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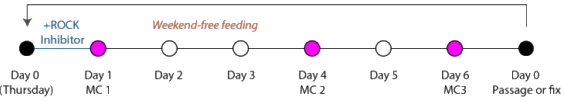

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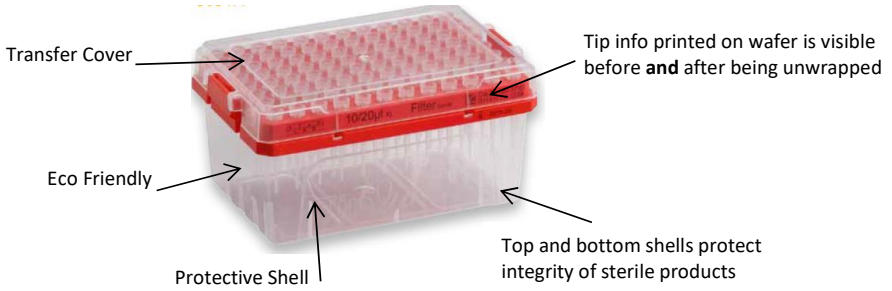

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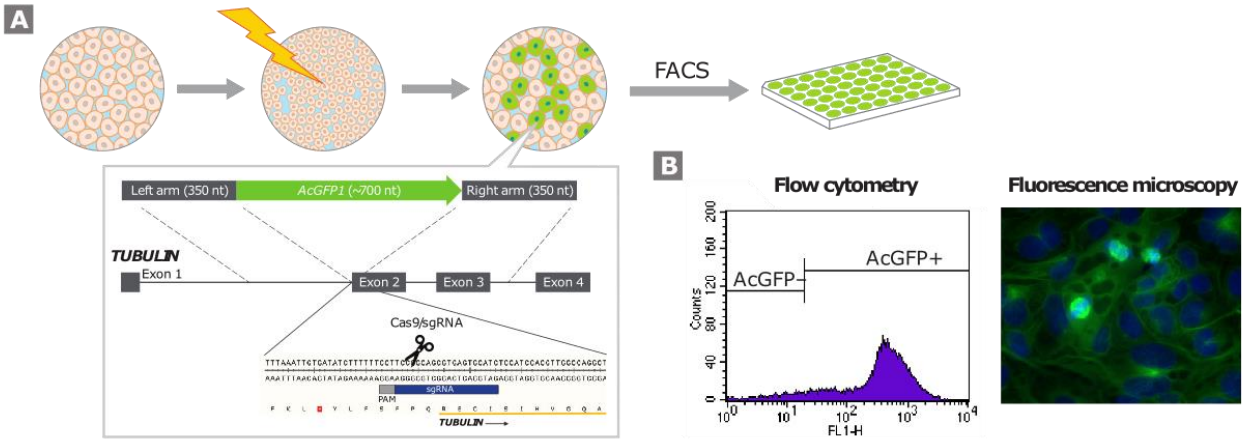

Panel A. Workf ow for targeted knockin of *AcGFP1* in the N terminus of tubulin. Cas9 ribonucleoprotein (RNP) complex and the donor template—a long ssDNA encoding *AcGFP1* with homology arms related to the *TUBA1B* site—were delivered using electroporation. Fluorescent cells resulting from a successful Homologous Directed Repair (HDR) were sorted and isolated as single cells using FACS. **Panel B.** AcGFP1+cells seeded using FACS were expanded to generate clonal cell lines, which were characterized by f ow cytometry, f uorescence microscopy, and Sanger sequencing (not shown) to verify the correct insertion of the template. A representative AcGFP1+clone is shown.

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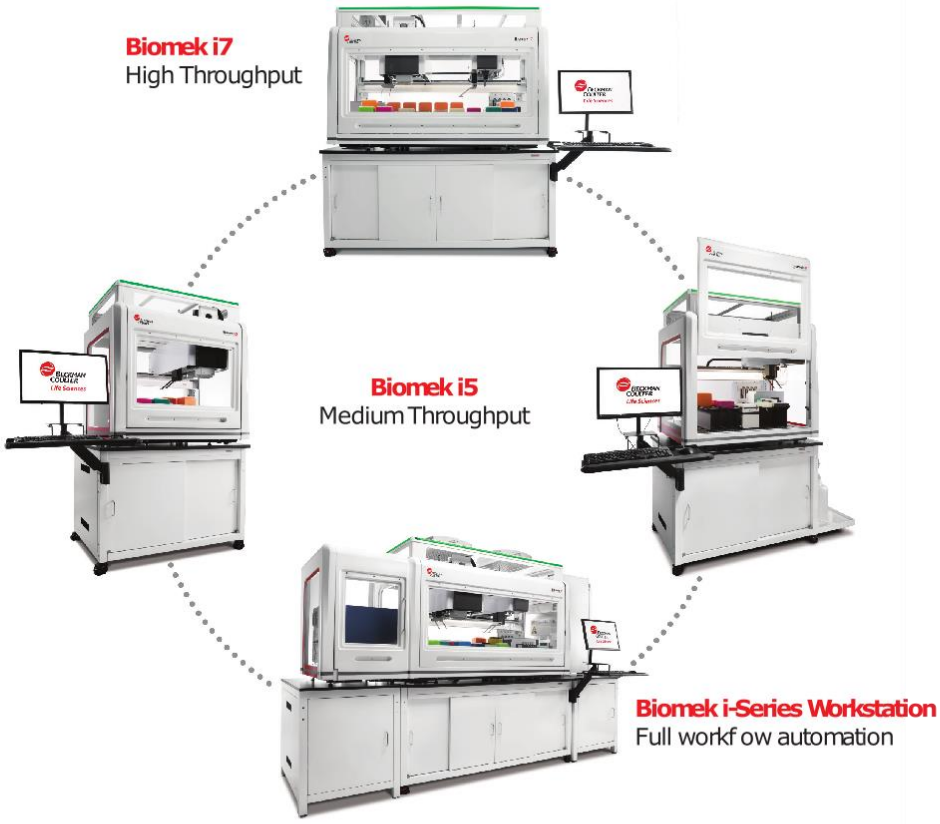

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PROGRAM

9:00 – 9:15 **CHRIS VAN GEET** (Vice Rector Biomedical Sciences Group, KU Leuven, Belgium)
"Welcome"

SESSION 1 Cellular Reprogramming
Session Chairs: Björn Heindryckx (UGent) - Vincent Pasque (KU Leuven)

09:15 – 09:50 **SIR JOHN GURDON** (University of Cambridge, UK)
"Nuclear reprogramming by Xenopus eggs and oocytes"
09:50 – 10:05 **ADRIAN JANISZEWSKI** (University of Leuven (KU Leuven), Belgium): Selected Oral
"Uncovering Mechanisms of Epigenetic Memory Reversal during Reprogramming to Induced Pluripotency"
10:05 – 10:20 **MICHELA BARTOCCETTI** (University of Leuven (KU Leuven), Belgium): Selected Oral
"Insights into late reprogramming: gene expression and DNA methylation dynamics define new stages surrounding the acquisition of naive pluripotency"
10:20 – 10:50 **COFFEE BREAK**

SESSION 2 Pluripotency, Model the Brain with Stem Cells, and Hematopoietic Stem Cell
Session Chairs: Mieke Geens (VUB) - Laurent Nguyen (University of Liege)

10:50 – 11:25 **AUSTIN SMITH** (University of Cambridge, UK)
"The Developmental Trajectory of Pluripotency"
11:25 – 11:50 **IRA ESPUNY-CAMACHO** (University of Milan, Italy)
"Human pluripotent stem cell-derived cortical neurons for disease modeling and brain repair"
11:50 – 11:55 **SPONSOR**: Filter Service
11:55 – 12:10 **ALESSANDRA QUARTA** (University of Antwerp, Belgium): Selected Oral
"The neural environment is a necessary, but not sufficient, prerequisite for directing iPSC-derived progenitors into the CX3CR1+CCR2- microglia-like phenotype"
12:10 – 12:25 **ADELINE ROSU** (University of Liege, Belgium): Selected Oral
"Translation stress induced by loss of Elongator activates a p53-dependent antitumor checkpoint in hematopoietic stem and progenitor cells"
12:25 – 12:45 **POSTER TEASERS**, selected from abstracts
12:45 – 14:45 **LUNCH AND POSTER SESSION**
14:00 – 14:15 **GENERAL ASSEMBLY**

SESSION 3 Regeneration and Transdifferentiation
Session Chairs: Claudia Spits (VUB) - Cedric Blanpain (ULB)

14:45 – 15:20 **ELLY TANAKA** (IMP Vienna, Austria)
"Coordination of signaling with stem cell dynamics during regeneration"
15:20 – 15:35 **JONATHAN BALDAN** (Free University of Brussels (VUB), Belgium): Selected Oral
"Human pancreatic adult acinar cells dedifferentiate towards an embryonic progenitor-like state in 3D suspension culture"
15:35 – 15:40 **SPONSOR**: Takara
15:40 – 16:10 **BREAK**

SESSION 4 Cancer Stem Cells and Skin Stem Cells
Session Chairs: Annelies Bronckaers (University of Hasselt) - Catherine Verfaillie (KU Leuven)

16:10 – 16:25 **MAUD DEBAUGNIES** (Free University of Brussels (ULB), Belgium): Selected Oral
"Understanding the mechanisms controlling EMT mediated therapy resistance in skin squamous cell carcinoma"
16:25 – 16:40 **SOPHIE DEKONINCK** (Free University of Brussels (ULB), Belgium): Selected Oral
"Defining the mechanisms leading to interfollicular epidermis expansion during post-natal development"
16:40 – 17:15 **MICHELE DE LUCA** (University of Modena, Italy)
"Combined cell and gene therapy of Epidermolysis Bullosa shed light on human epidermal stem cells"
17:15 – 17:25 **BEST POSTER AND TALK AWARD**
17:25 – 17:35 **CATHERINE VERFAILLIE** (BeSSCR president, University of Leuven (KU Leuven), Belgium)
"Concluding Remarks"

ORAL PRESENTATIONS

[6] Human pancreatic adult acinar cells dedifferentiate towards an embryonic progenitor-like state in 3D suspension culture.

Jonathan BALDAN, Isabelle HOUBRACKEN, Ilse ROOMAN, Luc BOUWENS

Vrije Universiteit Brussel

Presenting author: Jonathan BALDAN

The presence of adult stem cells in the pancreas is a debate of controversy. However, under well-defined conditions, differentiated cells may behave as facultative progenitors. Rodent pancreatic acinar cells are prone to reprogramming towards endocrine beta cells when exposed to defined extracellular factors in vitro and in vivo. Acinar-to-endocrine reprogramming required an initial dedifferentiation step that can be obtained in vitro by 3D suspension culture of murine acinar cells. We investigated if human acinar cells equally dedifferentiate in this defined culture condition. The initial human exocrine fraction is composed of 29.1 ± 2.6% CA19.9+ ductal cells and 70.7 ± 2.6% chymotrypsin+ acinar cells (n=5). FITC-conjugated UEA1 lectin was used to specifically label and non-genetically trace acinar cells. At day 4, FACS isolation confirmed the presence of acinar-derived UEA1+CA19.9- acinar cells, UEA1-CA19.9+ original duct cells and UEA1+CA19.9+ acinar-to-duct reprogrammed cells, representing respectively 39.2 ± 6.0%, 40.7 ± 3.1% and 6.2 ± 2.0% (n=5). The acinar derived UEA1+CA19.9- cells acquired an embryonic signature at mRNA level with expression of PDX1, SOX9, PTF1A, GP2, CD142, RBPJ and PARM1. CD142 and GP2 are embryonic surface markers characteristic of multipotent pancreatic progenitor cells (Kelly OG et al. 2011, Ramond C et al. 2017). At protein level, co-expression of CD142 with PDX1 and SOX9 was observed in the UEA1+ fraction confirming the qRT-PCR results. The CD142+ acinar-derived embryonic-like cells are proliferatively quiescent. Inhibition of TGF-beta signaling, through addition of ALK5iII, induced a 28-fold increase in KI67 labeling and upregulation of MYC, BIRC5 and UBE2C transcripts, i.e. proliferative and embryonic markers. We describe for the first time that human pancreatic acinar cells acquire a proliferative embryonic-like cellular state closely resembling multipotent pancreatic cells during development. This could find application in regenerative therapies for damaged pancreas tissue or for replacement of endocrine beta cells in diabetes.

[8] Insights into late reprogramming: gene expression and DNA methylation dynamics define new stages surrounding the acquisition of naive pluripotency.

Michela Bartoccetti, Xinlong Luo, Rita Khoueiry, Adrian Janiszewski, Jiayi Xu, Catherine Verfaillie, Vincent Pasque, Kian Peng Koh

KULeuven

Presenting author: Michela Bartoccetti

Transcription factor-mediated reprogramming of somatic cells to induced pluripotent stem cells progresses via sequential events to gain full features of pluripotency. The use of markers to isolate and characterize reprogramming intermediates is essential to explore the underlying mechanisms, but existing markers do not provide sufficient temporal resolution of events late in reprogramming. Here, we have generated murine transgenic lines harboring dual fluorescent reporters reflecting cell-state specific endogenous gene expression of the master pluripotency regulator Oct4 and the DNA dioxygenase Tet1. By assessing reprogramming intermediates based on dual reporter patterns, we identified a sequential order of Tet1 and Oct4 gene activation at proximal and naive-specific distal regulatory elements, beginning shortly after activation of NANOG, which signaled entry into pluripotency, and ending with activation of DPPA4, a late naive pluripotency marker. Transcriptome profiling of these sorted intermediates revealed five distinct cellular states reflecting transitions through first a gain of pattern specification processes, then a temporary stalling of cell migration and motility, followed by activation of meiotic genes and finally a restoration of cell motility and proliferation. Whole-genome base-resolution profiling of 5-methylcytosine showed global waves of DNA demethylation. Surprisingly, the first major wave of DNA demethylation precedes full gene activation of Tet1. Subsequently, a second wave occurred at specific loci to coincide with activation of meiotic and germline-specific genes, in a manner reminiscent of germline reprogramming. Loss of Tet1 is compatible with reprogramming towards full Oct4 gene activation, but generates iPSCs with epigenetic defects. Our results offer insights into the basis for epigenetic differences between embryo-derived and in vitro induced pluripotent stem cells. Overall, we demonstrate that the transcriptional logic of Tet1 expression can signal an epigenetic roadmap towards efficient reprogramming.

[19] Understanding the mechanisms controlling EMT mediated therapy resistance in skin squamous cell carcinoma.

Debaugnies M., Zocco M., Parent M-A., Brisebarre A., Latil M., Pastushenko I., Dubois C., Moers V., Sotiropoulou P., Blanpain C

ULB- Laboratory of Stem Cells and Cancer

Presenting author: Maud Debaugnies

The resistance of cancer cell to chemotherapy and radiotherapy is responsible of the death of most cancer patients. Different intrinsic mechanisms controlling intratumor heterogeneity like genetic, epigenetic, phenotypical states of tumor cells including EMT have been suggested to regulate resistance to therapy in cancer cells. Here, using a mouse model of skin squamous cell carcinoma (SCC) that undergo spontaneous EMT during tumorigenesis, we identified EMT tumor subpopulations that are differentially intrinsically resistant to a wide range of anti-cancer therapy including 5-FU, platinimum, taxane and radiotherapy in vivo and in vitro. Using genome wide transcriptional analysis, we identified a new candidate gene that may be responsible for the resistance to therapy. We confirm the role of this candidate using shRNA knock down in vitro. We performed proteomic analysis in order to identify how it mediates therapy resistance. Hopefully, the identification of such new potential target and the understanding of its mechanism of action will lead to an improvement in the efficiency of SCC therapy.

[20] Defining the mechanisms leading to interfollicular epidermis expansion during post-natal development

Sophie Dekoninck1, Edouard Hannezo2, Mariaceleste Aragona1, Sandrine Lenglez1, Charlotte de Neunheuser1, Souhir Gargouri1, Alejandro Sifrim3, Benjamin D. Simons4 & Cédric Blanpain1

(1)Laboratory of Stem Cells and Cancer, Université Libre de Bruxelles (ULB), Belgium (2)Institute of Science and Technology, IST Austria, Austria (3)Department of Human Genetics, University of Leuven, KULeuven, Leuven, Belgium (4)Cavendish Laboratory, Department of Physics, University of Cambridge, UK

Université Libre de Bruxelles

Presenting author: Sophie Dekoninck

The Interfollicular Epidermis (IFE) is a stratified epithelium constantly renewed and is a first barrier of defense for living organisms. The murine tail IFE contains different regions called scale and interscale and is a useful model to study skin Stem Cells (SCs). Previously, our group demonstrated that a hierarchy of slow-cycling SCs and Committed Progenitors (CP) maintained interscale compartment during adulthood whereas scale regions only contains CPs. While mouse tail IFE has been extensively studied during homeostasis nothing is known about the role of these two populations during postnatal growth. In this project, we use clonal analysis to trace isolated single basal cells and study their progeny during post-natal development from birth until adult. Our morphometric analysis conducted on the whole tissue reveals a linear expansion mainly achieved before postnatal day 30 (P30). Our clonal analysis shows that labeled IFE progenitors give rise to clones that expand overtime with an average basal footprint proportional to the whole tissue expansion demonstrating that the labeled population recapitulates the tissue growth. Mathematical analysis performed on clonal and proliferation data suggest that the linear growth is achieved through a constant imbalance toward self-renewal compensated by a decreasing proliferation rate. In sharp contrast with the homeostatic model in adult, cumulative clone size distribution rather fit with a single population of cells in interscale suggesting a more homogeneous basal compartment in post-natal development compared to adult. Interestingly, single cell RNA sequencing performed on basal progenitor during post-natal development versus adult confirms this observation.

[22] Translation stress induced by loss of Elongator activates a p53-dependent antitumor checkpoint in hematopoietic stem and progenitor cells

Adeline Rosu, Kevin Rouault-Pierre, Eve Ramery, Joan Somja, Francesca Rapino, Qiang Bai, Laurent Nguyen, Jan Cools, Dominique Bonnet, Alain Chariot, Pierre Close, Fabrice Bureau, Christophe J. Desmet

Liege University

Presenting author: Christophe Desmet

Tightly controlled protein synthesis is emerging as an essential requirement for the normal function, differentiation and fate decisions of hematopoietic stem and progenitor cells (HSPCs). Here, to reveal translational determinants of HSPC biology, we used mouse models of conditional inactivation of Elongator, an enzymatic complex that optimizes speed of translation elongation by catalyzing modifications of specific transfer RNAs. We report that inactivation of Elongator activity blocked HSPC differentiation and caused bone marrow failure through the activation of translation stress responses orchestrated by the transcription factors Atf4 and p53. In contrast, the maintenance, but not the reconstitution activity of Elongator-deficient hematopoietic stem cells, was spared. Deletion of p53 rescued Elongator-deficient HSPC function. Yet, Elongator inactivation prompted the development of p53-mutated hematologic malignancies and inactivation of p53 and Elongator synergistically promoted tumorigenesis. Inactivation of Elongator thereby reveals a p53-dependent quality control of translation that conditions antitumor fate decisions in HSPCs.

[40] Uncovering Mechanisms of Epigenetic Memory Reversal during Reprogramming to Induced Pluripotency

Adrian Janiszewski, Juan Song, Natalie De Geest, San Kit To, Florian Rambow, Greet Bervoets, Jean-Christophe Marine, Vincent Pasque

KU Leuven – University of Leuven, Department of Development and Regeneration, Leuven Stem Cell Institute, Leuven Cancer Institute

Presenting author: Adrian Janiszewski

The induction and reversal of gene silencing is central to the establishment and maintenance of cellular identity during development. Gene expression programs are induced by transcription factors, and remembered through cell divisions by epigenetic phenomena, which involve chromatin modifications and non-coding RNAs. However, how epigenetic memory of stable gene silencing can be reversed by transcription factors remains to be precisely revealed. Here, we will present new studies combining transcriptional profiling, epigenomic analyses and functional experiments to investigate the mechanisms by which chromatin and transcription factors mediate the reversal of epigenetic memory of gene silencing on the inactive X chromosome. We define the allele-resolution kinetics of transcriptional changes during the reprogramming of somatic cells into induced pluripotent stem cells (iPSCs). We will present evidence that genes reactivate in a hierarchical order during X chromosome reactivation. Gene reactivation is initiated before the activation of late naïve pluripotency genes and silencing of the long non-coding RNA Xist, and completed late in reprogramming. Epigenomic analyses reveal the enrichment of chromatin marks at cis-regulatory regions of genes that reactivate with different kinetics. We will show that late reactivated genes have lower starting levels of active marks such as H3K36me3. We will also present evidence that histone deacetylation acts as a barrier during X chromosome reactivation, while the histone acetyltransferase activity of the CREB binding protein is required. Our results lead us to hypothesize that a combination of chromatin states and hierarchical binding of pluripotency transcription factors dictate the kinetics of gene silencing reversal. We will test this hypothesis using ATAC-seq, ChIP-seq and single cell RNA-seq analyses. Altogether, we report for the first time the kinetics of X chromosome reactivation during reprogramming to iPSCs and provide insights into the mechanisms underlying the reversal of chromosome-wide epigenetic memory of gene silencing.

[63] The neural environment is a necessary, but not sufficient, prerequisite for directing iPSC-derived myeloid progenitors into the CX3CR1+CCR2- microglia-like phenotype.

