that upon exit from pluripotency, each lineage handles exogenous glutamine (Gln) differently to support their distinct requirements for fate acquisition. Glutaminase (encoded by GLS1 and GLS2),

converts Gln into glutamate, which can be processed for tricarboxylic acid (TCA) cycle anaplerosis. All lineage types repressed GLS2 expression, whereas GLS1 is upregulated only in meso- and endoderm and these lineage types perished in GLS1-inhibited conditions. This lineage-specific GLS1 sensitivity suggests meso- and endoderm could favor exogenous Gln processing for anaplerotic reactions to satisfy fate requirements, whereas ectoderm commitment is less dependent upon this route of Gln usage. How ectoderm is using exogenous Gln during its identity programming and the relation between the fate of Gln and dynamic hPSC cell state transitions has yet been fully elucidated. To examine Gln fate during hPSC differentiation, we profiled metabolite changes and quantified shifts during tri-lineage fate transition using mass spectrometry-based metabolomics and stable-isotope tracing. Here, we show that the metabolic regulation of Gln nitrogen (Gln-N), in the form of amine and amide groups, is distinctly altered during the progression of hPSC fate transitions. Gln amine fluxes revealed ectoderm uniquely skews incorporation of Gln-N away from TCA cycle anaplerosis. Instead, ectoderm shifts Gln-N towards nucleotide biosynthesis, demonstrated by high Gln amide flux into nucleotide precursors during early differentiation which resulted in a significantly higher abundance of nucleotides and their derivatives at the end of differentiation to possibly support the high proliferation rate observed in ectoderm. These data provide insight into how differential Gln-N disposition and utilization during hPSC differentiation may contribute to different hPSC fate transitions, with modulation of Gln-N being a potential approach to produce higher quality hPSC derivatives for studying early development and eventual therapeutic usage.

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Keywords: pluripotent stem cell, cell fate, metabolism

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ROLE OF EM-EXEM INTERACTIONS IN HUMAN BLASTOCYST DEVELOPMENT: INSIGHTS FROM EMBRYO MODEL

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The efficiency of human reproduction is considerably lower compared to other species, such as mice. Based on the accumulated knowledge from in vitro fertilization (IVF) technology, implantation is estimated to be a major bottleneck for the progression of a successful pregnancy. Despite the increasing demand for IVF technology, the success rate remains low (around 30%). A better understanding of peri-implantation development mechanisms is required to improve IVF technology and overcome infertility problems. Peri-implantation development of the human embryo is largely unknown due to limited access to the human embryo for ethical reasons and the limited number of applicable experimental technologies. To overcome these limitations in human peri-implantation development research, we have developed a stem cell-based blastocyst model called blastoids. Blastoids are composed of all three blastocyst lineages (epiblast, trophectoderm, and primitive endoderm) and recapitulate the morphological features of the human blastocyst. To validate the functional aspects of blastoids regarding implantation, we have developed an in vitro implantation assay using human endometrial organoid-derived cells. Blastoids attach to hormonally stimulated endometrial cells from the epiblast proximal region, known as the polar trophectoderm (TE). This is the same mode of interaction observed between the human blastocyst and endometrial cells in vivo. In this study, our aim is to reveal the mechanism regulating the direction of attachment toward the endometrial cells. Based on the local proximity between the polar TE and epiblast, we hypothesize that growth factors from the epiblast induce the differentiation of polar TE and confer the ability to attach. We performed a large screening of signaling factors secreted from the epiblast to induce differentiation of TE in a spatially regulated manner. We have confirmed the functional importance of these signaling pathways using the in vitro implantation assay.

Keywords: Blastoid, Cell-cell interaction, implantation

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THE UNEXPLORED ROLE OF EPICARDIAL CELLS IN HUMAN CARDIOMYOCYTE MATURATION

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The epicardium is a layer of epithelial cells that migrates to cover the primary heart tube by E12 in mice. The epicardium is pertinent for heart development as it secretes mitogens responsible for cardiomyocyte (CM) expansion and formation of the compact myocardium. These cells also populate the heart by undergoing an epithelial-to-mesenchymal transition into smooth muscle cells and cardiac fibroblasts. Developmental studies which ablate the epicardium results in a thin dilated ventricle and embryonic lethality. Developmental studies to date have highlighted the role of epicardial cells in CM proliferation but few have explored the impact of the epicardium in driving the process of CM maturation. This is because proliferation is thought to be dichotomous to maturation. Understanding, and recapitulating the process of CM maturation in development is integral to the creation of physiologically relevant stem cell models in vitro. This study utilizes human induced pluripotent stem cells to study the role of epicardial cells in CM maturation. 2D co-cultures and 3D engineered heart tissues were constructed and assessed for electrical, contractile and phenotypic maturation. In 2D co-cultures, epicardial cells increased CM proliferation (+7% from CM only control) and enhanced maturation. In particular, calcium handling in CMs was improved in the presence of epicardial cells in both 2D and 3D conditions as seen by an increased upstroke velocity and decreased time-to-peak. Patch clamp to characterize CM action potentials showed a favorable decrease in resting membrane potential and increase in upstroke velocity. Further, measures of contractile maturation including single cell force generation and sarcomere length of CMs were increased in epicardial co-cultures. These data support the hypothesis that the epicardium potentially plays a role in human CM proliferation, as well as maturation.

Funding Source: NIH NHLBI R01, HL160779
Keywords: hCardiomyocyte Maturation, Epicardial Cells, Cardiac Tissue Engineering

TOPIC: TIMING OF DEVELOPMENT

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BUILDING COMPUTATIONAL PIPELINES TO MEASURE MULTISCALE MECHANICS IN STEM-CELL BASED MODELS OF HUMAN GASTRULATION

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Growing evidence supports that, in addition to molecular cues, mechanical forces are an essential source of morphogenetic information, contributing both for shaping and patterning developing embryos. For instance, the inflation of the luminal cavity in mouse blastocysts results in trophectoderm stretching, which, in turn, controls embryo size and fate specification at the cell scale (Chan et al, 2019). In the same vein, mesenchymal stem cell differentiation was shown to be modulated by osmotic pressure. Despite this, measuring forces within living systems remains challenging. With this in mind, we characterized recently developed mechanosensing fluorescent probes called Flippers, whose fluorescence lifetime depends on lipid packing and on membrane tension. We benchmarked these probes across a range of biological systems, from encapsulated epithelial monolayers to mouse gastruloids and, more recently, human gastruloid discs. Since it was recently shown that tissue-scale mechanical properties can change abruptly during development in a manner reminiscent to unjamming transitions in physics, my goal is now to combine existing tools to measure mechanical forces across scales, from membrane tension, junctional dynamics or motile forces to tissue-scale stress. To do so, we are developing a computational image analysis pipeline to segment cell outlines in self-organizing human gastruloid discs and automatically extract a range of parameters, namely cell shape, motility and cell-cell rearrangements. These mechanical atlases will be correlated with force inference approaches, laser ablations and cell-intrinsic mechanical properties, namely plasma membrane tension using Flipper. Together, this multiscale approach will allow me to characterize the mechanical forces arising during cell fate acquisition and tissue patterning, but also to quantitatively test the contribution of different sources of cell-intrinsic forces to tissue scale dynamics and mechanical properties.

Funding Source: IMP Vienna
Keywords: Gastrulation, Biophysics, Mechanics

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DISSECTING STEM CELL HIERARCHIES IN HUMAN BRAIN DEVELOPMENT

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The functional study of stem cells is reliant on methods for prospectively isolating pure populations of distinct cell types, especially in primary human tissues where classical genetic tools are not available. The main challenge in prospective isolation lies in identifying combinations of cell surface markers that can discriminate functionally distinct stem and progenitor populations. Here, we introduce a general method for developing purification strategies by combining index sorting with single cell RNA-sequencing, which provides a one-to-one map between individual cells' surface markers (immunophenotype) and transcriptome. We apply this approach to fetal human brain tissue, and develop a method for prospectively isolating ten distinct neural stem and progenitor cell types, including radial glia, astrocytes, excitatory/inhibitory neurons, oligodendrocytes and their precursors (OPCs), as well as a newly-characterized glial progenitor cell (GPC) capable of giving rise to astrocytes and oligodendrocytes but not neurons. The lineage output of each population was validated through clonal in vitro differentiation and in vivo transplantation into the brains of neonatal mice. We further apply our method to develop prospective isolation strategies for other compartments of the fetal human brain, including vascular cells (endothelial cells, pericytes, fibroblasts) and immune cells (microglia, axon tract-associated microglia, perivascular macrophages). By barcoding purified vascular subsets, we were able to perform clonal tracing of defined starting populations, revealing unexpected plasticity between endothelial and mural lineages both in vitro and in vivo. Our method thus provides a framework for developing rigorous purification strategies in any tissue, facilitating subsequent functional studies. Such capabilities are essential for answering fundamental questions in stem cell biology involving lineage hierarchy/plasticity, cell type-specific roles in tissue organization, and genetic programs driving fate decisions.

Keywords: Neurodevelopment, Vascular Biology, Cell purification

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EFFECT OF PHOTOBIOMODULATION ON THE VIABILITY OF MULTIPOTENT MESENCHYMAL STEM CELLS FROM ADIPOSE TISSUE IN VITRO

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Skin wounds are a public health problem that affects patients' quality of life. Mesenchymal stem cells (MSC) are present in the normal physiological healing process and participate in a series of events that result in the restoration of injured tissue. Recent advances in cellular and molecular biology assist in tissue repair and the use of photobiomodulation leads to improvements in wound treatment, however the use of inappropriate parameters can affect this process. The aim of the study is to investigate the effect of different photobiomodulation (PBM) parameters on (MSC) cell viability. The study was approved by the Ethics and Research Committee of Hospital das Clínicas – FMRP/USP, CAAE 18691919.3.0000.5440, opinion nº 3.842.162. Mesenchymal cells derived from adipose tissue were subjected to photobiomodulation applications with the physical parameters: wavelength of 660 nm and 830 nm; power 100 mW; energy of 0.5 J, 2 J and 4 J. Analysis of mesenchymal stem cells was performed with MetaXpress® software 48 and 72 hours after irradiation, and statistical analyzes were performed with GraphPad Prisma® 7.0 software. The results obtained in the experiments showed that irradiation with both wavelengths was effective in increasing cell viability, with the 660 nm laser having an increase in cell viability in 48 hours compared to the 830 nm laser, and in a time of 72 hours with energy of 2 J and 4 J, the wavelength of 830 nm, there was greater cell viability than the 660 nm laser. Photobiomodulation is capable of stimulating cell regeneration when using the appropriate parameters and times.

Funding Source: São Paulo State Research Support Foundation (FAPESP), process no. 2019/09329-1
Keywords: Photobiomodulation, Mesenchymal stem cell, Cell viability

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PSEUDOHYPOXIA AS A PROMOTER OF THE PROLIFERATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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The pathophysiological basis of heart failure in humans lies in the heart's inability to regenerate. Adult cardiomyocytes (CM) perform mitosis after myocardial infarction, but their proliferation rate is extremely low for restoring normal cardiac function. Unlike adult CM, fetal CM can proliferate due to the uterine hypoxic microenvironment. Via Hypoxia-Inducible Factor 1α (HIF-1α), hypoxia induces glycolytic metabolism and Counteracts the Reactive Oxygen Species (ROS)-induced DNA damage, favoring CM proliferation. Systemic exposure to hypoxia has been proposed as a strategy to promote cardiac regeneration; However, its adverse effects on other organs limit its clinical use. Alternatively, a "pseudohypoxia" condition with stabilization of HIF-1α can be achieved pharmacologically using inhibitors of its degradation pathways, such as Roxadustat. We have proposed that Roxadustat mimics a hypoxic condition in CM, favoring a glycolytic metabolism and promoting cell proliferation. Considering that CM are a limited cellular source, Human Induced Pluripotent Stem Cells (iPSC) were used as a cardiomyocyte source. Western blot and RT-qPCR analysis showed that treatment of iPSC-CM with 50 µM Roxadustat stabilized the protein levels of HIF-1α and significantly increased the levels of glycolytic gene transcripts compared to untreated cells. Glycolytic rate and mitochondrial metabolism assays showed that Roxadustat increased extracellular lactate levels up to 4 times, decreased the oxygen consumption rate and increased the extracellular acidification rate. These results correlated with a decreasing ROS level. Finally, Roxadustat promoted DNA synthesis and cell division in a time-dependent manner, increasing the number of iPSC-CMs. These data show that Roxadustat mimics a hypoxic condition in iPSC-CM, favors a glycolytic and less oxidative metabolism, and promotes cell proliferation. This study is part of the first step to evaluate the regenerative potential of Roxadustat and iPSC-CM using a localized myocardial delivery strategy.

