# 15th Annual Meeting
# Dutch Society for
# Stem Cell Research

October 18th, 2024

University Medical Center Utrecht

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# CONTENT

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# 2024

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# Program 15th Annual Meeting of the Dutch Society for Stem Cell Research October 18th 2024, Roze Collegezaal, Heidelberglaan 100, AZU/UMC Utrecht

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| **9:00** | **Registration opens** |
| 9.30-09:50 | Coffee/tea |
| | **Session 1 Chair:** |
| 09:50-10:00 | Welcome |
| 10.00-10.15 | Myrddin Verheij (Post doc)<br>*Cultured type 3 innate lymphoid cells for adoptive transfer are differentially affected by commonly used drugs for graft-versus-host disease prophylaxis* |
| 10.15-10.30 | Evelyn Hanemaaijer (Post Doc)<br>*Multimodal single-cell analysis of human pediatric bone marrow reveals age-dependent differences in lymphocyte progenitor cell differentiation* |
| 10.30-10.45 | Liza Dijkhuis (PhD)<br>*Hematopoietic organoids from human induced pluripotent stem cells: mimicking hematopoietic development in a dish* |
| 10.45-11.15 | **Keynote lecture – Marella de Bruijn** MRC Weatherall Institute of Molecular Medicine, Oxford, UK<br>*Elucidating the birth of blood cells in mouse development* |
| 11.15-11.45 | Coffee/tea |
| | **Session 2 Chair:** |
| 11.45-12.00 | Eszter Varga (Post Doc)<br>*Transfusion-ready red blood cell generation From HUMAN Induced Pluripotent stem cells* |
| 12.00-12.15 | Imke Schuurman (PhD)<br>*Navigating Human Astrocyte Differentiation: Direct and Rapid one-step Differentiation of Induced Pluripotent Stem Cells to Functional Astrocytes Supporting Neuronal Network Development* |
| 12.15-12.30 | Anna Bertocci (PhD)<br>*Insights into iPSC population Dynamics: characterizing the "Village-in-a dish"model* |

12.30-13.30 Lunch

**Session 3 Chair:**

13.30-13.45 Marek van Oostrom (PhD)
*Coordination of mouse somitogenesis by coupling of the segmentation clock with cell cycle progression*

13.45-14.00 Saskia Heffener (PhD)
*Molecular basis of development of three hearts in cephalopods*

14.00-14.15 Daniel Krueger (Post Doc)
*Epithelial tension controls intestinal cell extrusion*

14.15-14.45 **Keynote lecture – Leo Otsuki,** IMBA, Austrian Academy of Science, Vienna, Austria
*Puzzling out patterning principles of limb regeneration in the axolotl*

14.45-15.15 Coffee/tea

**Session 4 Chair:**

15.15-15.30 Davide Cinat (PhD)
*Single-Cell Profiling Reveals a Pro-regenerative Function of Notch Signaling in Glandular Organoids*

15.30-15.45 Eline Koornstra (PhD)
*Identifying the regulatory potential of 7 million SNPs in human neural stem cells*

15.45-16.00 Marcella Dias Brescia (Post Doc)
*A simplified co-culture reveals altered cardiotoxic responses to doxorubicin in hPSC-derived cardiomyocytes in the presence of endothelial cells*

16.00-16.30 **Keynote lecture – Michiel Vermeulen** from Radboud university, Nijmegen and Netherlands Cancer Institute, Amsterdam
*Deciphering lineage specification during early embryogenesis in mouse gastruloids using multilayered proteomics*

16.30-16.45 Award ceremony

16.45-18.00 Drinks

# Keynote speakers

**Keynote lecture – Marella de Bruijn** MRC Weatherall Institute of Molecular Medicine, Oxford, UK
*Elucidating the birth of blood cells in mouse development*

**Keynote lecture – Leo Otsuki,** IMBA, Austrian Academy of Science, Vienna, Austria
*Puzzling out patterning principles of limb regeneration in the axolotl*

**Keynote lecture – Michiel Vermeulen** from Radboud university, Nijmegen and Netherlands Cancer Institute, Amsterdam
*Deciphering lineage specification during early embryogenesis in mouse gastruloids using multilayered proteomics*

**Marella de Bruijn**

Marella de Bruijn is Professor of Developmental Haematopoiesis at the MRC Molecular Haematology Unit of the MRC Weatherall Institute of Molecular Medicine (WIMM), Radcliffe Department of Medicine, at the University of Oxford, and is an associate of the Oxford Stem Cell Institute. She received her PhD from Erasmus University, Rotterdam, the Netherlands, where she trained in immunology. Her post-doctoral training was in developmental haematopoiesis with Profs Elaine Dzierzak and Nancy Speck at Erasmus University and Dartmouth Medical School, respectively. Marella's current research focusses on developmental trajectories, fate decisions, and gene regulatory networks at play during the emergence of the haematopoietic system in embryonic development.

**Leo Otsuki**

Leo Otsuki is a regeneration biologist fascinated by how we can harness, and extend, the patterning capabilities of progenitor cells for tissue regeneration. Leo carried out his PhD in the lab of Andrea Brand (Gurdon Institute, University of Cambridge, UK), where he investigated the genetic control of quiescence and proliferation in neural stem cells of the *Drosophila melanogaster* central nervous system. He next moved to the lab of Elly Tanaka (IMBA, Vienna, Austria) to study how positional information enables progenitor cells to reconstruct complex tissues during vertebrate limb and spinal cord regeneration. This relates, for example, to how a cell 'knows' how to make a thumb or little finger. To study these questions, Leo is using axolotls (regenerative salamanders) as a model. Inspired by the extensive transgenic toolkit available in *Drosophila*, Leo developed novel transgenic axolotls that fluorescently label positional information or cell proliferation directly in living tissues *in vivo*. His work has shown that, although positional information was previously thought to be fixed, it can be overwritten by transient external treatments, with lasting impacts on tissue patterning following repeated injuries. He is excited about the potential implications for synthetic tissue engineering. Towards this direction, Leo is developing a 3D tissue culture model using regenerative axolotl cells, which will be amenable to quantitative perturbations and synthetic studies that are difficult at present to perform *in vivo*.

**Michiel Vermeulen**

Michiel Vermeulen is a professor of Molecular Biology and investigates gene expression regulation in health and disease using integrative omics approaches. He is a pioneer in the application of quantitative interaction proteomics to identify and characterize proteins that specifically interact with epigenetic modifications. He has also published the first global interactomes for the mRNA modification m6A, ubiquitin linkages, and ADP ribose linkages. Additionally, he has conducted extensive research on chromatin-associated proteins, elucidating their roles in regulating embryonic and adult stem cell differentiation. With >190 scientific publications, many of which are featured in leading journals, he has made significant contributions to the field. Major obtained grants include NWO-VIDI (2009), NWO VICI (2021), ERC StG (2012), and ERC CoG (2017), in addition to various other national and international grants. In recognition of his achievements, he was elected as a member of Academia Europaea in 2019 and as member of EMBO in 2022.

# Abstracts - Invited speakers

Table of content (by order of appearance in the program):

**Cultured type 3 innate lymphoid cells for adoptive transfer are differentially affected by commonly used drugs for graft-versus-host disease prophylaxis**

Myrddin W. Verheij[1], Floor Baas[1], Ingrid Bulder[1,2], Jolien M.R. van der Meer[1], Marion Kleijer[1], Saïd Z. Omar[3,4], Nienke J.E. Haverkate[3,4], Mette D. Hazenberg[1,3,4,5,6], Bianca Blom[3,4], Carlijn Voermans[1,6]

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[4]Amsterdam Institute for Infection and Immunity, University of Amsterdam, Amsterdam, The Netherlands
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[6]Department of Hematology, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, the Netherlands

Type 3 innate lymphoid cells (ILC3) are important in tissue homeostasis. In the context of allogeneic hematopoietic stem cell transplantation (HSCT), we and others have shown that better reconstitution of NKp44$^+$ ILC3 after transplantation as well as a relatively high number of NKp44$^+$ ILC3 in the hematopoietic cell graft correlated with a reduced incidence of acute GvHD. Nonetheless, most ILC are depleted by remission induction chemotherapy and reconstitution of ILC after allogeneic HSCT is slow. To this end, we set up a culture protocol for the generation of IL-22-producing NKp44$^+$ ILC3 from hematopoietic stem and progenitor cells (HSPC) derived from cord blood (CB) or mobilized peripheral blood (MPB), with the aim of adoptively transferring ILC3 in allogeneic HSCT recipients. However, patients are typically treated with different immunosuppressive drugs for GvHD prophylaxis (e.g. methotrexate, mycophenolic acid, sirolimus, cyclosporin and ATG), and the potential effects of these drugs on adoptively transferred or developing ILC3 is unknown.
Here, we cultured ILC3 using CB- and MPB-derived HSPC in the presence of the drugs mentioned above. We found that the highest concentrations of mycophenolic acid (2,5 µM) and ATG (100 µg/ml) and all tested concentrations of methotrexate (4 nM, 20 nM, 100 nM) significantly inhibited the generation of ILC3. Interestingly, methotrexate did not seem to significantly affect fully differentiated ILC3 after 6 days of incubation.
Together, our data indicate that most of the commonly used immunosuppressants for GvHD prophylaxis do not seem to severely impact ILC3, showing promise for their application in adoptive transfer.