Alessandra Quarta1, Debbie Le Blon1, Tine D'Aes1, Evi Luyckx2, Somayyeh Hamzei Taj3, Sylvia Dewilde2, Vincent Pasque3, Mathias Hoehn2,5, Zwi Berneman1, Peter Ponsaerts1

1Laboratory of Experimental Hematology, Vaccine and Infectious Disease Institute (Vaxinfectio), University of Antwerp, Antwerp, Belgium 2Protein Chemistry, Proteomics and Epigenetic Signaling, University of Antwerp, Antwerp, Belgium. 3In-vivo-NMR Laboratory, Max Planck Institute for Metabolism Research, Cologne, Germany. 4Stem Cell Biology and Embryology, Department of Development and Regeneration, KU Leuven, Leuven, Belgium. 5Department of Radiology, Leiden University Medical Center, Leiden, Netherlands.

University of Antwerp

Presenting author: Alessandra Quarta

Differentiation of microglia from iPSC holds great potential for in vitro neurodevelopment and immunology research. Here, we present a novel protocol to obtain yolk sac-like CX3CR1+CCR2- macrophage progenitors from CX3CR1eGFP/+CCR2RFP/+ murine iPSC, which following co-culture with astrocyte-committed neural stem cells (aNSC) mature into a highly uniform population of CX3CR1+CCR2- iPSC-derived microglia that display typical ramified or amoeboid morphology and are able to colonize microglia-depleted organotypic brain slice cultures (mdOBSC). Phenotypical characterisation of these ramified CX3CR1+ iPSC-derived microglia compared to round shaped CX3CR1-CCR2- iPSC-derived macrophages demonstrated F4/80 expression by both cell populations, while the activation marker MHC-II was upregulated only by iPSC-derived macrophages upon LPS+IFNγ stimulation. This distinct MHCII expression pattern was strikingly similar to the expression pattern of MHCII on endogenous CX3CR1+CCR2- microglia and infiltrating CX3CR1+CCR2+ monocytes following experimental stroke in CX3CR1eGFP/+CCR2RFP/+ mice. In addition, in vitro LPS+IFNγ stimulated iPSC-microglia secreted a significantly lower amount of pro-inflammatory cytokines. Consistently with the concept that the neural environment is a key determinant for myeloid progenitors to adopt a microglia-like phenotype, CX3CR1- iPSC-macrophages, when cultured in the presence of aNSC or on mdOBSC, were subject to rapid conversion into CX3CR1+ amoeboid microglia-like cells with reduced expression of MHCII. However, co-culture with aNSC did not reduce the level of pro-inflammatory cytokines secretion by iPSC-macrophages. These findings suggest that although a neural environment is able to convert myeloid progenitors into cells morphologically and phenotypically similar to microglia, also retracing early microglia ontogenesis in vitro is essential to obtain iPSC-derived microglia-like cells that are functionally distinct from iPSC-derived macrophages.

POSTERS

[1] Robust protocol for feeder-free adaptation of frozen pluripotent stem cells using StemFlex

Jeffrey Aalders, Natasja van den Vreken, Björn Heindryckx and Jolanda van Hengel

Ghent University

Presenting author: Jeffrey Aalders

Background: Historically pluripotent stem cell (PSC) lines are cultured on mouse fibroblasts (MEFs). However protocols for downstream application with PSCs such as cardiomyocyte differentiation are often optimized for feeder-free cultures. PSCs can be adapted, but in some cases extensive spontaneous differentiation was observed when starting an important frozen ampule with PSCs on MEFs. Differentiation in feeder cultures is difficult to recognize and to remove, which led to loss of this sample. Objectives: Here we propose a direct feeder-free adaptation protocol from freezing using the novel StemFlex medium. Since StemFlex showed improved survival in other applications we hypothesized that it could help in the adaptation process. Methods: The new proposed adaptation protocol is compared with existing protocols such as a step-wise adaptation approach using gene expression analysis with RT-qPCR and immunohistochemistry. Results: The direct feeder-free adaptation protocol using StemFlex on Geltrex coating led to robust cultures in approximate two weeks, and expressing the pluripotent markers OCT4, SOX2 and NANOG. StemFlex could be changed to Essential 8 after one passage without decrease in growth, colony size or differentiation of the PSCs. Conclusions: StemFlex can be used to directly adapt PSCs from freezing without the need of MEFs. This protocol is easily implemented in labs that routinely perform feeder-free cultures allowing them to more easily start cultures of frozen PSCs of poor or unknown quality.

[2] Mapping mechanotransductive feedback between intrinsic and extrinsic mechanical forces in human pluripotent stem-cell derived organoids

Abdel Rahman Abdel Fattah, Brian Daza Jimenez, Gregorius Andika W. Rustandi, Jorge Barrasa Fano, Amandine Conte-Daban, Hans Van Oosterwyck, Peter Dedecker, Adrian Ranga

KU Leuven - Mechanical Engineering - Laboratory of Bioengineering and Morphogenesis

Presenting author: Abdel Rahman Abdel Fattah

Mechanical forces are increasingly acknowledged to play an important role in stem cell differentiation by engaging and providing feedback through mechanoresponsive signaling pathways. This is particularly relevant in multicellular three-dimensional (3D) contexts such as organoids, where the activation of mechanical forces has been shown to regulate patterning and morphogenesis. To date, several methods have been proposed to investigate extracellular and intracellular forces. However, these have often been implemented independently and largely in 2D. A major challenge remains in understanding the interplay between matrix and cell mechanics in 3D, which would require the implementation of multimodal mechanical force interrogation methods. The ability of human pluripotent stem cells (hPSC) to self-organize in 3D represents a compelling paradigm to explore the role of mechanotransduction in linking the behavior of multicellular aggregates to their microenvironment. Here, we implement a mechanomapping multimodal approach to study the magnitude and spatial distribution of mechanical forces for hPSCs in 2D and 3D. This enables the visualization and quantification of mechanotransduction occurring between hPSCs and a highly defined and tunable poly(ethylene) glycol (PEG)-based synthetic extracellular matrix. We use 1) traction force microscopy (TFM) to convey information on the mechanical stresses present in the matrix, and 2) mechanotransducing vinculin FRET biosensors to provide force information inside cells. We show that cells in 3D are generally under more tensile stress as indicated by overall lower FRET values compared to their monolayer counterpart. Additionally, differential vinculin localization in 2D and 3D suggests that cellular rearrangement may play an important role in the mechanotransduction of forces. Future directions include expanding the scope of this integrative approach to differentiating organoids, to elucidate how cellular structures collectively respond to external mechanical stimuli, and the link to subsequent fate specification and morphogenesis.

[3] Contrasting Wnt Signaling "dosage-dependent" effects on breast cancer cell proliferation.

Abreu de Oliveira, Willy Antoni; De Jaime-Soguero, Anchel; Escalona, Carmen, Lluis Viñas, Frederic.

Stem Cell Institute - KU LEUVEN

Presenting author: Willy Antoni Abreu de Oliveira

Growth arrest and induction of a dormant state allows cancer cells to become resistant to antimitotic chemotherapeutic treatments contributing to cancer relapse. Cancer dormancy remains a poorly understood process and the molecular mechanisms and cellular signals behind this reversible growth arrest are unknown. However, recent reports suggest that this state can be induced by chemotherapy and/or actively maintained by signals arising from the metastatic niche. Triple negative is the most aggressive of breast cancer subtypes. Despite the fact TNBC responds significantly better to chemotherapy than other breast neoplasms, it paradoxically has higher recurrence rates and poorer prognosis. Wnt signalling is significantly enriched in TNBC patients and correlates with poor outcome and relapse. Here we report that Wnt signalling activation induces a slow-cycling state in breast cancer cell lines which is reliant on β-catenin levels, suggesting a "dosage-dependent" balance between proliferation and dormancy. Moreover we report that this slow cycling state is accompanied by upregulated expression of stemness markers, enhanced chemotherapy resistance and, in vitro tumour initiating capacity. Our research highlights the role of Wnt signalling in chemotherapy resistance and recurrence and emphasises the importance of the development of specific and safe Wnt-targeting drugs for the treatment of TNBC.

[4] Unraveling the cellular dynamics of force mediated tissue expansion

Mariaceleste Aragona, Sophie Dekoninck, Souhir Gargouri, Yura Song, Cédric Blanpain

Laboratory of Stem Cells and Cancer, Université Libre de Bruxelles

Presenting author: MARIACELESTE ARAGONA

The skin is the outermost protective organ of the body, it acts as a barrier against external environment defending the animals from infections, trauma and water loss. Distinct populations of stem cells (SCs) reside in the skin epidermis and contribute to the homeostasis of the different epidermal compartments. The interfollicular epidermis (IFE) is a stratified squamous epithelium consisting of a single inner layer of proliferative cells, called the basal layer, expressing keratin14 (K14), and several suprabasal layers containing terminally differentiated cells. Resident basal SCs strongly adhere to their underlying basal membrane and maintain homeostasis of the IFE by continually replenishing the suprabasal cells that are shed from the skin surface. The ability of the skin to expand in response to mechanical forces has been used for decades in reconstructive and plastic surgery to repair birth defects and correct burn injuries. However, multi-disciplinary experimental approaches to decipher quantitatively and at the tissue scale level, the cellular and molecular basis that control the cellular dynamics leading to tissue expansion have never been undertaken so far in mammals. Here we performed lineage tracing and clonal analysis of the epidermis using Tamoxifen inducible K14CREER-RosaConfetti mice to study the contribution of SCs and progenitors during force mediated tissue expansion in the dorsal skin. The data demonstrate for the first time, in vivo, that stretching the skin results in proliferation and self-renewal of the basal cells and that tissue growth is driven by the activation of a specific SCs population. Transcriptional and epigenetic profiling identify the chromatin landscapes, gene regulatory networks and transcription factors that regulate force mediated tissue expansion.

[5] Organoids derived from inflammatory intestinal crypts of patients with ulcerative colitis lose their inflammatory transcriptional signature following ex vivo culture

Arnauts Kaline, Verfaillie Catherine MD PhD, Ferrante Marc MD PhD

SCIL, TARGID

Presenting author: Kaline Arnauts

Introduction: Ulcerative colitis (UC) is characterized by chronic inflammation of the colonic mucosa. Patient derived intestinal organoids provide an excellent tool to unravel the multifactorial mechanisms underlying UC. Organoids develop from stem cell-containing intestinal crypts and recapitulate many features of the source tissue. However, it remains unclear if organoids retain the inflammatory character of their origin. To address this, we isolated crypts both from inflamed and non-inflamed regions of the colon of UC patients, created organoids and compared the transcriptome of the isolated biopsies, crypts and the ex vivo cultured organoids. Material and methods: Fresh biopsies in both inflamed and non-inflamed segments were obtained during endoscopy from 7 patients with active UC (endoscopic Mayo score of ≥2) with an accessible border of inflammation. Crypts were isolated from fresh biopsies and cultured as organoids for 4 weeks with weekly mechanical splitting. RNA was extracted from biopsies, crypts and organoids after week 1 and 4. RNA sequencing was performed by the Lexogen QuantSeq 3′ mRNA-Seq Library Prep Kit FWD for Illumina. Results: PCA analysis of biopsies clearly demonstrated a separation between freshly isolated inflamed and non-inflamed biopsies, which was not the case in organoids from inflamed vs non-inflamed organoids after 4 weeks in culture. Initial analysis demonstrated that, compared to crypts from non-inflamed regions, crypts from inflamed regions expressed significantly higher levels of inflammatory marker genes. However, over-expression of some inflammatory markers (TNF-α, IFN-γ, IL-1ß) was lost already after one week in culture, whereas others (IL-8, IL-6, IL-1) were lost after 4 weeks only. Conclusion: We conclude that organoids lose their inflammatory transcriptional signature over time in culture. Therefore, we hypothesize that to mimic the inflammatory phenotype and create a physiological representative model, inflammatory components and/or immune cells should be added to the ex vivo culture system.

[6] Human pancreatic adult acinar cells dedifferentiate towards an embryonic progenitor-like state in 3D suspension culture.

Jonathan BALDAN, Isabelle HOUBRACKEN, Ilse ROOMAN, Luc BOUWENS

Vrije Universiteit Brussel

Presenting author: Jonathan BALDAN

The presence of adult stem cells in the pancreas is a debate of controversy. However, under well-defined conditions, differentiated cells may behave as facultative progenitors. Rodent pancreatic acinar cells are prone to reprogramming towards endocrine beta cells when exposed to defined extracellular factors in vitro and in vivo. Acinar-to-endocrine reprogramming required an initial dedifferentiation step that can be obtained in vitro by 3D suspension culture of murine acinar cells. We investigated if human acinar cells equally dedifferentiate in this defined culture condition. The initial human exocrine fraction is composed of 29.1 ± 2.6% CA19.9+ ductal cells and 70.7 ± 2.6% chymotrypsin+ acinar cells (n=5). FITC-conjugated UEA1 lectin was used to specifically label and non-genetically trace acinar cells. At day 4, FACS isolation confirmed the presence of acinar-derived UEA1+CA19.9- acinar cells, UEA1-CA19.9+ original duct cells and UEA1+CA19.9+ acinar-to-duct reprogrammed cells, representing respectively 39.2 ± 6.0%, 40.7 ± 3.1% and 6.2 ± 2.0% (n=5). The acinar derived UEA1+CA19.9- cells acquired an embryonic signature at mRNA level with expression of PDX1, SOX9, PTF1A, GP2, CD142, RBPJ and PARM1. CD142 and GP2 are embryonic surface markers characteristic of multipotent pancreatic progenitor cells (Kelly OG et al. 2011, Ramond C et al. 2017). At protein level, co-expression of CD142 with PDX1 and SOX9 was observed in the UEA1+ fraction confirming the qRT-PCR results. The CD142+ acinar-derived embryonic-like cells are proliferatively quiescent. Inhibition of TGF-beta signaling, through addition of ALK5iII, induced a 28-fold increase in KI67 labeling and upregulation of MYC, BIRC5 and UBE2C transcripts, i.e. proliferative and embryonic markers. We describe for the first time that human pancreatic acinar cells acquire a proliferative embryonic-like cellular state closely resembling multipotent pancreatic cells during development. This could find application in regenerative therapies for damaged pancreas tissue or for replacement of endocrine beta cells in diabetes.

[7] Acceleration of wound healing through ERK-mediated autologous micro graft treatment

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Presenting author: Martina Balli

Traumatic injuries, such as wounds and burns, are leading health and economical problems, hindering life quality of patients, necessitating long-term hospitalization and contributing to mortality. Incomplete understanding of molecular mechanisms of the wound healing process and its failure results in a lack of suitable therapies for impaired wound conditions and of acute wound repair acceleration. Improvement of the wound healing (WH) process denotes the goal of the tissue regeneration. Autologous micro graft (AMG)-based therapies represent an effective new treatment for WH, although, detailed mechanistic studies explaining their beneficial effect are still missing. Here, we show that AMG treatment increases cell migration and accelerates scratch closure in in vitro wound healing models, without affecting cell viability or cell proliferation. In accordance, comparative transcriptome analysis of AMG-treated and untreated cells shows enrichment in pathways involved in cell migration. AMG-based treatment induces Extracellular Signal-Regulated kinase (ERK) signalling pathway activation and increases significantly matrix metalloproteinase (MMPs) family expression and enzymatic activity. We demonstrate that MEK/ERK and MMPs activity inhibition reduces both AMG-dependent scratch closure in vitro as well as wound healing in vivo. Our results show for the first time that growth factors present in the soluble fraction of the AMG drive ERK activation, which in turn up-regulates matrix metalloproteinase (MMPs) expression and their enzymatic activity, resulting in accelerated cell migration and faster in vivo wound healing. Our study sheds light onto the molecular mechanisms of AMG-based treatment, leading to a better understanding of the physiology of the wound healing process, which in turn, will allow developing more effective therapeutic treatments for both acute wounds chronic WH disorders.

[8] Insights into late reprogramming: gene expression and DNA methylation dynamics define new stages surrounding the acquisition of naive pluripotency.

Michela Bartoccetti, Xinlong Luo, Rita Khoueiry, Adrian Janiszewski, Jiayi Xu, Catherine Verfaillie, Vincent Pasque, Kian Peng Koh

KULeuven

Presenting author: Michela Bartoccetti

Transcription factor-mediated reprogramming of somatic cells to induced pluripotent stem cells progresses via sequential events to gain full features of pluripotency. The use of markers to isolate and characterize reprogramming intermediates is essential to explore the underlying mechanisms, but existing markers do not provide sufficient temporal resolution of events late in reprogramming. Here, we have generated murine transgenic lines harboring dual fluorescent reporters reflecting cell-state specific endogenous gene expression of the master pluripotency regulator Oct4 and the DNA dioxygenase Tet1. By assessing reprogramming intermediates based on dual reporter patterns, we identified a sequential order of Tet1 and Oct4 gene activation at proximal and naive-specific distal regulatory elements, beginning shortly after activation of NANOG, which signaled entry into pluripotency, and ending with activation of DPPA4, a late naive pluripotency marker. Transcriptome profiling of these sorted intermediates revealed five distinct cellular states reflecting transitions through first a gain of pattern specification processes, then a temporary stalling of cell migration and motility, followed by activation of meiotic genes and finally a restoration of cell motility and proliferation. Whole-genome base-resolution profiling of 5-methylcytosine showed global waves of DNA demethylation. Surprisingly, the first major wave of DNA demethylation precedes full gene activation of Tet1. Subsequently, a second wave occurred at specific loci to coincide with activation of meiotic and germline-specific genes, in a manner reminiscent of germline reprogramming. Loss of Tet1 is compatible with reprogramming towards full Oct4 gene activation, but generates iPSCs with epigenetic defects. Our results offer insights into the basis for epigenetic differences between embryo-derived and in vitro induced pluripotent stem cells. Overall, we demonstrate that the transcriptional logic of Tet1 expression can signal an epigenetic roadmap towards efficient reprogramming.

[9] Elafibranor reduces characteristics of metabolically- and drug-induced fatty liver disease in human skin stem cell-derived hepatic cells

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Presenting author: Joost Boeckmans

Background: Fatty liver disease (FLD) is characterized by increased hepatic lipid load and encompasses multiple disease stadia, ranging from liver steatosis to life-threatening steatohepatitis, fibrosis, cirrhosis and cancer. Both metabolic syndrome and steatogenic drugs can induce FLD, giving rise to a similar clinical picture. Aim: The present study aims at evaluating whether human skin-derived hepatic cells (hSKP-HPC) are capable to assess the anti-FLD properties of elafibranor, a newly developed drug against non-alcoholic steatohepatitis (NASH) that is under clinical phase III evaluation. Methods: Human skin-derived precursors (hSKP) were first differentiated towards hepatic cells. Subsequently, hSKP-HPC were exposed for 24h either to a NASH-mimicking cocktail consisting of insulin, glucose, fatty acids and inflammatory cytokines, reflecting metabolically-induced NASH, or to sodium valproate, an anti-epilepticum known to induce steatosis. Elafibranor was concomitantly added to the exposed cells at high and low concentration. Lipid accumulation was assessed by fluorescence microscopy and flow cytometry using a stain for neutral lipids (BODIPY™). Inflammation was evaluated using antibody arrays (Abcam) for inflammatory cytokines. Transcriptomics study (Affymetrix Human Genome U133 plus 2.0 array) was conducted and subsequently pathway analyses were performed using Ingenuity Pathway Analysis. Drug-induced steatosis was preliminarily assessed by RT-qPCR (Applied Biosystems). Results: Metabolically-triggered hSKP-HPC showed an increase in lipid load and inflammatory cytokines. The lipid load was gradually decreased upon exposure to increasing elafibranor concentrations. Pathway analysis predicted 'chemotaxis' activation, which in an in vivo setting would suggest an increased recruitment of inflammatory cells in the liver. Elafibranor at the highest concentration reverted this effect. Sodium valproate-induced expression of lipogenic genes was also generally down-regulated upon addition of elafibranor. Conclusion: These preliminary data indicate that hSKP-HPC are capable to identify compounds with possible anti-FLD properties on distinct disease aetiologies and as such also represent a valuable human-relevant in vitro model to unravel their mechanism of action.

[10] Patient-derived organoids from endometrial disease capture clinical heterogeneity and are amenable to drug screening

Matteo Boretto, Lisa Perneel, Xinlong Luo, Bich Bui1, Aurélie Hennes, Hiroto Kobayashi1, Indra Van Zundert, Hiroshi Uji-i, Ruben Heremans, Nina Maenhoudt, Hilde Brems, Benoit Cox, Kian Peng Koh, Arne Vanhie, Ignace Vergote, Christel Meuleman, Joris Vriens, Carla Tomassetti, Dirk Timmerman and Hugo Vankelecom

KU Leuven

Presenting author: Matteo Boretto

Endometrial diseases present a major gynecological burden. Current research models fail to recapitulate human endometrial disease in nature and heterogeneity, thereby hampering scientific and clinical progress. Here we developed long-term expandable organoids from a broad range of endometrial pathologies. Organoids from endometriosis show disease-associated traits and reveal upregulation of specific WNT pathway components in advanced stages. Endometrial cancer-derived organoids recapitulate the genomic alterations and molecular characterization demonstrates faithful capturing of the cancer's subtype. The tumor organoids show grade-specific drug responses. Organoid cultures could also be derived from pre-cancerous pathologies like endometrial hyperplasia and Lynch syndrome with maintenance of inherited gene mutations. Analysis of the different disease organoids uncovered the presence of specific functional ion channels and metabolic peculiarities. Finally, endometrial disease organoids reproduce the original lesion when transplanted in vivo. Together, we developed multiple organoid models capturing endometrial disease diversity and providing new powerful research and drug discovery tools.