Funding Source: Postdoctoral Fellowship - Marie Skłodowska-Curie Actions (101027429 - PhyCaR)
Keywords: iPSCs-derived cardiomyocytes, Pseudohypoxia, Cardiac regeneration

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RECOMBINANT LAMININ PROTEINS PROVIDE A BIOLOGICALLY RELEVANT NICHE FOR PRIMARY CELLS IN VITRO, MIMICKING THE NATURAL NICHE FOR BOTH ADULT STEM CELLS AND PRIMARY CANCER CELLS

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Major efforts have been made on cell culture protocols for cellular therapies, however, studies on cell substrates for pathologically relevant cell cultures are less frequent. Cancer cell models in particular are often based on cell-cell contacts leaving out the essential cell-extracellular matrix (ECM) responses and despite that 85% of all cancers are epithelial cancer in direct contact with the ECM of the basement membrane. Laminins are a large ECM protein family enriched within the basement membranes, underling epithelial tissues, and an essential part of the stem cell niches. The laminins have multiple, often cell type–specific functions, such as adhesion, differentiation, migration, phenotype maintenance, and resistance to apoptosis. We have analysed the expression patterns of the laminin proteins, in healthy and cancer tissues. We can demonstrate that there is a shift in laminin gene expressions within multiple cancer forms compared to healthy tissues. Both on protein and on relative expression level, within multiple major cancer types such as, lung adenocarcinoma, renal cancers, pancreatic cancer, glioma, and melanoma. The different laminin proteins have different activities and their presence in a tumour can have positive or negative impact on 5-year survival probability, for example, in lung adenocarcinoma where high LAMA3 expression is associated with a negative 5-year survival vs high LAMA2 expression is associated with a positive 5-year survival (P=0,00051, n=494 resp. P=0,0027, n=500). We believe that these in vivo insights can be translated to improve in vitro conditions for primary cells. Cells from lung, skin, and gut, can be maintained in vitro without the need for serum or other undefined components when a biologically relevant laminin isoform is used as substrate, also with direct effects on the cell survival, proliferation and migration, parameters of major focus within drug development assays.

Keywords: Extracellular matrix, Adult stem cells, microenvironment

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TO INVESTIGATE THE IMPACT OF MIR-204 ON HUMAN ADSCS AND BREAST CANCER CELLS: IMPLICATIONS FOR CELL-ASSISTED LIPOTRANSFERS IN BREAST RECONSTRUCTION

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The safety of hADSCs in the BRCA microenvironment is impacted by their inherent immunogenicity and tumorigenicity. In addition, hADSC-CM harbours paracrine potential to affect the behaviour of cells in their microenvironment and induce adjacent cells to become motile (Rosner et al., 2022). Therefore, it is important to identify the underlying mechanisms of cell metastasis. MiR-204-5P play an important role in hADSC tumour-promoting inflammation and therefore may influence the ADSC-CM and BRCA interaction (Liang et al.,2021). hADSCs were isolated using informed patient consent and ethical consideration. MiR-204-5P was overexpressed in BRCA cells, MDA-MB-231 and T47D, using lentivirus transduction. BRCA over-expressing miR-204 (BRCAs-204+), MDA-MB-231-204+ and T47D-204+ were confirmed via RQ-PCR. hADSC CM was co-cultured with MDA-MB-231 204+ and T47D 204+ cells. Cell-cell-interactions were analyzed by migration, invasion, and angiogenesis using RQ-PCR. RNA was isolated using miRVana isolation. MiRNA and cytokine expression, were analysed using RQ-PCR and ELISA. MiR-204-5P was successfully over expressed in MDA-MB-231 and T47D ($p< 0.001$ t-test). Co-culture of BRCAs with hADSC-CM resulted in increased proliferation ($p< 0.01$ t-test) and migration ($p< 0.001$ ANOVA). MiR-204-5p overexpression inhibited viability ($p< 0.01$,t-test), proliferation ($p< 0.01$ t-test), and migration capacity ($p< 0.001$ t-test) in BRCAs. To mimic cell-assisted lipotransfers in breast reconstruction, hADSC-CM was co-cultured with BRCAs-204+. Migration and proliferation of BRCAs (MDA-MB-231-204+ and T47D-204+) were inhibited compared to normal BRCA co-culture with ADSC-CM and BRCAs overexpressing miR-204 alone ($p< 0.001$ ANOVA). In addition, migratory markers N-Cadherin, Vimentin, Twist and Snail were significantly inhibited following co-culture of hADSC-CM and MDA-MB-231 204+ and T47D 204+ cells ($p< 0.001$,ANOVA). Furthermore,miR-204-5p overexpression inhibited pro-inflammatory immunomodulators IL-6 ($p< 0.001$ ANOVA), IL-8 ($p< 0.001$ ANOVA), IL-10 ($p< 0.001$ ANOVA) and TGF- β ($p< 0.001$ ANOVA) which has further implications for ADSC transfer safety. MiR-204 overexpression decreases BRCA invasiveness and immune modulators which increases the safety and efficacy of hADSC-CM in the BRCA microenvironment.

Keywords: ADSCs, Breast cancer, miRNAs

TOPIC: TISSUE SELF-ORGANIZATION

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CILIOPATHY PROTEIN TMEM107 IN THE EYE DEVELOPMENT: INSIGHTS FROM RETINAL ORGANOID MODEL

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Primary cilia are projections from the cellular surface enriched with a number of receptors and signaling molecules. They act as a signaling hub which responds to various stimuli from the extracellular space. Several human diseases have been recently linked to malfunctions of primary cilia including retinopathies and other ocular defects. Here, we focus on the role of protein TMEM107, which is localized in a transition zone of a primary cilia and mutations in TMEM107 were uncovered in patients with Joubert and Meckel-Gruber syndromes. A mouse model with a complete knockout of Tmem107 displayed eye defects such as anophthalmia or microphthalmia where the retina differentiation was mostly affected. Physiological Tmem107 expression in the eye structures during prenatal mouse development corresponded with the sides of the phenotype and it was enhanced in the differentiating retina and optic stalk. Expression of key markers of this area such as SOX2 or PAX6 were downregulated in the distal part of the neural retina of Tmem107-deficient animals. Retinal organoids confirmed a crucial function of TMEM107 for retina development as the absence of TMEM107 leads to the loss of primary cilia on early stage organoids and outer segments on late stage retinal organoids. Moreover, TMEM107-/- retinal organoids exhibited downregulation of retina-specific genes associated with the failure of the generation of neural retina structures and cell types as well as the formation of cysts with accumulated liquid inside. The knock-out of TMEM 107 in the human ARPE-19 cell line uncovered the failure of the formation of primary cilia in retinal pigmented epithelial cells, aberrant upregulation of the SHH pathway demonstrated by the upregulation of GLI1, GLI2, PTCH1, and incapability of TMEM107-/- cells to respond to SAG treatment, because of already ectopically activated SHH signaling in these cells due to the absence of TMEM107. Altogether, our data suggest on multiple levels a key role of TMEM107 in early morphogenesis of vertebrate eye development, which is closely associated with ciliogenesis in differentiating retina.

Funding Source: This work was supported by the Czech Science Foundation (21-05146S)
Keywords: Retinal organoid, Ciliopathies, Shh

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DECIPHERING SIGNALLING INTERACTIONS BETWEEN MURINE EMBRYONIC AND EXTRA-EMBRYONIC TISSUES AT THE TIME OF IMPLANTATION

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For prenatal development to be successful, the embryo/foetus, crucially relies on the support of extra-embryonic organs such as the placenta and yolk sac. Precursors for these extra-embryonic tissues, namely the trophectoderm (TE) and primitive endoderm (PrE), arise at the blastocyst stage alongside the epiblast, and already then both fulfil key functions to enable developmental progression at the time of uterine implantation. While the trophectoderm facilitates attachment of the embryo to the uterus, the PrE guides the morphological changes of the epiblast during peri-implantation development. However, our understanding of the signalling interactions between the two extra-embryonic lineages and the embryonic lineage and how these interactions facilitate successful developmental progression is still incomplete. Here, we first present in silico predictions of signalling interactions between the three blastocyst lineages and show that both, the PrE and epiblast, are subjected to Wnt ligands secreted by the TE. In further 2D in vitro experiments, we could show that active Wnt signalling prevents the differentiation of PrE-like cells. Moreover, experiments using a 3D stem cell-based model of epiblast/PrE co-development indicate that Wnt signalling could prevent PrE cells from depositing a basal lamina that overlies the epiblast cells, thereby preventing the morphological changes necessary for the epiblast to mature. Given the role of Wnt activity in epiblast self-renewal, these results suggest Wnt activity as a regulator of the developmental progression of the blastocyst tissues. Secondly, we present a bottom-up approach aimed at understanding how the three blastocyst lineages support each other's development by building blastocyst-like structures (blastoids) from TE, embryonic, and PrE-like stem cells. Blastoids formed using current protocols fail to undergo developmental progression in utero. By optimising the ground state for each stem cell lineage and improving the signalling environments during blastoid formation, we hope to engineer blastoids with enhanced capacity for developmental progression. Collectively, the presented stem cell models and data will improve our understanding of the signalling interactions needed for developmental success at the time of blastocyst implantation.

Keywords: Primitive endoderm, Wnt signalling, Blastoids in utero development

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ETV4 IS A MECHANICAL TRANSDUCER LINKING CELL CROWDING DYNAMICS TO LINEAGE SPECIFICATION

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Dynamic changes in mechanical microenvironments, such as cell crowding, regulate lineage fates as well as cell proliferation. Although regulatory mechanisms for contact inhibition of proliferation have been extensively studied, it remains unclear how cell crowding induces lineage specification. Here, we found that a well-known oncogene, ETS variant transcription factor 4 (ETV4), serves as a molecular transducer that links mechanical microenvironments and gene expression. In a growing epithelium of human embryonic stem cells (hESCs), cell crowding dynamics is translated into ETV4 expression, serving as a pre-pattern for future lineage fates. A switch-like ETV4 inactivation by cell crowding derepresses the potential for neuroectoderm differentiation in hESC epithelia. Mechanistically, cell crowding inactivates the integrin-actomyosin pathway and blocks the endocytosis of fibroblast growth factor receptors (FGFRs). The disrupted FGFR endocytosis induces a marked decrease in ETV4 protein stability through ERK inactivation. Mathematical modeling demonstrates that the dynamics of cell density in a growing hESC epithelium precisely determines the spatiotemporal ETV4 expression pattern and, consequently, the timing and geometry of lineage development. Our findings suggest that cell crowding dynamics in a stem cell epithelium drives spatiotemporal lineage specification using ETV4 as a key mechanical transducer.

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Keywords: ETV4, Mechanotransducer, Cell fate decision

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HUMAN EMBRYONIC AND EXTRAEMBRYONIC INTERACTIONS SUPPORT DEVELOPMENTAL TRANSITIONS DURING THE SECOND WEEK

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One week after fertilization, human embryos implant into the uterus. This event requires the embryo to form a blastocyst, which consists of a sphere encircling a cavity lodging the epiblast that later forms the embryo proper, and the hypoblast cells that later form the yolk sac. Stem cells can form a blastocyst model that we called a blastoid. Here we show the critical role of locally produced signaling factors by the epiblast in facilitating the development of hypoblast cells. Using suspension culture, we also show that hypoblast cells continue to develop as human blastoids progress and support the development of the epiblast to form the amniotic cavity. Overall, this sequential and mutual support of extraembryonic hypoblast cells and epiblast cells drive a major developmental transition occuring in the second week of human embryogenesis.

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Keywords: Stem cell, Blastocyst, Hypoblast

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MACROPHAGE-DERIVED IL-1B CONTROLS METABOLIC REPROGRAMING IN DISTINCT FIBROBLAST PROGENITORS TO DETERMINE CELL FATE DURING WOUND HEALING

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Compare with facial skin, oral mucosa has a high regenerative capacity and rapid healing efficiency. To decipher the underlying molecular mechanisms, we created a parallel injury model for both the oral and facial skin within the same mice. Utilizing single-cell transcriptomics (scRNA-seq) analysis, we were able to illuminate the diverse cell populations and the intricate cell-to-cell interactions taking place at a single cell level. We identified a unique Il1rl1+ fibroblast subset that undergoes substantial transformations in both the oral mucosa and facial skin during the early phases of wound healing. Leveraging tools such as CytoTRACE, along with cell trajectory and RNA velocity analyses, we pinpointed this subset as fibroblast progenitors. In the aftermath of an injury, these progenitors are swiftly activated in the oral mucosa, maturing into fibroblasts that expedite the early stages of wound healing. Contrarily, in the facial skin, these cells tend to proliferate rather than differentiate, leading to a prolonged healing process when compared to the oral mucosa. Through the employment of GSEA and CellChat analyses, corroborated by flow cytometry and immunofluorescence staining, we demonstrated that IL-1β derived from macrophages augments oxidative phosphorylation in Il1rl1+ progenitors, steering them towards differentiation rather than proliferation, a process predominantly driven by glycolytic signals in skin. This points to a rapid response mechanism facilitated by heightened cell-cell interactions through IL-1β signals, thereby fostering accelerated tissue repair in the oral mucosa. We elucidated that the underlying mechanism involves a metabolic reprogramming in oral Il1rl1+ fibroblast progenitors driven by IL-1β/IL1R1, triggering the activation of the NFκB signaling pathway. This shift transitions the metabolic state from glycolysis to oxidative phosphorylation, favoring stem cell differentiation and consequently accelerating wound healing in the oral mucosa. Our studies offer fresh perspectives on therapeutic approaches, suggesting that targeting the IL-1β/IL1R1 axis and its downstream pathways could enhance skin wound healing and tissue regeneration following injuries, leveraging the insights gleaned from the rapid healing processes innate to oral wounds.

