ABSTRACT BOOK

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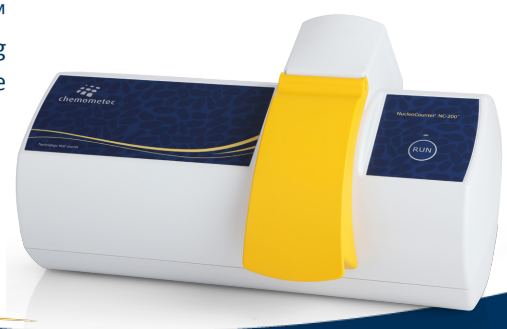

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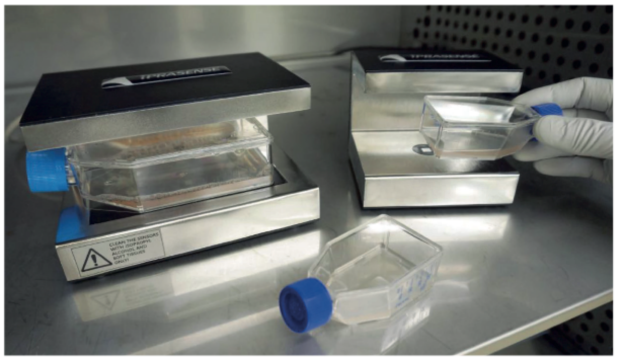

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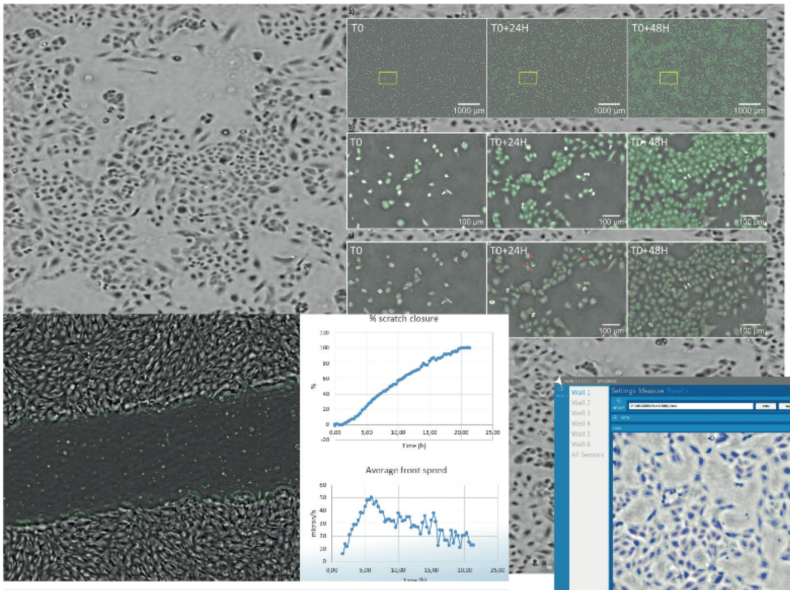

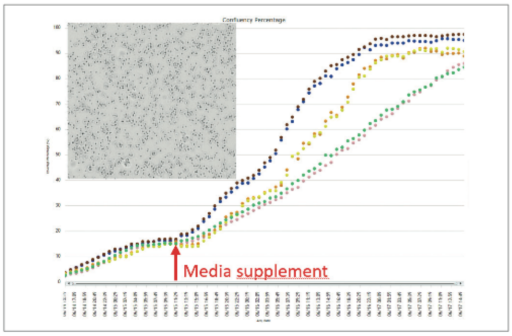

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PROGRAM

8:30 – 9:15 BERNARD ROGISTER (Vice-Dean of Medicine Faculty)
Welcome

Chairpersons: B. MALGRANGE – C. ALFANO

9:15 – 10:00 **MAGDALENA ZERNICA-GOETZ**
"Building life from stem cells to understand it"

10:00 – 10:05 Analis

10:05 – 10:20 **IVAN GLADWYN-NG**
"Stress-induced unfolded protein response contributes to Zika virus-associated microcephaly"

10:20 – 10:50 **SILVIA CAPPELLO**
"Dissecting molecular and cellular mechanisms of human migrating neurons"

10:50 – 11:20 Coffee break

Chairpersons: L. DELACROIX – D. HUYLEBROECK

11:20 – 11:50 **FIONA DOETSCH**
"From circuits to physiology: Novel regulators of adult neural stem cells"

11:50 – 12:05 **SAMIE PATEL**
"Glutamate uptake dysfunction in Duchenne Muscular Dystrophy (DMD) iPSCs derived Astroglia-like cells can be corrected by readthrough compounds"