**Multimodal single-cell analysis of human pediatric bone marrow reveals age-dependent differences in lymphocyte progenitor cell differentiation**

Evelyn S. Hanemaaijer1,‡, Konradin F. Müskens1,‡, Ireen J. Kal1‡ , Saikumar Jayalatha A.K. 1 , Brigit te Pas1 , Patrycja Fryzik1 , Nina Epskamp1 , Aleksandra Balwierz1 , Tito Candelli1 , Wim J. de Jonge1 , Thanasis Margaritis1,‡,*, Mirjam E. Belderbos1,$,‡,*

1 Princess Máxima Center for Pediatric Oncology, Utrecht, 3584 CS, The Netherlands

‡ These authors contributed equally

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$ Lead contact

Childhood poses unique, age-specific challenges to the hematopoietic system, including rapid growth and exposure to infections and environmental antigens. However, the evolution of the pediatric hematopoietic system during ageing remains incompletely understood. Here, we generated a comprehensive map of pediatric and adult bone marrow (BM), encompassing multiomic single cell (sc-) mRNA and surface protein expression (n=9, 90710 cells). We find young pediatric BM predominantly composed of B-lineage cells and transitions towards a higher prevalence of myeloid and T cells at around adolescence. Analysis of differential gene and protein expression revealed two transcriptionally and phenotypically distinct subsets within lymphoid progenitors (LyP): stable LyP (LyP-S) and B cell-biased LyP (LyP-B). LyP-B expresses transcription factors characteristic for B-cells, namely EBF1, BACH2 and PAX5, and had surface expression of CD127. In contrast, LyP-S expresses a more balanced transcriptional program, lacking the B-cell transcription factor expression, and had no surface expression of CD127. Remarkably, the relative abundance of LyP-B decreases from childhood to adolescence, corresponding with a systemic transition towards myeloid output. Preliminary results on the molecular mechanism supporting the LyP subtypes across different ages as well as spatial transcriptomics validating these pathways will be available at the DSSCR meeting. Overall, this study provides a comprehensive single-cell resource for understanding healthy hematologic development and provides a basis for future studies of early-life perturbations underlying hematologic diseases.

**Hematopoietic organoids from human induced pluripotent stem cells: mimicking hematopoietic development in a dish**

Liza Dijkhuis[1], Edurne Solabarrieta[1], Eszter Varga[1], Emile van den Akker[1], Gerald de Haan[1,2], Arthur Flohr Svendsen[1,2]

[1]Sanquin Research, Landsteiner Laboratory, Amsterdam, The Netherlands.
[2]Amsterdam UMC, Department of Clinical Hematology Amsterdam, The Netherlands.

To bridge the gap between the need and the availability of source material for hematopoietic stem cell (HSC) transplantations, novel approaches are needed. Alternatively, HSCs could be obtained from autologous human induced pluripotent stem cells (hiPSCs) to provide a patient-derived source, thereby overcoming this therapeutic bottleneck.
We aim to recapitulate *de novo* HSC generation using a minimal-cytokine input hiPSC-derived 3D differentiation model. We have optimized this system, which relies on spontaneously inducing embryoid-body structures, which organize into hematopoietic organoids, producing hematopoietic progenitor cells. Given the fact that these progenitors give rise to functional enucleated red cells (Varga, *in preparation*), we hypothesize that the protocol mimics *in vivo* hematopoietic development to some extent. We then explored whether this model can also be harnessed to obtain functional HSCs. Firstly, we immunophenotypically characterized these hematopoietic organoids and observed an early hematopoietic stem progenitor cell (HPSC)-like $CD34^{+}CD43^{+}$ population. Next, we performed a more in-depth and complete detection of populations within the organoids by scRNA-seq. We identified multiple non-hematopoietic (neural, stromal, and endothelial) in addition to hematopoietic lineages, the latter including an HSPC-like population in line with our flow cytometry data. This population shows transcriptomic enrichment of *in vivo* bona fide 'Nascent HSC signature', corroborating our hypothesis that throughout our 3D differentiation protocol, a small but robust population arises that resembles early definitive HSCs.
Finally, functional characterization of this HSPC population will shed novel insight into in vitro HSC generation, addressing fundamental biological questions but also will assist in paving the way to iPSC-derived next-generation therapies.

**Transfusion-ready red blood cell generation From HUMAN Induced Pluripotent stem cells**

Eszter Varga[1], Renuka Ramlal[1], Athina Chavli[1], Ruthmila Paskel[1], Eelke Brandsma[1], Marieke Klijn[2], Cees Haringa[2], Kerly Fu[1], Julien Karrich[1], Adrien Laurent[1], Richard A. Flavell[3,4], Derk Amsen[1], Marten Hansen[1], Marieke von Lindern[1] and Emile van den Akker[1]

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[2]TU Delft, Faculty of Applied Sciences, Department of Biotechnology, Delft, The Netherlands
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[4] Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT, United States

There is constant need of blood products that are generally rely on blood donations, although the world-wide demand does not meet availability. *In vitro* red blood cell (RBC) production would substantially supplement this need and would provide further benefits such as thoroughly screened products, possibility of genetic manipulation and therapeutic loading. IPSC is a promising cell source to derive transfusable RBCs and blood products due to their immortality, donor independency, availability in GMP-grade and as universal source (e.g. HLA-matched, blood group matched). However till to date the field lacks a sufficient iPSC differentiation protocol, which can reach required erythroid yield suffice transfusion, due to developmental immaturity, inefficient enucleation (5-25%) and traditionally applied small-scale, static culture conditions. RBC-transfusion products generally contain large cell quantity (10^11-12 cells/unit) and to be able to propagate that, feasible iPSC differentiation with high enucleation and scalable, suspension culturing allowing bioreactor applications is required. Here we describe a 3-phase iPSC to RBC differentiation platform and their translation process from static/adherent to dynamic/suspension culture condition, allowing scalability and eventual bioreactor application. Our optimized dynamic/suspension system yields ~4000 RBC/iPSC with a consistent 40-70% enucleation rate, thus a mini-transfusion (10^11 RBC, required in phase I trial) according to our calculation could be generated from 5,4x10^7 iPSC in bioreactors. The iRBCs derived here, exhibit *bona fide* function, as demonstrated by both *in vitro* and *in vivo* assays. Transfusion experiments with purified enucleated iRBCs into humanized MISTRG mice revealed comparable stability and clearance rates to native erythrocytes. Translation to bioreactors, including novel bioreactor design, culturing parameters, feeding regimen, in house GMP-grade media development is momentarily ongoing.
In conclusion, an efficient 3-phase iPSC-RBC differentiation and their translation to dynamic culturing described here for the first time, provides a bridge from small-scale static culturing to large-scale bioreaction RBC production facilitating clinical transfusion application.

**Navigating Human Astrocyte Differentiation: Direct and Rapid one-step Differentiation of Induced Pluripotent Stem Cells to Functional Astrocytes Supporting Neuronal Network Development**

Imke M.E. Schuurmans[1,2], Annika Mordelt[3,4], Katrin Linda[3,4,5], Sofia Puvogel[3,4], Denise Duineveld[3,4], Marina P. Hommersom[3,4], Lisa Rahm[3,4], Emma Dyke[3,4], Gijs-Jan Scholten[3], Caroline Knorz[3,4], Astrid Oudakker[3,4], Hans van Bokhoven[3,4], Lot D. de Witte[3,6], Clara D.M. van Karnebeek[2,7], Alejandro Garanto[1,3] and Nael Nadif Kasri[3,4]

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Astrocytes play a pivotal role in neuronal network development. Despite the well-known role of astrocytes in the pathophysiology of neurologic disorders, the utilization of human induced pluripotent stem cell (hiPSC)-derived astrocytes in neuronal networks remains limited. Here, we present a streamlined one-step protocol for the differentiation of hiPSCs directly into functional astrocytes without the need for ectopic gene expression or neural progenitor cell generation. We found that culturing hiPSCs directly in commercial astrocyte medium, was sufficient to differentiate hiPSCs into functional astrocytes within five weeks. Validation to varying extents across thirty hiPSC-lines demonstrated consistent astrocyte differentiation with minimal batch-to-batch variability. We confirmed astrocyte identity and functionality of the hiPSC-astrocyte monocultures by immunofluorescence, flowcytometry, RNA sequencing, glutamate uptake assays and calcium signaling recordings. Also in the context of disease, these astrocyte recapitulated biochemical parameters of several neurometabolic diseases. Optimization of the protocol enabled co-culture of hiPSC-astrocytes with Ngn2 hiPSC-derived neurons (iNeurons), promoting neuronal differentiation and synapse formation. Lastly, we used single-cell electrophysiology and multi-electrode arrays to confirm robust neuronal network development in 5-week-old hiPSC-astrocyte and iNeuron co-cultures. This protocol offers a rapid and efficient method to establish all-human astrocyte-neuron co-cultures, facilitating the investigation of cell-type-specific contributions to disease pathogenesis. While validated across multiple hiPSC lines, we aimed to develop a live document in bioRxiv, in which we invited other labs to follow the protocol and report their experiences. The first responses received reported similar results, highlighting the robustness of this protocol.

**Insights into iPSC population Dynamics: characterizing the "Village-in-a dish" model**

Anna Bertocci[1] , Mehrnaz Ghazvini[2] , Pierangela Chiafele[3] , Miao-Ping Chien[3] , Mike Broeders[1] , Joyce van Meurs[1,4], Roberto Narcisi[1]

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The use of induced pluripotent stem cells (iPSCs) for in vitro population genetics has gained popularity, enabling the modeling of complex diseases. However, traditional approaches are often time-consuming, expensive, and subject to technical variability. To overcome these limitations, the "village-in-a-dish" strategy has emerged, where iPSCs from multiple donors are cultured and differentiated together in a single dish. This platform increases throughput, reduces variability, and lowers costs, making it particularly useful for population genetic studies that require a high number of donors, as well as for investigating hard-to-access tissues, and developmental processes that are difficult to investigate in vivo. Despite its growing popularity, limited literature exists on the characterization of this approach. To address this gap, we investigated the effects of co-culturing cells from different donors in the same dish from multiple perspectives: qualitative, using cytoplasmic dyes to understand iPSC cluster composition; quantitative, by understanding each donor's contribution during culture; transcriptional, employing a single-cell selection pipeline to sort and sequence phenotypes of interest. Our results show that iPSC clusters may form from single or multiple donors, with varying compositions, and that the village setting does not affect the growth rates of the lines.