Funding Source: National Key R&D Program of China (No. 2022YFC2504200) National Natural Science Foundation of China (No. 82270960), Science & Technology Development Talent Project of Jilin Financial Department, Jilin, China (No. JCSZ2021893-35)
Keywords: Wound healing, metabolic reprograming, Macrophage

Poster Abstracts

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NEURAL PATTERNING IN THE DEVELOPING HUMAN SPINAL CORD: A GATEWAY TO ADVANCES IN STEM CELL THERAPY

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In the context of central nervous system (CNS) development, the current state of our knowledge falls short in terms of our ability to identify neural progenitor cells (NPCs) capable of generating distinct neuron or glial cell types, as well as pinpointing the precise locations where these cellular transitions occur. This is particularly important in cell transplantation of NPCs in regenerative medicine for restoring damaged neural circuits. Our study aimed to establish a comprehensive pipeline that augments our understanding of neural patterning (NP) within the developing human CNS. This endeavor holds the potential to shed light on the underlying mechanisms of neurodevelopmental disorders and open doors for regenerative medicine applications, notably in the treatment of conditions such as spinal cord injury (SCI), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS). To achieve our objectives, we leveraged two cutting-edge methodologies: Chromium single-cell RNA sequencing (scRNA-seq), and Visium spatial transcriptomics (ST). By applying these techniques to developing human embryonic spinal cord, we comprehensively explored the spatiotemporal distribution of distinct cell domains in human prenatal spinal cord samples spanning post-conceptional weeks 5 to 9; We identified single-cell populations associated with ST-defined cell domains, along with an examination of NPC fate commitment towards neuronal and glial cell lineages, both between and within these domains. The insights gleaned from these objectives will pave the way for the creation of human organoids, offering a novel platform to investigate the origins of specific neurons or glial cells in future regenerative medicine research.

Keywords: Neural Patterning, Neural Progenitor Cells, Spinal Cord Development

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ORGANOID CELL FATE DYNAMICS IN SPACE AND TIME

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Organoids are a major new tool to study tissue renewal. However, characterizing the underlying differentiation dynamics remains challenging. Here, we developed TypeTracker, which identifies cell fates by AI-enabled cell tracking and propagating end point fates back along the branched lineage trees. Cells that ultimately migrate to the villus commit to their new type early, when still deep inside the crypt, with important consequences: (i) Secretory cells commit before terminal division, with secretory fates emerging symmetrically in sister cells. (ii) Different secretory types descend from distinct stem cell lineages rather than an omnipotent secretory progenitor. (iii) The ratio between secretory and absorptive cells is strongly affected by proliferation after commitment. (iv) Spatial patterning occurs after commitment through type-dependent cell rearrangements. This “commit-then-sort” model contrasts with the conventional conveyor belt picture, where cells differentiate by moving up the crypt-villus axis and hence raises new questions about the underlying commitment and sorting mechanisms.

Keywords: Tracking, Organoid, Fate

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RECAPITULATING CRANIAL MYOGENESIS IN STEM CELL-BASED EX VIVO MODELS

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Cranial myogenesis follows a different developmental path compared to trunk myogenesis, both morphologically and molecularly. Muscles of the body differentiate from somite-derived cells, whereas most muscles in the head originate from rostral structures called pharyngeal arches. The cellular and molecular divergences among these muscle groups may contribute to the observed differential susceptibility of subsets of muscles to myopathies. We use mouse 3D gastruloids and other stem cells-derived systems as models that allow the study of tissue self-organization to investigate these diversities in vitro. Efficient differentiation protocols to obtain somite-like structures starting from mouse 3D gastruloids have been developed (Trunk-Like Structures, Somitoids, etc.), but not for head muscles. Our goal is to develop protocols to generate cranial muscles in mouse 3D gastruloids for ex vivo studies on their emergence and maintenance. First, we verified the expression of specific head mesoderm markers, such as Mesp1, Tbx1, Isl1, and Pitx2, in 3D gastruloids obtained with the standard protocol, that involves a single pulse of the Wnt agonist Chir99021, using both immunofluorescence and Hybridization Chain Reaction (HCR). Cells positive for Mesp1, one of the earliest head mesoderm markers, appear in 3D gastruloids at early timepoints after Wnt pulse and segregate from T/Bra+ cells (posterior mesoderm marker), localizing at the mid-to-anterior pole of elongated gastruloids. At later timepoints (around 144h-168h), we observed the expression of Isl1 at the anterior domain. However, in standard conditions, only a subset of gastruloids express Tbx1 and Pitx2 at 168h. We are currently testing different conditions to promote a robust cranial muscle fate by manipulating Nodal, Wnt and BMP pathways and by generating chimeric mouse 3D gastruloids.

Funding Source: ERC-2021-ADG - project STENIPATH
Keywords: Craniofacial muscle development, 3D Gastruloids, Tissue self-organization

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SINGLE SMALL MOLECULE DRIVES TROPHECTODERM FATE AND BLASTOID GENERATION FROM HUMAN NAÏVE PLURIPOTENT STEM CELLS

Chandrasekaran, Arun Pandian[1]**,** Alsolami, Samhan[2], Jin, Yiqing[1], Shakir, Ismail[1], Zhang, Yingzi[1], Li, Mo[1]

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Human naïve pluripotent stem cells (nPSCs) can differentiate into trophectoderm-like cells and then into blastocyst-like structures (blastoids), which mimic the segregation of lineages in the pre-implantation embryo. However, the ability of blastoids to recapitulate these features of early human development is primarily influenced by the use of several small molecule inhibitors or genetic perturbations. In this study, we found that a single small molecule can induce the formation of trophectoderm-like cells and promote the generation of blastoids from human nPSCs. Single-cell transcriptomics analysis further confirmed the presence of cells with the signatures of trophectoderm, epiblast, and primitive endoderm cell populations. We found that our blastoids can rederive into embryonic and extra-embryonic stem cells and further develop into structures resembling peri-implantation embryos in vitro. We also demonstrated that blastoids cultured in an extracellular matrix exhibit characteristics of early post-implantation development. Using chemical perturbations, we found that specific isoforms of protein kinase C play a critical role in forming blastoids induced by a single molecule. We also generated clonal blastoids from single nPSCs that resemble human blastocysts in terms of morphology, size, and lineage composition. Together, our work provides insights into generating blastoids from a single molecule without inhibiting the ERK, Nodal, or Hippo signaling pathways. Furthermore, our single nPSC-derived clonal blastoids could be a valuable model for studying human early development and pregnancy disorders.

Keywords: Naive human pluripotent stem cells, Human blastoids, Human early development

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THE INNER VALUES OF OCTOPUS NEURONS: DECIPHERING THE INTRINSIC PROPERTIES OF THE LATERAL LIP NEUROGENIC ZONE IN OCTOPUS VULGARIS

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Vertebrate nervous systems have often been used as a reference for large nervous system development. Cephalopods diverged 580 million years ago from the vertebrate lineage and are known for their stunning behavioural repertoire, like tool-manipulating skills and millisecond camouflage. Octopuses have evolved not only an interesting life history but also an underlying large nervous system. In this study, we are using Octopus vulgaris to deconvolve the evolutionary neurodevelopment and thus gain important insights into intrinsic properties of octopus neurogenesis. Previously, we described the lateral lip neurogenic zone (LLNZ), an embryonic neurogenic area. Strikingly, the LLNZ expresses evolutionary conserved transcription factors (TFs), like ascl-1 and neuroD, and gives rise to neurons migrating into the brain, similar to tangential or neural crest cell migration in vertebrates. Interestingly, we found a spatial patterning of the LLNZ based on the expression of evolutionary conserved transcription factors. Using 10X sc-multiomics (scRNA and scATAC) -sequencing of a mid-embryonic octopus we identified the forkhead-box TF orthologue fox-N1, the Sp-transcription factor orthologue sp-8 and the bHLH TF olig-3 marking sub-populations of ascl-1 positive cells. In vivo HCR revealed a division of the LLNZ into an outer (sp-8), central (fox-N1) and inner (olig-3) part. In order to study the intrinsic processes of octopus neurodevelopment, we established ocxplants, the first invertebrate neurogenic zone explants of Octopus vulgaris. We could reproduce the expression pattern for ascl-1, neuroD and elav ex vivo and interestingly found a similar tissue organisation as in vivo after 2 days of culture. This suggests an intrinsic self-organisation capacity of the LLNZ. Furthermore, ocxplants seem to de-differentiate within 4 days of culture into a neural-stem cell like state by upregulation of ascl-1 and sp-8, marking early neural stem cells. Thus we hypothesise that factors must be present driving the differentiation of the LLNZ in vivo. Taken together, we are using sc-multiomics and ocxplants to study the intrinsic features of octopus neurogenesis. We identified evolutionary conserved TFs and found that the octopus LLNZ can self-organise and de-differentiate ex vivo.

Keywords: octopus, sc-multiomics, self-organisation

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UNRAVELLING THE ROLE OF MTORC1 IN ADULT NEURAL STEM CELL QUIESCENCE DEPTH

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In the adult vertebrate brain, neural stem cells (NSCs) produce new neurons and glial cells that provide, throughout life, innate and adaptive plasticity under physiological and regenerative conditions. The majority of adult NSCs reside in quiescence (qNSCs), a reversible and actively maintained state of cell cycle arrest. This state is necessary for the long-term maintenance of NSCs, as their excessive activation results in the depletion of the NSC pool. Multiple lines of evidence further suggest that quiescence is heterogeneous with graded depth or length. Single-cell RNA-sequencing (scRNA-seq) data and in vivo functional assays revealed that NSCs are found in sub-states of deep or shallow quiescence. Nevertheless, the cellular and molecular mechanisms underlying the control of quiescence depth/length are poorly understood. In recent years, the mammalian target of rapamycin complex 1 (mTORC1) has emerged as an important regulator of NSC behavior both during development and in the adult brain. However, whether and how mTORC1 relates to quiescence heterogeneity in adult NSCs has not yet been well characterized. Using the adult zebrafish dorsal telencephalon, which harbors an extended monolayer of NSCs that are similar in nature and function to their mammalian counterparts, I have identified that the two downstream effectors of mTORC1, p-S6 S235/236 and p-4EBP1/2 T37/46, exhibit different and almost mutually exclusive patterns of expression in qNSCs. Our preliminary data further show that, while p-S6 S235/236 was present in qNSCs that were less likely to divide, p-4EBP1/2 was found in shallower, more committed qNSCs. In addition, Rapamycin-mediated inhibition of mTORC1 led to an increase in shallower quiescence in vivo suggesting a previously undescribed role of mTORC1 signaling in promoting the maintenance of a deeply quiescent state in adult NSCs. Using scRNA-seq, I now seek to uncover the quiescence-related genes that may be part of the response to Rapamycin treatment. I will also interrogate the interplay between mTORC1 and other key quiescence-promoting cues such as Notch3 signaling, in the control of quiescence. Altogether our results should provide important insights on the mechanisms underlying adult NSC maintenance.

Funding Source: This work is supported by the LabEX Revive

Keywords: Neural Stem Cells, Quiescence, mTORC1

Poster Abstracts

TUESDAY, 4 DECEMBER

All times in Central European Time (Vienna, Austria)

Poster Session III
3:15 PM – 4:00 PM

TOPIC: GENETIC PROGRAMS

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CD74 IS A REGULATOR OF HEMATOPOIETIC STEM CELL MAINTENANCE AND EXPANSION

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Hematopoietic stem and progenitor cells (HSPCs) are a small population of undifferentiated cells that have the capacity for self-renewal and differentiation into all blood cell lineages. These cells are the most useful cells for clinical transplantations and regenerative medicine. So far, it has not been possible to expand adult hematopoietic stem cells (HSCs) without losing their self-renewal properties. CD74 is a cell surface receptor for the cytokine macrophage migration inhibitory factor (MIF). Previously, we demonstrated that mice lacking CD74 exhibit an accumulation of HSCs in the bone marrow (BM) due to their increased potential to repopulate and compete for BM niches. Its absence leads to induced survival of these cells and accumulation of quiescent and proliferating cells. Furthermore, in in vitro experiments, blocking of CD74 elevated the numbers of HSPCs. In the current study, we aimed to translate these findings to human HSPCs and determine whether blocking CD74 is as effective in human and allows in vitro expansion of these cells. Our studies show that ex vivo culturing of mobilized peripheral blood cells from human donors with CD74 blocker elevates the number of viable HSCs. Thus, we suggest that blocking CD74 can lead to improved clinical insight into BM transplant protocols, enabling improved engraftment.