[11] A Subpopulation of Interstitial Stem Cells Controls Adipogenesis in the Skeletal Muscle

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Presenting author: Jordi Camps

Fat deposition in the skeletal muscle is a major complication in chronic muscle disorders, such as muscular dystrophy, and one of the main causes for the loss of mobility. Although multiple studies have investigated which cells give rise to adipocytes, our current knowledge about them and their role in pathology are still unclear. This is mainly because of the high heterogeneity of stem cell population in the skeletal muscle. Therefore, we used single-cell RNA-sequencing to decomplex stem-cell population in the skeletal muscle in healthy and dystrophic mice. We reveal the presence of three subpopulations in the major interstitial stem cell pool and show that the ratios of these subpopulations change in muscular dystrophy. One of these populations is marked by CD142 and is highly present in healthy but not in dystrophic muscle. Upon isolation of this population we show that it inhibits adipocyte differentiation in vitro by means of the secretion of Gdf10. In addition, we show that the ratio of this population determines the efficiency of adipogenesis. The discovery of these adipogenesis-regulatory cells in the skeletal muscle can aid our understanding of fat deposition and lead the way for potential treatments to sustain ambulation of muscular-dystrophy patients.

[12] Implication of the pancreatic δ-cells in the regeneration of β- cells in zebrafish

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Université de Liège

Presenting author: Claudio Carril Pardo

Diabetes occurs under insulin resistance and/or when the mass of insulin-producing pancreatic β-cells decreases by cell death. β-cell replacement constitutes a promising alternative to replenish the pancreas with functional β-cells. Several observations of pancreatic cell plasticity in adult mammals have led to the hope that triggering β-cell regeneration within the pancreas could be harnessed in future therapies. Notably, endocrine α-cells have been shown in mouse to convert to β-cells after massive destruction [1]. Using a chemogenetic model to induce selective destruction of β-cells, we recently showed that adult zebrafish duct cells display characteristics of embryonic pancreatic progenitors and that they can give rise to β-cells in physiological and induced diabetic condition [2]. In addition, recent observations in our laboratory revealed the appearance of bi-hormonal somatostatin/insulin (Sst/Ins) cells during β-cell regeneration in adult zebrafish. This suggests 1) that somatostatin-producing δ-cells can give rise to regenerated β-cells, or 2) that regenerated β-cells display different features (such as a mixed endocrine identity) as compared to the original ones. In this study we pretend to assess both possibilities by analyzing the regenerating β-cells at different time points during regeneration through immunohistochemistry and RNA sequencing approaches. 1. Thorel, F., et al., Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. Nature, 2010. 464(7292): p. 1149-54. 2. Ghaye, A.P., et al., Progenitor potential of nkx6.1-expressing cells throughout zebrafish life and during beta cell regeneration. BMC Biol, 2015. 13: p. 70.

[13] Translational control of mitochondrial biogenesis during hepatogenic differentiation

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Presenting author: Marino Caruso

Mitochondrial reprograming has previously been shown as regulating stem cell differentiation. Recently, our team characterized this mitochondrial remodeling in a model of human Bone Marrow Mesenchymal Stem Cell (hBM-MSC) hepatogenic differentiation [1]. Interestingly, a transcriptomic analysis performed at the early stage of hepatogenic differentiation not only confirmed the mitochondrial biogenesis, but also pointed out several elements suggesting a potential translational regulation of the mitochondrial reprograming. Among others, we noticed a transcriptional repression of 4E-BP1 (eukaryotic initiation factor 4E-binding protein 1), a protein regulating the cap-dependent translation and involved in the translational control of several nuclear-encoded mitochondrial mRNAs. Therefore, this project aims to investigate the translational control participating in mitochondrial biogenesis occurring during hepatogenic differentiation of hBM-MSC. First, we showed that 4E-BP1 protein level was effectively downregulated during hepatogenic induction and we are currently characterizing whether this downregulation has a functional impact on differentiation. Secondly, we are setting up a polysome fractionation of differentiating hBM-MSCs with the purpose to analyze the polysomes by RNA sequencing and characterize the differentially translated mRNAs during hepatogenic induction. Polysomes will also be analysed at the protein level by quantitative mass spectrometry in order to investigate whether hepatogenic differentiation induces a modification of the riboproteome. Altogether, this research project should shed light on the translational regulation that participates to the interplay between mitochondria, stem cells and differentiation. This work is funded by a FRIA grant of the FRS-FNRS (Foundation for Scientific Research), Belgium.

[14] Defining the mechanisms regulating the reactivation of multipotent cell fate of mammary gland stem cells

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Presenting author: Alessia Centonze

Mammary gland (MG) epithelium is composed of two lineages, basal (BCs) and luminal cells (LCs). The MG develops from embryonic multipotent progenitors that are replaced by unipotent basal and luminal stem cells (SCs) during postnatal development. However, in non-physiological conditions (eg: transplantation, oncogenic PIK3CA) BCs reacquire multipotency. In this project, we want to define the molecular mechanisms controlling the unipotency/multipotency switch of BCs upon different conditions. We found that LCs ablation activates multipotency of BCs. To understand the mechanisms regulating the cell fate switch upon LCs ablation, we performed bulk and single cell RNAseq of BCs to identify genes that are involved in BCs multipotency upon ablation. We have also profiled FACS isolated BCs following transplantation alone (multipotency in transplantation) or with LCs (unipotency in transplantation). By comparing the different gene signatures, we identified candidate genes that control BCs multipotency in the different experimental conditions associated with multipotency. The upregulation of many collagen genes in multipotent BCs leads us to hypothesize that the extracellular matrix (ECM) and its stiffness could be involved in the regulation of multipotency. To address this question, we will transplant BCs and LCs using different types and concentrations of purified collagens and hydrogels for which the degree of stiffness could be modulated and where components of the ECM can be added, to assess if different ECM and its stiffness modify the multipotency of BCs. Altogether, these experiments should allow to identify the mechanisms regulating SCs unipotency and multipotency in the MG, which may have important implications for other epithelia in which a similar fate switch is also observed and for tumor initiation and progression.

[15] Investigating genetic modifiers of hepatocellular carcinoma in iPSC derived hepatocytes and cancer lines with genome-wide CRISPR/Cas9 screening

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Presenting author: Namal Coorey

Hepatocellular carcinoma (HCC) is the third leading cause of cancer related deaths and accounts for 90% of primary liver cancers. HCC is the terminal event of chronic liver disease. It arises from acute or sub-acute liver insults that lead to ever increasing levels of hepatic fibrosis that culminate to cirrhosis and HCC. If diagnosed at the advance stages, the survival rates averages 12 months with rarely exceeding more than three years. Until recently, multi-kinase inhibitor, sorafenib was the only approved systemic medication for treatment of advanced HCC. In 2017, two additional multi-kinase inhibitors were included in the armamentarium. Therefore, there is a great-unmet need for new and effective therapeutics for HCC. Herein, investigated are novel targets for hepatocellular carcinoma by genome-wide CRISPR/Cas9 screening in iPSC derived hepatocytes-like cells (HLCs) and in HCC cell lines. To that end, the role of Glypican-3 (GPC3) in hepatocellular carcinoma and as a marker for HCC in genome-wide CRISPR/Cas9 screening. Employing the Brunello library of 73,178 barcoded guides, with coverage of four guides per gene, HLCs and HCC lines are assessed for GPC3 expression. The cells that show the greatest change in GPC3 levels by precision mutation with CRISPR/Cas9 mediated KO are determined and mutations are characterised by next generation sequencing of barcodes. Thereby, identifying genes when deleted that induce hepatocellular carcinoma in HLCs and genes when deleted that reduce the HCC phenotype in cancer lines. The role of these genes in HCC are further validated by the impact of these deletions on other known markers for HCC and non-malignant healthy cells. This two-pronged approach will give novel insight into both the progression of HCC and identify novel targets for treatment of HCC. With the ever expanding drugable genome, these targets will be invaluable in the development of novel therapeutics.

[16] From the pituitary gland to organoid modeling: There and Back Again

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Presenting author: Benoit Cox

The pituitary is the master endocrine gland, harboring stem cells of which the phenotype and role remain poorly characterized. Here, we established organoids from mouse pituitary as a novel research model to study pituitary stem cell biology. The organoids originate from the pituitary SOX2+ stem cells, are long-term expandable, retain a stemness phenotype during expansive culture and show specific hormonal differentiation ability after subrenal transplantation. Application of the protocol to transgenically injured pituitary in which the stem cells are activated, resulted in more numerous organoids. Intriguingly, these organoids display a cystic morphology whereas the organoids from undamaged gland are predominantly dense. Compared to the dense organoids, cystic organoids are more limited in expandability. Transcriptomic analysis revealed distinct epithelial phenotypes with cystic organoids composed of simple columnar/cuboidal epithelium and dense organoids (from undamaged pituitary) of stratified squamous epithelium. Further transcriptional profiling showed that the cystic organoid type more closely resembles the pituitary phenotype, at least to an immature state, and is capable of specific in vitro differentiation. Organoid characterization further exposed facets of pituitary stem cell regulatory pathways and advanced new injury-activated stem cell markers. Finally, comprehensive transcriptome analysis revealed striking similarities in biological processes between cystic organoids and ex vivo isolated stem cells as activated after injury. We suggest a stem cell 'injury signature' which advances a central role for immune/inflammatory processes. Further exploration may lead to a better understanding of the activation and regenerative response of pituitary stem cells following damage in the gland. Taken together, we established a novel organoid research model revealing new insights into pituitary stem cell identity and regulation. The organoid model will provide an important tool to decipher pituitary stem cell biology in both healthy and diseased gland.

[17] 3D human pluripotent stem cell-derived model of the neural tube in a synthetic extracellular matrix

Brian Daza Jimenez, Abdel Rahman Abdel Fattah, Gregorius Andika W. Rustandi, Adrian Ranga

KU Leuven

Presenting author: Brian Daza Jimenez

Recent advances in extracellular matrix bioengineering have enabled the generation of highly defined and biomimetic 3D models of morphogenesis and patterning. In particular, we have shown that single mouse embryonic stem cells (mESC) could generate a neural tube-like construct in tunable synthetic 3D microenvironments, thereby enabling the study of the role of the extracellular matrix in specifying dynamic changes in fate and pattern. However, to date, a human equivalent of this organoid model has thus far not been demonstrated, thereby limiting our understanding of early human neural tube development. Here, we have used human pluripotent stem cells (hPSCs) to generate single cell clonally-derived neuroepithelial cysts in synthetic poly(ethylene) glycol (PEG)-based extracellular matrices. These cysts acquire epithelial polarity within the first day of differentiation. Ventralizing the cysts gives rise to floor plate (FP) markers (FOXA2) while untreated cysts acquiring dorsal fate (PAX6). Recent findings suggest that even after inducing cysts ventrally, there is a temporal window before a cyst can start expressing FP markers. This transition from default dorsal to ventral can be important for correct patterning. These results show a different temporal mechanism than in the mouse system, but neuroectoderm expression is consistent with other studies of human cells in monolayer culture. We also observe that varying matrix stiffness modulates PAX6 expression, suggesting that cell-matrix plays a significant role in neuroectodermal differentiation. These results suggests that correct spatial organisation in these cysts is specified from the ventral to the dorsal aspect, but early expression of motor neuron precursors may suggests that before FP formation, motor neuron pathways may be involved in the transition to ventral fate. Future direction of this work include a more complete characterization of this novel human neural tube model and an investigation into the role of mechanically induced stress on patterning.

[18] Non-overlapping functions of the TCF/LEF family factors in mouse Embryonic Stem Cells

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Presenting author: Anchel de Jaime Soguero

Wnt/β-catenin signaling pathway exerts pleiotropic functions both in development and disease. While activation of Wnt signaling maintains self-renewal and stem cell homeostasis, its aberrant activation is usually correlated with some pathologies, such as cancer. The complexity of functions mediated by this pathway converges in the interaction of β-catenin with the TCF/LEF transcription factor family. In mammalians, an evolutionary diversification of this transcription factor family has come up with the existence of four different TCF/LEF proteins. Although well studied, whether they bind to the same DNA regions and therefore regulate the expression of the same target genes remains controversial. Using mouse Embryonic Stem Cells as a model, we described for first time that the most expressed TCF/LEF factors (Tcf3 and Tcf1) bind to different DNA binding motifs. While Tcf3 was found to repress transcription by binding for the promoters of pluripotency factors, upon Wnt activation Tcf1 induces the expression of several genes controlling cell cycle arrest, without altering pluripotency. Experiments of gain and loss of function of both TCF/LEF factors confirmed that different binding of Tcf1 and Tcf3 is translated in the regulation of different target genes and functions in mESCs. Altogether, here we show that Wnt signaling controls mESCs pluripotency and cell cycle through non-overlapping functions of distinct TCF/LEF family members.

[19] Understanding the mechanisms controlling EMT mediated therapy resistance in skin squamous cell carcinoma.

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ULB- Laboratory of Stem Cells and Cancer

Presenting author: Maud Debaugnies

The resistance of cancer cell to chemotherapy and radiotherapy is responsible of the death of most cancer patients. Different intrinsic mechanisms controlling intratumor heterogeneity like genetic, epigenetic, phenotypical states of tumor cells including EMT have been suggested to regulate resistance to therapy in cancer cells. Here, using a mouse model of skin squamous cell carcinoma (SCC) that undergo spontaneous EMT during tumorigenesis, we identified EMT tumor subpopulations that are differentially intrinsically resistant to a wide range of anti-cancer therapy including 5-FU, platinimum, taxane and radiotherapy in vivo and in vitro. Using genome wide transcriptional analysis, we identified a new candidate gene that may be responsible for the resistance to therapy. We confirm the role of this candidate using shRNA knock down in vitro. We performed proteomic analysis in order to identify how it mediates therapy resistance. Hopefully, the identification of such new potential target and the understanding of its mechanism of action will lead to an improvement in the efficiency of SCC therapy.

[20] Defining the mechanisms leading to interfollicular epidermis expansion during post-natal development

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Université Libre de Bruxelles

Presenting author: Sophie Dekoninck

The Interfollicular Epidermis (IFE) is a stratified epithelium constantly renewed and is a first barrier of defense for living organisms. The murine tail IFE contains different regions called scale and interscale and is a useful model to study skin Stem Cells (SCs). Previously, our group demonstrated that a hierarchy of slow-cycling SCs and Committed Progenitors (CP) maintained interscale compartment during adulthood whereas scale regions only contains CPs. While mouse tail IFE has been extensively studied during homeostasis nothing is known about the role of these two populations during postnatal growth. In this project, we use clonal analysis to trace isolated single basal cells and study their progeny during post-natal development from birth until adult. Our morphometric analysis conducted on the whole tissue reveals a linear expansion mainly achieved before postnatal day 30 (P30). Our clonal analysis shows that labeled IFE progenitors give rise to clones that expand overtime with an average basal footprint proportional to the whole tissue expansion demonstrating that the labeled population recapitulates the tissue growth. Mathematical analysis performed on clonal and proliferation data suggest that the linear growth is achieved through a constant imbalance toward self-renewal compensated by a decreasing proliferation rate. In sharp contrast with the homeostatic model in adult, cumulative clone size distribution rather fit with a single population of cells in interscale suggesting a more homogeneous basal compartment in post-natal development compared to adult. Interestingly, single cell RNA sequencing performed on basal progenitor during post-natal development versus adult confirms this observation.

[21] Postnatal deletion of Smad-interacting-protein 1 (Sip1, Zeb2) in progenitors or neuroblasts impacts olfactory bulb interneuron migration and differentiation

Deryckere A, Stappers E, Dries R, Peyre E, van den Berghe V, Conidi A, Zampeta F-I, Francis A, Bresseleers M, Stryjewska A, Vanlaer R, Maas E, Zwijsen A, van IJcken W, Grosveld F G, Nguyen L, Huylebroeck D, Seuntjens E

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Presenting author: Astrid Deryckere

Neurogenesis in the mouse brain is continued after birth in two distinct niches being the ventricular-subventricular zone (V-SVZ) lining the lateral ventricle and the dentate gyrus of the hippocampus. Neuroblasts produced in the V-SVZ migrate tangentially through the rostral migratory stream towards the olfactory bulb (OB) where they integrate and give rise to a variety of OB interneurons. Smad interacting protein 1 (Sip1/Zeb2) is a transcription factor that binds E2-box-type sequences and mutations in one allele cause Mowat-Wilson syndrome, a severe multi-spectrum neurodevelopmental disorder. Presence of Zeb2 has been found embryonically in the LGE and postnatally in the V-SVZ. We previously showed that conditional deletion of Zeb2 in either the LGE or V-SVZ results in a postnatal decrease in proliferation capacity as well as migration and differentiation defects. Moreover, we found that postnatal overexpression of Sox6 results in a similar phenotype. Here, we use Cre-drivers that target different populations of OB interneuron precursors to delete Zeb2 postnatally and evaluate the proliferative capacity of the V-SVZ and migration potential of OB interneurons.

[22] Translation stress induced by loss of Elongator activates a p53-dependent antitumor checkpoint in hematopoietic stem and progenitor cells

Adeline Rosu, Kevin Rouault-Pierre, Eve Ramery, Joan Somja, Francesca Rapino, Qiang Bai, Laurent Nguyen, Jan Cools, Dominique Bonnet, Alain Chariot, Pierre Close, Fabrice Bureau, Christophe J. Desmet

Liege University

Presenting author: Christophe Desmet

Tightly controlled protein synthesis is emerging as an essential requirement for the normal function, differentiation and fate decisions of hematopoietic stem and progenitor cells (HSPCs). Here, to reveal translational determinants of HSPC biology, we used mouse models of conditional inactivation of Elongator, an enzymatic complex that optimizes speed of translation elongation by catalyzing modifications of specific transfer RNAs. We report that inactivation of Elongator activity blocked HSPC differentiation and caused bone marrow failure through the activation of translation stress responses orchestrated by the transcription factors Atf4 and p53. In contrast, the maintenance, but not the reconstitution activity of Elongator-deficient hematopoietic stem cells, was spared. Deletion of p53 rescued Elongator-deficient HSPC function. Yet, Elongator inactivation prompted the development of p53-mutated hematologic malignancies and inactivation of p53 and Elongator synergistically promoted tumorigenesis. Inactivation of Elongator thereby reveals a p53-dependent quality control of translation that conditions antitumor fate decisions in HSPCs.

[23] The neuroregenerative effect of human dental pulp stem cells in vitro: revealing the potential of IGF-II

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Hasselt University

Presenting author: Yorg Dillen

Ischaemic stroke is a severe condition which is defined by loss of brain function due to impaired blood flow to the brain. Cell-based therapy is considered as a promising approach to minimize neurological damage and enhance functional recovery. The goal of this study is to evaluate the neuroregenerative effect of human dental pulp stem cells (DPSC) in vitro and identify the key paracrine factors mediating this effect. The effect of DPSC on the migration of NSC was investigated. Therefore, a transwell migration assay with mouse NSC was performed. The conditioned medium (CM) of DPSC was able to significantly increase the migration of NSC (n = 8). Various growth factors, including BDNF, NGF, GDNF, NT-3 and IGF-II were shown to be secreted by DPSC and their particular effect on NSC migration was investigated. IGF-II significantly attracted NSC in the transwell system (n = 7), revealing a potential role for IGF-II to stimulate the neuroregenerative process after stroke. To investigate the contribution of IGF-II to the stimulatory effect on NSC migration by CM of DPSC, the transwell migration experiments was performed while the function of IGF-II is inhibited. Preliminary experiments show a reduction in migration of NSC when the IGF-II function is blocked in the CM of DPSC. Taken together, our data reveal a promising role for IGF-II as a neuroregenerative strategy.

[24] THE SILENCE OF THE MUTANT: TOWARDS A TARGETED THERAPY FOR KCNQ2 ENCEPHALOPATHY

Nina Dirkx, Peter De Jonghe, Sarah Weckhuysen

VIB – UAntwerp Center for Molecular Neurology (CMN)

Presenting author: Nina Dirkx

KCNQ2-encephalopathy (KCNQ2-E) is a subtype of severe epilepsy, characterized by neonatal seizures, and by a severe developmental impairment. As in other epileptic encephalopathies (EEs), all currently available treatments purely target seizures, whereas the neurodevelopmental outcome is at least as devastating for the quality of life. There is thus a strong need for new therapies that target both aspects of the disease. Since KCNQ2-E is caused by dominant negative (DN) or gain of function (GOF) variants and KCNQ2-haploinsufficiency is known to cause benign familial neonatal epilepsy (KCNQ2-BFNE), in which patients develop perfectly normal, mutant allele specific silencing seems a promising approach for the treatment of KCNQ2-E. In this project, we will perform an in vitro proof of concept study for RNA interference as a treatment strategy for KCNQ2-E, using 2D neuronal cultures derived from human induced pluripotent stem cells (hiPSC) of 1 known GOF (R201H), 2 known DN (A294V and R560W) KCNQ2-E variants, as well as 1 KNCQ2-BFNE variant (K327G). We are generating co-cultures of excitatory and inhibitory hiPSC derived-neurons using forced viral-overexpression of transcription factors. Cultures will be characterized morphologically via immunocytochemistry, and electrophysiologically using microelectrode arrays and whole-cell patch clamping. Mutation specific shRNAs will be designed and validated in HEK cells. The most potent shRNAs will be used in neuronal cultures with the aim to revert the KCNQ2-E phenotype to the benign KCNQ2-BFNE phenotype. When successful, this approach could be extended to many other EEs with similar characteristics. Additionally, we are generating brain organoids to more precisely model the neurodevelopmental process. Whole-cell patch clamping, calcium imaging, characterization of the structural formation, synaptogenesis, neuron morphology and interneuronal migration will help gain insight into the underlying mechanism leading to developmental delay in KCNQ2-E.