**Coordination of mouse somitogenesis by coupling of the segmentation clock with cell cycle progression**

Marek van Oostrom, Yuting Li, Wilke H. M. Meijer, Tomas Noordzij, Erika Timmers,Charis Fountas, Jeroen Korving, Wouter M. Thomas, Benjamin Simons and Katharina Sonnen

Hubrecht Institute, Utrecht

In developing organisms a balance between differentiation and proliferation of cell populations exists. Even though an organism is growing exponentially, the underlying systems of development maintain a reproducibly robust patterning of tissues. Yet, how patterning is coordinated with growth to result in tissues with proper proportions of cell populations remains unclear. To study the relation between growth and patterning we use somitogenesis as a model.

Embryos of segmented animals undergo somitogenesis by periodically segmenting a piece of the presomitic mesoderm (PSM). This results in rhythmic differentiation of new somatic tissue. This differentiation is induced by rhythmic gene expression, known as the segmentation clock, and can be visualized as waves of oscillatory gene activity that run from posterior to anterior PSM. These end at the last somite formed and initiate new somite formation. During the time it takes for a segmentation clock wave to reach the anterior and form a single somite pair, the PSM cell population is maintained by proliferation. Within this project we aim to study the fundamentals of exactly this balance.

In our study, we track single cells in the mouse embryonic tail and extract quantitative data of proliferation and segmentation clock from lightsheet microscopy timelapses. We find correlations between the cell cycle and segmentation clock using Fucci reporters in the single PSM cells from the embryo. Subsequently, using microfluidics we entrain the signalling dynamics of the segmentation clock or cell cycle and show that there is bidirectional feedback coupling both processes. Lastly, we use a human in vitro system that resembles axial elongation known as ‘Axioloids’ to uncover the mechanism of the coupling between proliferation and segmentation clock.

**Molecular basis of development of three hearts in cephalopods**

Saskia Heffener [1], Rebecca R. Snabel [1], Karen Crawford [2], Caroline B. Albertin [3],
Gert Jan C. Veenstra [1,]

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Cephalopods are unique among molluscs with innovative and unique features such as a large nervous system, advanced vision, active camouflage and complex behavior. In an interesting case of convergent evolution with vertebrates, they have a dual closed circulation, contrary to other molluscs. The dual circulation is mediated by three hearts: two branchial hearts that pump oxygen-poor hemolymph to the gills, and a systemic heart that pumps oxygenated hemolymph to the soma.

We have explored the cell types and developmental origins of the three hearts of the longfin inshore squid *Doryteuthis pealeii*. We have data to support a common embryonic origin of the three hearts in paired cardiogenic primordia. We show how the circulatory system emerges from an early embryonic circulation governed by the yolk sac, and have documented gene expression patterns associated with the development of the three hearts. Distinct and coordinated patterns of differentiation, morphogenesis and migration contribute to the development of the branchial and systemic hearts. Using CRISPR-Cas9 we have knocked out key regulators such as GATA and NKX2 and show that they are essential for heart development. Using single-nucleus RNA and chromatin accessibility sequencing (snRNA-seq, snATAC-seq) we identify the cell type contributions of the branchial and systemic hearts and the regulatory motifs associated with their core transcription factor networks. Together these data shed important new light on the evolutionary innovations associated with heart development in squid.

**Epithelial tension controls intestinal cell extrusion**

Daniel Krueger†1*, Willem Kasper Spoelstra†2, Rutger Kok2, Dirk Jan Mastebroek1, Mike Nikolaev3‡, Marie Bannier-Hélaouët1,5‡, Nikolche Gjorevski 3‡, Matthias Lutolf3‡, Johan van Es1, Jeroen van Zon2*, Sander Tans2,4*, Hans Clevers1,5*§

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Cell extrusion is essential for self-renewal of the intestinal epithelium and must be executed without compromising the epithelial barrier. The prevailing view holds that cell extrusion is triggered by crowding-induced compression of cells at the intestinal villus tip. We show that tension, rather than compression, regulates cell extrusion in the intestinal epithelium. Combining optogenetic induction of tissue tension, genetic inhibition of myosin-II activity and local disruption of the basal actomyosin cortex within individual cells, we reveal that a supracellular and pulsatile actomyosin network generates tension throughout the intestinal villi, including the villus tip region. Mechanically weak cells with inability to maintain this tension drive their own extrusion. Our observations challenge the previous model for cell extrusion in intestinal homeostasis and maintenance of the epithelium's integrity.

**Single-Cell Profiling Reveals a Pro-regenerative Function of Notch Signaling in Glandular Organoids**

Davide Cinat1,2, Rufina Maturi2,3, Jeremy P. Gunawan1,2, Anne L Jellema-de Bruin1,2, Laura Kracht4 , Paola Serrano Martinez5 , Yi Wu6 , Abel Soto-Gamez1,2, Marc-Jan van Goethem1 , Inge R. Holtman2 , Sarah Pringle7 , Lara Barazzuol1,2, Rob P. Coppes1,2

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The establishment of organoid models has greatly advanced our understanding of the mechanisms regulating adult stem/progenitor cell dynamics and function. However, identifying these cells and their regulatory pathways in organs like the salivary glands, where stem/progenitor cells are scarce and often quiescent, remains challenging. Here, we performed single-cell RNA sequencing to profile cellular populations and signaling pathways in mouse salivary gland organoids (mSGOs) at different temporal stages and in response to radiation damage. Analysis of 7- and 11-day-old mSGOs revealed two potential salivary gland stem/progenitor cell populations, as well as basal duct cells, luminal duct cells, cycling cells, myoepithelial-like cells and pro-acinar cells, closely resembling in vivo tissue composition. Notably, bulk ATAC-seq and pseudotime analyses identified Sox9 and Itgb1-expressing cells as the most primitive populations within mSGOs. Cell-cell interaction analysis showed increased Notch signaling in these populations, which was further upregulated after irradiation. Interestingly, inhibition of Notch signaling using the γ-secretase inhibitor DBZ impaired organoid growth and induced premature differentiation in both mouse and human SGOs, underscoring Notch's critical role in maintaining self-renewal and preventing stem/progenitor cell exhaustion in regenerative conditions. These findings were extended to murine and human mammary and thyroid organoids, highlighting a conserved role of Notch signaling in glandular tissue regeneration. In summary, our data identified novel salivary gland stem/progenitor cell populations and revealed a pro-regenerative role of Notch signaling, suggesting its potential as a therapeutic target to improve radiotherapy outcomes.

This work was supported by the Dutch Cancer Society KWF Grant nr: 12092 and IBA Grant nr: PPP202

**Identifying the regulatory potential of 7 million SNPs in human neural stem cells**

Eline Koornstra1, Eva van Zanten2,3, Noud Klaassen4,5, Lize Meert1, Mariana Pelicano de Almeida1, Sander Lamballais2, Mike Dekker1, Miguel Martinez Ara4,5, Mathias Eder4,5, Hieab Adams3, Bas van Steensel4,5, Raymond Poot1

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Genome-wide association studies (GWAS) have identified thousands of single nucleotide polymorphisms (SNPs) associated with neurodevelopmental and psychiatric diseases. The large majority of these SNPs are located in non-coding regions of the genome and are therefore thought to contribute to disease risk by regulating transcription. However, identification of the causal variant remains challenging due to linkage disequilibrium, where neighboring SNPs are inherited together. Furthermore, the onset of many brain diseases during neurodevelopment necessitates the use of relevant cellular models to evaluate the regulatory impact of GWAS-identified SNPs. Massively parallel reporter assays (MPRAs) have emerged as a tool to analyze SNPs for their regulatory potential, and thereby aid in identifying causal SNPs. Here, we perform an MPRA called survey of regulatory elements (SuRE) in human neural stem cells (hNSCs) to assess the transcriptional regulatory potential of 7.1 million SNPs. We identify 7,002 putative regulatory SNPs (raQTLs), increasing the number of putative regulatory variants in neural cell types by an order of magnitude. raQTLs enrich for DNase hypersensitive sites, enhancers and transcription factor binding sites (TFBS), with some TFBS more sensitive to allelic changes than others. Overlapping SuRE data with eQTL data can pinpoint likely gene-regulatory variants. Similarly, overlap with GWAS for seven neuropsychiatric phenotypes reveals 187 raQTLs with a potential role in disease risk. Our SuRE data thereby provides a rich source to assign genetic variants relevant for disease.