Keywords: Hematopoietic stem and progenitor cells, CD74, ex vivo culturing

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CELL FATE DECISION MODEL WITH SELF-ORGANIZING STEM CELL SYSTEMS REVEALED CORE AND AUXILIARY REGULATORY PROGRAMS

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Single-cell transcriptomes of developing neural crest cells revealed the 3-step mechanism of the cell fate selection through the bifurcations: 1) coactivation of competing transcriptional programs, 2) gradual biasing and repelling of the programs, and 3) commitment to a specific fate. Gradual biasing is a critical process to resolve cell fate selection influenced by the cell's intrinsic state and environmental cues, like morphogenic molecules. The responses to the individual morphogenic factors are hindered during development as the cell integrates a multitude of signals. Responses to individual morphogens are nevertheless critical for mechanistic insight into developmental diseases like neurocristopathies. To obtain the morphogen-specific signatures of the cellular responses during human cell differentiation we adopted the protocol for culturing human neural crest cells on the micropatterned surfaces, subjecting them to the combinations of morphogens followed by single-cell RNA seq profiling. Clustering analysis and trajectory mapping revealed the stable structure of fate bifurcation with the first bifurcation splitting neural and non-neural fates. Subsequently, neural fates split into the neural crest and neural ectoderm, while non-neural fates split into placodes and non-neural ectoderm. The addition of FGF2 specified the neural crest cells into the mesenchymal state marked by TWIST1 or PRRX1 genes. Importantly, a pairwise comparison of regulons revealed core and auxiliary regulatory modules. Core modules promote the acquisition of crude cell fates like neuronal and non-neuronal, while auxiliary modules lead the cells to a specific state. For example, for neural crest cells SOX10 and MYCN represent the core regulatory program and PDLIM5, TBX20, and TRPS1 compose the auxiliary program activated during mesenchymisation of the neural crest cells after FGF2 addition. As a result, this approach allows for identifying individual morphogenic signatures as activated regulon modules during cell fate decisions. As a next step, we plan to systematically characterize the combinations of core and auxiliary programs leading to specific cell states during neural crest differentiation, and the competition of the regulatory programs, resulting in specific ratios of related cell fates.

Funding Source: ERC Synergy 'KILL OR DIFFERENTIATE', 856529, ERC-2019-SyG
Keywords: regulons, cell fate decisions, neural crest

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EFFICIENT SINGLE-CELL CLONING OF GENE-EDITED HUMAN IPSCS IN LOW-VOLUMES

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Genome-engineering of human iPSCs is often hampered by low single-cell cloning efficiency and high uncertainty of culture clonality, which can render the generation of high-quality cell lines laborious, expensive, and time-consuming. Here, we show how a novel microfluidic cell culture approach can be used to obtain clonal hiPSC lines with high efficiency as part of CRISPR/Cas9 genome-editing. By exploiting interfacial tension, small-scale cell culture chambers(GRIDs) can be fabricated on polystyrene dishes with an immiscible translucent fluorocarbon overlay. Each GRID-chamber operates with less than 1 µL of cell culture medium to cultivate single cells into clonal colonies. The chambers' unique optical properties allow for the identification of single cells directly after cell plating, ensuring upfront monoclonality assurance and downstream trace ability. Using a defined workflow as part of our automated Cloning Platform, we demonstrate high cloning efficiency across several genetically distinct hiPSC lines. Additionally, phenotyping of clonal hiPSC colonies revealed that cells maintained the expression of pluripotency markers and their genomic integrity.

Keywords: CRISPR-CAS9, iPSC, Single-Cell Cloning

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EPIGENETIC REGULATION OF COCHLEAR ORGANOIDS: EXPLORING CHD4'S ROLE IN SENSORINEURAL HEARING LOSS OF SIFRIM-HITZ-WEISS SYNDROME

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Sensorineural hearing loss (SNHL) remains a significant cause of functional disability worldwide, necessitating a deeper understanding of the underlying molecular mechanisms to develop more targeted therapies. Recently, pluripotent stem cell-derived inner ear organoids were established as a scalable and high-fidelity alternative for studying human auditory biology. Chromodomain helicase DNA-binding protein 4 (CHD4) is a crucial ATP-dependent chromatin remodeler, playing a central role in epigenetic gene regulation, DNA repair, and cell cycle progression. Several mutations were identified in the ATPase domain of CHD4 characterized under the syndrome of Sifrim-Heitz-Weiss (SIHIWES), a neurodevelopmental disorder manifesting hearing loss among other dysfunctions. In this study, we investigated the impact of CHD4 on cochlear organoid development using human pluripotent stem cells (hESCs). To achieve this, we initially established a PAX2-mCherry reporter hESC line, enabling us to efficiently isolate otic progenitors. Following that, CRISPR/Cas9 technology was employed to generate CHD4 p.G1003D mutant and CHD4 knockout (KO) hESC lines. We conducted RNA sequencing on otic progenitors isolated from cochlear organoids and performed immunohistochemistry on these organoids at various developmental stages. These analyses provided valuable insights into the specific role of CHD4 during the development of cochlear organoids. In our ongoing research, we plan to extend our investigation by performing ATAC-seq and CUT&RUN analyses on otic progenitors derived from 3D organoids. These additional experiments will further enhance our understanding of the epigenetic regulation orchestrated by CHD4 in the context of cochlear organoid development.

Funding Source: Funding source: This research was funded by the Fund for Scientific Research (R.FNRS.4649); Fonds Leon Fredericq (FLF-22/003); University of Liège (FSR-R.CFRA.3775).
Keywords: Inner ear, 3D organoids, epigenetics

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GENERATING MOUSE TOTIPOTENT CELLS IN VITRO

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Totipotency is the ability to generate any embryonic or extra-embryonic tissue, and in the strictest sense, to form a new individual. The zygote and two cell stage of mouse embryogenesis are totipotent. Recently, cells expressing totipotency markers were observed to emerge spontaneously in mouse pluripotent stem cell cultures. Many groups have identified transcription factors, epigenetic regulators, chromatin remodellers and signalling proteins which promote this transition. However, it remains unclear whether all these inducers generate cells which correspond to those in the early embryo.

Furthermore, it is not known whether these inducers act through a small set of common pathways, or whether they have unique and diverse mechanisms of action. In order to address this, we identified novel combinations of inducers which increase totipotency-associated marker expression several hundred-fold. This allowed us to cluster totipotent-like cells separately from pluripotent ones, using single-cell RNA sequencing. Crucially, only one cluster was formed and this clustered with totipotent cells from early mouse embryos, demonstrating the acquisition of a common state. Working with our single cell dataset and confirming the results of previous studies, we observed an increase in the sumoylation and ubiquitination machinery in the totipotent cluster. We also observed a shift in eukaryotic initiation factor profile of the cells, inducing a translational block also seen in 2-cell stage embryos, implying a tight control of protein translation and degradation in the totipotent-like state in vitro. We confirmed that cells transiting to totipotency tend to do so in G2/M phase of the cell cycle. Finally, we identified small molecule inhibitors which can induce and prolong this state at >75% efficiency in vitro. This allowed us to carry out the first proteomic study of mouse totipotent-like cells in vitro. In conclusion, we uncover new insights into the acquisition of totipotency in vitro, protein factors underlying its acquisition and a small molecule inhibitor combination which facilitates large-scale studies of totipotency.

Funding Source: ASHBi and CiRA core funding ASHBi Fusion grant JSPS short-term fellowship
Keywords: Totipotency, Mass spectrometry, Single-cell RNA sequencing

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GENERATION OF A 2C-LIKE CELL REPORTER IN HUMAN PLURIPOTENT STEM CELLS TO STUDY EARLY HUMAN DEVELOPMENT

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Transcriptomic analyses of naïve human pluripotent stem cell cultures have revealed the presence of rare cells resembling 8-cell stage embryos that emerge after the activation of the zygotic genome. These 8-cell-like cells (8CLCs) can be experimentally enriched and transcriptionally identified; however, their isolation and long-term culture remains challenging. In addition, 8CLCs do not represent developmental stages prior to zygotic genome activation (ZGA). In this work, we aimed to identify genetic elements that can be used to mark cells that bear resemblance to early human embryonic cells prior to major ZGA. We first analyzed expression data of preimplantation human embryos and identified transposable elements that exhibit higher expression levels in human 2-cell embryos compared to subsequent developmental stages. We then generated fluorescent marker- containing reporter systems based on these transposable elements and introduced them to the CLYBL safe harbor region. We showed that the reporters are functional and can be experimentally activated using a CRISPR/Cas9-based activation system. These novel reporter cell lines provide a platform to investigate the presence of 2C-like cells within human pluripotent stem cell populations and may facilitate identification of factors that may induce human 2C-like cells.

Funding Source: This work was funded by TUBİTAK project number 121Z292
Keywords: 2C-like cells, iPSC, Genetic reporter

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A DEGRON-BASED APPROACH TO MANIPULATE EOMESODERMIN FUNCTIONS IN THE CONTEXT OF THE DEVELOPING MOUSE EMBRYO

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Eomesodermin (Eomes) is one of the earliest-expressed T-box transcription factors (TFs) in mammalian development. In mice, it is essential in maintaining the trophectoderm (TE) lineage, which later gives rise to the placenta. Accordingly, Eomes-null embryos arrest at peri-implantation due to premature differentiation and failure of expansion of the TE. During gastrulation, Eomes is expressed in the primitive streak (PS) and required for specification of numerous mesodermal progenitors, as well as the definitive endoderm. However, its multiple roles within the short timespan of gastrulation hamper efforts to dissect its unique functions within each lineage. We thus sought to exploit fast-acting degron technologies to generate an Eomes-degron allele, which inducibly degrades the tagged protein upon administration of small dTAG molecules, as well as a (viable and fertile) mouse line harbouring the same allele. We demonstrate that degron-tagged Eomes is fully functional in embryoid bodies, and dTAG treatment results in rapid protein degradation and an Eomes-null phenocopy. Ex vivo embryo treatment similarly results in rapid Eomes

degradation. In utero dTAG treatment, however, most often produces partial and tissue-variable Eomes degradation, which we attribute to Eomes' divergent expression dynamics in each tissue. We therefore propose that long-term ex vivo embryo culture, permitting precise embryo staging and rapid Eomes depletion (and recovery) constitutes a powerful system to study this dynamically-expressed developmental TF. We further exploited this degron system to study Eomes' functions in trophoblast stem cells (the in vitro TE equivalent), where it may function to promote the stem cell state by partnering with chromatin remodellers.

Funding Source: This work was supported by a Wellcome Trust studentship (AMB) and Principal Research Fellowship (EJR).

Keywords: Eomesodermin, degron, mouse

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COMPREHENSIVE CHROMATIN PROTEOMICS RESOLVES FUNCTIONAL PHASES DURING STEM CELL DIFFERENTIATION AND IDENTIFIES CHANGES IN REGULATORY COMPONENTS

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The establishment of cellular identity is driven by transcriptional and epigenetic regulators of the chromatin proteome - the chromatome. Comprehensive analyses of the chromatome composition and dynamics can therefore greatly improve our understanding of gene regulatory mechanisms. Here, we developed an accurate mass spectrometry (MS)-based proteomic method called Chromatin Aggregation Capture (ChAC) followed by Data-Independent Acquisition (DIA) and analyzed chromatome reorganizations during major phases of pluripotency and neuromesodermal progenitor (NMP) differentiation. This enabled us to generate a comprehensive atlas of proteomes, chromatomes, and chromatin affinities of the respective differentiation states, and to pinpoint the specific binding and rearrangement of regulatory components. These comprehensive datasets combined with extensive analyses identified novel phase-specific factors and provide a detailed foundation for an in-depth understanding of mechanisms that govern the phased progression of pluripotent and multipotent stem cells. The technical advances reported here can be readily applied to other models in development and disease.