12:05 – 12h10 Zeiss

12:10 – 12:25 **RITA KHOUEIRY**
"The many functions of TET1 in the early post-implantation mouse embryo"

12:25 – 12:45 Poster teasers

12:45 – 14h45 Lunch and poster session

Chairpersons: V. PASQUE – R. VANDENBOSCH

14:45 – 15:30 **ALI BRIVANLOU**
"Molecular analysis of embryonic stem cells"

15:30 – 15h45 **JOÃO FRADE**
"Controlled ploidy reduction of pluripotent 4n cells generates 2n cells during mouse embryo development

15:45 – 15:50 Chemometec

15:50 – 16:05 **SILVIE FRANCK**
"MSH2 knock-out human pluripotent stem cells as model for CTG repeat instability in myotonic dystrophy type 1"

16:05 – 16:35 **CÉDRIC MAURANGE**
"Regulation of neural stem cell self-renewal during development and tumorigenesis in Drosophila"

16:35 – 17:00 Coffee break

Chairpersons: B. LAKAYE – A. VAN KEYMEULEN

17:00 – 17:15 **CHRISTOPHER LANG**
"Investigating the roles of Ror2 in mouse skin tumorigenesis and its clinical relevance with human skin tumors"

17:15 – 17:30 **ADRIANA SÁNCHEZ-DANÉS**
"Defining the cell populations responsible for skin cancer initiation and relapse following therapy"

17:30 – 18:00 **FRÉDÉRIC LLUIS**
"Targeting the pleiotropic effects of WNT pathway in pluripotency"

18:00 Concluding remarks and General Assembly

Oral Presentations

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

Adriana Sánchez-Danés, Mélanie Liagre & Cédric Blanpain

Laboratory of Stem Cells and Cancer, Université Libre de Bruxelles (ULB)

PRESENTING AUTHOR: ADRIANA SÁNCHEZ-DANÉS

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 use the 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 lead to 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 progenitors, were competent to initiate tumour formation upon oncogenic hedgehog signalling. Interestingly, this difference was due to the hierarchical organization of tumour growth in oncogene-targeted stem 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 tumours (Sánchez-Danés et al., Nature, 2016). To 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 used to 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 mediates BCC 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 identify Lgr5 as a marker expressed in the murine and human resistant tumor cell population upon Smoi administration. Finally, we demonstrate that combination of Smoi administration with Lgr5 lineage ablation leads to BCC eradication, constituting a clinically promising approach to overcome resistance to therapy and cure BCC.

Controlled ploidy reduction of pluripotent 4n cells generates 2n cells during mouse embryo development

João Frade, Paola Cortes, Umberto di Vicino, Shoma Nakagawa, Neus Romo, Frederic Lluis and Maria Pia Cosma

Center for Genomic Regulation (CRG) - The Barcelona Institute of Science and Technology and Universitat Pompeu Fabra

PRESENTING AUTHOR: JOÃO FRADE

Cells with high ploidy content are common in mammalian extra-embryonic and adult tissues. Cell fusion is known to generate polyploid cells during development and tissue regeneration in mammals. Besides, reprogramming of somatic cells to progenitor or pluripotent cell states has been achieved through cell fusion in vivo and in vitro and has potential applications in regenerative medicine. However, whether increased ploidy can be tolerated in embryonic lineages remains unknown. Here we show that pluripotent, tetraploid cells can contribute to the mouse embryo. We found that heterotypic fusion-derived tetraploid cells, when injected in a recipient mouse blastocyst, contribute to the embryo upon ploidy reduction and can generate diploid cells in vivo. Parental chromosome segregation in pluripotent tetraploid cells occurs through tripolar mitosis and can be random but also non-random. Tetraploid-derived diploid cells are not aneuploid; instead, they maintain the correct number of parental chromosomes and show a differentiated phenotype. 4n-to-2n ploidy reduction events occurred with no apparent chromosome loss or subsequent daughter cell death, as seen by karyotype analysis and time-lapse live microscopy. Overall, we discovered controlled genome reduction in pluripotent tetraploid cells, a mechanism that can ultimately generate diploid cells during mouse embryo development. These results not only unveil unexpected functions in mouse development but also they should be taken into consideration for cell therapy-based tissue regeneration approaches.