**A simplified co-culture reveals altered cardiotoxic responses to doxorubicin in hPSC-derived cardiomyocytes in the presence of endothelial cells**

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Abstract

Cardiotoxicity is a significant challenge in cancer therapies, particularly with doxorubicin, a widely used anthracycline known for its broad anti-cancer spectrum but life-threatening cardiac side effects. There is a critical need for more predictive in vitro models to understand doxorubicininduced cardiotoxicity and patient-specific drug responses. In this study, we used human pluripotent stem cell (hPSC)-derived cardiomyocytes (hPSC-CMs), cardiac fibroblasts (hPSC-cFBs) and endothelial cells (hPSC-ECs) to investigate the cardiotoxic effects of doxorubicin in two-dimensional mono- and multi-cell type cultures. By mimicking the cumulative dose effect seen in patients through repeated doxorubicin treatments and using a machine learning-based in silico image analysis tool, we could precisely quantify caspase 3/7 activity as an early toxicity marker and identify hPSC-CMs in multi-cell type cultures. This innovative approach allowed continuous monitoring of apoptosis from phasecontrast images, revealing that hPSC-ECs showed higher sensitivity to doxorubicin than isogenic hPSC-CMs or hPSC-cFBs and significantly enhanced cardiomyocyte toxicity in co-culture. In contrast, dermal fibroblasts differentiated from the same hPSC line showed no toxic response under the same treatment regimen. These results challenge the conventional focus on cardiomyocytes as the primary target of drug-induced cardiac damage. Our findings not only highlight the complex interplay among different cardiac cell types in mediating the toxic effects of doxorubicin, but also demonstrate the potential of AI-enabled tools to advance personalized drug screening and safety assessments

# Abstracts – Submitted abstracts

**HeLLO: Hepatocyte-like liver organoids for drug toxicity prediction**

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Using whole transcriptome and cell identity analysis, we previously found that many liver *in vitro* models do not fully represent hepatocytes, rendering the cells incapable of modeling a substantial proportion of liver functions. We developed an improved liver organoid culture protocol to allow establishment, expansion, and biobanking of hepatocyte-like liver organoids (HeLLOs) from any liver tissue. Whole transcriptomic analysis showed that HeLLOs displayed high liver resemblance in important hepatic functions such as drug metabolism, fatty acid metabolism, and bile secretion. Accordingly, HeLLOs display high enzymatic activities for drug metabolism and fatty acid metabolism and robustly recapitulate functional bile acid transport compared to the established liver organoid model. Furthermore, we demonstrated that HeLLOs predicted hepatotoxic drugs with twice higher sensitivity than 2D cultured primary human hepatocytes. We believe that HeLLOs represent an interesting model for drug toxicity prediction especially in the early phase of drug development where high throughput and reliable models are needed.

**Modelling human kidney malformation using iPSC-derived organoids model: anything but BOR-ing**

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Background
Congenital abnormalities of the kidney and urinary tract (CAKUT) are the main cause for pediatric kidney failure. This umbrella term embraces several rare diseases, amongst which branchio-oto-renal (BOR) syndrome is one of the most widely known yet still poorly understood. This syndrome affects both inner ear and kidney development, and is most commonly caused by mutations in the EYA1 gene, although penetrance mechanisms are unknown. Around 40% of BOR patients have a hypodysplastic kidney phenotype, however there is currently no human in vitro model to study this disease.

Aim
We aimed to investigate the capability of induced pluripotent stem cell (iPSC)-derived kidney organoids to model BOR syndrome, since they recapitulate kidney development and present nephron structures.

Methods
We generated iPSC lines from BOR-syndrome patients and generated kidney organoids. We performed immunohistochemical analysis, mRNA and protein quantifications at several time points during development and compared them to healthy controls.

Results
We found key genes in kidney development were downregulated in patient organoids, specifically at the developmental stage of metanephric mesenchyme. Moreover, patient-derived organoids showed reduced nephron structures compared to controls.

Conclusion
This research demonstrates for the first time the effects of BOR-causing mutations on human tissue, proving kidney organoids offer a novel avenue to study developmental kidney disease in vitro.

**Label free prediction of submerged primary airway epithelial cell staining**

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Fluorescence microscopy is a powerful technique for identifying cells or cellular structures. Researchers use fluorescence microscopy to analyse the topology, location and distribution of cells or cell structures and is both highly specific and highly sensitive. A large challenge however is that fluorescence microscopy is usually performed on fixed cells and has a maximum of 4-5 immunofluorescent markers. With the use of Artificial Intelligence (AI), we attempt to predict fluorescent staining on non-fixated and living cell cultures based on bright field microscopy. Automatic staining predictions in airway epithelium will allow for repeated measurements in a single well over long periods of time. Primary human airway cells are widely used to study various airway diseases, so we use airway basal stem cells obtained from healthy patients and patients with respiratory diseases such as cystic fibrosis, Primary ciliary dyskinesia. Here we developed convolutional neural networks (CNN) models to predict staining in conventional air-liquid interface cultures and submerged airway cultures. By stacking model predictions a theoretical unlimited amount of markers can be predicted on a single bright field image. We show that the developed AI models are able to predict cell stainings in airway cultures to a very high accuracy.

**Identifying cross-lineage dependencies of cell-type specific regulators in gastruloids**

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Correct gene expression levels in space and time are crucial for normal development. Advances in genomics enable the inference of gene regulatory programs that are active during development. However, this approach cannot capture the complex multicellular interactions that occur during embryogenesis. Compared to model organisms such as fruit flies and zebrafish, the growth of mammalian embryos in utero further complicates the analysis of cell-cell communication during development. However, in vitro models of mammalian development such as gastruloids can overcome this limitation. Using time-resolved single-cell chromatin accessibility analysis, we have delineated the regulatory profile during gastruloid development and thereby identified the critical drivers of developmental transitions. We observed that gastruloids develop from bipotent progenitor cells driven by the transcription factors OCT4, SOX2 and TBXT, and differentiate along two main branches. A mesoderm branch characterized by the TF MSGN1, and a spinal cord branch characterized by CDX1, 2, 4 (CDX). Consistent with our lineage reconstruction, ΔCDX gastruloids fail to form spinal cord. Conversely, Msgn1 ablation inhibits the development of paraxial mesoderm, as expected. However, this also abolished spinal cord cells, which is surprising given that MSGN1 is not associated with differentiation along this branch. Therefore, formation of paraxial mesoderm is required for spinal cord development. To validate this, we generated chimeric gastruloids using ΔMSGN1 and wildtype cells, which formed both spinal cord and paraxial mesoderm. Strikingly, ΔMSGN1 cells specifically contributed to spinal cord, suggesting that inter-tissue communication between paraxial mesoderm and spinal cord is necessary for the formation of the latter. Our work has important implications for the study of inter-tissue communication in development and how gene regulatory programs are functionally executed to form complex multicellular developmental structures.

**The roles of the Transcription Factors Atf3, Zfp711, and Bcl6b in the Early Stages of the Hemato-endothelial Development**

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**Abstract**
The role of three transcription factors (Atf3, Zfp711, and Bcl6b) that are upregulated early in the earliest stages of hematopoietic development were studied during early development using a knockout of each gene in mouse embryonic stem cells and their differentiation in vitro. The results show that loss of Atf3 affects the differentiation of many cell types including hematopoietic precursor cells immediately after the hemato-endothelial lineages split from the cardiac lineages and at later stages of development. Loss of Zfp711 only has no effect at the earliest stages of development but affects primarily the development of late mesoderm cell types. In contrast Bcl6b which is known to be important in specific types of endothelium has no effect at the earliest stages of development.
**Keywords:** Atf3, Zfp711, ZNF711, Bcl6b, Hematopoietic Development, Mesodermal Development, Erythro-Myeloid Progenitors, Endothelial-to-Hematopoietic Transition

**Adipose-Derived Stem Cells in Urethral Stricture Treatment: A Systematic Review of Preclinical Evidence**

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Abstract

Background: Adipose-derived stem cells (ADSCs) represent an abundant and accessible source of mesenchymal stem cells, recognized for their anti-inflammatory and pro-angiogenic properties. Urethral stricture disease (USD) is a common issue in men that impairs urine and seminal fluid flow, lowering quality of life. USD is characterized by excessive extracellular matrix (ECM) deposition due to inflammation and hypoxia, and may benefit from ADSC-based therapies by promoting tissue regeneration and repair. This review examines ADSC efficacy in preventing or reducing fibrosis, managing inflammation, and promoting vascularization in USD.

Methods: A systematic literature search was conducted in PubMed and Embase (May-June 2024) using related keywords. Studies were selected based on predefined criteria without restrictions on study subject or publication date. Outcomes of interest included the efficacy of ADSCs in mitigating fibrosis, promoting angiogenesis, and reducing inflammation. The SYRCLE risk of bias tool was used to assess study quality.

Results: Nine animal studies were included, all utilizing induced urethral stricture models. Interventions ranged from fat grafting and adipose derived stromal vascular fraction (AD-SVF) suspension injections to advanced delivery techniques and genetic modifications. Consistent beneficial effects were observed across methods, including more organized ECM, decreased collagen I and III expression, and increased expression of both angiogenic factors and anti-inflammatory markers. However, fat grafting showed contrasting results regarding angiogenic factors.

Conclusion: ADSC therapies show promise for USD treatment by addressing inflammation, fibrosis, and angiogenesis. This approach may reduce stricture recurrence and improve urethral reconstruction outcomes. However, more research is needed for clinical application in humans.

**Single-cell multimodal sequencing and spatial transcriptomics to dissect hematopoietic stem cell failure in pediatric MDS-RCC bone marrow**

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Pediatric myelodysplastic syndrome with refractory cytopenia of childhood (pMDS-RCC) is a poorly understood bone marrow (BM) disease, characterized by ineffective hematopoiesis and risk of progression to acute myeloid leukemia. To investigate the molecular, cellular and spatial mechanisms underlying pMDS-RCC, we applied single-cell proteogenomic analysis on diagnostic BM samples from pMDS-RCC patients (n=6, 44747 cells) and healthy children (n=7, 68094 cells).

Analysis of cell type composition revealed significantly decreased frequencies of hematopoietic stem and progenitor cells (HSPCs) and myeloid cells, and significantly increased frequencies of T-cells in pMDS-RCC compared to healthy controls. While T-cell frequencies were increased, their activation and exhaustion status appeared normal, suggesting that their accumulation is likely due to factors other than increased proliferation.

Interestingly, cell cycle analysis revealed a substantial increase in the percentage of HSPCs in the G2M phase in pMDS-RCC, potentially indicating a differentiation defect. To explore the molecular pathways underlying this defect, we compared differentially expressed genes. Several genes related to HSPC maintenance were significantly downregulated in HSPCs (e.g. AREG, SRGN) of pMDS-RCC HSPCs. Importantly, cell-cell interaction inference revealed a lack of both known (e.g. KIT-KITL) and novel interactions between pMDS-RCC HSPCs and the BM stroma, pointing towards a defect in supporting signaling within the pMDS-RCC BM.