[25] Modeling Alström Syndrome-Associated Cardiomyopathy: "A Deaf Heart" Promotes Proliferation

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During the perinatal period, cardiomyocyte (CM) proliferation abruptly declines and the majority of CMs undergo cell cycle arrest. Postnatal cell cycle arrest is a crucial event for heart maturation to prevent cardiac dysfunctioning. Alström Syndrome (ALMS) is a recessive autosomal ciliopathy, caused by mutations in the ALMS1 gene, in which, in some rare cases, CM cell cycle arrest does not occur. ALMS has a systemic clinical phenotype, characterized by sensorineural hearing loss, type 2 diabetes mellitus and fibrosis in multiple organs, including the heart. Cardiomyopathy implications have been observed in approximately 70% of the ALMS patients. Interestingly, a clinical case study, describing two siblings with neonatal heart failure, reported a rare pediatric situation of mitogenic cardiomyopathy with identification of ALMS1 gene mutations (Shenje et al., Nat Commun. 2014). Here, we reprogrammed human somatic cells, isolated from ALMS patients with known ALMS1 gene mutations, towards induced pluripotent stem cells (iPSCs), generating a human iPSC-derived CM disease model. We observed persistent proliferation of diseased CMs, suggesting a dysregulation of the postnatal cell cycle arrest due to ALMS1 gene mutations. In addition, higher percentage of ALMS1-deficient CMs was observed in the G2/M cell cycle phase compared to control cells. Moreover, gene and protein expression levels of ALMS1-deficient CMs revealed significant down-regulation of late-stage CM markers and, interestingly, abnormal WNT/β-Catenin and YAP/TAZ signaling signatures. These findings could be relevant for unraveling novel disease mechanisms, as well as interactions between ALMS1 protein and both WNT/β-Catenin and YAP/TAZ signaling pathways, eventually pointing out new therapeutic targets to counteract disease onset or progression.

[26] Muscle-derived mesenchymal stem cells can be differentiated into cardiomyocytes in vitro.

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Background: Mesenchymal stem cells can be isolated from equine skeletal muscle, offering an alternative to commonly used bone marrow-derived mesenchymal stem cells. Equine muscle-derived mesenchymal stem cells (mdMSC) have already been differentiated into endothelial cells. Aims: To differentiate equine mdMSC into cardiomyocytes. Methods: Equine mdMSC have been achieved from a muscular micro-biopsy. They have been grown by explant culture and isolated using a Percoll discontinuous density gradient. Cells have been expanded during several passages, then the culture medium has been removed and differentiation into cardiomyocytes has been conducted using 10nM phorbol myristate acetate (PMA) for 48h. Seven days after PMA removal, immunofluorescence against cardiac troponin T has been performed. Results: After 2 weeks of explant culture, mdMSC have been isolated. A small amount of muscle (15-20mg) has allowed reaching approximately 60 millions mdMSC in 6 weeks. Cells cultured in PMA were positive for cardiac troponin T, confirming their differentiation into cardiomyocytes, whereas mdMSC in culture medium weren't. No morphological modification has been observed. Conclusions: A considerable amount of pluripotent equine mdMSC can be readily achieved in a micro-invasive manner. mdMSC can be a good alternative to bone marrow-derived mesenchymal stem cells whose sampling is painful and whose production yield is low. Cardiomyocytes differentiated from mdMSC could be used after myocardial infarction. This work is part of a wider project aiming to obtain cells of the cardiovascular system from mdMSC. Indeed, mdMSC and cells obtained by differentiation represent a valuable research material and offer original and promising therapeutic prospects in cardiovascular sciences. Next step will aim to obtain valvular interstitial cells. These cells, combined with endothelial cells that have already been differentiated, could support the development of hemocompatible tissue-engineered heart valve. Keywords: muscle-derived mesenchymal stem cells, cardiomyocytes, phorbol myristate acetate, cardiac troponin T

[27] Uncovering regulators of human embryonic stem cell differentiation bias towards definitive endoderm

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

[28] The effect of microstructural alterations of fibrin and self-assembling peptide hydrogels on Dental Pulp Stem Cells behavior

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Introduction Humans lack the ability to naturally regenerate their own tissues. To overcome this limitation, tissue engineering strategies utilizing combinations of biocompatible scaffolds, growth factors, and stem cells to mimic natural morphogenesis, are currently in development. Amongst those strategies are the regenerative endodontic procedures, that can be defined as biologically-based processes designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex. Objectives To evaluate the effect of the inclusion of a novel experimental molecule (COAM) on the microstructural properties of fibrin and self-assembling peptide hydrogels. To evaluate the influence of the microstructural alterations on Dental Pulp Stem Cells (DPSCs) behaviour. To identify the most suitable hydrogel for further in vivo experiments. Materials and Methods Biophysical characterization for the hydrogels with (control) and without (test) COAM was performed using; 1- atomic force microscopy (AFM) and scanning electron microscopy (SEM) to determine the influence on the microstructure (roughness average, fiber length, thickness, straightness and alignment), 2- the stiffness of the hydrogels was measured using a nanoindentator. DPSCs were encapsulated in hydrogels with and without COAM and the cell viability was determined at 1, 4 and 7 days. Results and Conclusion The inclusion of COAM didn't alter the microstructure of the hydrogels on the fiber level. Young's elastic modulus for fibrin hydrogel at 3.5mg fibrinogen concentration was 742 Pa (±204) for the control group and 683 Pa (±73.4) for the test group. Cell viability in the fibrin hydrogel was; 1- control, 92.3% (D1), 91.6% (D4), 90.8% (D7), 2- test, 90.6% (D1), 91.2% (D4), 88.0% (D7). For the Self assembling peptide hydrogel; 1- control, 66.9% (D1), 55.8% (D4), 54.1% (D7), 2- test, 68.3% (D1), 60.1% (D4), 53.9% (D7). Statistically significant differences ($P<0.05$) were present between the cell viability in fibrin hydrogels vs self assembling peptide hydrogels. In this study fibrin hydrogel shows superior outcomes compared to self assembling peptide hydrogel.

[29] DEFINING THE CELL OF ORIGIN OF BREAST CANCER

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Presenting author: Marco Fioramonti

Breast cancer consists of heterogeneous tumours classified into different subtypes. The mechanisms causing this heterogeneity are unknown. Oncogenic Pik3ca(H1047R) expression in mammary basal cells (BCs) induced the formation of luminal-like tumours, while its expression in luminal cells (LCs) gave rise to luminal-like and basal-like tumours. This demonstrated that the cell of origin of breast cancer plays a role in the determination of tumour histotype. Moreover, both BCs and LCs, usually unipotent during adulthood, became multipotent after expression of Pik3ca, generating LCs and BCs, respectively. Activation of multipotency seems to be essential for Pik3ca-driven tumorigenesis. In the adult mammary gland, LCs represent an heterogeneus population composed by oestrogen receptor positive (ER+) and ER- cells. Lineage tracing experiments showed that ER- LCs are maintained by a distinct pool of stem cells (SCs). Using a doxycycline-inducible ER-rtTA mouse model, we showed that ER+ cells are sustained by lineage-restricted SCs that exclusively contribute to the expansion and maintenance of their lineage. The presence of different independent subpopulations of LCs suggests that they could act as cell of origin of different types of breast cancer. We used Notch1, ER and K8 promoters to induce Pik3ca expression and trace ER-, ER+ and all LCs, respectively. Thanks to this, we were able to study the plasticity of LCs subpopulations upon Pik3ca expression. Our results indicated that both ER+ and ER- LCs give rise to BCs after Pik3ca expression, but only ER+ cells give rise to ER- cells and not vice versa. We will perform Single Cell RNA-sequencing in order to characterize the mechanisms leading to this multipotency. Furthermore, our study will clarify if all LCs are able to give rise to breast cancer, and if the cell of origin within LCs has an impact on the tumour outcome.

[30] MSH2 knock-out human pluripotent stem cells as model for CTG repeat instability in myotonic dystrophy type 1

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Human pluripotent stem cells (hPSC) are a powerful tool to model repeat instability in myotonic dystrophy type 1 (DM1). The mismatch repair pathway, especially MSH2, has been linked to repeat instability in MSH2 knock–out mouse models and MSH2 knock-down human cell models. We recently constructed a MSH2 knock-out DM1 hPSC model, by CRISPR/Cas9 editing. The complete absence of MSH2 allows us to define the role of MSH2 in repeat instability to a greater extent than in human MSH2 knock-down models. Repeat instability was measured by PacBio sequencing of PCR fragments spanning the repeat, allowing an accurate assessment of the repeat length. Our preliminary data shows that the CTG repeat in two of our MSH2-/- hESC models seems to stabilize and even might contract compared to the unstable repeat in MSH2+/+ hESC models of the same line. The expansion in MSH2+/+ hESC lines continues to expand over long term culture and have a wide repeat size distribution compared to their MSH2-/- lines in which the repeat lengths are less heterogeneous and cluster around a particular CTG expansion. Our results suggest that MSH2 drives repeat instability in DM1 hPSCs and that a lack of MSH2 could stabilize the CTG repeat and shifts towards a contraction bias. In addition, the methylation status of the CCCTC-binding factor (CTCF1) site, flanking the CTG repeat, loses its methylation gradually overtime, concomitant with CTG repeat stabilization in our MSH2-/- hESC models as analysed by massive parallel sequencing. We hypothesize that our observations lead to the following chronological order of events: MSH2 knock out halts the expansion bias observed in MSH2+/+ hESC lines which eventually leads to small CTG repeat contractions and upstream CTCF1 demethylation upon reaching a threshold CTG repeat size.

[31] C9ORF72 repeat expansions cause axonal transport defects in iPSC-derived motor neurons

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The hexanucleotide repeat expansion (HRE) GGGGCC in C9orf72 is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Repeat-associated non AUG (RAN) translation of the GGGGCC expansion results in five different dipeptide repeat proteins (DPRs: poly GA, poly GP, poly GR, poly PR and poly PA), which have been proposed to play a crucial role in C9orf72 HRE-induced cytotoxicity. Defective axonal transport is an early perturbed event occurring across several models of familial ALS, indicating that it may be a key initiating contributor to the selective vulnerability of motor neurons. At present, very little is known about the effect of the C9orf72 HRE on axonal transport and the mechanism underlying it. Here we used induced pluripotent stem cell (iPSCs)-derived motor neurons (MNs) differentiated from multiple C9orf72 ALS/FTD patients and controls in order to investigate the effect of the HRE on axonal transport and the contribution of poly-PR on this phenotype. No difference in the ability to differentiate into mature, functional and Islet1/ChAT positive motor neurons was observed between control and C9orf72-positive cultures. C9orf72 iPSCs-derived MNs showed RAN translation pathology and an increase in p62/SQSTM1 levels compared to control. Moreover, we found that the C9orf72 expansion resulted in a significant decrease in the number of motile mitochondria along the processes and this phenotype seemed to be more pronounced over time. In addition, treatment with synthetic DPR protein (PR)20 decreased the number of motile mitochondria in iPSCs-derived control motor neurons. Together, our data suggest that C9orf72 HRE cause axonal transport impairment in human-derived motor neurons and show that poly-PR may play a role in the mechanism underlying the axonal transport deficit observed in the C9orf72 iPSCs-derived MNs.

[32] DISEASE MODEL FOR GRN-LINKED FTLD USING PATIENT- AND ENGINEERED STEM CELLS.

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We created an iPSC based model for FTLD caused by mutations in the progranulin gene (GRN), including isogenic mutant and wild type lines. For this, we seamlessly inserted the GRN gene mutation (IVS1 +5G>C) into a normal donor iPSC line (using site specific homologous recombination as described in Garcia Leon et al, Alzheimer's & Disease, 2018) and created iPSC lines from patients with GRN-mediated FTLD, wherein we conditionally overexpressed a single copy of the tetracycline inducible GRN gene in the AAVS1 locus (Ordovas et al., Stem Cell Reports). Sequencing confirmed the introduction of the GRNIVS1+5G>C mutation and GRN expression levels, assessed by RT-qPCR, confirm GRN haploinsufficiency throughout cortical neuron differentiation. Conditional overexpression of PGRN to correct PGRN haploinsufficiency in patient derived iPSC lines was confirmed by qRT-PCR and Western blot. Patient- and engineered stem cells were characterized, differentiated into cortical neurons using published methods (Shi et al. Nature Protocols, 2012) and evaluated defects both at the iPSC stage and at neuronal progenitor/neuron stage (DIV40). These functional studies identified that PGRN haploinsufficient iPSC and DIV40 neuronal progeny displayed cytoskeletal dysfunction. We also subjected iPSC and DIV40 and DIV70- progeny to RNA-sequencing. Transcriptome analysis of the patient derived and engineered iPSC and neuronal progeny revealed the presence of deranged cytoskeletal transcriptional pathways both in undifferentiated iPSC and in their neuronal progeny. In conclusion, by investigating patient derived and genome engineered GRNIVS +5G>C PSC and cortical neurons, we demonstrated PGRN-linked deranged cytoskeletal abnormalities in iPSC as well as their cortical neural progeny, both functionally and at the transcriptome level. Further validation of these pathways is ongoing.

[33] DNA Hypermethylation drives EMT-mediated resistance to various cancer therapies

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Overcoming therapeutic resistance is one of the major hurdles in cancer care. The process wherein epithelial cells switch to a mesenchymal state (epithelial-to-mesenchymal transition or EMT) often underlies this acquired resistance to cancer therapy. The precise mechanisms driving EMT are however not fully elucidated. Here, we explore whether DNA methylation changes are possibly involved in EMT-mediated therapeutic resistance. We selected 10 pairs of cell lines consisting of a 'parental' cell line and a treatment 'resistant' cell line obtained after low-dose chronic exposure of the parental line to a targeted therapy (n=7) or chemotherapy (n=3). Based on changes in gene expression and cellular morphology, we observed that 8 resistant lines had undergone EMT. When transfecting resistant cell lines with miRNA-200, a known inhibitor of EMT, expression of EMT markers was reversed and resistance to therapy disappeared, indicating that EMT is essentially underlying the resistance. Resistant cells accumulated a remarkably high number of DNA methylation changes, and significantly more CpGs than expected based on random chance underwent methylation changes in >1 cell line. Importantly, promoters of epithelial marker genes were consistently more hypermethylated, while promoters of mesenchymal marker genes were hypomethylated in resistant cells. Mechanistically, we observed the majority of these methylation changes to be mediated by the demethylating TET enzymes. To prove that methylation changes were causal to EMT-driven therapeutic resistance, we exposed resistant cells to a non-cytotoxic dose of the demethylating agent 5-aza-2'-deoxycytidine. Interestingly, upon DNA demethylation, as confirmed by LC-MS, resistant cell lines lost their mesenchymal phenotype and became sensitive again to the corresponding therapy. Based on these findings, we propose DNA methylation as a novel mechanism underlying EMT-mediated therapeutic resistance of tumors.

[34] Modelling the dynamics of the hepatocyte-specific transcriptional network

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Presenting author: Claude Gérard

Characterizing the dynamics of the gene regulatory network (GRN) driving hepatocyte development is essential for understanding how cell fate decisions are made, and for optimizing in vitro production of hepatocytes. The GRN driving hepatocyte differentiation comprises several liver-enriched transcription factors (LETFs). Current functional studies cannot predict how each factor quantitatively controls the expression of all other members of the GRN. Our goal is to develop a computational tool to capture the temporal dynamics of the GRN and to predict how quantitative variations of individual GRN members impact on the global function of the network. We measured LETF expression at several stages in developing hepatocytes, using total RNA from whole liver and FACS-purified hepatocytes, from wild-type and mutant mouse embryos. Our measurements were used to calibrate a mathematical model which is based on a set of kinetic equations describing the temporal expression of the GRN members throughout development. The mathematical model was validated by comparing the in silico-predicted and experimentally-measured effect of a miRNA inhibiting LETFs. We currently generate mouse hepatoblasts in vitro by forced expression of LETFs and verify if the GRN obtained in vitro matches with that from developing liver. We will adapt the mathematical model to provide a tool for improving LETF-driven differentiation of human hepatocytes in vitro.

[35] Investigating the molecular signaling of Lgr5 engagement on Wnt modulation

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Lgr4 and Lgr5 receptors are GPCRs family members, and have been reported to be co-expressed in adult intestinal stem cells (SC). Moreover, by binding its ligands R-spondins, Lgr5 strongly potentiate the canonical Wnt/ß-catenin pathway but does not signal in a «classical» GPCR-dependent manner. Moreover, similarly to the glycoprotein hormone receptors, to which they are phylogenetically related, Lgrs have a long hinge region connecting the seven transmembrane domains with the LRRs. Moreover, Lgr4 and Lgr5 play a role as Wnt modulators in the intestine and other tissues, but the molecular mechanisms are still unclear. To get further understanding about their biological function regarding Wnt signalling modulation in SC, we are focusing on in vitro/ex vivo studies to dissect molecular signalling associated with Lgr4/5 receptors "activation/engagement" and on in vivo studies using conditional Lgr loss-of-function mouse lines in homeostasis, regenerative and oncological models. The goal being to determine whether Lgr4 and Lgr5 could represent valuable drug targets to better control this pathway. On the knowledge of glycoprotein hormone activation and protein alignments, we have generated several mutants, lying between the hinge region and the transmembrane domains. They have been tested,regarding the level of Wnt/ßcatenin activation, by performing TOP/Flashluciferase assays following transient transfection in HEK293T. Taking into account that these mutants are similarly expressed at the cell membrane, only one, located in the hinge region, potentiates Wnt activity in absence of mRspo1, at the highest Wnt3a concentration. Following these first data and to further investigate the signaling cascade, we will test the impact of others Rspo on the Wnt/ß-catenin pathway and on classical GPCR ones. In addition, lentivirus infection of organoids, driving the expression of the Lgr5 mutant, should be interesting to address its potential impact on stem cells self-renewal and differentiation.

[36] Extracellular vesicle - derived microRNAs improve mesoangioblasts treatment in muscle wasting condition

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Skeletal muscle is a dynamic and plastic organ endowed with an intrinsic capability of growth and regeneration both in physiological conditions, as well as in case of injury. Chronic muscle illnesses, generally caused by genetic and acquired factors, lead to deconditioning of the skeletal muscle structure and function, and are associated with a significant loss in muscle mass. At the same time, progressive muscle wasting is a hallmark of aging. We recently showed that mesodermal iPSC-derived progenitors can regenerate striated muscles and combining RNA-seq and miRNA-seq data, we defined microRNA (miRNA) cocktail that promote myogenic potential of those myogenic progenitors (Giacomazzi et al, 2017). Moreover, given the paracrine properties of myogenic stem cells, extracellular vesicles (EVs) -derived signals have been implicated both in the pathogenesis of degenerative neuromuscular diseases and as a possible therapeutic target (Hervera et al, 2018). In this work we have screened the content of EVs from animal models of muscle hypertrophy and muscle wasting associated with a chronic disease and aging. Analysis of the transcriptome, protein cargo and miRNAs has allowed us to identify a hypertrophic miRNAs signature amenable for targeting muscle wasting. We have tested this signature in vitro on mesoangioblasts (MABs), adult vessel associated stem cells, given their relevance for treating muscle loss, and we have observed an increase in myogenic differentiation. Furthermore, injections of miRNA treated MABs in aged mice has resulted in an improvement in skeletal muscle features, such as muscle weight, strength, cross-sectional area and fibrosis. We provided evidence that the EV-derived miRNA signature we have identified enhance potential of myogenic stem cells. Our results are embedded in the emerging field of combining EV and stem cells for regenerative medicine purposes, as we have employed miRNAs technology and stem cell therapy to target skeletal muscle decay and loss of function.

[37] MICAL2 effects on physiological and pathological myogenic fate

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Filamentous-actin (F-Actin) is one of the crucial components of contractile myofiber units. F-Actin binds the dystrophin-associated glycoprotein complex (DGC), mechanical link between the cytoskeleton and the extracellular matrix of muscle fibers. Disassembly of this protein complex renders the sarcolemma vulnerable to contraction-induced injury, leading to progressive fiber damage, membrane leakage and cell death. F-Actin interacts with Microtubule Associated Monooxygenase, Calponin And LIM Domain Containing 2 (MICAL2), capable to make oxidation-reduction reactions due to its FAD domain. Indeed, MICAL2 modifies actin subunits and promotes actin turnover by severing disaggregation and preventing repolymerization. In this study, we focus on the role of MICAL2 in skeletal, cardiac and smooth muscle differentiation. In particular, we found that murine mesoangioblasts, vessel associated stem cells, express high levels of MICAL2 when differentiated into smooth muscle cells. Adult and embryonic hearts also express MICAL2, that is again upregulated in murine embryonic stem cells and human induced pluripotent stem cells during differentiation into cardiomyocytes. Similarly, MICAL2 increases during myogenic differentiation of myoblast cell-line and primary skeletal muscle stem cells. Intriguingly, loss of function studies of MICAL2 expression resulted in impaired myogenic differentiation on both stem cell types. On the contrary, gain of function experiments show a positive impact of MICAL2 on skeletal muscle commitment. Finally, in acute and chronic skeletal muscle regeneration MICAL2 appears very highly expressed in regenerating nuclei, indicating its importance in these pathological conditions. Taken together these data demonstrate that modulations of MICAL2 have an impact on muscle differentiations. Further experiments are necessary to discern if the absence of MICAL2 affects also smooth and cardiac muscle differentiation. Moreover, understanding the role of MICAL2 in myogenic commitments and being able to modulate its expression might help the capacity to regenerate of all the muscle cell types.