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

Silvie Franck*, Lise Barbé*, Simon Ardui, Dominika Dziedzicka, Fien Vanroye, Pierre Hilven, Stella Lanni, Christopher Pearson, Joris Vermeesch, Karen Sermon

Vrije Universiteit Brussel

PRESENTING AUTHOR: SILVIE FRANCK

Human pluripotent stem cells (hPSC), either embryonic stem cells (hESC) or induced pluripotent stem cells (iPSC) are a powerful tool to model repeat instability in DM1. Mismatch repair genes, especially Msh2, play an important role in inducing repeat instability in DM1 as shown in knock out mouse models. Also, human cell models with a downregulation of MSH2 induced by shRNA indicate MSH2 as a possible instability modifier. However, remaining MSH2 protein levels in down regulation experiments in human cell models and methods to measure instability with a limited resolution probably only yielded partial answers. To fully understand the role of MSH2 in repeat instability in human models, we knocked out MSH2 in three DM1 hESC lines and two hiPSC lines derived from two different DM1 patients using CRISPR/Cas9 systems. Repeat instability in hPSCs was measured by PacBio sequencing of long range PCR fragments spanning the repeat, allowing accurate and complete assessment of the TNR length. Our preliminary data shows that the first MSH2 wild type DM1 hESC line has a wide repeat size distribution compared to its MSH2 mutant line 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. However, our other MSH2 knock-out hPSC lines still need to be analysed and could probably strengthen this first observation. We show that hPSC are excellent models for DM1, as we have reiterated the previously observed role of MSH2 in TNR instability. Differentiation of our MSH2 knock-out hPSC lines into disease-relevant tissues or manipulation of other instability modifiers will expand the model.

Stress-induced unfolded protein response contributes to Zika virus-associated microcephaly

Ivan Gladwyn-Ng[1,*], Lluis Cordon Barris[1,*], Christian Alfano[1,*], Catherine Creppe[1,*], Thérèse Couderc[2,3,*], Giovanni Morelli[1,4], Nicolas Thelen[1], Michelle America[1], Bettina Bessières[5,6], Férechté Encha-Razavi[5], Maryse Bonnière[5], Ikuo K. Suzuki[7], Marie Flamand[8], Pierre Vanderhaeghen[7,9], Marc Thiry[1], Marc Lecuit[2,3,10,] and Laurent Nguyen[1]

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[10]Paris Descartes University, Sorbonne Paris Cité, Division of Infectious Diseases and Tropical Medicine, Necker-Enfants Malades University Hospital, Institut Imagine
** co-authors*

PRESENTING AUTHOR: IVAN GLADWYN-NG

Accumulating evidence support a causal link between Zika virus (ZIKV) infection during pregnancy and congenital microcephaly. However, the mechanism of ZIKV-associated microcephaly remains unclear. We combined analyses of ZIKV-infected human fetuses, cultured human neural stem cells and mouse embryos to understand how ZIKV induces microcephaly. We show here that ZIKV triggers ER stress and UPR in the cerebral cortex of infected postmortem human fetuses as well as in cultured human neural stem cells. After intracerebral and intraplacental inoculation of ZIKV in mouse embryos, we also show that it triggers endoplasmic reticulum stress in embryonic brains in vivo. This perturbs a physiological unfolded protein response within cortical progenitors that controls neurogenesis. Thus, ZIKV-infected progenitors generate fewer projection neurons that eventually settle in the cerebral cortex whereupon sustained ER stress leads to apoptotosis. Furthermore, we demonstrate that administration of pharmacological inhibitors of UPR counteracts these pathophysiological mechanisms, and prevents microcephaly in ZIKV-infected mouse embryos. Such defects are specific to ZIKV as they were not observed upon intraplacental injection of other related flaviviruses in mice.

The many functions of TET1 in the early post-implantation mouse embryo.

Rita Khoueiry, Abhishek Sohni, Bernard Thienpont, Xinlong Luo, Michela Bartoccetti, Harm Kwak, An Zwijsen, Diether Lambrecht and Kian Peng Koh

Stem Cell Institute

PRESENTING AUTHOR: RITA KHOUEIRY

The mammalian TET enzymes erase DNA methylation by reiterative oxidation of 5-methylcytosine (5mC). Both Tet1 and Tet2 are highly expressed in mouse embryonic stem cells (ESCs), inner cell mass and PGCs, suggesting that the role of Tet1 is likely subdued by Tet2 in naive pluripotency. While TET enzymes have been intensely studied as major epigenetic regulators in early pre-implantation stages, remarkably little is known about their physiological roles and the extent of their functional redundancy following embryo implantation. Here, we define a pre-streak embryonic stage where TET1 has unique non-redundant functions. Our analysis shows that TET1 regulates numerous genes defining differentiation programs in the epiblast and extra-embryonic ectoderm (ExE). In the epiblast, loss of TET1 results in increased expression of primitive streak markers suggestive of precocious differentiation. In the EXE, loss of TET1 upregulates metabolic genes enriched in oxidative phosphorylation pathways. In vitro, ablating TET1 affects the methylation status of primed epiblast cells. However, TET1 represses the majority of epiblast target genes independently of methylation changes, in part through regulation of the gene encoding the transcriptional repressor JMJD8. Importantly, our examination of two Tet1 mutant strains shows a sub-penetrance embryonic defects from E8.5-E9.5 onwards in homozygous-null mutant mice with B6 background, most prominently as failure of anterior neuropore closure and brain exencephaly; the phenotype increased in severity and penetrance (>95%) upon out-breeding to CD1. These observations recapitulate phenotypes of neural tube defects, which are often influenced by multifactorial genetic modifiers in mouse KO models. Collectively, our study reveals an interplay of catalytic and non-catalytic activities of TET1 and a gene repressive effect essential for normal development. Finally, our work suggest that TET1 might be a major epigenetic factor underpinning the developmental origin of several diseases including neural tube defects.