Keywords: Chromatin proteome, Transcription and Epigenetics, Mass Spectrometry-based proteomics

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MULTI-DONOR HUMAN CORTICAL CHIMEROIDS REVEAL INDIVIDUAL SUSCEPTIBILITY TO NEUROTOXIC TRIGGERS

Antón-Bolaños, Noelia[1], Faravelli, Irene[1], Faits, Tyler[2], Andreadis, Sophia[1], Trattaro, Sebastiano[1], Kastli, Rahel[1], Adiconis, Xian[2], Di Bella, Daniela[1], Tegtmeyer, Matthew[2], Nehme, Ralda[2], Levin, Joshua[2], Regev, Aviv[3], Arlotta, Paola[1]

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Inter-individual genetic variation affects the susceptibility to and progression of many diseases. Efforts to study the molecular mechanisms mediating the impact of human genetic variation on normal development and disease phenotypes are limited, however, by the paucity of faithful cellular human models, and the difficulty of scaling current systems to represent multiple individuals. Here, we present human brain "Chimeroids", a highly reproducible, multi-donor human brain cortical organoid model generated by the co-development of cells from a panel of individual donors in a single organoid, while maintaining fidelity to endogenous tissue. By re-aggregating cells from multiple single-donor organoids at the neural stem or committed progenitor cell stage, we generate Chimeroids in which each donor produces all cell lineages of the cerebral cortex, even when using pluripotent stem cell lines with notable growth biases. We leveraged Chimeroids to investigate inter-individual variation in susceptibility to neurotoxic stressors that exhibit high clinical phenotypic variability: ethanol and the anti-epileptic drug valproic acid. Individual donors varied in both the penetrance of the effect on target cell types, and the molecular phenotype within each affected cell type. Our results show that human genetic background may be an important mediator of neurotoxin susceptibility and introduce Chimeroids as a scalable system for high-throughput investigation of the contribution of human genetic variation to brain development and disease.

Keywords: Brain Cortical Organoid models, Development and disease, Human genetic variation

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SALL3 MEDIATES THE LOSS OF NEUROECTODERMAL COMMITMENT POTENTIAL IN HUMAN EMBRYONIC STEM CELLS WITH CHROMOSOME 18Q LOSS

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Human pluripotent stem cell (hPSC) cultures are prone to genetic drift, as cells that have acquired specific genetic abnormalities experience a selective advantage in vitro. These abnormalities are highly recurrent in hPSC lines worldwide, but currently, their functional consequences in differentiating cells are scarcely described. An accurate assessment of the risk associated with these genetic variants in both research and clinical settings is therefore lacking. In this work, we established that one of these recurrent abnormalities, the loss of chromosome 18q, impairs neuroectoderm commitment and affects the cardiac progenitor differentiation of human embryonic stem cells (hESC). We show that downregulation of SALL3, a gene located in the common 18q loss region, is responsible for failed neuroectodermal differentiation. Knockdown of SALL3 in control lines impaired differentiation in a manner similar to the loss of 18q, while transgenic overexpression of SALL3 in hESC with 18q loss rescued the differentiation capacity of the cells. Finally, we show by gene expression analysis that loss of 18q and downregulation of SALL3 leads to changes in the expression of genes involved in pathways regulating pluripotency and differentiation, including the WNT, NOTCH, JAK-STAT, TGF-beta and NF-kB pathways, suggesting that these cells are in an altered state of pluripotency.

TOPIC: TISSUE MECHANICS

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BIOMANUFACTURING IN LOW-EARTH ORBIT (LEO): ACCELERATING DISCOVERIES IN SPACE FOR CLINICAL APPLICATIONS ON EARTH

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The International Space Station (ISS) National Laboratory sponsors fundamental and applied research in low-Earth orbit (LEO) enabling in-space biomanufacturing for terrestrial benefit and supporting NASA in the commercial development of space. The ISS offers access to the unique benefits of a microgravity laboratory, producing breakthrough research and new and improved products and technologies by creating demand to pioneer a path forward for the transition to commercial LEO platforms before the end of ISS operations.

The ISS National Laboratory is a unique research platform off the planet, leveraging microgravity to better understand biology and explore novel opportunities for biomedical technology advancement. Space accelerates innovation in the translation of research on stem cells and organoids for use in disease models, and in the discovery, development and manufacture of drugs and biological materials for use on Earth in regenerative medicine and disease treatment. The unique properties of microgravity can enhance biomanufacturing processes, including protein crystallization, cell culture, drug formulation, enabling the production of high-quality biological products with improved purity and efficacy.

The integration of these disciplines onboard the International Space Station (ISS) provides unprecedented opportunities to advance in-space biomanufacturing, translating basic research into clinical applications. The unique environment onboard the ISS can accelerate discoveries, advance our knowledge of human health, and create tangible benefits for terrestrial applications.

Keywords: microgravity, stem cells, biomanufacturing

Poster Abstracts

TOPIC: GENETIC PROGRAMS

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UNRAVELLING THE METABOLIC INFLUENCE OF SPIC, A KEY PLAYER IN PLURIPOTENCY AND CELLULAR HOMEOSTASIS

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Metabolic reprogramming is a fundamental determinant of pluripotency and developmental processes. Understanding the intricacies of metabolic regulations is crucial for insights into development and the origins of congenital metabolic disorders. Here, we explore the influence of Spic, an ETS transcription factor highly expressed in ground state pluripotency, on stem cell metabolic states. Our transcriptomic and epigenomic analyses revealed Spic's role in regulating genes related to metabolic pathways, such as methionine cycle and lipid biosynthesis. To deepen our understanding of Spic's impact on metabolism and homeostasis, we used metabolomic assessments in Spic overexpressing and knockout cells while examining mitochondrial activity. Our initial findings suggested Spic's involvement in acyl chain remodelling and phosphatidylcholine production which affect cells signalling, methylation capacity, and energy production in stem cells. To understand the magnitude of Spic's role in cellular metabolism, we aim to further investigate the dynamics of lipids retention, secretion, composition, and rate of ATP production in Spic loss and gain of function models. Moreover, we will explore the impact of disrupting Spic regulated pathways on ground state pluripotency. This research sheds light on Spic's role in governing pluripotency and metabolism, clarifies its regulatory functions within stem cell biology and its potential therapeutic implications for metabolic diseases.

Keywords: Stem cell metabolism, Spic, Ground state pluripotency

TOPIC: MORPHOGEN GRADIENTS

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ROLE OF PDGFRA+ CELLS AND A CD55+ PDGFRA-LO FRACTION IN THE MOUSE GASTRIC MESENCHYMAL NICHE

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PDGFRA-expressing mesenchyme provides a niche for intestinal stem cells. Corresponding compartments are unknown in the stomach, where structurally distinct corpus and antral glandular epithelia have related niche dependencies. Previous studies considered antrum and corpus as a whole and did not evaluate niche functions. Using high-resolution imaging, transcriptional profiling, and in vitro assays for gastric gland growth, we identify regional subpopulations and supportive capacties of purified mouse corpus and antral PDGFRA+ cells. Corpus and antral PDGFRAHi sub-epithelial myofibroblasts are principal sources of BMP ligands, and two molecularly distinct pools distribute asymmetrically along antral glands but together fail to support epithelial growth in vitro. In contrast, PDGFRALo cells that are strategically positioned beneath gastric glands and express CD55 enable corpus and antral spheroid growth in the absence of other cells or soluble factors. Grem1+ CD81+ CD55+ intestinal trophocytes support intestinal crypt and gastric gland growth in vitro and gastric CD55+ PDGFRALo cells encompass a small Grem1+ fraction. Grem1+ cell ablation in vivo impairs intestinal but not gastric stem cells, implying that CD55+ stomach cell activity derives largely from other subpopulations. Our study provides insights into spatial, molecular, and functional organization of gastric mesenchyme and the spectrum of signaling sources for epithelial support.

Keywords: gastric mesenchyme, Pdgfra cells, BMPs Grem1 Rspo

Poster Abstracts

TOPIC: TIMING OF DEVELOPMENT

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CRISPR/CAS9 AND PIGGYBAC TRANSPOSON-BASED CONVERSION OF A PATHOGENIC BIALLELIC TBCD VARIANT IN A PATIENT-DERIVED IPSC LINE ALLOWS CORRECTION OF PEBAT-RELATED ENDOPHENOTYPES

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Induced pluripotent stem cells (iPSCs) have been established as a reliable in vitro disease model system and represent a particularly informative tool when animal models are not available or do not recapitulate the human pathophenotype. The recognized limit in using this technology is linked to some degree of variability in the behavior of the individual patient-derived clones. The development of CRISPR/Cas9-based gene editing solves this drawback by obtaining isogenic iPSCs in which the genetic lesion is corrected, allowing a straightforward comparison with the parental patient-derived iPSC lines. Here, we report the generation of a footprint-free isogenic cell line of patient-derived TBCD-mutated iPSCs edited using the CRISPR/Cas9 and piggyBac technologies. The corrected iPSC line had no genetic footprint after the removal of the selection cassette and maintained its "stemness". The correction of the disease-causing TBCD missense substitution restored proper protein levels of the chaperone and mitotic spindle organization, as well as reduced cellular death, which were used as read-outs of the TBCD KO-related endophenotype. The generated line represents an informative in vitro model to understand the impact of pathogenic TBCD mutations on nervous system development and physiology.

Keywords: iPSCs, CRISPR/Cas9, Tubulinopathies

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DECODING THE DEVELOPMENT AND POTENTIAL OF NEURAL STEM AND PROGENITOR CELLS IN THE HUMAN CENTRAL NERVOUS SYSTEM

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The spinal cord comprises the caudal region of the central nervous system and is responsible for conveying and processing motor and sensory information between the brain and the periphery. Any injury to the human spinal cord may result in irreservable functional loss due to the limited regenerative potential of the spinal cord. There is currently no cure for such conditions. Therefore, understanding how neurons and glia are generated from neural stem and progenitor cells (hNPCs) during development, and how hNPCs maintain or lose their stem cell potential during development and aging in the human spinal cord is an important yet understudied topic. Our recent work used single-cell and spatial omics has revealed the cell diversity and cell type specification of the developing human spinal cord during the first trimester. In this study, to further revealed the genetic and epigenetic regulation of human neural and stem progenitor cells for their self-renewal, differentiation and aged-related changes of stem cell potential, we used single-cell multiome-seq, spatial transcriptomics and epigeneomics to profile the spatiotemporal gene expression and regulations from developmental and adult human spinal cord at different ages. We revealed how different subtypes of human neural stem cells are genetically regulated during differentiation into different neurons and glia during development, and lost their stemness during aging. Furthermore, we observed chromatin accessibility of neural stem cells in the adult spinal cord stem cells, suggesting a therapeutic potential to recruit such cell population for stem cell therapy after injuries. Thus, we delineate spatiotemporal genetic regulation of human spinal cord across life span and leverage these data to gain insights into future therapeutic possibilities.

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Keywords: Neural stem and progenitor cells, single-cell and spatial omics, Neurodevelopment

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EPICARDIAL AND EPICARDIAL-DERIVED CELLS ENHANCE MATURATION OF HIPSC-DERIVED CARDIOMYOCYTES VIA DIRECT CONTACT

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In cardiac tissue engineering, an important goal is the enhancement of cardiomyocyte maturity, a critical step toward imitating functional heart tissue in vitro. One promising method to achieve this lies in understanding the interplay between support cells and cardiomyocytes within co-culture systems. Several studies have shown that epicardial and epicardial-derived cells promote the maturation of cardiomyocytes, but the mechanism of action is unclear. Here we evaluated whether direct contact of epicardial and epicardial-derived cells with cardiomyocytes was necessary to drive cardiomyocyte maturation. To this end, we co-cultured epicardial and epicardial-derived cells with cardiomyocytes in the presence or absence of a transwell barrier for 9 days. Following co-culture, the calcium handling capabilities of the cardiomyocytes were assessed using the calcium-sensitive dye Rhod-2 AM. In all direct co-culture conditions, we observed an increase in downstroke velocity, a significant increase in average maximum amplitude and upstroke velocity, along with a significant decrease in time to peak compared to the transwell conditions. This indicates better calcium handling and therefore increased functional maturation of the cardiomyocytes when in direct contact with epicardial or epicardial-derived cells. These findings can guide future research toward more effective strategies for cultivating mature cardiomyocytes in vitro.

Funding Source: NIH NHLBI R01, HL160779
Keywords: Cardiomyocyte Maturation, Epicardial Cells, Cardiac Fibroblasts

TOPIC: GENETIC PROGRAMS

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IN SITU HYBDRISATION IN HUMAN FETAL RETINA AND MANIPULATION OF RETINOIC ACID SIGNALLING IN HPSC-DERIVED RETINAL ORGANOIDS SHOWS CYP26A1 BUT NOT FGF8 IS EXPRESSED DURING HUMAN MACULAR DEVELOPMENT

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The macula is a specialised region of the retina adapted for high acuity vision, which is unique to humans and primates. Compared to the peripheral retina, the macula contains a high density of cone photoreceptor cells permitting colour vision, as well as particular synaptic configuration to allow for rapid phototransduction. However, we have a poor understanding of the genetic programs underlying macular development. Existing 3D retinal organoid models typically reflect the peripheral retina, with a high rod:cone ratio. The formation of the cone-dense high acuity area of the chick, a region analogous to the fovea, is regulated in part by inhibition of retinoic acid (RA) signalling, through RA catabolising enzymes Cyp26a1/c1, leading to upregulation of growth factor Fgf8. Of note, CYP26A1 expression has been demonstrated in the presumptive human macula at post-conception week (PCW) 7. However, a comprehensive analysis of CYP26A1 & FGF8 expression across human macular development has not been carried out. We used RNAscope in situ hybridisation in human fetal retina samples across the developmental time window of macular development (PCW5-10) to investigate the precise timing of CYP26A1 & FGF8 expression. We found CYP26A1 was first expressed at PCW6, with expression peaking at PCW7, and persisting to PCW10, at which point cone precursor markers RXRγ/OTX2 are expressed. However, no FGF8 expression was detected in the developing macula region across this time period, indicating conservation for RA signalling in macular formation, but divergence in downstream mechanisms. Manipulation of RA levels in 3D human pluripotent cell-derived retinal organoids by dosing with RA receptor inhibitor AGN193109 during photoreceptor specification resulted in increased mRNA levels of cone markers RXRγ/ARR3 and reduction in rod marker NRL, while dosing with recombinant FGF8 protein resulted in no change in photoreceptor gene expression, reflecting our findings of CYP26A1 & FGF8 expression in human macular development in vivo. These findings help to better understand the genetic pathways underlying human macular development, and indicate a degree of evolutionary divergence in its formation compared to cone-rich structures in other species.