[38] Development of organoids from human tooth as novel research model and tool towards disease modeling and regenerative replacement therapy

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Presenting author: Lara Hemeryck

Tooth loss, mostly as a consequence of oral disease, is a major health problem worldwide. Replacing missing or lost teeth with a biological tooth would be an interesting alternative to the current standard implantation of synthetic materials. Organoids, defined as self forming 3D in vitro reconstructions of a tissue, provide a powerful means to pursue this goal. In this study, we aim at developing organoids from dental tissue acquired following extraction of human wisdom teeth. At present, we succeeded in establishing organoids from the dental follicle of multiple extracted wisdom teeth. The organoids were found expandable for more than 8 passages. Gene expression analysis showed strong expression of dental stem cell markers. Using immunohistochemistry and transmission electron microscopy, two distinct layers were observed within the tooth organoids: an outside layer mainly composed of actively proliferating (stem) cells and an inside layer with more non-dividing cells. Given the importance of epithelium-mesenchyme interactions during tooth development, we also aim at developing hybrid organoids containing both epithelial and mesenchymal dental tissue. Currently, we are testing different culture conditions to define the optimal medium for maintaining both dental tissues in balance. Next, we will examine the differentiation capacity of the tooth organoid models obtained. Taken together, our study will generate an innovative human tooth in vitro model that will be highly valuable for studying tooth development and pathology and may pave the way towards tooth regenerative replacement therapies.

[39] Preconditioned Neurons with NaB and Nicorandil, a Favorable Source for Stroke Cell Therapy

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Poor survival of stem cells in the harsh microenvironment at the site of stroke, especially during the acute phase of injury, remains a serious obstacle to achieve the desired prognosis. We hypothesized that the combined treatment of neural stem cells (NSCs) with small molecules would precondition them to become robust and survive better as compared to the native non-preconditioned cells. Mouse ganglionic NSCs were isolated, cultured and characterized. The cells were preconditioned by treatment with NaB and Nicorandil and transplanted in an experimentally induced stroke model. Sham-operated animals without treatment or animals with experimental stroke treated with basal medium, native NSCs, NSCs preconditioned with NaB or Nico alone were used as controls. The tissue samples and cells with different treatments were used to measure BDNF level and the activity of PI3K, APE1 and NFkßp50 both in vitro and in vivo respectively. Additionally, survival of the cells and recovery indices for stroke were studied. The combined treatment with NaB+Nico resulted in increased BDNF level and higher PI3K, APE1 and the downstream NFkB activation which were blocked by pre-treatment with their respective inhibitors. Donor cell survival increased post engraftment as assessed by BrdU immunostaining and reduced TUNEL positivity at the site of engraftment. There was a reduction in pro-inflammatory cytokines and infiltration of both GFAP+ and CD68+ at the injury site. There was a reduction in the infarct size and neurological function was preserved in the preconditioned cell treatment group. Our preconditioning approach with small molecules effectively improved the survival as well as the functionality of NSCs.

[40] Uncovering Mechanisms of Epigenetic Memory Reversal during Reprogramming to Induced Pluripotency

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Presenting author: Adrian Janiszewski

The induction and reversal of gene silencing is central to the establishment and maintenance of cellular identity during development. Gene expression programs are induced by transcription factors, and remembered through cell divisions by epigenetic phenomena, which involve chromatin modifications and non-coding RNAs. However, how epigenetic memory of stable gene silencing can be reversed by transcription factors remains to be precisely revealed. Here, we will present new studies combining transcriptional profiling, epigenomic analyses and functional experiments to investigate the mechanisms by which chromatin and transcription factors mediate the reversal of epigenetic memory of gene silencing on the inactive X chromosome. We define the allele-resolution kinetics of transcriptional changes during the reprogramming of somatic cells into induced pluripotent stem cells (iPSCs). We will present evidence that genes reactivate in a hierarchical order during X chromosome reactivation. Gene reactivation is initiated before the activation of late naïve pluripotency genes and silencing of the long non-coding RNA Xist, and completed late in reprogramming. Epigenomic analyses reveal the enrichment of chromatin marks at cis-regulatory regions of genes that reactivate with different kinetics. We will show that late reactivated genes have lower starting levels of active marks such as H3K36me3. We will also present evidence that histone deacetylation acts as a barrier during X chromosome reactivation, while the histone acetyltransferase activity of the CREB binding protein is required. Our results lead us to hypothesize that a combination of chromatin states and hierarchical binding of pluripotency transcription factors dictate the kinetics of gene silencing reversal. We will test this hypothesis using ATAC-seq, ChIP-seq and single cell RNA-seq analyses. Altogether, we report for the first time the kinetics of X chromosome reactivation during reprogramming to iPSCs and provide insights into the mechanisms underlying the reversal of chromosome-wide epigenetic memory of gene silencing.

[41] The phosphatase regulator NIPP1 restrains inflammation in murine skin

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NIPP1, for Nuclear Inhibitor of Protein Phosphatase 1 (PP1), belongs to the large group of regulatory interactors of PP1 (RIPPOs) and acts as a scaffold for the recruitment of a subset of nuclear PP1 substrates. To study the in vivo function of NIPP1 in epidermal homeostasis, we generated a skin-specific NIPP1 knockout (SKO) through excision of a floxed Ppp1r8 fragment by Keratin-14 driven expression of Cre-recombinase. SKOs gradually developed a thickened epidermis, due to hyperproliferation of basal cells. To characterize the underlying molecular mechanism, we performed comparative transcriptomic analysis of the tail epidermis isolated from control (CTR) and SKO mice using RNA sequencing. The 846 differentially expressed genes were highly enriched for genes that were associated with immune response and inflammation. Ingenuity Pathway Analysis (IPA) analysis also hinted at increased NF-κB signalling in the SKOs. Accordingly, we found an increased expression of multiple NF-κB targets, including various cytokines (e.g. Ccl22, Cxcl9, Tnf and Ltb), and an enhanced recruitment of CD45-positive immune cells in the SKOs. Moreover, the epidermal hyperproliferation in SKOs was rescued by administration of the NF-κB inhibitor dexamethasone, validating the contribution of inflammation to the observed epidermal hyperproliferation phenotype. To gain more insights into the nature of the cells that contributed to the production of cytokines, we isolated hair follicle stem cells (HFSCs) by fluorescence activated cell sorting (FACS). Various cytokines were dramatically upregulated in isolated HFSCs from SKO tail epidermis, accounting for the activation and infiltration of immune cells. Moreover, a siRNA-mediated knockdown of NIPP1 in cultured epidermal HaCaT cells also resulted in an upregulation of cytokines. Thus, the depletion of NIPP1 in HFSCs results in the cell autonomous expression of cytokines. In conclusion, our data suggest that NIPP1 limits the initiation of inflammation in murine skin.

[42] Molecular phenotyping of neurons derived from CMT2 patient-iPSC lines

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Introduction Our understanding of disease mechanisms in Charcot-Marie-Tooth (CMT) neuropathy has expanded significantly owing to successful generation of disease models recapitulating the patient phenotype. Nonetheless, several human therapeutic trials have failed despite promising data derived from cellular and mouse models, leaving the urgent need for treatment unresolved. The development of iPSC technology seems promising to fill-in the gap between pathogenesis and in vivo phenotypes and may facilitate the identification of more reliable therapies. Methods We reprogrammed fibroblasts derived from four CMT2 patients with different causal mutations in the MFN2, HSPB8, HSPB1 and NEFL genes using Sendai-virus transductions. We differentiated these iPSC lines, along with healthy controls, into spinal motor neurons (sMN) using an established protocol (Guo W et al., 2017). A label-free shotgun proteome study was undertaken to find common differential proteomic signatures of patient over control, followed by RT-qPCR for the subsequent validations. Results We successfully generated sMN with an efficiency of almost 95% from both patients and controls. The proteome analysis uncovered 413 differentially regulated proteins (-log p-value>1.3) common to all genotypes. The majority of identified proteins play a role in the cytoskeleton or extracellular matrix and are vesicle associated proteins or localized to membrane bound organelles (ER and Golgi apparatus). Importantly, we observed alterations in protein expression relevant for neurodegeneration (MMP14), calcium signalling (FBN1, FBN2, PDGFRB, ATL3), actin-binding (Filamins, MYH9, TAGLN2, PALLD, ACTN) and protein quality control (HSPA2, ATL3). Furthermore, these proteins were validated at the mRNA level. Currently, we are characterizing these sMN using a high-content microscopy screening to identify phenotypic hallmarks common to CMT2, which will aid in establishing future assays for finding new therapeutic strategies. Conclusions Our data provides insights into the molecular mechanism of axonal CMT, potential biomarkers, therapeutic targets and cellular phenotypes in iPSC-derived models relevant for different CMT subtypes.

[43] Gains of chromosome 12p results in failure to exit pluripotent state and reduced differentiation capacity during hepatic differentiation of human pluripotent stem cells

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Differentiated derivatives of human pluripotent stem cells (hPSC) hold great promise in regenerative medicine. However, a small subpopulation of cells, known as residual undifferentiated stem cells (rSC), frequently fail to differentiate and can jeopardize the safety of clinical applications through the formation of teratoma tumors. Determining the cause of rSC formation could help improve the safety of hPSC in the future. In this study we performed a colony formation assay at several time points during hepatic differentiation (HD) of three genetically normal hPSC lines, resulting in numerous rSC sublines. These rSC lines underwent a second round of HD and were analyzed for the presence of definitive endoderm (DE) and hepatic markers at early (SOX17) and late timepoints (HNF4α). A majority of rSC sublines either retained their WT phenotype, differentiating normally, or exited the pluripotent state but failed to robustly differentiate to DE or HD. A subline of VUB14, VUB14_rSC2, not only failed to robustly differentiate but retained a high proportion of OCT4+ cells after 8+ days in HD medium. aCGH of these cells revealed that they carried an 11.76Mb gain at 12p13.33p31. We compared VUB14_rSC2 to two additional lines, ERB5 and VUB19_DM1, which carried similar gains on the p-arm of chromosome 12. The same phenotype was observed in all three sublines, a significant reduction in differentiated cells with a high proportion of cells maintaining OCT4 expression. Further analysis shows no deficiency in ectoderm differentiation, demonstrating that the loss of differentiation capacity is specific to (mes)endoderm. Notably, the pluripotency factor NANOG is present within the minimal gain of chr12 and is overexpressed in all three sublines. Given the known genetic mosaicism present in hPSC and the recurrent and potentially dangerous character of aberrations like the ones on chromosome 12, further work on mutations causing this rSC phenotype is warranted.

[44] Gelatin Microspheres Releasing Transforming Growth Factor Drive In Vitro Chondrogenesis of Human Periosteum Derived Cells in Micromass Culture

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Presenting author: Abhijith Kudva

For cartilage tissue engineering, several in vitro culture methodologies have displayed their potential for the chondrogenic differentiation of mesenchymal stem cells (MSCs). Micromasses, cell aggregates or pellets, and cell sheets are all structures with high cell density that provides for abundant cell-cell interactions, which have been demonstrated to be important for chondrogenesis. Recently, these culture systems have been improved via the incorporation of growth factor releasing components such as degradable microspheres within the structures, leading to enhanced chondrogenesis. Herein, we incorporated different amounts of gelatin based microspheres releasing transforming growth factor β1 (TGF-β1) into micromasses composed of human periosteum derived cells (hPDCs). PDCs, which are a MSC-like cell population and possess multipotent capability at the single cell level, are located within the inner cambium of the periosteum. Their ease of isolation and high proliferative capabilities make them an attractive stem cell choice for cartilage tissue engineering. The aim of this research was to investigate hPDC micromasses with chondrogenic stimulation by TGF-β1 delivery from the degradable microspheres in comparison to exogenous supplementation with TGF-β1 in the culture medium. Microscopy images showed that the gelatin microspheres could be successfully incorporated within hPDC micromasses without interfering with the formation of the structure, while biochemical analysis and histology demonstrated increasing DNA content at week 2 and accumulation of glycosaminoglycan and collagen at weeks 2 and 4. Importantly, similar chondrogenesis was achieved when TGF-β1 was delivered from the microspheres compared to controls cultured with TGF-β1 in the medium. Of note, increasing the amount of growth factor within the micromasses by increasing the amount of microspheres added did not further enhance chondrogenesis of the hPDCs. These findings demonstrate the potential of using cytokine releasing, gelatin microspheres to enhance the chondrogenic capabilities of hPDC micromasses as an alternative to supplementation of the culture medium with growth factors.

[45] Synthetic matrices for iPSC derived liver organoids

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Current pluripotent stem cell (PSC) hepatocyte differentiation protocols generate cells that fall short of mature liver cells. Therefore, we aimed to generate liver organoids using PSC derived parenchymal and non-parenchymal cells in synthetic 3D matrices, as synthetic matrices allow fine-tuning the microenvironment by modifying functionalisation groups and stiffness. The hypotheses we wished to address was does co-culture of different PSC-derived liver cells (hepatocytes, stellate (SCs), endothelial (ECs) and Kupffer cells (KCs)), in a matrix that mimics biophysical and biochemical features of liver improve maturation and function with ultimate goal to create better models for in-vitro toxicity screening, drug metabolisation, and liver studies. We functionalised 4-arm-polyethylene-glycol (PEG) blocks by different adhesion ligand peptides and an MMP cleavable cross-linker, and created hydrogels also with varying mechanical properties (1K—6KPa). An initial screen using > 200 microenvironments using PSC-hepatocyte progeny identified two unique microenvironments that improved the hepatocyte phenotype and function by ± 50-fold. As this was confirmed in replicate studies, the top unique microenvironment was subsequently used for co-culture of PSC-hepatocytes combined with PSC-derived SCs (as described in Col et al., 2018), PSC-ECs (directed differentiation by transcription factor ETV2) and PSC-macrophages (Claes et al., under review). All four cell populations were incorporated into organoids developing within these hydrogels as qRT-PCR detected transcripts for various markers of all four cell types in the coculture for >4 weeks. However, this did not allow quantification of the frequency of the different cells, nor if true improved differentiation was occurring. Studies are ongoing and will be presented that further characterise the liver organoids for precise frequency of different cells persisting for =/>4 weeks, the spatiotemporal organization of the different cell populations, and the relative maturation, i.e. functional properties, of the cells.

[46] Decoding the activated stem cells of the neonatal pituitary: exploration of Hippo signaling and organoid modeling

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The pituitary gland is known to undergo a vivid maturation process immediately after birth, coinciding with an activated state of its stem cell (SC) compartment. Here, we started to characterize the SCs of the neonatal pituitary both during homeostasis and upon damage. We previously discovered that the adult pituitary can restore damage and that SCs are likely involved in this regeneration, as supported by their increase in number and activated nature in response to injury. Here, we observed that the neonatal pituitary SC compartment also expanded upon pituitary damage. Since Hippo signaling is well-known to regulate tissue SC activation and regeneration, we examined the expression of Hippo components YAP/TAZ in the pituitary. Expression was observed in both neonatal and adult gland, although showing varying characteristics. YAP is mainly present in the nucleus of SOX2+ SCs, whereas TAZ is primarily located in the cytoplasm of parenchymal cells. YAP/TAZ expression appears upregulated after damage in the adult pituitary, suggesting a role for Hippo signaling in SC activation (and regeneration), while in the neonatal gland only TAZ appears stronger expressed following injury. To study pituitary SC behavior in vitro, an organoid model was recently developed from adult gland. Here, we applied this protocol to neonatal pituitary, and found that organoid formation is more efficient and requires less grow factors than organoid development from adult pituitary, correlating with the activated nature of the SC compartment in the neonatal gland. Early pituitary markers were found to be expressed in the organoid cells, but hormone expression was absent. Formation efficiency further increased when starting from damaged neonatal pituitary and these organoids could be partially differentiated into hormone-expressing cells. Now, we will further characterize this new neonatal pituitary organoid model, eventually providing a powerful in vitro tool to decode pituitary SC biology and regenerative potential.

[47] Dental pulp stem cells and leukocyte- and platelet-rich fibrin for articular cartilage repair

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Presenting author: Melissa Lo Monaco

Osteoarthritis (OA) is a degenerative and inflammatory condition of synovial joints with irreversible loss of cartilage matrix. Dental pulp stem cells (DPSCs) can be differentiated into cartilage-producing cells and secrete numerous growth factors associated with tissue repair and immunomodulation. Moreover, leukocyte- and platelet-rich fibrin (L-PRF) has recently emerged in regenerative medicine due to its growth factor content and fibrin matrix. We hypothesize that DPSCs and L-PRF improve the functional outcome in OA via immunomodulation and stimulation of (endogenous) cartilage regeneration. We evaluated whether L-PRF enhances the chondrogenic differentiation of DPSCs. Furthermore, we examined the paracrine effects of DPSCs and L-PRF on chondrocyte survival and proliferation. First, human DPSCs (hDPSCs) were subjected to a chondrogenic differentiation system, with or without L-PRF conditioned medium (CM) and exudate and compared to human bone marrow-mesenchymal stem cells (hBM-MSCs) via (immuno)histology. Secondly, mice primary chondrocytes were cultured with either hDPSC CM, L-PRF CM or exudate. The effect on chondrocyte survival and proliferation after 24h, 48h and 72h was evaluated. Preliminary data suggest that L-PRF does not increase the expression of chondrogenic markers after differentiation (n=1). Differentiated hBM-MSCs express aggrecan and collagen II, whereas differentiated hDPSCs only express collagen II. Secondly, hDPSC CM and L-PRF CM appear to have a pro-survival (n=4) and proliferative (n=3) effect on chondrocytes in vitro. Future in vitro studies will evaluate the effect of secreted factors of hDPSCs and L-PRF on primary healthy and OA chondrocyte survival, proliferation, OA-related gene expression and matrix production. The ultimate goal is to investigate the in vivo regenerative capacity and paracrine effects of ovine DPSCs and L-PRF in an ovine model of OA. We will therefore isolate DPSCs from sheep and confirm the MSC characteristics of the isolated cells according to the guidelines of The International Society for Cellular Therapy.

[48] Interplay of Tet1, Oct4 and Nodal inputs regulate embryonic lineage fate balance

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Ten-Eleven-Translocation (TET) proteins are DNA dioxygenases that convert 5-methylcytosine to 5-hydroxymethylcytosine and further oxidized intermediates in DNA demethylation. In mouse embryonic stem cells (ESCs), Tet1 and Tet2 regulate 5hmC production and cell lineage specification, but the molecular mechanisms remain to be clarified. Here, we showed that loss of Tet1 in independent strains of ESCs consistently resulted in skewing towards mesendoderm (ME) fate during embryoid body (EB) differentiation, concomitantly with reduced neuroectoderm lineage potential. While these phenotypes are associated with hyperactive Nodal signals via Smad2/3 phosphorylation, they are exacerbated in hybrid and outbred genetic backgrounds compared to C56BL/6 congenic strain and furthermore, accompanied by sustained expression of Oct4 during differentiation. Supplementation of Nodal signaling inhibitor SB431542 or knockdown of Oct4 or during serum-free differentiation restored the balance of ME marker gene expression in TET1-deficient cells. Moreover, the lineage skewing phenotypes of Tet1-deficient cells can be rescued to similar extents by wild-type and catalytic mutant Tet1, suggestive of non-catalytic regulation by Tet1 involving other strain-dependent modifier genes. In particular, dynamic molecular interactions between Tet1 and Oct4 began in the earlier peri-implantation stages of epiblast differentiation. In naive ESCs, Tet1 and Oct4 co-occupy predominantly promoter regions. Upon differentiation into gastrulation-primed epiblast-like cells (EpiLCs), a dramatic repatterning of Tet1 and Oct4 genomic occupancy occurs such that de novo Tet1 bound sites are enriched for genes associated with germ-layer lineage determination and overlapped with Oct4 at intergenic regions. Overall, our results define both cooperative and antagonistic interactions between Tet1 and Oct4 at developmental loci critical for early embryonic cell fate decisions.

[49] Development of organoids from ovarian cancer as novel research model to decipher pathogenic mechanisms and therapy resistance

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Epithelial ovarian cancer (EOC) is the most lethal gynecological cancer in women, with only 30-40% of patients surviving beyond 5 years of diagnosis. A major reason for the high mortality rate is that in more than 70% of the patients, the disease is not discovered until advanced stage when the cancer has already metastasized. In addition, the majority develops recurrent disease which shows increasing resistance to chemotherapy. Mechanisms underlying EOC pathobiology and chemoresistance are poorly understood, and therapy efficacy and patient survival have not significantly improved over the last 40 years. The key reason for this standstill is the lack of reliable and clinically translatable study models for EOC. In our study, we aim to establish organoids from human EOC. Organoids represent 3D in vitro reconstructions of the tissue of origin, thus providing powerful new tools to model and study human diseases. Moreover, organoids show long-term expansion capacity while retaining their characteristics. EOT biopsies were dissociated, embedded in 3D Matrigel, and cultured in standard cancer organoid medium. So far, organoids were obtained from several clinical EOT samples. Currently, we are optimizing the culture medium through careful selection and dosing of multiple signaling and growth factors in order to achieve optimal efficiency of development and growth of EOC organoids. Gene expression analysis of the organoids showed expression of EOC, epithelial-mesenchymal transition (EMT) and WNT pathway markers. The different organoid lines established will be thoroughly compared to the original tumor to validate whether they reproduce the (immuno-)phenotype and capture disease heterogeneity. Taken together, we will create an organoid biobank covering multiple patients with EOC (and diverse subtypes) that will allow to decipher mechanisms underlying the cancer and guide us to new therapeutic targets which will be tested using the organoids as drug screening platform.