Investigating the roles of Ror2 in mouse skin tumorigenesis and its clinical relevance with human skin tumors

Christopher Lang, Anthony Veltri, Wen-Hui Lien

de DUVE Institute (UCL)

PRESENTING AUTHOR: CHRISTOPHER LANG

Throughout life, skin epithelium requires constant turnover and this regeneration process is fine-tuned by signaling cues from the microenvironment. When these signaling are deregulated, tumor development may be induced. Receptor tyrosine kinase-like orphan receptor 2 (Ror2), one of major receptors involved in non-canonical Wnt signaling, activates the JNK/AP1 and/or calcium/PKC pathways to modulate tissue polarity and cell motility. To investigate the roles of Ror2 in skin tumorigenesis, we first analyzed human squamous cell carcinomas tissues from patients and found higher expression of ROR2 and JNK activity in tumors. We then utilized Ror2 conditional knockout (cKO) mouse model and DMBA/TPA carcinogenesis protocol to study the functional significance of Ror2 in tumorigenesis. We found that Ror2 cKO mice developed fewer tumors and grew tumors in a slower rate, indicating the requirement of Ror2 for tumor formation and progression. Moreover, depleting Ror2 in carcinogen-induced tumors led to tumor regression, suggesting the essential role of Ror2 in tumor maintenance. Our results together indicate a crucial role for Ror2 in skin tumor initiation, progression/maintenance, as well as unveil a clinical relevance of Ror2 function in human skin tumors.

Glutamate uptake dysfunction in Duchenne Muscular Dystrophy (DMD) iPSCs derived Astroglia-like cells can be corrected by readthrough compounds

Samie Patel, Keimpe Weirda, Ruben Boon, Jonathan De Smet, Ann Swijsen, Robin Duelen, Philippe Van Damme, Liesbeth DeWaele, Joris De Wit, Maurilio Sampaolesi, Gunnar Buyse, Catherine M Verfaillie

Stem Cell Institute Leuven (SCIL)

PRESENTING AUTHOR: SAMIE PATEL

Duchenne Muscular Dystrophy (DMD) is a genetic condition that affects boys and is characterized by a progressive muscle weakness and eventual death. In addition, there is also evidence that DMD is associated with decreased cognitive performance. Astrocytes play critical roles in maintaining a permissive niche for proper development of synapses, neurite extension and overall evolution of neuronal architecture during brain development. We hypothesized that defects in DMD astrocytes may be related to the cognitive deficits seen in patients with DMD. In this study, we created induced pluripotent stem cells (iPSCs) from DMD patients carrying six different mutations along the 79 DYS exons. We demonstrated that although the DMD iPSC lines can be efficiently differentiated into astroglial progenitors and subsequent quiescent S100β-expressing astrocytes, gene expression profiles from DMD iPSC-astrocyte progenitors differed from those in normal iPSC-astrocytes. In addition, the cytoskeletal morphometry of DMD astrocytes was severely altered. Compared with healthy iPSC-astrocytes, DMD iPSC-astrocytes display an impaired glutamate clearance function and consistently elevated release of glutamate in the culture medium., Moreover, we have preliminary evidence that DMD iPSC-astrocytes induced excitotoxicity in healthy neuronal stem cells could be rescued by (i) NMDA receptor blockers to prevent binding of excess glutamate on early neurons, (ii) or restoration of dystrophin protein in deficient astrocytes by using read through compounds (RTC) such as Gentamicin, which eliminated almost all the spontaneous accumulated glutamate in culture medium. Future experiments are targeted at delineating the mechanisms underlying these defects by assessing interaction between glutamate transporters on glial membrane to underlying dystrophin protein or the associated complex via co-immunoprecipitation and super-resolution microscopy. Finally, our high throughput system could provide a rapid, translatable platform to evaluate efficacy of readthrough compounds (RTC) and their eventual transition to the clinic.