Funding Source: Fight for Sight
Keywords: Macula, Retinal organoid, Retinoic acid

Poster Abstracts

TOPIC: TIMING OF DEVELOPMENT

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FROM PLURIPOTENT STEM CELLS TO INTERVERTEBRAL DISC PROGENITOR CELLS: A RECONSTRUCTION BASED ON SINGLE-CELL TRANSCRIPTOMICS

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Notochordal cells (NC) are a rare cell-type present in all vertebrates, that plays many important signaling functions during embryonic development i.e., the correct regionalization of the central nervous system and axial skeleton formation. NC also play a role in the formation of the intervertebral disc and subsequently reside at its center, gradually disappearing during childhood. There is currently no effective treatment for disc degeneration, which is a prominent cause of chronic low back pain. NC have been demonstrated to be crucial regulators in disc homeostasis maintenance and thus hold great potential for cell-based therapies. This warrants the resolution of knowledge gaps concerning the molecular controls of NC specification and maturation, during embryogenesis and at different stages of life. By translating fundamental knowledge from mouse developmental biology to human induced pluripotent stem cells (iPSCs) research, we recently defined the fine balance of canonical WNT/β-catenin and NODAL/ SMAD2/3 signaling necessary to enhance differentiation and establish a stable population of notochord-like cells (NLC) with specific molecular features of embryonic notochord. Our current work describes an extended reference molecular signature for notochord established from in vitro (human stem-cells) and in vivo (human native notochord) RNA sequencing at single cell resolution, which allowed us to delineate the transcriptomic landscape associated with notochordal lineage specification. Research efforts are pursued to identify the trajectories and gene regulatory networks (including the scenic differential regulons: CREB3L2, FOS, JUNB, SOX9) linked to notochordal fate decision. We provide evidence that selective inhibition of TGF-β pathway reduces the cellular heterogeneity observed during the differentiation process in vitro and associated with the emergence of the notochordal lineage. In addition, we identified lineage markers and validated specific cell-surface markers (CD109 and CD166) as potential tools to allow purification of NC for further functional studies. This work provides transcriptomic reference that will serve as a resource for notochord identification in human systems, various notochord-related diseased-tissues modeling, and facilitate biomedical research.

Funding Source: Financial support from EU Horizon 2020, "iPSpine" and the French Society of Rheumatology, "Spherodisc".

Keywords: Notochordal cells, Single-cell transcriptomics, cell fate specification

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REWIRING NUCLEOTIDE METABOLISM TO SUPPORT PLURIPOTENT STEM CELL FATE TRANSITIONS IN EARLY DEVELOPMENT

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Successful embryogenesis requires temporal shifts in the use of nutrients that fuel anabolic and bioenergetic pathways to support distinct cell needs for specific fate acquisitions. One of the earliest decisions in embryonic development is modeled by a primed-state human pluripotent stem cell (hPSC) to either self-renew or transition into ectoderm, endoderm, or mesoderm lineages. Each lineage is distinguished by unique patterns of growth and proliferation, with an hPSC shift to ectoderm accompanied by higher growth rates and mass

accumulation than transitions into the other two lineages. Nucleotide synthesis is known to support proliferation by providing substrates for DNA replication and transcriptome expression, yet the role of nucleotide metabolism in hPSC trilineage differentiation is not fully understood. To assess shifts in nucleotide metabolism during hPSC fate transitions, we directed hPSCs into specific lineage types under nutrient-balanced media conditions and employed a multi-omics analytical approach. RNA-seq and immunoblot analyses displayed upregulated transcript and protein levels of metabolic enzymes involved in de novo nucleotide synthesis during the shift from hPSC to ectoderm, but a decrease in these components for transitions to endo- or mesoderm. Metabolomics data revealed an increase in nucleotide levels from early to end of ectoderm differentiation, unlike the other two lineages. Notably, and distinct to only ectoderm fate, is that media footprint analysis showed a high uptake of aspartate (Asp), an essential nitrogen donor in de novo nucleotide biosynthesis. However, hPSCs directed into ectoderm under exogenous Asp withdrawal did not affect cell viability or impede fate commitment, suggesting sufficient intracellular Asp levels satisfy the requirements to progress ectoderm fate acquisition. These data suggest that, unlike endo- or mesoderm, as hPSCs transition to ectoderm, there is elevated de novo nucleotide synthesis activity to support the high proliferative needs as cells progress to ectoderm fate. Further probing this metabolic vulnerability can provide insight into how nucleotide metabolism can be targeted to understand and possibly control hPSC fate transitions in early development and enhance hPSC differentiation protocols for biomedical applications.

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Keywords: pluripotent stem cell, cell fate, metabolism

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SYNCHRONIZING OSCILLATIONS AND DIVISIONS: UNDERSTANDING THE BIDIRECTIONAL COUPLING OF THE SEGMENTATION CLOCK AND CELL CYCLE

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Coordinated regulation of cell proliferation and differentiation is pivotal for the formation and patterning of multicellular structures. During vertebrate development, the process of somitogenesis captures the intricate balance of these cellular events. Here, several molecular oscillators and spatial gradients temporally pattern the formation of somites. The details of how the patterning and proliferation of the presomitic mesoderm (PSM) is regulated are less understood. Here we investigate the interactions between the cell cycle and the segmentation clock to unravel the coupling of cell proliferation and patterning during somitogenesis. Employing a 3D zebrafish embryonic tissue model and SiMView lightsheet microscopy, we present evidence for the bidirectional coupling of the phase dynamics between the cell cycle and the segmentation clock. The findings unveil a spatial period gradient of the cell cycle along the anterior-posterior (AP) axis. Cells positioned closer to the posterior of the embryo exhibit faster cell cycle oscillations, whereas those at the anterior end display a slower pace. As the cell cycle is inhibited, the segmentation clock period elongates and becomes progressively desynchronized over time. Furthermore, we reveal a precise regulation mechanism governing the number of posterior progenitor cells and their rate of differentiation into the PSM, maintaining a consistent 1:3 ratio of progenitors to PSM cells throughout development. We propose a comprehensive framework wherein the spatially coupled phase dynamics of the cell cycle and segmentation clock play a crucial role in preserving the ratio and distribution of cell types, along with their proliferation rates during somitogenesis.

Funding Source: National Science Foundation Graduate Research Fellowship National Institute of General Medical Sciences (NIGMS) Advanced Imaging Center, Janelia Research Campus Gordon and Betty Moore Foundation Howard Hughes Medical Institute
Keywords: Somitogenesis, Cell-cycle, Patterning

Poster Abstracts

TOPIC: TISSUE MECHANICS

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DECIPHERING THE ROLE OF PROGENITOR AND STEM CELLS IN EXOCRINE PANCREAS REGENERATION IN ZEBRAFISH

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Exocrine pancreas displays an outstanding capacity for regeneration and cell fate plasticity. Using zebrafish models for exocrine cell ablation, we previously discovered a novel rare cell population displaying features of immature exocrine pancreas cells and identified these cells as a source of tissue regeneration after virtually complete removal of mature acinar cells. To better understand this progenitor pool, we have now established novel transgenic tools in zebrafish for conditional ablation and fluorescence-based lineage tracing. In addition, we have developed FACS protocols to enrich for acinar progenitor cells from conditional-ablated transgenic zebrafish larvae to enable the transcriptomic characterization of these cells under quiescent and regenerative conditions using single-cell RNA-seq. Furthermore, we have initiated a pharmacological investigation of the signaling pathways involved in the exocrine regeneration of zebrafish larvae, with an initial focus on pathways known to be involved in transient acinar to ductal metaplasia (ADM) and pancreatic cancer (PDAC). Towards this end, we selected small-molecule inhibitors of ADM- and/or PDAC-associated pathway components (KRAS, MTOR, WNT & MAPK) to investigate which of these might also play a key role in acinar cell regeneration after near-complete ablation. Quantification of sectioned and dissociated double-labeled pancreas from ptf1a:GFP/ela3l:E2Crimson zebrafish suggest that the proportion of ptf1a+/ela3l- progenitor cells is reduced from 5% in the larval pancreas to < 0.2% in the adult exocrine pancreas. To enable efficient FACS-sorting of these rare cells, we established an ela3l:mScarlet ablation line with bright and stable fluorescence that does not form aggregates and is easier to detect. To trace ptf1a+ cells during normal pancreas development and exocrine regeneration, we developed a Cre/lox based reporter system for genetic cell labeling (ptf1a:CreERT2; hsp70:Cytbow). Finally, we have established a line for conditional ablation of ptf1a+ cells to determine if these cells are the only source for exocrine regeneration. We are now carrying out a focused screening using inhibitors of key exocrine regeneration/proliferation pathways to interrogate the potential molecular mechanism(s) of exocrine cell regeneration.

Keywords: exocrine pancreas, adult stem cells, regeneration

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REGULATION OF SIZE AND SHAPE OF THE NOTOCHORD DURING MOUSE DEVELOPMENT

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The notochord is a rod-like organ that extends along the anterior-posterior body axis during embryonic development in vertebrates. This organ plays a central role in pattern formation by secreting signalling molecules that spread into the surrounding tissues. The dimensions of the notochord are relevant for its secretory function, yet how the size and shape of the notochord are controlled is poorly understood. To address this, we used deep tissue clearing to visualize and measure the notochord size and shape between E8.5 and E10.5 of mouse development. Our data revealed that the notochord cross-sectional area is constant along the trunk of the embryo and increases in size in the posterior region of the embryo. The notochord maintains this funnel-like shape across developmental time as it increases its length. To understand how this is achieved, we studied the cell behaviours that affect notochord size and shape. We found that spatially non-uniform cell division rates in the notochord account for its increase in size over time. Live imaging of embryo explants further revealed that notochord cells undergo active cell migration, which leads to directional displacement of notochord cells relative to the neighbouring somites. Perturbation experiments suggest that cell migration is essential for maintaining the correct diameter and length of the notochord. Using biophysical modelling, we are currently investigating how cell proliferation, active cell migration and the deposition of notochord basement membrane interact to regulate notochord size and shape. These results will provide new insight into how the extension of the notochord is coordinated with other tissues while at the same time ensuring correct gradient formation of notochord-produced morphogens.

Keywords: notochord, mouse development, morphogenesis

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SPECTRIN GOVERNS CELL SHAPE AND FATE DURING EPIDERMAL DEVELOPMENT

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To generate the skin barrier, epidermal stem cells/progenitors detach from the underlying basement membrane, change their shape, and differentiate. However, the mechanisms that orchestrate cell shape and fate are poorly understood. Here, we show that spectrin, the membrane cytoskeleton protein that regulates the mechanical properties of many cell types, governs epidermal cell shape and, unexpectedly, epidermal differentiation and barrier formation. Spectrin alpha, non-erythrocytic 1 (encoded by Sptan1), was detected in all the epidermal layers, and its levels were upregulated upon epidermal differentiation. In-utero depletion of Sptan1 did not alter the epidermal stem cell/progenitor cell shape or proliferation but hindered their differentiation. Namely, their ability to exit the cell cycle, change shape, and form a functional barrier. Seeking a mechanism, we show that spectrin regulates cell shape by boosting cortical actomyosin levels and contractility and cell differentiation by enhancing the activity of the EGFR-TRPV3-transglutaminase pathway. We also demonstrated that actomyosin contractility was required for the EGFR-TRPV3-transglutaminase pathway activity. Our results highlight spectrin's critical structural and regulatory functions in epidermal barrier formation and demonstrate how the two functions are executed in a physiologically relevant mammalian tissue.