This study provides the first comprehensive single-cell view of the pMDS-RCC BM, revealing cellular features linked to BM failure. Ongoing studies aim to spatially characterize these pathways in BM biopsies and validate targetable factors *in vitro*.

**Assessing the risk of tumorigenesis of pluripotent stem cell therapies**

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Human pluripotent stem cell (hPSC)-based therapies are rapidly reaching clinical application as potentially curative treatments for numerous human diseases, including type 1 diabetes. However, despite the initiation of clinical trials, there are still safety concerns particularly related to the risk of tumorigenesis. This is partly due to the accumulating evidence that hPSCs recurrently acquire (epi)genetic aberrations during long-term culture (i.e., culture adaptation), ranging from single-gene mutations to chromosomal copy number variations (CNVs), the latter most frequently within chromosome 1, 12, and 20. These recurrent CNVs are thought to arise due to increased expression of driver genes located in these specific chromosomal regions which confer a selective advantage. Concerningly, these aberrations are reminiscent of human cancers which has prompted extensive research into whether they are correlated to malignant transformation. Malignant human germ cell tumors (hGCTs) are of particular interest in this field as they arise from the deregulation of the pluripotent cells of the early embryo and share similar CNVs including amplifications of chromosome 12. We aim to investigate the underlying dynamics of the observed CNVs shared by hPSCs and human cancers (focused on hGCTs). This will involve targeted CRISPR-based alterations of the copy numbers of chromosome 1, 12 and 20 and modulating the expression levels of their putative driver genes in hPSC and hGCT cell lines. Additionally, the phenotypic effects of these alterations will be assessed, including their impact on a preclinical hPSC-derived model currently being developed as a treatment for type 1 diabetes (i.e., hPSC-derived pancreatic islets).

**Modeling the heart-brain axis using innovative organ-on-chip technology**

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The heart-brain axis regulates the heartbeat but it is also involved in pathologies such as arrhythmia and stress-induced cardiomyopathy. Current models lack translational value and/or physiological resemblance. Here we developed a heart-nerve-brain chip capable of recapitulating key (patho)physiological aspects of the human heart-brain connection with the use of human pluripotent stem cells (hPSCs). Cardiac tissue, vagus nerve cells and hippocampal brain organoids were implemented on a microfluidic chip.

A first generation chip was designed with two separated chambers for heart and brain and a channel for the vagus nerve utilizing micro milling. Both chambers were connected to the nerve channel via microchannels. Indeed, nerve tissue showed innervation of both organoids, validated by immunohistochemistry. Tissues were viable on chip for at least two weeks.

Because of these promising results, a second microfluidic chip was generated using photolithography. It is able to accommodate not only heart, brain and vagus (parasympathetic) nerve, but also has a channel to include sympathetic nerve tissue. Previously, receptors for both sympathetic and parasympathetic nerve stimulation were observed on the cardiac tissue. A disbalance between sympathetic and parasympathetic activity is often involved in cardiac arrhythmias, making this chip a highly relevant disease model. Furthermore, the new chip allowed neurite outgrowth without cell bodies passing through, thus achieving compartmentalization. The results presented here strongly substantiate the potential of our heart-brain axis on-a-chip for disease modelling and will be a powerful tool to further our understanding of the axis and its related pathologies.

**Human induced pluripotent stem cell-derived corneal organoids to dissect cornea development and disease**

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The cornea is the transparent tissue at the front of the eye, essential for protection and light refraction. It is composed of three main cellular layers: the outer epithelium, which acts as a protective barrier against outside insults; the stroma, providing support and transparency, and the inner layer endothelium, regulating hydration.

We aim to generate corneal organoids from human iPSCs and establish a new 3D in vitro model to be used for understanding cornea development and disease. We will use single cell RNA sequencing to elucidate the distinct cell types and pathways involved in this process.

Results show that, 2 months into differentiation, early cORG have a transparent “bubble-like” morphology, similar to other published reports, with expression of progenitor markers PAX6 and TP63 also confirmed by immunocytochemistry. Preliminary single cell RNAseq data shows the presence of progenitor cell populations of the 3 main cell types of the cornea, as well as other ocular cell types, like retinal or pigmented cells. Later timepoints are currently being analysed.

Our results suggest that cORG can mimic the human cornea development in vitro. We are now applying these models to a rare blinding disorder named aniridia, caused by haploinsufficiency of *PAX6,* which codes for an essential transcription factor for eye development. Aniridia-derived cORG will allow us to understand the role of PAX6 in cornea development and establish a valuable model for drug screening and development of novel therapeutic approaches.

**UMCG Functional Genomics Center (UMCG FGC)**

Marialucrezia Losito

The discovery of protocols to derive induced pluripotent stem cells (iPSCs) from individual patients has opened up unique opportunities to study genetic disease and to screen for new therapeutic interventions. The advent of CRISPR-Cas9 technology is expediting the possibility to efficiently generate patient-derived iPSC models or revert disease-causing mutations in patient-derived iPSCs.

The UMCG Functional Genomics Center (UMCG FGC) is an expertise centre that supports, trains, and advises researchers with iPSC- and CRISPR-Cas9-related experiments. Since 2016, the FGC has been involved in over 140 projects from more than 60 different research groups, generating over 120 human iPSC cell lines (starting from several types of human somatic cells) and providing full characterization of iPSC clones, with the additional option to introduce genetic modification using CRISPR-Cas9.

One of the major current aims of the centre is to optimize, strengthen and standardize iPSC generation and CRISPR genome engineering protocols, and to further increase the throughput of the iPSC pipeline by using automated procedures, which will lead to large-scale production of iPSC disease models in a time- and cost-effective manner. In combination with CRISPR genome engineering, this will enable the high-throughput generation of patient-specific stem cells with matched isogenic controls. In addition, the centre has unique access to cutting-edge single-cell DNA and RNA sequencing technologies, which will help phenotype disease models and to map heterogeneity in the cell populations thus improving treatment stratification and moving towards the development of personalized medicine.

Please contact us if you need assistance with your iPSC/CRISPR-related projects or if you want to take advantage of our high throughput iPSC generation pipeline.

**Effect of aneuploid cells on in vitro human embryo morphology**

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Early human embryo development can partially tolerate chromosomal abnormalities. After in vitro fertilization, up to 60% of pre-implantation embryos display chromosomal mosaicism, a mix of cells with different chromosomal constitution. While transferring mosaic embryos is associated with lower implantation rates and miscarriage risks, it can still result in healthy births. This study examines the developmental potential of mosaic embryos and the effect of aneuploid cells on early development. Trophectoderm biopsies were obtained from human embryos donated for research on embryonic day 5 (E5), and further cultured until day 8 (E8) in a time-lapse incubator, followed by immunofluorescence analysis of lineage markers. Shallow NextGeneration Sequencing assessed the chromosomal content of biopsies at E5 and the entire embryo at E8. Results showed that in only half of the 58 samples, the chromosomal content observed in the E5 biopsy could be confirmed at E8. The cytogenetic status correlated with total cell count and embryo size. Embryos found to be mosaic at E5 but diploid at E8, had comparable diameters to consistently diploid embryos. However, E8 aneuploid and aneuploid-mosaic embryos showed significant reductions in cell counts compared to diploid embryos. Cells positive for Epiblast and Hypoblast markers showed an altered peripheral or scattered spatial distribution. Time-lapse imaging also revealed striking differences in the developmental dynamics of abnormal (mosaic and aneuploid) versus normal embryos during this critical peri-implantation period. Overall morphological differences and pre-implantation dynamics offer insights into the impact of aneuploid cells in the developmental potential of mosaic embryos.

**Profiling of the Proximal Interactome of the Epigenetic Polycomb Repressive Complexes Reveals Wide Functional Crosstalk in Mouse Pluripotency**

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Abstract

Tight control of gene expression, achieved through the concerted action of transcription factors and chromatin modifiers, is critical for embryonic development. Polycomb repressive complexes PRC1 and PRC2 are multiprotein epigenetic complexes that play essential roles in establishing and maintaining cell identity. Recent findings suggest that activity of these complexes can be modulated by proteins that bind PRC1 and PRC2 weakly, transiently or that interact with these complexes through ancillary proteins. To identify such proteins, we profiled the proximal interactome (proxeome) of the PRC1 and PRC2 catalytic subunits RNF2 and EZH2, respectively, in mouse embryonic stem cells (mESCs) using endogenously (mini)TurboID tagging. This revealed >100 proteins proximal to PRC1 and PRC2, which mainly comprise transcription factors, transcriptional regulators and RNA binding proteins. Notably, the EZH2 proxeome included both PRC complexes, while the RNF2 proxeome only identified PRC1 subunits. More than half of the PRC2 proximal proteins are shared with PRC1, revealing the molecular constitution of Polycomb chromatin domains. We identified pluripotency-associated transcription factors as proximal to the PRC complexes, including NANOG, for which follow-up studies showed an intriguing interplay with PRC2. Finally, we compared the dynamic PRC2 and PRC1 proximal interactomes between ground-state mESCs, serum-cultured mESCs and embryoid bodies. We find a range of stage-specific interactors, which might be associated with PRC1 and PRC2 redistribution as observed during early mouse embryogenesis. Altogether, our comprehensive analysis uncovers the dynamic PRC1 and PRC2 proxeome during development, and their interplay. It showcases how epigenetic factors interact with key transcription factors to maintain cellular identity.