[50] Role of Trop2-expressing stem cells in intestinal epithelial regeneration

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Inflammatory bowel diseases (IBDs) are chronic gastrointestinal disorders with complex molecular etiology characterized by sub-mucosal accumulation of inflammatory cells and severe damage of the epithelial layer, resulting in development of refractory ulcers. Recent studies point to the importance of epithelial cells for efficient "mucosal healing" and maintenance of long-term remission in these relapsing-remitting diseases. Thus, it is essential to get better knowledge of the molecular and cellular mechanisms associated with epithelial repair in experimental models of IBD, to improve mucosal healing in patients in the future. Recently, our group has provided evidences that damage of the mouse adult stomach activates partial re-expression of a "de-differentiation" genetic program in the epithelium leading to the appearance of regenerating "fetal-like" cells characterized by the expression of the Trop2 cell surface marker. In the present study, we have extended this analysis to the small intestine using the same model of injury involving acute stem cell ablation. Trop2 expressing cells were transiently detected in the injured tissues as early as 24 hours after damage, but not in control tissues; suggesting that Trop2 re-expression might be a sign of general regeneration process in the digestive tract of the mouse. To understand if this applies to human pathologies like IBDs, we are currently analyzing human biopsies from Crohn's disease and ulcerative colitis for Trop2 expression. Preliminary data indicate that Trop2 cells can be detected in such pathologies meanwhile there are barely found in control intestine. Tofurther dissect the cellular events leading to epithelial repair after the injury in the mouse model, a new mouse line Trop2-GFP-IRES-CreERT has been generated. Dr. Koo lab). The validity of this new line for lineage tracing experiments is currently being tested on adult and embryos where the Trop2 marker identifies fetal progenitors.

[51] Gain of 20q11.21 in human pluripotent stem cells impairs TGFβ-dependent ectodermal commitment

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Gain of 20q11.21 is one of the most common recurrent genomic aberrations in human pluripotent stem cell (hPSC) cultures. While it has been established that the survival advantage of the abnormal cells is due to the overexpression of the anti-apoptotic gene Bcl-xL, little is known about its impact on the differentiation capacity of the cells. Here we show that mutant hPSC have an impaired ectoderm commitment, caused by a deregulation of TGFβ signaling. RNA sequencing of mutant and control hPSC lines, along with a line transgenically overexpressing Bcl-xL, show that overexpression of Bcl-xL alone is sufficient to cause the majority of the transcriptional changes induced by the gain of 20q11.21, irrespective of the size of the mutation. Bcl-xL overexpression also results in a strong downregulation of CHCHD2, a mitochondrial membrane protein that modulates TGFβ signaling. Moreover, the differentially expressed genes in mutant and Bcl-xL overexpressing lines are enriched for genes involved in TGFβ- and SMAD-mediated signaling, and neuron differentiation. Finally, we show that this altered signaling has a dramatic negative effect on ectodermal differentiation, while the cells maintain their ability to differentiate to mesendoderm derivatives. Together, these findings demonstrate the damaging effects of genetic abnormalities on the use of hPSC in research or in a clinical context, and stress the importance of thorough genetic testing of the lines prior to their use.

[52] Characterization of NR2F2, an essential regulator of squamous cell carcinoma progression and maintenance

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Skin squamous-cell-carcinoma (SCC) is one of the most frequent cancers in Human. Expression profile analysis of epithelial cells isolated from benign and malignant squamous skin tumors in mouse has revealed that NR2F2, a nuclear receptor transcription factor, is among the rare genes upregulated specifically in malignant SCC, suggesting a function in tumor progression. NR2F2 is absent in normal epidermis and benign tumors and begins to be expressed in SCC. To determine the role of NR2F2 in the malignant transition of SCCs we used genetic approaches to modulate its expression in different models of skin tumors, observing that the deletion of NR2F2 in the mouse epidermis inhibits tumor progression. Moreover, the conditional ablation of NR2F2 in chemically induced tumors is able to induce the regression of existing SCC and prevent the formation of secondary tumors upon grafting, indicating an essential role in tumor maintenance and propagating capacity. Although the precise molecular mechanism remains to be elucidated, the transcriptional profiling and the histological characterization of NR2F2 ablated tumors suggest that NR2F2 might play an important role in remodeling the tumor microenvironment, also through an interaction with the immune system. In collaboration with the pathology department of the Erasme hospital we are validating the relevance of this gene to human tumors. NR2F2 is expressed in several types of human SCC, in focal or diffuse pattern. We are addressing its functional relevance by using CRISPR to delete NR2F2 in human SCC cell lines. Our results suggest that the function of NR2F2 is conserved, as the NR2F2-KO lines exhibit impaired tumor forming ability upon grafting in immunodeficient mice. NR2F2 represents an ideal target for the development of novel therapies in SCC treatment; hence our findings could potentially have important therapeutic implications.

[53] Extracellular vesicles from human dental pulp stem cells as proangiogenic strategy in tooth regeneration

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Tooth loss remains a major health issue since current therapies cannot regenerate damaged dental tissues such as pulp and enamel. Successful pulp regeneration depends on angiogenesis, which is key for oxygen and nutrient supply. Proangiogenic features have already been assigned to mesenchymal stem cells (MSC) of the dental pulp. So far, paracrine factors, including VEGF, have been identified as responsible angiogenic mediators. However, more recent studies indicate that extracellular vesicles (EVs) produced by bone marrow MSC (BMMSC) also have the potential to induce neovascularisation. Therefore, we compared the angiogenic properties of EVs from dental pulp stem cells (DPSC) with those of BMMSC. EVs were isolated from serum-free conditioned medium (CM) of DPSC and BMMSC after 48h by differential ultracentrifugation. EV size and concentration were measured by Nanoparticle Tracking Analysis (NTA) and purity was confirmed by Western blot with enrichment of classical EV markers CD9, CD63, CD81 and Annexin II and absence of non-EV marker mitochondrial Bax. The functional effect of EVs on the migration of endothelial cells (HUVEC), as a key step in angiogenesis, was studied in a transwell system. EVs from DPSC induce HUVEC migration (n=8). However, this effect was less compared to BMMSC EVs (n=6), which might be caused by the lower EV yield from DPSC as measured by NTA. An antibody array revealed lower expression of proangiogenic factors (e.g. VEGF, MCP1 and angiopoietin-1) and enrichment of antiangiogenic factors (e.g. TIMP1) in EVs compared to EV-depleted CM. Our preliminary data show promising in vitro proangiogenic effects of DPSC EVs. In the future, we will analyse the potential of DPSC and BMMSC EVs to induce blood vessel growth in ovo and ultrastructural analysis of both EV types will be performed. Acquired insights have positive implications for pulp regeneration and diseases associated with insufficient angiogenesis, including stroke.

[54] Impact of pluripotency state and Activin A supplementation on the derivation of primordial germ cell-like cells from human embryonic stem cells

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Background Primordial germ cells(PGCs) are the diploid precursors of gametes. Human primordial germ cell-like cells (hPGC-LCs) can be obtained in-vitro from pluripotent embryonic stem cells (hESC) by culturing them as embryoid bodies (EBs) in the presence of bone-morphogenetic protein-4 (BMP4), Stem-Cell Factor (SCF), Erythrocyte Growth-Factor (EGF), human leukaemia-inhibitor factor (hLIF) and Rho-Kinase inhibitor (ROCKi). In mouse, it's a prerequisite to start from ESC in a 'naive' state of pluripotency through the epiblast-like condition to obtain PGCs efficiently. In human, the impact of pluripotency on PGC formation efficiency remains unclear. Methods We cultured 2 in-house derived hESC lines in 4 distinct pluripotent states: (i) primed, (ii) Wnt-i (Wnt-pathway inhibited), (iii) '4-inhibitor (4i) and (iv) RSeT© naïve condition; and differentiated them to form hPGC-LCs. To improve the efficiency of germline formation, we tested the impact of Activin A supplementation in the conventional differentiation media. Fixed EB's were stained for markers of pluripotency like OCT4 and the early-germline such as SOX17 and PDPN. EBs from each replicate and cell-line were imaged as z-stacks on a Leica SP8 confocal and analysed using ImageJ/FIJI. Positive cells were quantified and subjected to statistical analysis. Results The hESC formed PGC-LCs with colocalised expression of OCT4, SOX17 and PDPN after 4,25 days of culture. By quantifying the number of triple positive hPGCLCs in the EBs, we demonstrated that using the conventional differentiation medium, hESC from the '4i' condition were most competent to form PGC-LCs. Activin-A supplementation significantly increased the yield of hPGCLCs in the 4i condition in one of the cell lines. In the other cell line 'primed' hESC differentiated with Activin A supplement exhibited higher competency towards germline. Conclusion The pluripotency state of hESC affects germline competence. This competency can be significantly enhanced by supplementing the differentiation media with Activin A. Future Perspectives This study based on confocal imaging should be supported by the transcriptome data of the obtained hPGCLCs compared to the profiles of early human PGCs harvested in-vivo. In-vitro maturation of hPGCLC to express late-stage germ-cell or meiotic markers leading to gametogenesis would further clarify the most efficient method of germline formation.

[55] Exploring the contribution of gene dosage effects of 17q gain genes on hESC and neuroblastoma proliferation

Mus, L.*1,2 , Denecker, G.*1,2, Van Haver, S.1,2, Zeltner, N.3, Ogando, Y.4, , Sanders, E.1,2, Jacobs, E.1, Popovic, M.5, Van Neste, C.1,2, Vanhauwaert, S.1,2, Durinck, K.1,2, Menten, B.1, De Preter, K.1,2, Heindryckx, B.5, Studer, L.3, Roberts, S.*4 and Speleman, F.*1,2 *equally contributed 1

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Cancer hallmarks such as uncontrolled cell growth and genome instability are driving forces of tumorigenesis. By default, DNA replication poses a threat to dividing cells as replication stress (RS) can cause DNA lesions. Oncogenes such as MYC(N) cause RS owing to increased use of replication origins and nucleotide depletion. Neuroblastoma (NB), a MYCN-driven pediatric tumor arising from sympatho-adrenergic progenitor (SAP) cells, presents with a low mutation burden and highly recurrent patterns of DNA copy number alterations including 17q gain. Cancer cells and human embryonal stem cells (hESCs) share similarities including short G1/S-phase imposing constitutive RS and genomic instability. Using integrated data-mining of more than 300 NB transcriptomes, we obtained evidence for 17q gain-driven dosage effects for several genes implicated in control of RS. Furthermore, 17q gain is also amongst the most frequently observed gains in cultured hESCs. Based on these findings, we hypothesize that 17q gains are strongly selected for in both NB and hESC to overcome excessive RS and ensure sustained proliferation. To test this hypothesis, we have performed co-culture of parental hESC with a silent genome (hESCno) and derived hESC with 17q gain (hESC17q) and show evidence for a selective advantage for hESC17q over hESCno. We are performing additional experiments and monitoring of expansion of hESC17q over hESCno using shallow-whole-genome-sequencing, digital PCR and FISH under different culture conditions including serum deprivation and naïve-to-primed transitioning. In addition, transcriptome/proteome analysis, DNA-combing assays and immunodetection for pCHK1, pRPA32 and γH2AX as RS markers will be performed to monitor the effects of 17q gain on RS resistance. To test our hypothesis for the role of dosage-sensitive RS resistor 17q genes in NB tumor progression, we set up a collaborative effort towards establishing a novel hESC-derived NB xenograft model using inducible MYCN-overexpressing 17q-gained hESC lines upon hSAP differentiation to NB progenitors.

[56] Antisense oligonucleotide-based correction of deep-intronic ABCA4 splice mutations using patient-derived fibroblasts and photoreceptor precursor cells

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Introduction: Stargardt disease (STGD1) is one of the most common inherited retinal diseases. It can be caused by over 600 different coding mutations in the ABCA4 gene, explaining 70% of the cases. We and others reported non-coding, deep-intronic mutations in ABCA4 causing STGD1. Two of these (c.4539+1100A>G & c.4539+1106C>T) are neighboring mutations that create a cryptic splice donor site, leading to inclusion of a 68- or 112-bp pseudo-exon and subsequent premature termination of ABCA4 protein synthesis. Here, we aimed to correct aberrant splicing caused by these mutations with antisense oligonucleotides (AONs), using patient-derived fibroblasts and photoreceptor precursor cells (PPCs). Methods: We designed five AONs, targeting different regions of the cryptic donor site. Subsequently, these were transfected in control and patient-derived (c.4539+1106C>T) fibroblasts, and subjected to RNA isolation and RT-PCR analysis. Furthermore, we generated induced pluripotent stem cells (iPSCs), using the CytoTune iPS 2.0 Sendai Reprogramming Kit (Newcells Biotech), to obtain PPCs using a 2D-differentiation protocol. Results: Two out of five AONs lead to a total or partial rescue of normal splicing in patient-derived fibroblasts (c.4539+1106C>T), in a dose-dependent manner. A sense oligonucleotide and a mutation-specific AON for c.4539+1100A>G were used as controls and did not show any restoration of normal splicing. This confirms the specific effect of the AONs and indicates that one mismatch is enough to abolish AON efficacy. The most potent AONs were selected for rescue experiments at the RNA and protein level in iPSC-derived PPCs. Conclusion: Overall, we describe the ability of AONs to correct aberrant splicing resulting from deep-intronic mutations in ABCA4. Furthermore, we show the importance of iPSC differentiation into PPCs, as this cell type is otherwise not accessible. This offers important therapeutic options for inherited retinal diseases leading to blindness.

[57] Improvement of hepatic differentiation of human skin-derived stem cells by 3D-scaffolds and fluidics

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Human skin precursors (hSKP) are multipotent adult stem cells with high proliferation capacity in vitro. Previously, our lab demonstrated that hSKP can differentiate towards hepatic cells by sequential exposure to growth factors and cytokines in a two-dimensional (2D) setup. Yet, the obtained hepatic cells (hSKP-HPC) show a mixed phenotype of immature and mature hepatocytes. The aim of this study is to improve the hepatic functionality of hSKP-HPC by culturing the cells in a 3D-scaffold imitating the 3D-architecture of the in vivo human liver. Furthermore, the circulating medium for cell culture is supplied through a microfluidic device mimicking the hepatic blood flow. The biocompatibility of different photo-crosslinkable polymers, including synthetic poly(ethylene glycol), poly(ε-caprolactone)-based compounds as well as natural compounds and OrmoComp® (a benchmark material) was evaluated by culturing hSKP on 2D-sheets. Assessment of the compound stability, cytotoxicity and induction of apoptosis showed that OrmoComp® was the most biocompatible photopolymer for culturing hSKP. This compound was also the most suitable for the fabrication of 3D-scaffolds with hexagonal baskets that mimic the 3D-orientation of hepatocytes in vivo. Scaffolds with dimensions in the range of 700 μm2 could be made by high-precision two-photon polymerization. The integration and distribution of hSKP in these scaffolds was confirmed by fluorescence microscopy. hSKP-HPC cultured in a microfluidic device under continuous flow (0.4 dyn/cm2 shear stress) for 6 days, acquired a more polygonal morphology and exhibited a higher glycogen storage than cells cultured in 2D. Transcriptomic analysis showed an upregulation of endoderm-specific genes and early hepatic enzymes and a downregulation of pluripotent markers. Further perspectives include the integration of the 3D-scaffolds inside the microfluidic chip to replicate the micro-physiology of the liver more accurately.

[58] Comparison of three differentiation protocols to create iPSC-derived cardiomyocytes

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Cardiomyocytes derived from induced pluripotent stem cells (iPSC-CMs) offer an attractive platform for cardiovascular research, including disease modelling, drug toxicology testing and development of regenerative therapies. Patient-specific iPSC-CMs are extremely useful to study disease pathogenesis and have a huge potential for evaluation of disease prognosis and development of personalized treatment. Several monolayer-based serum-free protocols have been developed for the differentiation of iPSCs to cardiomyocytes, but their performance has not yet been compared on the same iPSC-lines. In this study, we tested three independent protocols (published by Lian et.al.(1); Burridge et.al.(2); and one adapted from Lian et.al.) for iPSC-CM differentiation on six iPS cell lines: three lines from control individuals and three from patients carrying a mutation in the SCN5A gene. This gene encodes the cardiac voltage-gated sodium channel (Nav1.5) and the loss-of-function mutation causes the cardiac arrhythmia - Brugada syndrome. We performed molecular characterization of the obtained iPSC-CMs with immunostaining for cardiac specific markers (α-actinin, Troponin I, Nav1.5, Nkx2.5) and investigated morphology, beating ability and survival of the cardiomyocytes. Based on our observations, we concluded that two of the tested protocols are resulting in a high percentage of properly differentiated iPSC-CMs, as well as showing satisfying survival and cell quality after dissociation. As our main goal is the electrophysiological characterization of the patient cell lines, as a further step we will perform patch-clamp experiments to evaluate and compare the action potential (AP) and sodium current characteristics of the iPSC-CMs derived by these two protocols. (1) Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/β-catenin signaling under fully defined conditions – X. Lian et.al.; Nat. Protoc., 2013, 8(1): 162-175 (2) Chemically Defined and Small Molecule-Based Generation of Human Cardiomyocytes - P.W. Burridge et.al.; Nat. Methods, 2014, 11(8): 855-860

[59] Unraveling the role of pituitary stem cells during pituitary tumorigenesis and regeneration

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The pituitary gland represents the regulatory core of our hormonal system and steers fundamental body processes like growth, metabolism, sexual maturation and stress. Dysfunction of the pituitary, hypopituitarism, can therefore cause severe and life-threatening health problems. Our group developed a mouse hypopituitarism model in which growth hormone-expressing (GH+) cells are killed by diphtheria toxin (DT) treatment. Interestingly, the ablated cells are partially restored in the following months, thereby showing that the adult gland has regenerative capacity. Moreover, the local stem cells are activated and appear involved in the regeneration process. Also during tumor development in the pituitary, the stem cells appear activated. In this study, we investigate the behavior of stem cells during regeneration and tumor development in the pituitary. To examine the role of pituitary stem cells (characterized by SOX2 expression) in regeneration, we developed a transgenic mouse model in which both GH+ cells and SOX2+ stem cells are ablated by DT treatment. Currently, we are assessing the ablation efficiency of both cell types and are examining the influence of SOX2+ cell ablation on the GH+ cell regeneration. Recently, our lab developed organoids from adult mouse pituitary as novel model to study stem cell biology. Organoids are three-dimensional structures developing from tissue stem cells. They reproduce the tissue of origin and are long-term expandable. Organoids can also be established from diseased tissue like tumors. Here, we aim at developing organoids from pituitary tumor. At present, we successfully established organoids from the pituitary of the dopamine receptor D2 (Drd2) knock-out mouse model in which tumors develop. We are now characterizing these organoids and will eventually set up an organoid model for human pituitary tumor. Unraveling the role of stem cells in pituitary regeneration and tumorigenesis may eventually contribute to improved clinical management of damage- and tumor-based pituitary problems and deficiencies.

[60] Immunomodulatory properties of kidney stem/progenitor cells derived from urine of preterm neonates

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Background: Mesenchymal stromal cells (MSC) have immunomodulatory potential along with self-renewal and ability to differentiate towards cells of mesodermal origin. They inhibit proinflammatory cytokines and regulate immune cells. We recently described human kidney stem/progenitor cells (nKSPC) isolated from urine of preterm neonates, born before completing nephrogenesis. nKSPCs have regenerative paracrine effects and differentiate into functional kidney epithelial cells. This study aimed to investigate whether nKSPCs can also convey impactful immunomodulatory effects. Methods: Mixed lymphocyte reaction (MLR) was performed to investigate the potential of nKSPCs to supress T-cells proliferation. nKSPCs or bone marrow MSCs were added to the MLR at different ratios. Only PBMCs were allowed to proliferate, while the other cells were treated with mitomycin-C to hinder proliferation. 3H-thymidine incorporation was used to assess proliferation. The release of immunomodulatory cytokines was measured during the 5 days of co-culture using a MSD U-plex. Expression of genes related to immunomodulatory effects were analysed by qPCR after priming nKSPCs with 200 IU/mL IFN-γ or 100 μg/mL poly I:C, a TLR3 stimulator, for 24h. Results: nKSPCs were as efficient as MSCs in suppressing T-cells proliferation. The secretion of TNF-alpha decreased in the presence of nKSPCs or MSCs. The release of IL-6, a blocker of pro-inflammatory activities was significantly increased by the co-cultures. Suppression of T-cell proliferation is related to up-regulation of indoleamine 2,3-dioxygenase (IDO). Incubation of nKSPCs with IFN-gamma drastically increased expression of IDO, while poly I:C had higher impact on the release of IL-6 and IL-8; suggesting that the immunosuppressive effects of nKSPCs is conferred by up-regulation of IDO probably activated by JAK-STAT1 signalling pathway. Conclusions: Besides their potential to differentiate into functional kidney cells and regenerative paracrine effects, nKSPCs present immunosuppressive properties which shape them an ideal source of cells for kidney-targeted regenerative medicine.