Keywords: spectrin, actomyosin, differentiation

TOPIC: TISSUE SELF-ORGANIZATION

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UNCOVERING EPIGENETIC AND METABOLIC REGULATORS OF LINEAGE FATE DURING HUMAN PRE-IMPLANTATION DEVELOPMENT

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Understanding the fundamental principles underlying lineage fate and patterning in the early mammalian embryo remains a key question in developmental biology. While many studies have focused on the role of transcription factors (TFs), emerging evidence underscores an interplay between metabolism and chromatin states that have the potential to alter transcriptional outcomes, adding a layer of complexity and regulation to these events. Although valuable insights have been extrapolated from rodent studies, it is pivotal to distinguish between the mechanisms that are shared between mammal species, and those unique to humans. Notwithstanding, due to a lack of methods to efficiently perturb gene expression at early stages of human development, our understanding of the mechanisms orchestrating human embryo patterning remains largely elusive. To this end, we developed CRISPRi Blastoids - a tractable in vitro model to knock-down genes of interest (GOI) in the context of a 3D model of early human development. Using a human embryonic stem cell (hESC) line with Dox-inducible expression of catalytically inactive Cas9 (dCas9), we established a tool that allows an inducible knock-down (KD) of target genes during blastoid formation. Furthermore, by adjusting the timing and duration of Dox treatment, the perturbation can be finely tuned, expanding the range of possibilities of this tool. Drawing upon transcriptomic data of human embryos and in vitro models, we have selected a set of candidate target genes to uncover epigenetic and metabolic barriers towards specification. Taken together, this model allows us to dissect the function of single genes during the first days of development, and will also enable us to interrogate how genetic, epigenetic and metabolic regulation is intertwined.

Keywords: CRISPRi, Blastoids, Epigenetics

Poster Abstracts

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ANEUPLOIDY AS A FIRST GENETIC HIT THAT ENHANCES THE TUMORIGENIC CAPACITY OF DIFFERENTIATED HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) are increasingly implicated in cell-based regenerative therapies and are considered as potential treatments for injuries and chronic conditions. However, hPSCs in culture frequently acquire chromosomal abnormalities (CA), raising concerns about the safety of their usage in therapy. Strikingly, the aberrations found in the hPSCs are similar to those identified in cancers, and they confer cells a growth advantage and reduce cell death, but it is unknown if these abnormalities can prime cellular oncogenic transformation in differentiated cells. This lack of knowledge is due to the shortage of appropriate research models and insufficient systematic studies. In this work, we hypothesize that these mutations represent a first hit in the oncogenic process, enhancing the ability of differentiated hPSCs to transform upon an oncogenic hit. We used an in vitro organoid-based model of brain tumorigenesis, in which we subject a set of genetically balanced hESC lines (hESCcontrol) (VUB01, VUB02, VUB03, VUB04, VUB07, VUB14 and VUB19) and hESC lines with well-characterized CA (hESCCA) (gains of 1q, 12p, 17q, 20q and losses of 18q) to directed mutagenesis, in order to study the potential genetic defects boosting the tumorigenic potential of hPSC-derived cells. Thus far, our findings show that hESCs with recurrent CA, such gains of 20q11.21 (commonly found in glioblastomas), 1q24.2 and 17q, are more likely to form tumorous overgrowth in brain organoids after mutagenic transformation by overexpressing cMyc than organoids formed with genetically balanced hESCs (95-100% of hESCCA-organoids showed transformed outgrowths compared to 20-30% in control). Yet, organoids formed using hESCCA with gain of 12p or losses of 18q, had lower transformation rate (20-40%) than others CA. Interestingly, hESC with CA, in particular gains of 12p, 20q and losses of 18q, formed organoids with unstructured and disorganized shapes compared to control organoids, suggesting that these genomic aberrations not only enhance their transformation capacity but also affect the ability of hESC to form normal brain organoids. Our work provides new insight on the functional impact of CAs in hPSC and valuable information to assess the long-term risks of transplanting hPSC-derived cells with genetic defects.

Funding Source: Fonds Wetenschappelijk Onderzoek FWO
Keywords: aneuploidy in oncogenesis, Human pluripotent stem cells, hPSCs-brain organoid to study tumorigenesis

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CELLULAR MECHANICS AND SELF-ORGANIZATION DURING AXES FORMATION IN MOUSE GASTRULOIDS

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Early development has been studied from genetic and biochemical perspectives for decades. However, gene regulatory networks (GRNs) and signaling pathways alone cannot fully explain how a coordinated body plan arises from a homogeneous pool of pluripotent cells in the early mammalian embryo. In our work, we want to add a biophysical perspective to this classical view of developmental dynamics. We aim at understanding the role of forces, material properties and tissue mechanics during early mammalian development, using mouse gastruloids. These multi-cellular aggregates of mouse embryonic stem cells self-organize into embryo-like structures, comprising the three major germ layers and a coordinate body plan including anterior-posterior, dorso-ventral and medio-lateral directionality. This allows us to study the formation of a global coordinate system in a minimal in vitro system without extraembryonic tissues. We therefore consider our findings relevant to the inherent properties of mammalian cells to self-organize in the absence of external clues, rather than being species-specific to mouse development. Combining physics and engineering approaches, our team has characterized anterior-posterior axis development and identified a link between gene expression state and visco-elastic properties of differentiating cells in gastruloids. Furthermore, we have shown that the ground state of cells (naive vs primed pluripotency) can determine germ layer proportions and shape of differentiated gastruloids. Perturbation of essential signaling pathways (Mek/Erk or Tgf-β) during differentiation influences these properties, which hints at a connection between GRNs/signaling and cellular behaviors such as cell migration multicellular rearrangements. In the following, we want to examine more deeply how mechanics and signaling influence each other to promote symmetry breaking and primary body axes formation on a molecular, cellular and tissue level.

Keywords: Axes formation, Tissue mechanics, Mouse gastruloids

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DIFFERENCES IN QUIESCENCE DEPTH ALONG THE NEURAL LINEAGE CONTRIBUTE TO THE MAINTENANCE OF ADULT NEUROGENESIS IN THE ADULT ZEBRAFISH TELENCEPHALON

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Stem cell (SC) activation results in cell divisions that are self-renewing and/or generate further committed progenitors. Tissues experiencing high cell turnover require frequent SC activation to maintain homeostasis compared to others with less turnover, in which activation is infrequent. In tissues where cell turnover is low, SC activation is balanced by a reversible state of cell cycle arrest known as quiescence, G0 in the cell cycle. In the adult vertebrate brain, 95% of neural SCs (NSCs) are quiescent at any given time. This low activation frequency is essential for NSC maintenance, and therefore neurogenesis, over the lifetime, providing a highly relevant model system for the study of quiescence regulation. Quiescence is a transcriptionally dynamic and heterogeneous cell state with durations ranging from days to months. In addition, differences in quiescence “depth”, or sensitivity to activating cues, have been described. While cells in deeper quiescence sub-states are likely to experience longer quiescence phases, the relationship between quiescence depth and duration has yet to be fully explored. Using scRNA-seq in NSCs isolated from the zebrafish telencephalon, we recently identified distinct quiescence sub-states thought to reflect cells with different quiescence depths. To interrogate the relationship between quiescence depth and duration, we first identified cells from each sub-state in situ using smRNA-FISH. We then inferred the relative quiescence duration of each sub-state using BrdU-labelling and the co-expression of cell cycle markers, combined with the analysis of morphological features associated with quiescence durations of different lengths. We found that quiescence depth and duration are indeed linked, with cells in deeper sub-states having longer quiescence phases than those in shallower sub-states. NSC and neural progenitor (NP) markers were then used to position each sub-state along the neural lineage, elucidating the relationship between quiescence depth and commitment. This work demonstrates that while quiescence is a feature of both NSCs and NPs, shallower sub-states are over-represented among further committed progenitors compared to more deeply quiescent NSCs that divide less frequently but maintain a greater capacity for self-renewal.

Keywords: quiescence, neural stem cell, adult neurogenesis

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ENDOGENOUS NODAL DIVERGES WNT SIGNALING INTERPRETATION FROM DEFINITIVE ENDODERM TO POSTERIOR MESODERM IN GEOMETRICALLY CONSTRAINED HUMAN PLURIPOTENT CELLS

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The Wnt pathway is crucial in embryonic development, guiding processes such as gastrulation, mesendoderm differentiation, and axial elongation. However, the exact mechanisms of how Wnt coordinates these diverse stages yielding different outcomes remain not fully understood. Axial elongation occurs through the generation of Neuromesodermal progenitors (NMPs). These progenitors are maintained in the tailbud of the embryo by FGF8 and WNT3a. Indeed, NMP-like cells are induced when human embryonic stem cells are exposed to these ligands under standard culture. In contrast, when the same protocol is performed in geometrically constrained colonies, these self-organize into an intricate 3D structure, featuring a ball of epiblast disk-like cells (SOX2+, OCT4+, NANOG+, ECAD+) on top of layers of definitive endoderm (DE) (SOX17+, FOXA2+, GATA6+). Surprisingly, when confined colonies are exposed to increasing Wnt doses, signaling levels as measured with live GFP::ß-catenin are elevated, however, these elevated Wnt signals do not induce mesoderm or posteriorize the responding cells. By manipulating signaling pathways, we found that on micropatterns, elevated endogenous NODAL signaling together with the exogenous WNT drives cells to DE. The ability of WNT to induce NMPs and their specialized descendants (CDX1+, CDX2+, and TBX6+ or SOX1+) is restored only when WNT activation is combined with Nodal inhibition. Furthermore, measuring live Nodal dynamics revealed that the first 24 hours of TGF-ß activation are crucial for regulating the decision between endoderm and somitic-mesoderm. Altogether, these findings highlight the role that spatial control and ligand dynamics play in regulating the early fate decisions of pluripotent cells.

Funding Source: CONACYT 41944 and NSF MCB-2135296.
Keywords: WNT NODAL signaling dynamics, micropatterned human embryonic stem cells, endoderm and mesoderm cell fate decision

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HUMAN MICROGLIA CONTRIBUTE TO VIRAL-MEDIATED INFLAMMATION AND IMPACT NEURONAL ACTIVITY IN RETINAL ORGANOIDS

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Viral infection-induced inflammation during pregnancy has been associated to malformation of the fetal brain and to long-term behavioral consequences in adulthood such as schizophrenia. Microglia, the brain resident immune cells respond to inflammatory signals and at the same time are actively involved in neuronal development. Yet, little is known about how an inflammatory environment affects microglia during human embryonic development and which consequences this has on neuronal circuit formation and function. Human induced pluripotent stem cells (hiPSC) provide a unique opportunity to generate brain region-specific models and to study their neuronal organization and connectivity. Recently, we have shown that we can differentiate hiPSC into functional microglia-like cells (iMG) and retinal organoids (RO). Combining both models allows us to overcome the limitation that organoids commonly lack microglia and to generate microglia-assembled retinal organoids (iMG-RO). Here, we show that iMG successfully integrate into OTX2+- and Recoverin+-labeled retinal cups and colonize synaptic layers. However, iMG presence has no immediate impact on the neuronal activity at baseline condition as determined with calcium imaging. To simulate a viral-mediated inflammatory environment, we apply poly(I:C) to iMG-RO. As anticipated, iMG show an activated phenotype leading to an inflammatory signature, which alters the calcium peak amplitude but not the frequency of neurons. To identify whether we can rescue the poly(I:C)-mediated effects, we apply the anti-inflammatory drug Ibuprofen, which can be taken during the first half of pregnancy. The treatment dampens iMG activation and mitigates inflammatory gene upregulation in RO. Remarkably, only in the presence of iMG in RO, Ibuprofen treatment rescues the increased calcium peak amplitude suggesting that microglia are critical involved in resolving this effect. This provides first insights into how inflammation during pregnancy might lead to neurological phenotypes in human.

Keywords: viral-infection, microglia, retina

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INTEGRATED MOLECULAR-PHENOTYPIC PROFILING REVEALS TUNEABLE MODULATORS OF MORPHOLOGICAL VARIATION IN STEMBRYOS

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Three-dimensional models of mammalian embryo development (stembryos) hold great promise in basic and applied research. However, considerable phenotypic variation despite identical culture conditions limits their potential. The biological processes underlying this seemingly stochastic variation are poorly understood. Here, we investigated the roots of this phenotypic variation by intersecting transcriptomic states (molecular fingerprints) and morphological history (phenotypic fingerprints) of individual stembryos across stages modelling post-implantation and early organogenesis. Through machine learning and integration of time-resolved single-cell RNA-sequencing with imaging-based quantitative phenotypic profiling of hundreds of structures, we identified features predictive of the stembryo end-state. We revealed that balanced metabolic activity at early timepoints is strongly associated with harmonious production of somitic and neural tissues. This, in turn, correlates with high WNT and FGF signalling pathway activity, suggesting that variable activity of a FGF-glycolysis-WNT loop underlies phenotypic heterogeneity. Finally, we show that chemical modulations of the metabolic profile tune stembryo phenotype. Altogether, we provide a broadly applicable framework to chart and predict phenotypic variation in organoid systems, which can be leveraged to identify and control underlying biological processes.