**The conserved Wnt target gene *Eva1c* encodes a Slit/Robo coreceptor required for intestinal stem cell survival and function**

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Stem cells maintain tissue integrity through constant proliferation and differentiation. In the intestinal crypt, several signaling pathways are necessary for proper homeostasis and stem cell functioning, including the Wnt and Slit/Robo pathways. The canonical Wnt signaling pathway has been studied intensively and is known to activate a transcriptional program in the stem cells that enables their stem cell potential. The Slit/Robo pathway, however, has received little attention, despite the fact that Slit and Robo are both exclusively expressed in the crypts and required for stem cell maintenance. We have recently shown that in the nematode *C. elegans*, the Wnt and Slit/Robo pathways are functionally linked through the Wnt-dependent expression of EVA-1, a co-receptor that is essential for Slit/Robo signaling. Here, we use mouse small intestinal organoids to study the mammalian EVA-1 ortholog EVA1C in intestinal stem cells. Similar to *C. elegans*, we find that *Eva1c* expression is Wnt-dependent, indicating that it is a Wnt target gene conserved from nematodes to vertebrates. We show that *Eva1c* is specifically expressed in stem cells in the crypt domains of differentiating organoids. Moreover, conditional deletion of *Eva1c* strongly reduces organoid growth and differentiation, a phenotype consistent with a defect in Slit/Robo signaling. Furthermore, bulk RNA-sequencing shows that loss of *Eva1c* induces organoid dedifferentiation to a fetal-like state. Based on these results, we propose a model in which the Wnt-dependent expression of EVA1C restricts Slit/Robo signaling activity to intestinal stem cells, that is required for their survival and function.

**Understanding the role of TP53 in the malignant behavior of human pluripotent stem cells**

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The malignant potential of human pluripotent stem cells (PSC) is not fully understood and its assessment currently relies on the evaluation of the cells' behavior *in vivo* upon their engraftment into mice (teratoma assay). Resulting tumors are histologically identical to human (malignant) Germ Cell tumors (hGCT), and thus the malignant potential of the engrafted lines are assessed according to hGCT pathology. *In vitro* however, long-term culture of PSCs can lead to (epi)genetic drift, potentially activating processes that resemble malignant transformation. Here we investigated the relevance of *TP53* mutations in the malignant transformation of hPSC, a key gene regulating cell cycle control and (epi)genetic stability and often found mutated in PSCs due to culture adaptation by comparing the implications of its depletion in hPSC and hGCT. With this goal, *TP53* knockout (KO) cell lines were generated through CRISPR-Cas9 technology of representatives of an embryonic (H9) and an induced (Lu07) PSC line, as well as previously two hGCT lines (2102Ep, NCCIT). Phenotypically, *TP53*-KO cell lines displayed proliferation rates similar to those of their isogenic wild-type counterparts, despite showing a greater colony formation capacity and ability to grow in an anchorage-free environment. We demonstrate that despite the loss of *TP53* expression and related depletion of *P21* expression (both mRNA & protein), the KO lines did not show changes in expression of the pluripotency and malignancy-associated miRNA-371 and miRNA-373. Similarly, we did not observe changes in the expression levels of pluripotency markers (*OCT4, SSEA3, TRA-1-60, NANOG*) in hPSCs, and bulk RNAseq did not show difference in expression of malignancy-associated genes. We also explored the effect of *TP53* depletion on cisplatin, gemcitabine and navitoclax sensitivity, DNA-damaging agents commonly used in clinics for the treatment of hGCTs. Loss of *TP53* led to increase of cisplatin and gemcitabine resistance of PSC lines, similar to clinical observations in hGCTs. Overall, this study shows that *TP53* mutations in PSCs *in vitro* can lead to a greater resilience of the cells and causing clinically relevant malignancy-associated traits, while not necessarily triggering their full oncogenic transformation.

**Long-term iPSC-derived retinal organoid maturation is required to model autosomal dominant retinitis pigmentosa caused by the common P23H rhodopsin variant**

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# Retina-on-Chip (RoC-ME) consortium

Retinitis Pigmentosa (RP) is an inherited disease causing progressive vision loss due to rod photoreceptor death. Rhodopsin (RHO) is a photopigment crucial for visual function. The dominant-negative c.68C>A (p.Pro23His, known as P23H) RHO variant is the most recurrent mutation underlying autosomal dominant RP with no therapy currently available. We generated iPSC-derived retinal organoids from control, and CRISPR/Cas9 gene-edited P23H-RHO iPSCs, to create a human disease model for this variant and study the molecular and cellular phenotype. We found that P23H-RHO retinal organoids followed a similar developmental pattern as control organoids and did not display any disease phenotype up to 210 days (DIV210). Interestingly, RHO protein was not localized at the outer segment-like structures both in control and P23H-RHO retinal organoids. Only after DIV280, we detected proper localization of RHO in control retinal organoids. Contrarily, in P23H-RHO organoids, the majority of RHO remained mislocalized. This resembled the mislocalization pattern that was previously reported in several knock-in rodent models and postmortem P23H-RHO human retinas. We also investigated the presence of oxidative and ER stress, as well as apoptotic markers at different time points. In summary, we developed a human-based retinal model for autosomal dominant RP. Our results highlight the importance of timing in modeling retinal diseases using retinal organoids and the challenges to fully recapitulate the disease phenotype. These findings contribute to the generation of novel in vitro retinal disease models that will help to further understand the underlying molecular mechanism, and accelerate the development of novel therapeutic interventions.

**Microtubules, pulling forces and delayed abscission in stem cells**

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The proper execution of metazoan development is dependent on the timely orchestration of division and differentiation. During the final step of cell division, termed cytokinesis, the cytoplasm is partitioned in two, leading to the formation of a cytoplasmic bridge that connects the daughter cells. Typically, cytokinesis is directly followed by abscission, the physical severing of the bridge. Pluripotent stem cells remain connected through cytoplasmic bridges for hours, delaying abscission. Upon exit from pluripotency, abscission speeds up. Currently, the mechanism that controls abscission speed remains unknown. Pulling forces on the bridge have been proposed to act as regulators of abscission. However, the origin of these pulling forces and how they affect the abscission machinery remains elusive. In this study we seek out to investigate how mechanical forces affect microtubule organization on the cytoplasmic bridge and its implication for abscission. As differences in mechanical forces may lead to changes in bridge morphology, we started out by characterizing microtubules throughout the maturation of cytoplasmic bridges in pluripotent mESCs. Our preliminary data revealed two distinct microtubule disappearance phenotypes prior to abscission. Currently, we are investigating the mechanism for these differences in microtubule remodeling.

**Mesenchymal Stem Cells Dampen Inflammatory Pathways and Enhance Chemotaxis in CD4+T Cells**

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Inflammation is a biological response orchestrated by immune cells to address harmful stimuli. When inflammation becomes prolonged or uncontrolled, it results in significant immune cell infiltration at the site of inflammation. CD4+ T cells are crucial in the progression of inflammatory processes, making the regulation of CD4+ T cell responses essential for resolving inflammation. Umbilical cord mesenchymal stem cells (MSC) are a promising therapeutic option for managing prolonged inflammation due to their immunomodulatory properties. We previously demonstrated that MSC induce an anti-inflammatory memory response in TCR-stimulated CD4+ T cells. The induction of a memory response by MSC is essential for ensuring that the immune system can effectively resolve inflammation, provide long-term protection, and mount a quicker response to previously encountered antigens. This rapid and targeted response is crucial in preventing the recurrence of excessive inflammation and in maintaining immune homeostasis over time. To further explore the mechanisms underlying this response, we performed bulk RNA sequencing on TCR-activated CD4+ T cells co-cultured with MSC. Our results showed that MSC induce the expression of chemotaxis-related genes in CD4+ T cells following TCR stimulation, such as CCL5, CXCL10 and GBP1. Additionally, MSC suppress the NF-κB and ERK signalling pathways, but not the p38-MAPK pathway. Notably, there was an increase in TNIP1 gene expression in MSC and T cell co-cultures which is intriguing since TNIP1 can shut down multiple signalling pathways simultaneously. Our results highlights a new avenue for exploring the immunomodulatory functions of MSC.

**Organoid screen identifies P53 reactivation for the attenuation of radiation-induced senescence.**

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Abstract

Background: Radiotherapy treatment significantly improves the survival of head and neck cancer patients. However, it often involves unavoidable irradiation of normal tissues surrounding the tumor, including the salivary glands. We have previously shown radiation preferentially induces cellular senescence in the salivary gland stem/progenitor cell niche contributing to hyposalivation. Clearance of senescent cells using senolytic drugs has been shown to potentally represent a therapeutic way to ameliorate radiotherapy-induced xerostomia.

Objective: To develop an efficient method to screen for potent senolytic drugs in a salivary gland organoid model and test for regeneration capacity following senolytic treatment.

Materials & Methods: (Sham-)irradiated salivary gland organoids were treated with different doses of candidate senolytic drugs. As a measure of senolytic activity a Caspase-3/7 fluorogenic substrate was added together with the selected drugs. Increases in salivary gland organoid apoptosis following treatment, inferred from Caspase-3/7 activity, were detected in real-time using an IncuCyte S3. Drug concentrations that efficiently induced apoptosis in irradiated cells but were not or less toxic to unirradiated cells were selected to further verify the elimination of senescent cells and corresponding changes in the organoid forming efficiency (OFE). The top candidate was tested in vivo using C57BL/6 mice treated 8-weeks post IR.

Results: Of the senolytics tested, Nutlin3a, an MDM2 inhibitor and P53 activator, increased organoid self-renewal capacity after irradiation and had the most favorable toxicity profile. Accordingly, treatment with Nutlin3a resulted in a reduction of senescent markers in irradiated organoids and a transcriptional increase in p53 target genes. Localized injection of Nutlin3a in irradiated mouse salivary glands elicited a regenerative response and resulted in a robust increase in saliva production.