[61] Glutamate-spillover by dystrophin-deficient astrocytes leads to neuronal excitotoxic stress in vitro which can be corrected by read-through agents

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Duchenne muscular dystrophy (DMD) causes aside from muscle degeneration also cognitive defects. We hypothesized that loss of dystrophin from DMD astrocytes might be related to these cognitive defects. Like DMD myocytes, DMD PSC-derived astrocytes displayed cytoskeletal abnormalities, defects in Ca2+ handling and nitric oxide signaling, increased levels of reactive oxygen species (ROS) and increased mitochondrial ROS stress. In addition, confirmed by in-depth transcriptomics studies between DMD astrocytes and available genomic data from Autism Spectrum Disorder (ASD) patient population, we observed shared dysfunctional pathways and brain regions affected. Most importantly, defects in glutamate handling in DMD astrocyte caused significantly decreased neurite outgrowth and hyper-excitability of normal PSC-derived neurons. Read-through molecule mediated restoration of dystrophin expression in DMD astrocytes harboring a premature stop codon mutation, significantly corrected the defective astrocyte glutamate handling and prevented associated neuronal toxicity. In conclusion, we propose a role for dystrophin deficiency in defective astrocytic glutamate handling, causing defects in neuronal development and function, which may lay at the basis of the neurocognitive defects seen in DMD patients. In conclusion, we demonstrate that • Loss of dystrophin from DMD patient iPSC-derived astrocytes causes defects in astrocyte glutamate handling. • Excess glutamate produced by DMD patient iPSC-derived astrocytes causes toxicity towards normal cortical neuronal progeny. • Such defects are in line with recent observations that increased glutamate concentrations is found in DMD human and mouse brain. • The transcriptional changes seen in DMD patient iPSC-derived astrocytes are consistent with dysfunctional pathways seen in brains from ASD patients. • As glutamate channel or NMDA blockers can selectively inhibit DMD astrocyte mediated neuronal toxicity, these inhibitors hold exciting clinical potential to treat the cognitive defects seen in DMD. • Lastly, for the subpopulation of patients currently treated with PTC124 for myogenic defects, it will be of great interest to determine effects on cognitive function of this intervention.

[62] Microwave Assisted Hydrothermal Synthesis of Hydroxyapatite on Multiwall Carbon Nanotubes Promotes Osteogenic Differentiation of Pre-Osteoblasts

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BIOFABICS

Presenting author: Jennifer Patterson

Mimicking the structure of bone, where hydroxyapatite (HAp) nanocrystals are deposited along collagen fibers via biomineralization by cells, pushes research towards sophisticated composite scaffolds incorporating nanosized features. However, biomineralization approaches to create HAp-based materials are time-consuming and often fall short of their natural counterparts. Carbon nanotubes (CNTs) are known for unique physical, mechanical, and electrical properties, and they mimic collagen with their fibrillar shape and dimensions. Herein, CNTs are rapidly coated with HAp and evaluated for their ability to stimulate osteogenic differentiation of pre-osteoblasts. To prepare HAp, calcium (Ca) and phosphate (P) sources were dissolved in 0.1 M NH_4OH and mixed to reach a Ca/P ratio of 1.67. The mixture was heated at 200 °C for 10 minutes in a high pressure microwave. This protocol was translated to oxidized CNTs to create a HAp coating. Cytocompatibility and osteogenic differentiation of MC3T3-E1 pre-osteoblasts exposed to HAp powder or HAp coated CNTs over 28 days were evaluated using Presto Blue and alkaline phosphatase (ALP) assays and phalloidin/DAPI, Alizarin red, and picrosirius red staining. FTIR, XRD, EDX, and ICP-OES confirmed high purity, hexagonal HAp was formed with a near stoichiometric Ca/P ratio of 1.66. Cell metabolic activity increased from 7 to 28 days, and at 28 days, it was significantly higher for cells treated with the HAp-CNT medium than the controls. After adding ascorbic acid and dexamethasone, ALP activity increased up to 14 days and decreased again after 21 days. After 28 days, calcium content was significantly higher for cells in HAp and HAp-CNT containing medium compared to negative references. In conclusion, CNTs coated with high purity, hexagonal HAp with near stoichiometric Ca/P ratio were prepared and exposed to pre-osteoblasts. The cells proliferated and underwent osteogenic differentiation. As future work, the HAp-coated CNTs could be incorporated into polymer scaffolds to induce bone formation.

[63] The neural environment is a necessary, but not sufficient, prerequisite for directing iPSC-derived myeloid progenitors into the CX3CR1+CCR2- microglia-like phenotype.

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Differentiation of microglia from iPSC holds great potential for in vitro neurodevelopment and immunology research. Here, we present a novel protocol to obtain yolk sac-like CX3CR1+CCR2- macrophage progenitors from CX3CR1eGFP/+CCR2RFP/+ murine iPSC, which following co-culture with astrocyte-committed neural stem cells (aNSC) mature into a highly uniform population of CX3CR1+CCR2- iPSC-derived microglia that display typical ramified or amoeboid morphology and are able to colonize microglia-depleted organotypic brain slice cultures (mdOBSC). Phenotypical characterisation of these ramified CX3CR1+ iPSC-derived microglia compared to round shaped CX3CR1-CCR2- iPSC-derived macrophages demonstrated F4/80 expression by both cell populations, while the activation marker MHC-II was upregulated only by iPSC-derived macrophages upon LPS+IFNγ stimulation. This distinct MHCII expression pattern was strikingly similar to the expression pattern of MHCII on endogenous CX3CR1+CCR2- microglia and infiltrating CX3CR1+CCR2+ monocytes following experimental stroke in CX3CR1eGFP/+CCR2RFP/+ mice. In addition, in vitro LPS+IFNγ stimulated iPSC-microglia secreted a significantly lower amount of pro-inflammatory cytokines. Consistently with the concept that the neural environment is a key determinant for myeloid progenitors to adopt a microglia-like phenotype, CX3CR1- iPSC-macrophages, when cultured in the presence of aNSC or on mdOBSC, were subject to rapid conversion into CX3CR1+ amoeboid microglia-like cells with reduced expression of MHCII. However, co-culture with aNSC did not reduce the level of pro-inflammatory cytokines secretion by iPSC-macrophages. These findings suggest that although a neural environment is able to convert myeloid progenitors into cells morphologically and phenotypically similar to microglia, also retracing early microglia ontogenesis in vitro is essential to obtain iPSC-derived microglia-like cells that are functionally distinct from iPSC-derived macrophages.

[64] Model specific dependency of EMT for metastasis

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Metastasis is the terminal disease of oncological patients, it can occur from weeks to years following tumour resection and is incurable. Epithelial- to- mesenchymal transition (EMT) was observed in several types of tumours and is correlated with poor prognosis, resistance to anti-cancer therapy and metastasis. However, several studies that challenged the role of EMT in metastasis have different interpretations of their observation, therefore the importance of EMT in metastasis remains elusive. The discrepancies observed are due to the models that were used with forced over-expression or knock-out (KO) of EMT transcription factors (TFs) at non- physiological levels. These models ignore that another EMT-TF could take over the function of the knocked-out TF, but also that EMT-TFs are tissue-specific and non-redundant. Is EMT important for metastatic dissemination? Does EMT occur at the primary tumour or in the blood? What is the phenotype of circulating tumour cells (CTCs)? Do all CTCs have the same potential to colonize the distant organ? Is mesenchymal- to- epithelial transition (MET) important for the formation of overt metastases? To address those questions, we used two genetic mouse models of skin tumours of different degrees of EMT (from mesenchymal to fully differentiated) regardless the forced over-expression or KO of EMT-TFs. We observed that EMT is important for the initial stages of metastatic dissemination in spontaneous model, contrastingly to the grafted model where EMT is dispensable for metastases. However, in both models the presence of CTCs is associated with lung metastases and CTCs present mesenchymal phenotype at the early stage of EMT. Moreover, we observed that MET is needed to form overt metastases. Our findings can open new approaches for the treatment of cancer, precisely determine which cells and at which stage of cancer disease to target.

[65] In vitro mESC-derived Papillary Thyroid Carcinoma (PTC) model to study mechanisms underlying Nis downregulation

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Expression of the sodium iodide symporter (NIS) is required for efficient iodide uptake. Since most differentiated thyroid cancer expresses NIS, radioactive iodide is routinely utilized to target remnant thyroid cancer cells after thyroidectomy. It is known that BRAF mutations impair Nis expression and radioiodine refractoriness of thyroid cancer, but the underlying mechanism remains undefined. Recently we have developed a thyroid cancer in vitro model where Braf-oncogene can be transiently induced, by tamoxifen (Tam) incubation, in functional thyroid follicles derived from mouse embryonic stem cells. Thus, we took advantage of this newly generated model to study mechanisms underlying Nis downregulation and investigate ways to reactivate this molecule. We have observed that BrafV637E mutation has direct effect on Nis downregulation, once Nis expression is partially recovered when Tam treatment is interrupted. In addition, to investigate the pathways involved in such effect, we have treated Braf-induced cells with inhibitors of MAPK, PI3K, histone deacetylase (HDAC: VPA and SAHA), DNA methyltransferase (5-AZA-2'deocytidine and RG108) and N-acetyl cysteine (NAC), isolated and/or in combination. We have seen that most of drugs had weak effect when incubated isolated, although some combinations seem to completely restore Nis mRNA levels to those observed in the control (thyroid cells). More specifically, VPA co-treatment with MAPK and PI3K inhibitors, or SAHA showed positive effect on Nis reactivation. In addition, the association of MAPK and NAC leaded to significant Nis levels recovering. Furthermore, confocal images demonstrated Nis membrane staining in Braf-induced cells and some follicular structures recovery could be observed. Thus, our data suggests that Braf-oncogene impacts Nis expression via mechanisms involving HDAC overexpression and intracellular oxidative stress. Moreover, some combinations of drugs might restore Nis levels being an alterative to treat radioiodine refractory tumors.

[66] Stem cells and Ghrelin peptides to counteract sarcopenia

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Presenting author: Flavio Lorenzo Ronzoni

Sarcopenia is a complex syndrome defined as the irreversible loss of skeletal mus-cle mass and functionality in aged individuals that results in frailty, mobility disor-ders, and loss of independence [1]. The pathology is characterized by muscle atro-phy and impaired muscle regeneration. The mechanisms involved in its develop-ment are not fully understood, although hormonal changes, inflammation, insulin resistance and nutritional deficiencies are surely involved in. In addition, we and other authors showed that aging affect progenitor myogenic cells, including meso-angioblasts (adult vessel-associated stem cells) [2] that are unable to counteract sarcopenic phenotype. Acylated and unacylated ghrelin (AG and UnAG, respectively) are circulating pep-tides codified by the ghrelin gene. By acting through its receptor GHSR1a, AG stimulates appetite, adiposity, a strong release of growth hormone (GH) and has a broad anti-inflammatory activity. UnAG does not bind to GHSR1a however, similar to AG has an anti-atrophic effect on skeletal muscle [3]. Our preliminary results show that murine mesoangioblasts treated with recombinant UnAG or AG were able to differentiate spontaneously forming contractile myotubes. In addition, in murine embryonic stem cells and human mesodermal induced plu-ripotent stem cells subjected to myogenic differentiation, the presence of recombi-nant proteins resulted in improved myogenic commitment. Taken together our results show that both AG and UnAG are potent myogenic in-ducers on adult and pluripotent stem cells.

[67] Epigenetic and Genetic Effects of Chromosomal Sex on Reprogramming to iPS Cells and Pluripotency

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Presenting author: Juan Song

Pluripotency can be established from somatic cells by reprogramming approaches and also captured from early embryos. However, how chromosomal sex affects reprogramming processes and the molecular and cellular properties of pluripotent stem cells remains largely unclear. We have recently isolated isogenic XY male and XX female mouse induced pluripotent stem cells (iPSCs). Here, we will present new studies combining transcriptional profiling, chromatin profiling, DNA methylation analysis, pluripotency exit measurements, growth and genetic analyses, and functional experiments to investigate the transcriptional, epigenetic and genetic effects of sex on the induction, maintenance and exit from pluripotency. We will show that the transcriptional landscape, open chromatin landscape, global DNA methylation, exit from pluripotency and cellular growth differ in XX female iPSCs compared with XY male iPSCs, partly mimicking early mammalian embryo development. We will present evidence that X chromosome loss in female iPSCs resolves sex-specific differences but does not restore imprint methylation. We will show that global erasure of DNA methylation and delayed pluripotency exit can be molecularly uncoupled in XX female embryonic stem (ES) cells through manipulation of the X-linked MAPK inhibitor Dusp9, but not Zic3 and Tfe3. We will also present evidence that the open chromatin landscape of iPSCs is modulated by X chromosome dosage at thousands of chromatin regions and reveal the transcriptional regulatory logic by which X chromosome dosage influences pluripotency. Defining the mechanisms regulating the establishment, maintenance and exit from pluripotency in vitro and in vivo and understanding how these mechanisms are influenced by X chromosome dosage will have important implications for development, cell fate reprogramming studies and regenerative medicine.

[68] Defining the enhancer landscape of early cardiac progenitors

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Mesp1 is a key transcription factor that promotes the specification of the first and second heart fields and of the various cardiovascular cell lineages during development. The mechanisms by which Mesp1 differentially regulates distinct target genes in a specific temporal and spatial manner is currently unknown. To address these questions, we performed RNA-seq, ChIP-seq and ATAC-seq on differentiating mouse embryonic stem cells (ESCs) in which Mesp1 expression can be induced by doxycycline administration, in order to study the subsequent temporal changes in the transcriptional and chromatin landscape. Bioinformatic analysis of these datasets provided us with a genome-wide overview of Mesp1-bound enhancers, including direct target genes, kinetics of expression, and a list of potential cofactors of Mesp1. Using CRISPR-Cas9, we then knocked-out several Mesp1-bound enhancers regulating key cardiac genes. This resulted in reduced expression of these genes during ESC differentiation with and without Mesp1 overexpression. Several of these enhancers, including some close to key first and second heart field genes (e.g. Hand1, Hoxb1), were further validated using a reporter assay. We thereby showed that these enhancers are sufficient to activate gene expression in a specific Mesp1+ population. Using this reporter assay and in vivo single-cell RNA-seq data of Mesp1+ cells we have previously generated, but also new in vitro single-cell RNA-seq of differentiating ESCs we will generate, we will attempt to isolate and characterize subpopulations of Mesp1+ cardiac progenitors expressing either first (Hand1) or second (Hoxb1) heart field genes, but also cardiac progenitors with biased fate towards cardiomyocyte (Hand1) or endothelial (Notch1) lineages.

[69] Mechanisms regulating metastasis-initiating cells in squamous cell carcinoma

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Presenting author: Magdalena Sznurkowska

Metastasis is the leading cause of death in cancer patients. To form metastasis, tumor cells need to leave the primary tumor, reach the blood flow, colonize distant tissues, and establish secondary tumors. In this project, using state of the art lineage tracing in mouse models of skin SCCs presenting different degree of EMT, we will assess the importance of EMT in regulating metastasis initiation, and the mechanisms leading to tumor circulating cells. Transcriptional profiling including single cell analysis coupled with gain and loss of gene function will be used to unravel the importance of MET, as well as the mechanisms controlling lymph node versus lung tropism in skin SCC metastasis. This study will provide key insights into the molecular mechanisms that regulate metastasis-initiating cells and control organ colonization in spontaneous metastasis from genetically induced mouse cancer models.

[70] Unravelling the Cellular and Transcriptional Logic of Reprogramming to Totipotency

Adrian Janiszewski, Eszter Posfai, Sarita Panula, Tine Pardon, Mouna El Bakkali, Irene Talón, Natalie De Geest, Alexander Murray, John Paul Schell, Nicolas Ortega, Fredrik Lanner, Jannet Rossant, Vincent Pasque.

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Totipotency is the unbiased ability of a cell to differentiate into any cell type of the body, in addition to extra-embryonic cell types. This property characterizes cells of pre-implantation embryos, and is rapidly lost upon cell divisions to form the pluripotent epiblast and the two extra-embryonic lineages; the trophectoderm and the primitive endoderm. Distinct stem cell types; embryonic stem cells (ESCs), trophoblast stem cell (TSCs) and extra-embryonic endoderm (XEN) stem cells, have been derived from all three linages of the late pre-implantation embryo, which can form any cell type of that respective lineage. Cell fate restriction mechanisms prevent switching from one cell lineage to another. Nuclear transfer experiments have demonstrated the possibility of inducing totipotency. However, whether the totipotent state can be induced in vitro remains unclear. Recent studies have reported the derivation of extended pluripotent stem cells (EPSCs), which can form both embryonic as well as extra-embryonic lineages. Here, we will present results from transcriptional profiling and differentiation experiments to investigate the transcriptional effects of reprogramming ESCs into EPSCs, as well as the ability of EPSCs to activate extra-embryonic genes. We will show that the transcriptional state of EPSCs is rapidly induced during the conversion of ESCs into EPSCs, and involves changes in the expression of developmental genes. We will present evidence that EPSCs transcriptionally differ from totipotent cells of the pre-implantation embryo, and maintain silencing of 4-16 cell stage embryo marker genes. Furthermore, when exposed to trophectoderm differentiation conditions, EPSCs maintain silencing of trophectoderm markers, demonstrating that EPSCs do not readily differentiate toward the extra-embryonic lineage. Defining the mechanisms regulating the establishment and maintenance of specific stem cell states, and understanding how to reprogram stem cells from one lineage into another, will have important implications for development and regenerative medicine.

[71] Spatial and temporal regulation of basal multipotency during prostate postnatal development

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The prostate epithelium originates from the urogenital sinus during embryogenesis and starts to branch after birth, forming a ductal tree composed of luminal and basal cells by the end of puberty. Lineage tracing experiments demonstrated that the prostate postnatal development is mediated by multipotent basal progenitors as well as unipotent progenitors whereas adult maintenance is ensured by two types of unipotent basal and luminal progenitors. Here we aim to determine the cellular hierarchy that governs prostate development combining quantitative clonal lineage tracing experiments and whole mount (WM) 3D imaging. We performed clonal analysis administrating low doses of tamoxifen to newborn (P1) and 2-week-old (P14) K5CreER/Rosa-confetti mice to specifically target basal cells. During early development (P1), we observed overtime patches of basal and/or luminal cells from the same color along the elongating ducts. To perform quantitative analysis, we mapped the position and the composition of the different patches. After mathematical clone reconstruction, we demonstrated that unicolor patches within a duct were fragments of one clone. Confocal analysis of the prostate WM later during the development (P14) revealed the existence of clones containing both basal and luminal cells mainly in distal regions (tip) of the elongating ducts. Taken together, these data suggest that during early postnatal development multipotent basal cells mediate growth of the distal region of the ducts as well as ductal elongation whereas later, basal multipotency remains active mostly to the distal region of the elongating ducts. Our study uncovers the spatiotemporal regulation of basal multipotency during prostate postnatal development. Further experiments will allow us to better understand the molecular mechanisms that regulate this process.

[72] Hydroxyapatite Coated and Patterned 3D Carbon Nanotube Structures to Mimic Bone and Promote Osteogenic Differentiation

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Bone fractures are one of the most common traumatic injuries, and about 10% of the fractures result in non-union. Bone transplantation, metal prosthetics, and polymer composites have been used as bone substitutes, but they have not met expectations for reasons including donor shortage, immune rejection, and mechanical and structural mismatch. Therefore, herein, we developed a carbon nanotube (CNT) honeycomb structure and successfully functionalized it with hydroxyapatite (HAp) to mimic the native microarchitecture of bone. Our process started by lithographically patterning a catalyst layer on a silicon wafer, which was transferred to a CVD furnace for CNT synthesis. Oxygen species were introduced on the patterned CNT structure by UV-ozone, and the structure was aggregated using capillary forces. The honeycomb structure was placed in a solution of calcium (Ca) and phosphate (P) sources in a Ca/P ratio of 1.67 and then heated at 200 °C for 10 minutes in a high pressure microwave reactor. The HAp-coated honeycomb structures were seeded with MC3T3-E1 pre-osteoblasts to look at relevant cell growth and potential for osteogenic differentiation. The synthesis of near stoichiometric and hexagonal HAp was confirmed by ICP-OES and XRD, and EDX showed the presence of the HAp coating on top of and in the cavities of the honeycomb structure. Nanoindentation showed a Young's modulus in the range of stiffness of native bone tissue. After 28 days, the seeded pre-osteoblasts had spread and were found within the cavities of the honeycomb structure. Osteogenic differentiation was confirmed by Alizarin red and picrosirius red staining, which revealed the formation of a Ca-rich collagenous matrix, and increased alkaline phosphatase activity was measured. Overall, this work presents a new method for fabricating bone scaffolds to promote the osteogenic differentiation of progenitor cells using a 3D hybrid CNT-HAp composite that addresses mechanical and structural limitations of previous scaffolds.

[73] Regulation of the LIN28/Let-7b axis to induce human stem cell derived hepatocyte-like cell maturation

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In vitro models to study liver diseases and toxicology rely on the use of primary human hepatocytes (PHHs) and hepatoma cell lines, which both have major limitations. Therefore, scientists are investigating alternative sources of hepatocytes such as pluripotent stem cells (hPSCs). The Verfaillie lab has generated protocols to develop hepatocyte-like cells (HLCs) from hPSCs; however, such HLCs are not fully mature yet. We demonstrate that the LIN28/Let-7b axis, which is important during liver development, is aberrantly expressed in hPSC-HLCs. LIN28 A and B, a family of RNA-binding proteins, is highly expressed in hPSCs, fetal liver and cancer, which results in low expression levels of mature microRNA Let-7b. By contrast, PHHs do not express LIN28A and B, leading to high levels of Let-7b. We hypothesize that correct modulation of this axis will induce hPSC-HLC maturation. Therefore, we have generated hPSC lines that contain, in the Adeno-Associated Virus Integration Site 1 locus, a tetracycline-mediated inducible knockout of LIN28A and LIN28B as well as a hPSC line with an inducible overexpression of miRNAs Let-7b, as well as two other miRNAs involved in this axis, miR-125b and miR-26a. We demonstrate that inducible knockout of LIN28 and overexpression of the miRNAs in undifferentiated hPSCs causes a decrease in hPSC proliferation rate. Additionally, we differentiated hPSCs towards hepatocyte-like cells in the presence of doxycycline to overexpress the 3 miRNAs. Unfortunately, no effects were seen from miRNA overexpression on hepatic differentiation. Evaluation of transgenic miRNA levels demonstrated, however, that only one of the miRNAs was significantly increased, suggesting that the integration of a single copy into the genome might not be sufficient. We will test this hypothesis by overexpressing the miRNAs using lentiviral vectors at an MOI of 3-4, to incorporate multiple miRNA copies followed by repeat differentiations.