Funding Source: This work was supported by the Max Planck Gesellschaft, a Bf3R grant, an EIC Pathfinder grant (Horizon-EIC-2021-PathfinderChallenges-01 101071203, SUMO) to Jesse Veenvliet
Keywords: Embryo Model Systems, Metabolism, Somitogenesis

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FULL-LENGTH LAMININS CRUCIAL FOR RECREATING THE CELLULAR NICHE IN TISSUE MODELS

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Laminins are an extracellular matrix (ECM) protein family of around 16 different tissue-specific-isoforms and serve a very important role in the formation and maintenance of the basement membrane (BM) architecture, thus they are vital for tissue homeostasis. Intact laminins are essential, since mutations in genes encoding laminins can cause a wide spectrum of diseases sometimes called “laminopathies”. Mutations in different domains of the laminin protein result in BM weakness and a series of muscle; kidney nerve and eye disorders result. Namely, several human congenital diseases are caused by laminin chain mutations, such as Pierson syndrome and epidermolysis bellusa. The biological effects of laminins are vastly mediated by cell surface receptors which link laminin matrices to intracellular signalling pathways. Therefore, laminins are not only relevant for cell adhesion but further vital for different physiological functions. These functions include cell survival, migration, differentiation, cell maturation, polarization, and organization of specialized cell type in different tissues and organs, such as brain, pancreas, vasculature, lung, liver, kidney, muscle and skin. In vivo, the majority of laminin receptors are integrins and non-integrins including dystroglycan, syndecans and luthern blood group glycoprotein. Furthermore, laminins have a high binding affinity to growth factors (GF). Such interactions between GFs and the ECM are essential for controlling GF release kinetics in vivo, robustly modulating tissue morphogenesis. Mimicking the cell microenvironment in vitro with full-length laminins is crucial for the development of successful differentiation protocols, predicable disease models and effective gene editing. Our internal studies confirm the important role of full-length laminins. We could show higher efficiency on ES cell cultivation, faster proliferation rate with standard morphological appearance, compared to truncated laminin. In conclusion, laminins have a key role in development and tissue morphogenesis. Full-length laminins are highly suitable matrixes for recreating the natural cell niche in vitro for a variety of tissues.

Funding Source: BioLamina
Keywords: Laminin, Microenvironment, Development

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INVESTIGATING TRISOMY 21-ASSOCIATED CEREBELLAR DEVELOPMENTAL ALTERATIONS USING HUMAN CEREBELLAR ORGANOIDS

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Trisomy 21 (TS21) is the main cause of Down syndrome (DS), the most common chromosomal abnormality affecting approximately 1 in 1000 newborns worldwide. DS patients present a significantly smaller cerebellum, which can contribute to intellectual disability and speech impairment. Our main question is how this extra copy of chromosome 21 (HSA21) affects cerebellar development, translating into a reduced volume in DS individuals. To understand cerebellar dysfunction, we are generating cerebellar organoids from healthy and TS21-derived iPSC lines, which can replicate the early stages of human cerebellar development. We analyzed the transcriptomic profiles of cerebellar organoids carrying a triplication of HSA21 and compared with healthy controls. RNA-seq data analysis suggests a significant global gene expression alteration, with ~3000 differentially expressed genes throughout the genome. Cellular component analysis of significant differentially expressed genes demonstrated a collagen-enriched extracellular matrix (ECM) in TS21 organoids as well as a down-regulation of actin-related genes. This data correlates with the lumen formation defect that we observed in the TS21 condition on early committed cerebellar organoids. Our results show a significant impact of TS21 on crucial biological mechanisms that may affect early cerebellar developmental stages.

Funding Source: Great Ormond Street Hospital (GOSH) Charity and Wellcome-funded Human Developmental Biology Initiative (HDBI).
Keywords: cerebellar development, cerebellar organoids, trisomy 21

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PATIENT DERIVED CORTICO-STRIATAL ASSEMBLOIDS FOR MODELING MITOCHONDRIAL DISEASE

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Defects in the mitochondrial respiratory chain (RC) underlie a spectrum of human conditions typically affecting the central nervous systems (CNS). One of the most severe manifestations of mitochondrial disease in children is Leigh syndrome (LS) that can affect 1/36,000 newborns. LS causes symmetric lesions in CNS and specifically in the striatum, leading to psychomotor regression. Among RC components, complex I (CI) is the most frequently affected in LS. Here, we focus on two mutations in the CI nuclear gene NDUFS4. We generated iPSCs from two LS patients carrying nonsense NDUFS4 mutations (c.316C>T and c.20C>G). Using CRISPR-Cas9, we introduced these two mutations into a control iPSC line. In all mutant iPSCs (patient- and CRISPR-derived), we observed a decrease in expression of the NDUFS4 protein and lower CI activity. We previously found that LS impairs the development of unguided cerebral organoids by disrupting neuro-morphogenesis. We now aim to address the region-specific CNS defects of LS by generating cortical (COR) and striatal (STR) brain organoids. We confirmed that COR and STR organoids express mature cortical and striatal markers by day 70. Bulk RNA sequencing revealed an up-regulation of inflammatory markers in mutant COR and STR organoids compared to isogenic control organoids, with particular accumulation within STR organoids. To investigate the connectivity between these different brain regions, we established COR-STRI assembloids. We added a synapsin reporter adeno-associated virus to COR from day 65-70 before co-culturing with STR. After 2 weeks of culture, we observed neural projections from COR to STR, and we are currently addressing potential differences in mutant organoids. We next plan to use high-density multi-electrode array (HD-MEA) to investigate the functionality of cortico-striatal assembloids, and to add iPSC-derived microglia to address the impact of neuroinflammation. Combining these various techniques, we hope to gain a deeper understanding of the striatal-specific defects and the overall neuronal pathology of LS to possibly identify innovative targets of interventions.

Keywords: Assembloid, Mitochondrial disease, neuroinflammation

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PERI-IMPLANTATION EMBRYOGENESIS AND REGULATION

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Connecting preceding blastocyst formation and following gastrulation respectively, peri-implantation embryogenesis is a key biological event during mammalian development. The embryo undergoes a series of cellular and molecular regulatory processes from pre- to post-implantation transition. In this presentation, we will discuss in vitro and in vivo models, omics measurement and molecular marker identification to explore the ingenious linkages among molecular program, lineage specialization, and polarity formation from a perspective of multidimensional molecular regulation. Relevant studies potentially provide clues to understand cell fate and regulation of embryo development, as well as the possible causes of habitual abortion and infertility.

Keywords: peri-implantation embryo, anterior-posterior axis, anterior visceral endoderm

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SCULPTING FATE WITH LIGHT: SPECIFYING ORGANIZING SIGNALING CENTERS WITH SPATIAL COORDINATES IN HUMAN PLURIPOTENT STEM CELLS

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Organizing centers secrete morphogens that specify the emergence of germ layers and the establishment of the body's axes during embryogenesis. While traditional experimental embryology tools have been instrumental in dissecting the molecular aspects of organizers in model systems, they are impractical in human in-vitro model systems for dissecting the relationships between signaling and fate along spatial coordinates. To systematically study human embryonic organizer centers, we devised a collection of optogenetic ePiggyBac vectors to express a photoactivatable Cre-loxP recombinase, allowing the systematic induction of organizer structures by shining blue light on human pluripotent stem cells (hPSCs). We used a light stimulus to geometrically confine BMP4 and SHH expression in hPSCs, resulting in active signaling and fate transitions. These fate transitions led to the self-organization of proximal-distal patterns resembling anteroposterior and dorsoventral embryonic axes with a priori-defined spatial coordinates. Surprisingly, we reveal that BMP4 signaling depends on a specific tissue architecture to induce mesodermal populations during anteroposterior axis formation. scRNA-seq

analysis established that BMP4 and SHH light-induced hPSCs self-organized the cellular populations of the human gastrula and neurula, respectively. In all, we have shown that combining light-inducible expression of morphogens with hPSCs recapitulates the molecular logic of embryonic organizing signaling centers in vitro.

Funding Source: EMBO LTF-254-2019 and Robertson Therapeutic Development Fund.
Keywords: morphogens, optogenetic, spatial-coordinates

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SELF-ORGANIZATION OF DORSAL-VENTRAL PATTERN IN MOUSE AND HUMAN NEURAL TUBE ORGANOIDS

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During embryonic development, organizers induce cell fate patterning in surrounding tissues by secreting morphogen signals, such as SHH. Remarkably, organizers can form by a process of 'self-organization' in organoids, in the absence of embryonic axis registration or directional induction. This has implications for engineering and regenerating correctly patterned tissues, and provides an opportunity to study complex, regulative biology under defined conditions. Single mouse pluripotent stem cells can form 3D neural tube (NT) organoids. Timely addition of retinoic acid (RA) induces scattered precursors that self-organize into a localized floorplate organizer, which expresses SHH and thus drives ventral-dorsal NT patterning like in vivo. Here, we study the mechanism by which global RA application to a clonal structure triggers symmetry breaking, how self-organization ensues, and why there is a temporally restricted window-of-competence for RA to induce and organize SHH expression. Furthermore, we establish analogous human NT organoids, which also self-organize SHH-expressing floorplate in response to a precisely timed RA pulse. This provides a 3D model for critical stages of human nervous system formation, and allows us to investigate how self-organizational processes are adapted for human-specific developmental size and timing. Our findings suggest a conserved mechanism between mouse and human species that scales temporally but not spatially.

Keywords: Self-organization, Neural tube, Symmetry breaking

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THEORY OF COLLECTIVE CELL FATE DECISIONS IN INTESTINAL ORGANOIDS

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To form a functionally complex organ, cells sense external signals and integrate them with their intrinsic properties to determine their cell fate. A prime example of cell-cell interactions driving organ patterning are intestinal organoids, in which a population of uncommitted progenitors undergoes a symmetry breaking event to result in stem cell niche formation and crypt-villus axis emergence. Previous work showed that this symmetry breaking is driven by Dll1/Notch lateral inhibition, but also requires heterogeneous activity of Yap1. However, a conceptual framework to understand how cell-cell communication and cell-intrinsic heterogeneity result in complex fate choices is lacking. To bridge this gap, we develop a biophysical model of collective cellular decisions, combining lateral inhibition, heterogeneous cellular states, and active epithelial mechanics. Using a set of perturbation experiments including Yap1 inhibition and overexpression to constrain the model, we make key quantitative predictions for cellular states during symmetry-breaking, including a prepatterned Dll1 state based on Yap1 activity. By combining high-throughput imaging and single-cell omics, we confirm our predictions and reveal how cells integrate the cell- and tissue-scale signals into the prepatterned Dll1 state via FoxA transcription factors, through extensive epigenetic remodeling of secretory progenitor cells. Taken together, we demonstrate how minimal biophysical models can make key predictions for stem cell fate choices in complex multicellular systems. This approach reveals how cells sense multimodal signals including biochemical and metabolic signals and integrate this information with tissue-level mechanical properties to take the first cell fate decision, break symmetry and properly pattern intestinal organoids.

Keywords: Cell Fate Decisions, Intestinal Organoids, Biophysical Modelling

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USING OF PATIENT-SPECIFIC IPSC-DERIVED COLON ORGANOIDS FOR EARLY DIAGNOSIS OF COLORECTAL CANCER

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Cancer is a multifactorial disease with a high mortality. Colorectal cancer (CRC) causes approximately 700.000 deaths/year worldwide. Although certain mechanisms behind CRC propagation can be partially explained by genetic factors, exact cancer initiation mechanism has not yet been revealed. Age and environmental factors play a particularly prominent role in the formation of sporadic CRC. However, it remains unknown how and why certain colon cells are affected by such factors. The most reliable diagnostic method is the pathological examination of biopsies taken during a colonoscopy. However, since the symptoms of CRC patients are usually detected at more advanced stages, it is not practical to apply such an invasive method for early diagnosis in individuals who do not yet have any complaints. For this reason, it is of paramount importance to develop new methods for the early diagnosis of sporadic CRC development. Here, we investigated whether iPSC-derived colon organoids from CRC patients could serve as a tool for early diagnosis. We reprogrammed iPSCs from erythroid progenitor cells of both primary, metastatic CRC patients, and control individuals, using Sendai vectors. After confirming the pluripotency of the cells, we generated colon organoids using a three-step intestinal differentiation protocol. To verify that these colon organoids contained all the necessary cell types, we conducted tests for CHGA, Villin, KLF5, FOXA2, Lysozyme, and LGR5. Transcriptome analysis was carried out after the 10th passage of colon organoids. Our findings revealed an enrichment of the WNT signaling pathway in CRC organoids compared to control. Moreover, genes associated with extracellular matrix organization and protein digestion and absorption exhibited differential expression in colon organoids from cancer patients, both with and without metastasis, compared to control. This suggests that shared gene clusters undergo distinct changes in colon organoids among cancer patients, enabling predictive assessments prior to cancer development. Metastatic CRC patients displayed differential gene expression associated with epithelial-mesenchymal transition (EMT) when compared to primary CRC patients. This implies that colon organoids could potentially offer insights into the metastatic potential of CRC patients.

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Keywords: induced pluripotent stem cells (iPSC), colon organoids, Colorectal cancer

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