Conclusion: We conclude screening for drugs that show senolytic activity without hampering stem cell expansion (as measured by OFE) may be a promising methodology for the identification of compounds with potential applications in post-radiotherapy regenerative medicine.

Using iPSC-derived retinal organoids and RPE to study lipid-related retinal disorders

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Lipids are essential for visual function. Yet, there are significant knowledge gaps regarding the specific role of different lipids in the normal and diseased retina. While murine models have been traditionally used for such studies, we aim to assess the suitability of human-based systems, specifically iPSC-derived retinal organoids (ROs) and retinal pigment epithelium cells (RPE), in modelling lipid-related retinal disorders.

For that, we differentiated control iPSC lines into RPE and ROs using both a rapid direct differentiation protocol (~140 days) and a 2D/3D combination protocol (~240 days). At defined time points, we harvested ROs and RPE samples for lipidomic analysis. In parallel, human retinal samples were collected for comparative purposes. This work is still ongoing, however preliminary results from a pilot study demonstrated that we can detect a wide range of lipids, including disease-associated lipid species, in ROs.

To further evaluate these models, we selected Sjögren-Larsson syndrome (SLS) as a proof-of-concept. Using CRISPR/Cas9, we generated two isogenic iPSC lines harboring two recurrent SLS mutations in the *ALDH3A2* gene and differentiated them into ROs and RPE. Additionally, patient-derived fibroblasts are currently being reprogrammed into iPSCs. Both SLS ROs and RPE differentiated successfully, exhibiting low ALDH3A2 protein levels. Currently, we are investigating possible molecular and biochemical phenotypes, by evaluating lipid content and cell-type specific markers.

Overall, these human-based models may provide valuable insight into lipid-related retinal disorders, facilitating the identification of novel disease mechanisms and potential therapeutic targets.

**Integration of hiPSC-derived macrophages in a 3D vessel-on-chip model**

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Perivascular macrophages (PVMs) are integral for tissue homeostasis and pathophysiology and are commonly defined as a subpopulation of tissue-resident macrophages that are either in direct contact or in close proximity to the abluminal (outer) surface of the blood vessels. Here, we integrated M0-like macrophages in a 3D vessel-on-chip (VoC) model comprised entirely of human induced pluripotent stem cell (hiPSC)-derived cells (hiPSC-ECs, hiPSC-VSMCs and hiPSC-M0s) within a fibrin hydrogel. We observed that vascular cells reproducibly formed interconnected networks with no apparent differences in their morphology when co-cultured with hiPSC-M0s; however, the vessel diameter was decreased, indicating a more capillary-like vascular network. Proliferation of hiPSC-M0s was only observed in medium supplemented with the hematopoietic growth factor MCSF. hiPSC-VSMCs behaved similarly in different VoC cultures, with no significant differences in the percentage of cells localized close to the vascular network. The majority of hiPSC-M0s were found in close association with the microvessels, resembling PVMs in vivo. In addition to that, immunofluorescent analysis highlighted the expression of key PVM markers in hiPSC-M0s (CD68, CD163 and LYVE1). Finally, we performed a quantitative cytokine-release assay, which indicated that the presence of hiPSC-M0s restricted fibrin-induced vascular inflammation. Thus, integrating hiPSC-M0s in a 3D vascular microenvironment promotes the acquisition of a more PVM identity, both phenotypically (interaction with the vascular network and PVM marker expression) and functionally (dampening of inappropriate inflammation). This multicellular microfluidic platform could be potentially used to further elucidate the role of the PVM population, especially in diseases involving vascular inflammation.

**Potential malignancy of stem cells: identification and characterization of York sac elements in experimental teratomas**

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Concern about the safety of pluripotent stem cell-based therapies, predominantly related to potential malignant transformation, is a big drawback in bringing these therapies to the clinic. The teratoma assay—a method in which stem cells are xenografted into immunocompromised mice—is currently the accepted (gold) standard for risk assessment. A pluripotent stem cell line is considered malignant when embryonal carcinoma-like cells are present in the xenograft. Elements of yolk sac tumor(-like cells) might also be present, but the significance of these elements for the safety of stem cell products is unclear. Human germ cell tumors containing yolk sac elements are considered highly malignant. This study aims to develop tools for identifying and further characterizing yolk sac tumor-like elements in experimental teratoma (xenograft-derived or *in vitro* generated) and in primary germ cell tumors. To this end, single-cell RNA expression profiles will be obtained from previously generated paraffin-embedded samples. This eliminates the need to re-perform xenograft assays, reducing costs, time and animal lives. Preliminary data shows that RNA of sufficient quality can be obtained from 20-year-old samples. The final goal is to find new transcriptional or molecular markers for stem cell-derived yolk sac-like elements and identify malignancy-related molecular pathways of value for risk stratification of pluripotent stem cell products.

**Towards an improved erythroid production from iPSC**

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Objectives: Rare but severe anemia's such as Sickle cell anemia and Diamond Black anemia syndrome are debilitating diseases that of yet have for many patients no cure available. Thus, new treatments are very much needed. Induced pluripotent stem cells (iPSC) are a promising source of new potential treatments as they can be differentiated into many different cell types, including erythrocytes. We have formulated a protocol that shows differentiation of human iPSC into hematopoietic stem and progenitor cells (HSPCs) and terminally differentiated effector cells such as erythrocytes. Unfortunately, so far *in vitro* differentiation has proven a significant challenge. This is mainly due to developmentally immature/non-adult hematopoiesis, mimicking aspects of spatio-temporal, independent hematopoietic waves during fetal development. Definitive waves of hematopoiesis originate from hemogenic endothelium (HE) in a process termed endothelial to hematopoietic transition (EHT). This occurs both in the yolk sac, producing erythro-myeloid progenitors (EMPs), and in the AGM-region, where the first hematopoietic stem cells (HSCs) are generated. Improved understanding of iPSC differentiation will provide ways to resemble definitive hematopoietic waves. This project aims to unravel the differentiation process and identify factors involved in the erythroid specification.

Methods and results: Directed hematopoietic specification from iPSC was induced by differentiation of iPSC colonies using specific growth factors and cytokines that support the formation of mesoderm, endothelial and hematopoietic cells in a temporal manner. Single cell RNA sequencing of disrupted iPSC derived hematopoietic organoids revealed the presence of hemogenic endothelium, endothelial (CD73+) and hematopoietic cells (CD43). To track endothelial (CD73+) or hematopoietic (CD43+) differentiation from HE within the differentiating hematopoietic organoid, specific reporter constructs have been generated. These constructs have been nucleofected in K562 cells which resulted in a knock-in efficiency of 3,98% and 4,90% for CD43 and CD73 constructs, respectively. Subsequently, iPS cell lines have been successfully generated with these constructs. In combination with a Cas9 expressing iPSC line, we will perform a CRISPR library screen to identify genes/TFs that control the erythroid differentiation during iPSC-derived hematopoiesis.

Conclusion: There is a need for new therapies and increased insight for rare anemia's. iPSC derived hematopoietic red blood cells but in future also hematopoietic stem cells could provide both a new treatment as well as new model systems to study those diseases. We will study iPSC derived hematopoiesis and erythropoiesis through the newly developed reporter cell lines.

**Endogenous tagging using a STRAIGHT-IN self-complementing split fluorescent protein approach in human induced pluripotent stem cells**

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Endogenous gene tagging of fluorescent reporters in human induced pluripotent stem cells (hiPSCs) is a valuable research tool to not only study protein expression and localization in undifferentiated cells, but also in hiPSC-derived differentiated cell types. However, generating these hiPSC lines is cumbersome as it requires cloning of a targeting construct, plasmid delivery often together with sgRNA-Cas9 RNP complexes targeting a gene of interest (GOI), followed by proper tag integration through inefficient homology-directed repair (HDR). Here, we utilized a self-complementing split fluorescent protein approach to efficiently and rapidly tag proteins in hiPSCs. Using our previously established STRAIGHT-IN platform, we inserted the large fragment of mNeonGreen2 (mNG2), encompassing the non-fluorescent first ten β-strands ($mNG2_{1-10}$) under a constitutive promoter into the *AAVS1* locus. Next, the eleventh β-strand of mNeonGreen2 ($mNG2_{11}$) was integrated at the N- or C-terminus of a GOI using CRISPR/Cas9 gene-editing. Molecular cloning is not required as $mNG2_{11}$ (48 nt) can be encoded within a standard, commercially synthesized single-stranded oligodeoxynucleotide (ssODN) donor template. In a proof-of-principle experiment we tagged the *HIST1H2BJ* gene at the C-terminus with $mNG2_{11}$ with very high efficiency. After clonal isolation, (dd)PCR analysis, and Sanger sequencing we confirmed the homozygous integration of $mNG_{11}$. Fluorescent microscopy showed hiPSCs with green nuclei, which is in agreement with nuclear H2B histone localization. In summary, we have developed an hiPSC line that can be utilized to swiftly and efficiently tag proteins with mNeonGreen2 enabling expression and functionality studies. We are currently expanding the color palette of self-associating split fluorescent proteins.

**The Balance Between Myocardial Strength and Mass**

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**Background**: The lack of understanding about true regeneration of the human heart is reflected in the current medical therapies for heart failure, that do not aim to cure the underlying deficit of functional cardiomyocytes. Whereas cell turnover in the adult heart is extremely low, during development embryonic signaling pathways regulate the robust increase in myocardial mass through cardiomyocyte duplication. Orchestration of these cell fate decisions require temporal and stage-specific specification and growth of the various myocardial compartments. This involves a tight regulation of the local cardiomyocyte proliferation and is directed by a network of signaling pathways.

**Hypothesis**: We investigate the contribution of the CHIR99021 (CHIR) small molecule via the Wnt/GSK3β/β-Catenin/TCF axis and Insulin via the Insulin/PI3K-AKT/FOXO route on the stage-specific effects for proliferation of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs).