POSTERS

[1] Unravelling the cellular dynamics of force mediated tissue expansion

Mariaceleste Aragona, Sophie Dekoninck, Cédric Blanpain

Laboratory of Stem Cells and Cancer, Université Libre de Bruxelles (ULB)

PRESENTING AUTHOR: MARIACELESTE ARAGONA

Distinct populations of 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 basement 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 forced tissue expansion in the dorsal skin. The data collected so far are demonstrating for the first time, in vivo, that stretching the skin results in basal cells proliferation and that tissue growth is driven by the activation of a specific SCs population.

[2] Expression of adhesion molecules in human blastocysts attaching in vitro

Aberkane Asma, De Paepe Caroline, Essahib Wafaa, Sermon Karen, Tournaye Herman, Verheyen Greta, Van de Velde Hilde

Vrije Universiteit Brussel

PRESENTING AUTHOR: ASMA ABERKANE

Human embryo implantation is initiated by attachment of the blastocyst to endometrial epithelial cells. Molecular pathways regulating this process in humans are hardly known. Here for we aimed to investigate adhesion molecules potentially playing a role in human embryo attachment in vitro. This study was approved by both Local Ethical Committee and Federal Ethical Committee for research on human embryos. The blastocysts were donated for research after patients' informed consent. Gene expression of 84 adhesion molecules (TaqMan Array Card) was analysed in 24 day 6 human vitrified-warmed blastocysts. Fifteen blastocysts were co-cultured individually for 12 hours in an in-vitro adhesion model using the endometrial Ishikawa cell line. Following co-culture, 8/15 unattached (U-ATT) and 7/15 attached (ATT) blastocysts were harvested. The remaining 9 blastocysts were harvested without co-culture (CONTROL). Statistics were performed using Prism. For immunohistochemistry (IHC), day 6 blastocysts were co-cultured for up to 24 hours (day 7) and analysis was performed on three CONTROL, three day 6 ATT and four day 7 ATT blastocysts. Out of 84 analysed genes, 31 were expressed in all blastocysts. For five genes, the expression was significantly different between CONTROL, U-ATT and/or ATT blastocysts. Eight additional genes were only expressed in co-cultured blastocysts. Out of these eight genes, two were expressed in both U-ATT and ATT blastocysts, whereas the other six genes were mainly upregulated in the ATT blastocysts, e.g. Tenascin C (TNC) [$P=0.018$] and Thrombospondin-1 (THBS1) [$P<0.001$]. IHC confirms expression of TNC protein starting from day 6 ATT embryos and is increased in day 7 ATT blastocysts. Present data sheds new light on the genomic regulation of human embryo adhesion. Adhesion molecules in human embryos are significantly influenced by co-culture and attachment to endometrial epithelial cells. More research is ongoing to confirm the role of these genes during human embryo implantation.

[3] Culturing conditions select for one type of progenitor in three-dimensional embryonic ganglionic eminence spheroids.

T. Aerts, A. Pancho, E. Seuntjens

KU Leuven, Research Group of Developmental Neurobiology

PRESENTING AUTHOR: TANIA AERTS

In the current study, we aimed to develop an interneuron stem cell culture derived from the ganglionic eminences (GEs) of embryonic mice. The GEs are a transient progenitor region present in the subpallium of the developing mammalian brain. They are the main source of cortical interneurons and comprise the medial ganglionic eminence (MGE), the lateral ganglionic eminence (LGE) and the caudal ganglionic eminence (CGE). Within the GE, primary progenitor cells (PPCs) reside in the ventricular zone (VZ), while the subventricular zone (SVZ) and mantle zone (MZ) contain intermediate progenitor cells (IPCs) and immature interneurons, respectively. Isolation of GE cells of E13.5 mice and subsequent cultivation in the presence of EGF and FGF induces the formation of spheroids that can be kept over several passages. To characterize this spheroid culture, we investigated the effect of culturing conditions on the expression of progenitor markers. We isolated cells separately from the MGE, CGE and LGE of E13.5 Dlx5/6-Cre-Ires-GFP mice. In these mice, the Dlx5/6 intergenic enhancer drives expression of GFP. Flow cytometry experiments at 0 days in vitro (DIV) revealed that the cells were a mixture of PPCs (Sox2+ and Nestin+), IPCs (Nestin+ and Dlx5/6+) and immature interneurons (Dlx5/6+), concurrent with the isolated regions present in the GE (VZ, SVZ, MZ). After 4 DIV, more than 94% of cells were PPCs, indicating that culturing selects for one type of progenitor cells. In addition, qPCR experiments confirmed that this identity was maintained up to passage ten. The cells are amenable for Crispr/Cas9-mediated genome editing and could be used for gene function studies in cortical interneuron development, as a cheap in vitro drug screening platform or to study “interneuronopathies” such as epilepsy.

[4] Temporal dynamics of Tet1 and Oct4 reactivation identify novel stages in late reprogramming.