[74] ER lipid defects in neuropeptidergic neurons impair sleep patterns in Parkinson's disease

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VIB and KU Leuven

Presenting author: Jorge S Valadas

Parkinson's disease patients report disturbed sleep patterns long before motor dysfunction. Here, in parkin and pink1 models, we identify circadian rhythm and sleep pattern defects and map these to specific neuropeptidergic neurons in fly models and in hypothalamic neurons differentiated from patient iPSC. Parkin and Pink1 control the clearance of mitochondria by protein ubiquitination. While we do not observe major defects in mitochondria of mutant neuropeptidergic neurons, we do find an excess of endoplasmic reticulum-mitochondrial contacts. These excessive contact sites cause abnormal lipid trafficking that depletes phosphatidylserine from the ER and disrupts the production of neuropeptide-containing vesicles. Feeding mutant animals phosphatidylserine rescues neuropeptidergic vesicle production and acutely restores normal sleep patterns in mutant animals. Hence, sleep patterns and circadian disturbances in Parkinson's disease models are explained by excessive ER-mitochondrial contacts and blocking their formation or increasing phosphatidylserine levels rescues the defects in vivo.

[75] Closing the neural tube: the epistatic interaction of TET1 with nutrient metabolism

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Neural tube defects (NTD) are among the most common birth defects worldwide. In the 1990s, supplementation with folate vitamins has been shown to reduce recurrence of NTDs up to 70%, but it is unclear why. The complex disease etiology involves many hereditary and environmental factors. Our group has observed recently that homozygous loss of Tet1 in outbred mice is embryonically lethal due to the development of exencephaly, an early stage of cranial NTD. Here we describe the NTD phenotype of Tet1-KO mice and reveal potential targets of TET1 in the early embryo. The penetrance of NTDs in Tet1 null embryos exhibit distinct strain differences, being >75% in CD1 outbred mice, but reduced (±25%) in C57BL/6J (B6) congenics, and absent in 129SvEv/B6 F1 hybrids. The Tet1-KO phenotype does not respond to folic acid (FA) supplementation or deficiency in CD1 and B6 mice, respectively. In epiblast-like cells (EpiLCs), loss of TET1 in genetically heterogenous CD1 strain in comparison with loss in a 129/B6 F1 hybrid backgrounds, revealed candidate strain-dependent modifier genes, several of which are involved in differentiation processes and potentially involved in neural tube closure. In future studies, we will evaluate the role of these candidate TET1-interaction genes in neural tube closure and determine whether other nutrients such as methionine (like FA, also a folate-one-carbon-metabolism intermediate) or vitamin C (a TET co-factor) will affect NTD penetrance rates. These studies will lead to better treatment or preventive measures, such as nutrient supplementations, for high-risk pregnancies.

[76] Functional expression of pain-sensing TRP channels in iPSC-derived sensory neurons

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Transient Receptor Potential (TRP) channels in somatosensory neurons detect sensory stimuli from the periphery and they play a central role in various forms of acute and chronic pain. Since access to viable human sensory neuronal cells is limited, we explored the possibility of using hiPSC-derived sensory neurons as a human in vitro model system to study pain-related TRP channels. We used a previously established protocol using dual SMAD inhibition to drive differentiation of hiPSCs into sensory neuron-like cells. While expression of canonical markers of sensory neurons had already been validated, an in-depth characterization of TRP channels in these neurons was still lacking. In this study, we used qPCR, Ca2+-microfluorimetry and patch-clamp experiments to evaluate the expression and function of sensory TRP channels. Our results not only confirm functional expression of TRPV1, TRPM8 and TRPA1, but also demonstrate for the first time a strong functional expression of TRPM3, a rather newly described nociceptor channel involved in acute and inflammatory pain. Previous work showed that TRPM3 activity is modulated by μ-opioid receptors (μOR) in animal models. In short, activation of peripheral μORs inhibits TRPM3 via a cascade involving Gβγ proteins, and thereby attenuates TRPM3-dependent pain. These findings might explain how opioids reduce the activity of peripheral pain-sensing nerve cells, and introduce TRPM3 as a direct drug target to combat (neuropathic) pain with fewer central side effects. We are currently evaluating this process in the hiPSC-derived sensory neurons, to establish whether a similar interplay between opioid signaling and TRPM3 is operational in a human context. To conclude, we obtained sensory neurons derived from hiPSCs, and validated the functional expression of TRP channels important in somatosensation and pain. These hiPSC – derived sensory neurons have the potential to become a leading model for researching cellular neurophysiology and (neuropathic) pain perception in humans.

[77] Tox4 Modulates Cell Fate Reprogramming to iPS Cells

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Reprogramming somatic cells into induced pluripotent stem cells (iPSCs) by ectopic expression of Oct4, Klf4, Sox2 and c-Myc holds promise for understanding the regulation of cellular identity and for applications in regenerative medicine and disease modelling. However, the factors and mechanisms involved in reprogramming remain largely unclear. We previously showed that during reprogramming, cells transit through distinct cellular states characterized by the sequential activation of EZH2, NANOG and DPPA4. To elucidate the molecular players underlying transitions between these cell states, we performed a siRNA screen during the reprogramming of somatic cells into iPSCs. We identified Tox4 as a novel factor that modulates cell fate, using the efficiency of reprogramming to iPSCs as an assay. We found that Tox4 depletion during reprogramming reduced the emergence of late reprograming intermediates (DPPA4 positive), as well as intermediary (NANOG positive) and also early reprogramming intermediates (EZH2 positive). Reducing Tox4 impeded reprogramming irrespective of the reprogramming culture conditions used. Knockdown of somatic Tox4 suggests a broader role of this transcription factor in cell fate change. We show that TOX4 assembles into a multimeric form, consistent with a role in regulating transcriptional programs underlying the control of cellular identity. Our study reveals Tox4 as a novel regulator of cell fate and reprogramming to induced pluripotency. Future experiments include single cell transcriptome and epigenomic analyses to reveal how Tox4 modulates gene regulatory programs and chromatin states, and testing whether Tox4 is also involved in direct conversion of fibroblasts into induced neurons.

[78] Pituitary regeneration and aging: role of pituitary stem cells and YAP/TAZ signaling

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The pituitary gland is the key orchestrator of our endocrine system, translating central and peripheral inputs into precise hormone production. Previous work showed that the young-adult mouse pituitary holds regenerative capacity following transgenically induced injury, and that the pituitary stem cells are activated and likely involved in this regeneration. However, the ability to regenerate was found to subside with increasing age. Here, we aim at deciphering the mechanisms of pituitary regeneration and its decline with aging, thereby focusing on the role of the stem cells and the involvement of the Hippo (YAP/TAZ) pathway. In the adult gland, YAP and TAZ expression was found in different cell types and subcellular localizations, with YAP mainly detected in the nucleus of pituitary stem cells (as characterized by SOX2 expression) and TAZ localized in the cytoplasm of hormone-expressing cells. After injury, both components appear upregulated which is currently being quantified. To investigate the functional importance of the Hippo pathway in pituitary stem cell regulation, we are analyzing the effect of YAP/TAZ inhibition in vitro using pituitary organoid cultures. In aged pituitary, the SOX2+ cell compartment appears less activated upon injury than in the younger gland. In analogy, we found that the capability of pituitary stem cells to generate pituispheres in vitro decreases with age. The observations suggest that there is a decrease in stem cell functionality with increasing age which may underlie the pituitary's regenerative decline. Currently, we are comparing transcriptomes from young and aged pituitary SOX2+ stem cells to look for altered molecular pathways. Remarkably, stem cells from old pituitary are still capable of efficiently generating organoids when seeded in a Matrigel drop and supplemented with WNT3A, suggesting that the old stem cells can regain proliferative capacity under particular culture conditions.

[79] Mapping of skeletal progenitors in embryonic limb cartilage and their application in developmental bone engineering

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Presenting author: Louca Verbeeck

The healing of large bone defects remains a major unmet medical need. A strategy to repair these is cell based bone tissue engineering. Our developmental engineering approach consists of the in vitro manufacturing of a living cartilage tissue construct (callus-like) that upon implantation forms bone in vivo by recapitulating a developmental endochondral ossification process. Key to this strategy is the identification of the cells and cell sources to produce such cartilage intermediates efficiently. To provide a scientific basis for this, we developed a cell selection strategy based on published skeletal stem cell markers implemented on cells released from embryonic limb cartilage and analyzed for their potency in an in vivo ectopic bone formation assay. Femurs from 14.5dpc mouse embryos were isolated, dispersed into single cell suspensions and live sorted using a panel of previously published surface markers for skeletal stem cells and progenitors. Sorted populations were encapsulated in collagen or alginate hydrogels and assessed for cartilage and bone formation in nude mice. After three weeks, skeletal tissue formation was evaluated by nano-CT, histology, and molecular marker analysis. We investigated in these assays two cell populations purified from the femurs: CD51+CD105-CD90-CD200- progenitors and CD51+CD105-CD90-CD200+ skeletal stem cells. Both populations formed bone in both gels. Collagen implanted cells formed a complete ossicle with bone marrow, while the alginate implanted cells created small bone organoids scattered across the alginate hydrogel. Our data indicate the ability of these cells to continue their developmental path and form bone in vivo in an ectopic environment. This cell selection and isolation approach could be further explored as a tool to purify and select for postnatal adult stem cells from different sources with a high biological potency for bone formation.

[80] Prdm12, a novel key regulator of the Nerve Growth Factor-TrkA signalling pathway, is required for nociceptive sensory neuron development

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In human, many cases of congenital insensitivity to pain are caused by mutations of components of the NGF-TrkA signalling pathway, required for survival and specification ofnociceptors, and which plays a major role in pain processing. Prdm12 is a newly discovered epigenetic regulator essential for pain sensing in human, but its exact function remains unknown. Here, we show in mouse somatosensory ganglia that Prdm12 is restricted to developing and adult nociceptors. Analysis of Prdm12 mutants reveals its requirement for the survival and maturation of nociceptors, at least in part through the initiation and maintenance of the expression of TrkA. Prdm12 overexpression promotes TrkA expression in frog ectodermal explants when combined with the neurogenin1/2 transcription factors, and in human pluripotent stem cell derived neuronal progeny. Together, our results establish Prdm12 as novel key regulator of TrkAexpression that control nociceptor specification, and suggest it is a novel druggable pain target.

[81] Putative role of lysosomes in cell differentiation of bone marrow derived human mesenchymal stem cells

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Bone marrow-derived mesenchymal stem cells (BM-MSC) are able to differentiate into several cell types such as adipocytes, osteoblasts, chondrocytes and hepatocyte-like cells. In this work, we are investigating a potential role of lysosomes in the hepatogenic differentiation process. In an attempt to characterize the lysosomal population, we performed flow cytometry analyses with the Lysotracker Deep Red probe, Western blot analyses with antibodies against lysosome-associated membrane proteins and enzymatic assays. We did not observe any major difference between differentiated and undifferentiated cells, suggesting that the lysosomal population remains quite stable during the differentiation process. However, we observed an increase both in the abundance and in the activity of cathepsin B during the early phase of hepatogenic differentiation, but not in the adipogenic and osteogenic differentiation processes. Cathepsin B is a lysosomal cysteine protease with endopeptidic and carboxypeptidic activities. To determine whether cathepsin B plays a role in the hepatogenic differentiation of BM-MSC, we used CA074Me, a specific membrane-permeable inhibitor of cathepsin B. Despite a complete inhibition of cathepsin B, the upregulation of TDO2 (tryptophan 2-3 dioxygenase) and AAT (alpha-1 antitrypsin), two hepatogenic markers, was not affected. This needs to be confirmed using shRNA but this result suggests that cathepsin B is dispensable for the hepatogenic differentiation of BM-MSC. Cathepsin B could act intracellularly (intralysosomal proteolysis or apoptosis) but also extracellularly (extracellular matrix degradation and remodeling). As only a low cathepsin B activity was detected in the culture medium of differentiated cells, cathepsin B could exert its action intracellularly. Further studies are required to determine the biological significance of cathepsin B increased activity during BM-MSC hepatogenic differentiation.

[82] Spatio-temporal analysis of human preimplantation development reveals dynamics of EPI/TE

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In vitro fertilization (IVF) cycles represent 3% of births in western countries with an average efficiency of the procedure below 27%. This low rate of development is attributed to suboptimal e culture conditions and lack of knowledge of the biological processes of human embryo development. Recent technological progresses such as single-cell (sc)RNAseq and CRISPR-CAS9-mediated out have allowed unprecedented gain of knowledge of human preimplantation development. However, we still lack a framework linking developmental stage, the main criteria used in IVF practic molecular signatures. Here, we propose a human preimplantation development model linking transcriptomics analysis and precise developmental stage. This model is based on pseudotime mo of scRNAseq data with time-lapse staging. We show that trophectoderm (TE) / epiblast (EPI) lineage specification in human occurs at the blastocyst stage, just before expansion. We valida immunofluorescence the expression profile of novel markers enabling precise staging of human preimplantation embryos, such as NR2F2 which highlights the transition from speci mature/committed TE. This allowed us to unravel molecular sequence of events pacing human preimplantation development. We further expanded our analysis to correlate cell fate specificatio morphogenesis. Altogether, our study clarifies the first lineage specification event in human and provides a browsable framework to realize spatio-temporal mapping of events underlying lineage specification.

[83] Acinar cells in the neonatal pancreas grow by self-duplication and not by neogenesis from duct cells

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Pancreatic acinar cells secrete digestive enzymes necessary for nutrient digestion in the intestine. They are considered the initiating cell type of pancreatic cancer and are endowed with differen plasticity that has been harnessed to regenerate endocrine beta cells. However, there is still uncertainty about the mechanisms of acinar cell formation during the dynamic period of early po development. To unravel cellular contributions in the exocrine acinar development we studied two reporter mouse strains to trace the fate of acinar and duct cells during the first 4 weeks of life received tamoxifen on the day of birth and were followed during the next four weeks. In the acinar reporter mice ElastaseCreER R26-YFP, the labelling index of acinar cells remained unchanged the neonatal pancreas growth period (35.3 ± 5.4% YFP+ acinar cells (n = 7) at 1-week of age vs 35.4 ± 5.6% YFP+ acinar cells at week 4 (n = 10) ($p > 0.05$)), evidencing that acinar cells are formed duplication. In line with this, duct cell tracing in Hnf1bCreER R26-LacZ mice did not show significant increase in acinar cell labelling (0.02 ± 0.02% X-gal+ acinar cells at 4-weeks of age (n = 4)), exc duct-to-acinar cell contribution during neonatal development. Immunohistochemical analysis confirms massive levels of acinar cell proliferation in this early period of life, as more than 40% of am cells are positive for Ki67 at week 1. Further, also increase in acinar cell size contributes to the growth of pancreatic mass. Moreover, the mean number of acinar nuclei per acinus remains co throughout neonatal development. We conclude that the growth of acinar cells during physiological neonatal pancreas development is by self-duplication (and hypertrophy) rather than neog from progenitor cells as was suggested before.

[84] Integrative proteomics reveals a protective role of PRC2 for the ground state pluripotent epigenome

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The ground state of pluripotency is defined as a basal proliferative state free of epigenetic restriction, represented by mouse embryonic stem cells (ESCs) cultured with two kinase inhibitors (so "2i"). Through comparison with serum-grown ESCs, we identify epigenetic features characterizing 2i ESCs by proteome profiling of chromatin including post-translational histone modification most prominent difference is H3K27me3 and its enzymatic writer complex PRC2 that are highly abundant on eu- and heterochromatin in 2i ESCs, with H3K27me3 redistributing outside canonica targets in a CpG dependent fashion. Using PRC2-deficient 2i ESCs, we identify epigenetic crosstalk with H3K27me3, including significant increases in H4 acetylation and DNA methylation. This su that the unique H3K27me3 configuration protects 2i ESCs from preparation to lineage priming. Interestingly, removal of DNA methylation in PRC2-deficient 2i ESCs lacking H3K27me3 using 5-azac hardly affected ESC viability and transcriptome, suggesting that ESCs are independent of both major repressive epigenetic marks.

[85] Defining the cell populations responsible for skin cancer initiation and relapse following therapy

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The identification of specific cell type from which cancer arises and the cancer cell population that resists upon therapy leading to tumor relapse constitute the main topics of our research. We u basal cell carcinoma (BCC), the most frequent cancer in humans, as a cancer model for our studies. To uncover the cancer cell of origin in BCC and the changes in the cellular dynamics that l tumor initiation, we assessed the impact of oncogenic hedgehog signalling activation in distinct cell populations and their capacity to induce BCC. We found that only stem cells, and not proge were competent to initiate tumour formation upon oncogenic hedgehog signalling. Interestingly, this difference was due to the hierarchical organization of tumour growth in oncogene-targete cells, characterized by an increase of symmetric self-renewing divisions and a higher p53-dependent resistance to apoptosis, leading to rapid clonal expansion and progression into invasive tumo study the cancer cell population that mediates BCC relapse upon therapy, we treated two different genetic BCC mouse models with a Smoothened inhibitor (Smoi), the most commonly drug u treat locally advanced and metastatic BCC. The mechanism by which Smoi leads BCC regression and emergence of resistant tumor cells are currently unknown. We found that Smoi mediat regression by promoting epidermal differentiation and that during the course of Smoi administration, some BCC become resistant to therapy mimicking the situation found in humans. We identi as a marker expressed in the resistant tumor cell population upon Smoi administration. Finally, we demonstrate that combination of Smoi administration with Lgr5 lineage ablation leads eradication, constituting a clinically promising approach to overcome resistance to therapy and cure BCC.

[86] Neurorepair in a new gerontology model; the African turquoise killifish

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Neuron loss aWer brain injury and disease-associated neurodegeneration are major causes of death and disabilities in the world. Despite intensive research, there are still no effective treat available. The lack of success may be related to the exclusion of the factor age in many investigations, although aging clearly negatively impacts the capacity for recovery upon neuron lo neuroregeneration. Our main goal is to test the efficacy, validity, and predictive value of a new animal model, the short-lived African turquoise killifish, to elucidate and combat the impact of ag neurorepair. The killifish shares remarkable aging phenotypes with humans, such as increased GFAP expression, cellular senescence and neurodegeneration. Hereto, we first have optimized wound injury model, targeting the telencephalon. By visualizing the cellular response to the injury we discovered that killifish can regenerate lost neurons aWer injury, like other teleosts. Compar young and old fish (6 weeks of sexually mature versus 18 weeks representing the last quarter of life) revealed that young adult fish can recover faster upon wounding compared to aged particular, we observed a reduced proliferation capacity of SOX2+- Gs+ - BLBP+ stem cells in aged fish aWer injury. In young fish the proliferation reaches a maximum at 2-3 days post injury and his no longer revealed a lesion site by 23 days, while proliferation in aged fish stays permanently low, with the stab wound still visible 23 days post-injury. A differential investigation of the mo response to stab wounding consists of a proteomics screening for injury- and aging-related cell surface proteins to prioritize potential targets towards rejuvenating the extracellular environment brain of aged fish. A parallel transcriptomic approach aims at unraveling the stem cell diversity in the telencephalon and their specific contribution to the observed neuroregeneration process.

[87] Urine-derived kidney progenitor cells in nephropathic cystinosis: the key to kidney regeneration ?

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Background Nephropathic cystinosis is a rare lysosomal storage disorder, caused by bi-allelic mutations in the lysosomal cystine/proton cotransporter cystinosin (CTNS). The kidney is the first an severely affected organ, characterized a general proximal tubular dysfunction early in life, followed by progressive glomerular damage leading to end-stage renal disease at adolescence. Recen demonstrated an excessive loss of proximal tubular cells (PTEC's) and podocytes in urine of cystinosis patients. Therefore we hypothesized that in compensation for this cell loss, ongoing regen might happen which could be reflected by the presence of kidney progenitor cells in the urine of cystinosis patients. Methods Kidney progenitor cells were isolated and cultured from fresh samples of cystinosis patients prior to kidney transplantation (cystinotic uKPC's). Quantification of uKPC's in urine was performed via qPCR. The cystinotic uKPC's were characterized by qPCR and Specific uKPC clones differentiated to PTEC's and podocytes, as demonstrated by qPCR and IF, followed by functional assessment. Complementation of CTNS in cystinotic uKPC's was perform transduction with a self-inactivating lentiviral vector containing a CTNS-3HA transgene. Results We demonstrated a significant increased loss of kidney progenitor cells in urine of cystinosis p compared to controls (progenituria). Potent uKPC clones showed expression of mesenchymal stem cell and nephron progenitor markers. Some demonstrated the capacity of exclusive differen towards a functional PTEC or Podocyte. Transduction with a CTNS-3HA containing LV- vector was shown to be successful. Conclusion We demonstrated the presence of kidney progenitor cells urine of cystinosis patients, of which some can differentiate to a functional PTEC or Podocyte. Moreover, these uKPC's can be complemented with CTNS via LV-vector transduction. Hence, uKPC's serve as a future therapeutic tool in regenerative medicine once the rescue of the healthy phenotype following CTNS complementation has been proven successful.