**Goals**: Our study provides novel insights into the molecular signaling pathways that control the interplay between proliferation and maturation of cardiomyocytes during heart development.

**Methods**: We performed a combinatory screening for Akt (Insulin) and Wnt (CHIR99021) pathway activators in our previously described expansion media resulting in four treatment groups consisting of: Insulin-/CHIR99021-, Insulin+/CHIR99021-, Insulin-/CHIR99021+ and Insulin+/CHIR99021+. Next we performed automated sarcomere analysis, mitochondria function analysis, contractility assays, RNA sequencing and molecular experiments for TCF and FOXO in hiPSC-CMs.

**Results**: In this combinatory screen for Insulin and CHIR99021 we found synergistic effects on promoting proliferation of immature hiPSC-CMs, whereas the absence of these pathway activators led to rapid cell cycle exit, and metabolic (mitochondria) and structural maturation (sarcomeres) of hiPSC-CMs. Moreover, we uncover an interplay between Insulin and FOXO signaling that mediates this synergistic effect on the proliferation and/or maturation of immature hiPSC-CMs.

**Conclusions**: We provide novel insights in the cues to maintain cardiomyocyte proliferation versus maturation via an interplay between the Insulin/Akt/FOXO and Wnt/GSK3β/β-catenin/TCF signaling routes. The understanding of embryonic signaling routes would be of importance for future regenerative strategies in heart failure.

**Building an iPSC-based 3D Neuromuscular Junction on a Chip**

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The neuromuscular junction (NMJ) is a highly organised synapse that facilitates communication between a motor neuron and muscle fiber. Impairment of neuromuscular transmission may lead to muscle weakness, paralysis, or even death. In fact, NMJ defects are a common hallmark observed in many neuromuscular disorders, including amyotrophic lateral sclerosis, Duchenne muscular dystrophy, and Myasthenia Gravis. Therefore, the NMJ has been widely studied in a variety of models. However, many existing models have limitations: 2D cell cultures lack structural context and do not allow for the study of muscle contraction and function, while animal models often fail to accurately represent human genetic backgrounds and exhibit different pathophysiological characteristics when modelling neuromuscular diseases. We hypothesize that a human iPSC-derived 3D NMJ on a chip model can bridge this gap and can propel *in vitro* NMJ and neuromuscular disease research to the next level. To this end, we have developed an innovative chip design that supports the co-culture of human motor neurons and 3D muscle bundles. To optimize the functional (i.e. contraction), structural (i.e. morphology), and developmental outcomes (i.e. gene and protein expression) of the 3D NMJ model, we have optimized culture conditions and chip design. Our data show that the number of motor neurons, type, timing and combination of neurological growth factors and chip design significantly improved the longevity, strength and maturity of the model. At the conference we'll present the model as is optimized thus far with the ultimate ambition to use it for disease modelling and drug screening.

**EP300 facilitates human trophoblast stem cell differentiation**

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During early human placenta development, cytotrophoblasts differentiate into extravillous trophoblast and syncytiotrophoblast. Defective trophoblast differentiation may result in severe pregnancy complications, including early-onset fetal growth restriction and pre-eclampsia. The incidence of these complications is increased in pregnancies of fetuses affected by Rubinstein-Taybi syndrome, a developmental disorder predominantly caused by heterozygous CREBBP or EP300 mutations. Although the acetyltransferases CREBBP and EP300 are paralogs with many overlapping functions, the increased incidence of pregnancy complications is specific for EP300 mutations. We hypothesized that these complications have their origin in early placenta development and that particularly EP300 plays an important role in that process. We therefore investigated the role of EP300 and CREBBP in trophoblast differentiation, using human trophoblast stem cells (TSCs) and trophoblast organoids. We found that knockdown of EP300, and not CREBBP, blocked differentiation of TSCs into both the syncytiotrophoblast and extravillous trophoblast lineages. In fact, it resulted in proliferation of TSC-like cells under differentiation-inducing conditions. By transcriptome sequencing we found that several ligands for the EGF receptor were upregulated upon EP300 knockdown. This upregulation could partially explain the inhibitory effect of EP300 knockdown on differentiation, as blocking the EGF receptor rescued trophoblast differentiation to a large extent. These findings suggest that EP300 facilitates trophoblast differentiation by interfering with at least EGFR signaling, whereas its paralog CREBBP is not as important in this process. This points towards a crucial role for EP300 in early human placenta development, and may explain the increased incidence of complications seen in pregnancies of EP300-mutated fetuses.

**Unravelling The Fate and Impact of Aneuploidy on Early Human Embryo Development using Mosaic Blastoids**

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Only 30% of in vitro fertilization (IVF) cycles result in live birth, with chromosomal abnormalities significantly contributing to implantation failure and early pregnancy loss. Up to 90% of pre-implantation embryos are mosaic, characterized by the presence of cells with distinct karyotypes. There is limited insight into the risks posed by specific aneuploidies; even mosaic embryos with a high proportion of aneuploidies can result in healthy live births. The fate and impact of specific aneuploidies, as well as the mechanisms by which some embryos can compensate, remain unclear.

We use human blastoids, stem-cell-derived models of the blastocyst. Our improved blastoids include hypoblast and, in combination with an optimized post-implantation culture system, can accurately mimic peri- and early post-implantation development up to primary yolk sac formation. These blastoids exhibit morphogenetic behavior similar to human embryos, making them a robust model for our studies. Furthermore, we generated mosaic blastoids and observed that aneuploidies may interfere with developmental progression. To study multiple (specific) aneuploidies, we aim to use genetic barcoding, enabling high-throughput tracking of aneuploidies throughout early development.

This approach allows us to study how specific aneuploidies affect early human development and reveal when and how embryos may recover, providing insights for improving IVF success.

**Frequency encoding regulates cell type composition in the small intestine**

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Intestinal homeostasis requires tight regulation of stem cell maintenance and commitment to absorptive and secretory cells, two key intestinal lineages. While major signalling pathways critical for this control have been identified, how they achieve such a tight balance in cell type composition remains unclear. Here, we uncover dynamic expression of Hes1, a direct NOTCH target, in intestinal stem and progenitor cells, and investigate its role in vivo and in vitro. A knock-in reporter4 reveals distinct, cell-specific period lengths in Hes1 oscillations that form a gradient along the crypt-villus axis. Whereas secretory precursors oscillate at low periods, absorptive precursors oscillate at higher periods before transitioning to a differentiated state. To test the function of different frequencies, we innovated a microfluidic system that modulates Hes1 oscillations in organoids. We find that varying the oscillation frequencies modulates secretory cell differentiation: While 90-min oscillations promote Paneth cells, 130-min oscillations increase formation of other secretory subtypes. Moreover, low-frequency oscillations support stemness and a proliferative state. Our study provides the first clear evidence that information for tissue homeostasis in the intestine is encoded in the temporal dynamics of signalling components.

**Integrin-activating Yersinia protein Invasin sustains long-term expansion of primary epithelial cells as 2D organoid sheets**

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Matrigel®/BME®, a solubilized basement membrane-like preparation, is especially rich in the Extra-Cellular Matrix (ECM) protein laminin-111. This hydrogel supports the formation and long-term growth of epithelial 3D organoids from adult stem cells (ASC)[1,2]. Laminin-111 activates integrin β1 complexes, thus preventing anoikis and driving epithelial polarization. Here, we address if a bacterial integrin-activating protein can mimic Matrigel/BME. Enteropathogenic *Yersinia* bacteria invade human gut epithelial cells using their outer membrane protein Invasin. This protein binds and activates a diversity of integrin β1 complexes, including the laminin-111-specific α6β1 receptor. A recombinant 25 kDa C-terminal integrin-binding fragment of Invasin allowed adhesion of gut epithelial cells, when coated on culture plates. Upon addition of organoid growth factor medium, the epithelial cells grew out in 2D and could be expanded and passaged long-term. Polarization, junction formation and generation of various intestinal cell types (i.e. enterocytes, goblet cells, Paneth cells, and enteroendocrine cells) was stable over time. Sustained expansion of multiple other human-, mouse-, and even snake epithelia was accomplished under comparable conditions. The 2D culturing format holds advantages over the 3D 'in gel' format in terms of imaging, accessibility of basal and apical domains and automation for high throughput screening approaches. Invasin represents a fully defined, affordable, versatile, and animal-free complement to Matrigel/BME.

**Leveraging cell type-specific eQTLs to uncover miRNA-driven post transcriptional gene regulation in pancreatic islets**

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Post transcriptional gene regulation is an important way for cells to control mRNA levels and in turn cell identity. One of such mechanisms of gene regulation involves the binding of microRNAs (miRNAs) to the 3' untranslated region (UTR). Predicting gene-miRNA regulatory networks based on sequence complementarity alone faces challenges. We developed a computational method that uses single cell RNA sequencing (scRNA-seq) data to identify population-level 3'UTR genetic variants that behave as cell type-specific cis-expression quantitative trait loci (cis-eQTLs) and leveraged this information to infer miRNA-mediated gene regulation. We applied our approach in pancreatic beta cells from non-diabetic (ND) and type 2 diabetes (T2D) donors, which is a heterogeneous disease of beta cells caused by environmental and genetic factors. We found novel and known eQTLs in beta cells, which are dysregulated during T2D. Downregulation of 12 islet miRNAs in T2D uncovered by small RNA sequencing explained the behaviour of 15 eQTLs specific for beta cells of ND donors. Among those, we found and experimentally validated that miR-127-5p regulates PTEN expression in beta cells via the novel eQTL rs701848. Furthermore, we linked rs701848 to the activation status of the EGFR-PI3K-AKT signaling axis and the insulin secretory capacity of primary human islets. Our results demonstrate how disease-mediated changes can help identify miRNA-eQTL interactions in homeostasis.