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

KU Leuven

PRESENTING AUTHOR: MICHELA BARTOCCETTI

Induced pluripotent stem cells (iPSCs) are reprogrammed in vitro from somatic cells by forced expression of transcription factors including the master pluripotency regulator OCT4. The Ten-Eleven-Translocation TET DNA oxygenases mediate active DNA demethylation during reprogramming, but their precise stage-specific contributions remain unclear. Both Oct4 and Tet1 are regulated by distal and proximal cis regulatory domains active respectively in naive and primed pluripotent states. Here, we have generated murine transgenic lines harboring dual fluorescent reporters reflecting cell-state specific expression of Oct4 and Tet1, to investigate the dynamics of Tet1 and Oct4 reactivation during 4-factor reprogramming of mouse embryonic fibroblasts (MEFs) to iPSCs. By distinct coupling of mCherry reporters driven either by a naive-specific Tet1 distal promoter or by total Tet1 gene activity, with GFP reporters driven by naive-specific or total Oct4 gene expression, we used time-course live cell imaging and flow cytometry to identify a deterministic trajectory of events marked by temporally distinct stages of Tet1 and Oct4 gene activation. First, Tet1 is activated prior to Oct4 by proximal regulatory elements at both genes, followed almost immediately by a second phase of activation of distal regulatory domains, in which naive-specific Tet1 gene activity again clearly precedes that of Oct4. The sequential order is conserved in different reprogramming methods and media conditions, and occurs after entry into pluripotency marked by NANOG protein expression but prior to induction of DPPA4. By sorting stage-specific reprogramming intermediates based on dual reporter patterns, we have found transcriptomic changes that define new players in primed-to-naive transitions in the late phase of reprogramming. In both embryonic differentiation and reprogramming, full Tet1 gene activation involving its distal regulatory locus, rather than that of Oct4, may be a hallmark of naive pluripotency reflective of the pre-implantation epiblast.

[5] Elafibranor restores lipogenic gene expression in a human skin stem cell-derived non-alcoholic fatty liver disease (NAFLD) model

Joost Boeckmans, Alessandra Natale, Karolien Buyl, Joery De Kock, Vera Rogiers, Tamara Vanhaecke* & Robim M Rodrigues* *Equally contributing last authors

In Vitro Toxicology and Dermato-Cosmetology, Faculty of Medicine & Pharmacy, Vrije Universiteit Brussel

PRESENTING AUTHOR: JOOST BOECKMANS

Non-alcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease. It ranges from uncomplicated hepatic steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and cancer. NASH is the first stage in the spectrum of NAFLD which is associated with increased health risk. No anti-NASH drug has been approved so far as discovery and development are largely hampered by the lack of adequate human-based in vitro systems that recapitulate the human pathophysiology. Hepatic cells derived from human skin precursors (hSKP-HPC) represent a unique in vitro model for the investigation of drug-induced NAFLD. In the current study, hSKP-HPC exposed to insulin (100 nM) and glucose (4500 mg/L) served as an in vitro steatosis model. NASH conditions were created by subsequently exposing the steatotic hSKP-HPC to sodium oleate (65 µM) and palmitic acid (45 µM) in combination with a pro-inflammatory fibrotic cytokine cocktail (50 ng/mL TNF-α + 25 ng/mL IL-1β + 8 ng/mL TGF-β). Gene expression analysis revealed a typical lipogenic gene expression profile concordant with human data: key lipogenic genes (ACC, FAS and SREBP-1c) were upregulated in the steatosis stage, but downregulated in the NASH model (24h exposure; one-way ANOVA, $p<0.05$). Gene expression changes were accompanied by an increased lipid load and caspase-3/7 activity in the NASH condition and a gradual increase in ATP production (72h exposure). As a proof-of-principle, the phase III anti-NASH compound elafibranor (Genfit) was tested in both in vitro systems. In the steatotic setup, elafibranor (10 µM) restricted the increased expression of SREBP-1c, ACC and FAS (24h treatment; student t-test, $p<0.05$). Under NASH conditions, elafibranor induced the expression of ACC and FAS and tended to reverse NASH to a steatotic stage. These data show that hSKP-HPC exhibit the potential to serve as a platform for anti-NAFLD drug discovery.

[6] The nutrient microenvironment is an essential driver of hepatocyte differentiation and maturation in vitro

Ruben Boon, Ilaria Elia, Manoj Kumar, Laura Ordovas, Guy Eelen, Matthew Bird, Philip Roelandt, Matteo Rossi Tine Tricot, Jonathan De Smedt, Marta Aguirre Vazquez, Thomas Vanwelden, François Chesnais, Adil El Taghdouini, Etienne Sokal, David Cassiman, Christian Lange, Peter Carmeliet, Sarah-Maria Fendt and Catherine Verfaillie

KU Leuven

PRESENTING AUTHOR: RUBEN BOON

Pluripotent stem cells (PSCs) hold an enormous potential for studying development and disease in a human context. Especially in the field of toxicology, PSCs are highly needed to generate liver cells that accurately reproduce human metabolisation and drug toxicity. The major hurdle in applying PSC technology in any context is the difficulties in steering their fate to a certain lineage in the unnatural environment that is cell culture. This is especially the case for hepatocytes since even primary cells immediately lose all their characteristics in an in vitro setting. We here show that the nutrient microenvironment is an essential component determining the functionality of PSC-derived hepatocyte-like cells (HLCs). We found that the differentiation of PSCs towards the hepatic lineage is characterized by the persistence of a stem cell-like metabolic profile; a high dependency on glucose and a failure to induce mitochondrial activity. Based on whole genome RNAseq data, we identified several hepatic and metabolic transcription factors that where lacking in HLCs. Although overexpression of these regulators from the AAVS1 safe harbor locus was able to induce HLC maturation to some extent, we found the nutrient composition in standard culture media to be limiting for the acquisition of full differentiation. We demonstrated that nutrients further activate hepatic and metabolic transcription factors, and are required for the induction of gluconeogenesis, mitochondrial activity and xenobiotic drug metabolism. This is, we believe, the first study showing nutrient engineering to be a vital tool for steering PSC differentiation towards functional HLCs. The combination of both nutrient and genome engineering allowed us to establish an HLC based high throughput screening system that accurately predicts hepatocyte toxicity, even of drugs requiring metabolization, and this at clinically relevant sensitivity.

[7] The good, the bad and the ugly: an endometrial tale.

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The endometrium represents the inner mucosal lining of the uterus. During a woman's reproductive life-time, this tissue undergoes more than 400 cycles of growth, degeneration and regeneration. The powerful regenerative capacity makes the endometrium a fascinating unique natural model of tissue damage and regeneration. Despite being a key organ for mammalian reproduction, little is known about the mechanisms that control its homeostasis, prominent remodeling and disease pathogenesis. Endometrial diseases are the first gynecological burden and primary ground of infertility. Research has long been hampered by the lack of reliable in vitro models. In our lab, we established an organoid model which faithfully recapitulates endometrial biology and disease. These organoids form under WNT-activating culture conditions and show clonogenic expansion potential. Ciliated and secretory cells are detected demonstrating that different cell types can be generated from the same stem/progenitor cell. The organoids can be cultured for more than 8 months retaining genomic stability and hormonal responses. Transcriptomic profiling advanced candidate markers of adult stem cells in the endometrium. Furthermore, organoids allowed to explore the contribution of the WNT pathway to the physiological hormonal responses. We further established organoids from diseased conditions recapitulating the entire spectrum of endometrial pathologies which will ultimately lead to the generation of a biobank to genomically and transcriptomically explore endometrial pathogenesis. Taken together, the endometrial organoid model will help understanding the fundamental pathways of endometrial biology and regeneration, and how these pathways are hijacked during disease pathogenesis. In addition, the organoid biobank will serve as drug-screening platform for better disease management. Matched organoids established from healthy and diseased endometrium within the same patient will help to develop drugs which specifically target the diseased endometrium.

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

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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 of BC fate. We found that LCs ablation activates multipotency of BCs. To understand the mechanisms regulating the cell fate switch upon LCs ablation, we will perform 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 such as Zeb1, Sox10, Axl, Neat1 and Runx2 that control BCs multipotency in the different experimental conditions associated with multipotency. The functional role of these genes will be studied using genetic loss of function in vivo following lineage ablation and transplantation. The upregulation of many collagen genes in multipotent BCs leads us to hypothesize that the extracellular matrix 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, the experiments should allow the identification the mechanisms regulating SC 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.

[9] Unravelling the pathophysiological mechanisms of LIS1-associated human cortical malformations

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LIS1 is a keystone protein in neuronal development that controls various biological activities, such as cellular transport, proliferation of neuronal progenitors and neuronal migration. Functional disruption of this protein is responsible for Miller Dieker syndrome (MDS), characterized by cortical malformations such as different lissencephalic grade of severity and subcortical band heterotopia. In order to decipher the pathological mechanisms resulting from LIS1 mutation, we engineered dorsal cortical progenitors (DCP) from CRISPR/Cas9 edited human embryonic stem cells (hESC) that express reduced levels of LIS1 protein. This cellular model will be used to uncover the molecular pathways and the correspondent cellular functions dysregulated when LIS1 is not properly expressed in early cortical progenitors. The candidate genes connection to LIS1 will be confirmed in this model by gain of function (GOF) or lose of function (LOF) experiments and screened in the exome of different lissencephalic patients. This study will bring into light the pathophysiology of lissencephaly with new molecular pathways associated to LIS1 disruption that can be target by drugs to prevent, rescue or ameliorate the prognosis of this disease.

[10] The WNT/LGR5 pathway in pituitary regeneration and organoid modeling

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PRESENTING AUTHOR: BENOIT COX

The pituitary is the master endocrine gland essential for normal body physiology. Our group recently discovered that the adult pituitary is capable of regeneration. Using a transgenic mouse model, growth hormone (GH)-expressing cells are decimated by treatment with diphtheria toxin (DT), but they considerably regenerate 4-5 months later. We observed that the pituitary stem cells promptly react to the inflicted injury by proliferating and co-expressing GH, thereby implying their involvement in the regenerative process. Gene expression analysis showed upregulation of WNT/LGR5 pathway components in the pituitary upon the DT-induced damage. LGR5, a co-receptor of the WNT pathway, has been identified as a stem cell marker in multiple organs, and intriguingly also in injured tissues like the liver. WNT/LGR5 activation is instrumental to grow organoids, three-dimensional self-organizing structures that mimic the organ of origin and allow robust long-term ex vivo culturing. By testing several conditions, we were able to develop organoid structures from pituitary cells in a WNT-dependent manner, expandable for multiple passages. Moreover, organoids developed from the pituitary stem (SOX2+) cells, not from the other cells. The organoids are immature, as evidenced by persistent SOX2 expression, but show gene transcription of some early-pituitary markers. Remarkably, different organoid morphologies, either cystic or dense, were obtained when starting from the transgenically damaged or control pituitary tissue, respectively. The two organoid types exhibit distinct WNT pathway component signatures and show dissimilar proliferative capacity. The observed differences may be correlated with the activation status of the pituitary stem cells in the damaged versus control tissue. Currently, we are exploring several protocols to achieve organoid differentiation towards the different pituitary hormonal cell types. Taken together, pituitary organoids provide a powerful and novel in vitro platform to explore pituitary biology, regeneration and disease, and the involvement of stem cells in these processes.

[11] Derivation of cochlear cells from pathological and isogenic human iPSCs for modeling hereditary hearing loss

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PRESENTING AUTHOR: AMANDINE CZAJKOWSKI

Alström Syndrome (AS) is a human autosomal recessive genetic disorder characterized by numerous clinical symptoms including deafness. AS is caused by mutations in ALMS1 gene encoding for ALMS1 protein located at the basal body and implicated in ciliogenesis, cell cycle and proliferation. Knowing that ALMS1 is express in the inner ear, we are interested in understanding the unknown mechanisms involving this protein in the genetic deafness in AS patients. To develop a model as close as possible to the human pathology, we use human induced pluripotent stem cells (hiPSCs) generated from healthy and AS patients. To exclude patient linked epigenetics and differentiation defects, we are trying to correct the genomic mutation in the AS hiPSCs to generate isogenic hiPSCs using the CRIPSR/Cas9 system. Recently it was demonstrated that mouse and human embryonic stem cells can be directed to an otic fate. Using a stepwise protocol, we demonstrate that healthy hiPSCs (waiting for isogenic hiPSCs) can generate a population of cells with gene and protein expression profiles consistent to the ones of otic progenitor cells (OSCs). When co-cultured with mouse feeder cells, human OSCs are able to differentiate into hair cells (HCs). We successfully apply the differentiation protocol to AS hiPSCs generated from AS patients. We are currently confirming gene expression pattern and testing HCs functionality. Experiments to study the proliferation and the ciliogenesis at different stages of the differentiation are ongoing. Thanks to the isogenic hiPSCs we'll be able to confirm the observed defects are due to the ALMS1 mutation.

[12] Bird's eye view of the landscape of histone modifications in the ground state of mouse embryonic stem cells.

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PRESENTING AUTHOR: LAURA DE CLERCK

Transient pluripotent states of the embryo can be stably captured in vitro in the form of embryonic stem cells (ESCs). Mouse ESCs cultured in serum-free media supplemented with two kinase inhibitors (2i) are in a uniform condition of self-renewal termed the 'naïve' ground state. Through comparison with serum-grown ESCs, we identified distinctive post-translational histone modifications (hPTMs) by mass spectrometry (MS). Histones were extracted from the cells and subjected to propionylation and trypsin digestion prior to MS analysis. 44 individual hPTMs and 148 hPTM combinations on histone H3 and H4 were identified and quantified with high reproducibility ($R > 0.95$) across replicates. Unique features of 2i ESCs include: high levels of H3K27me3, together with histone clipping and H4 hypo-acetylation.

[13] Canonical Wnt pathway activation triggers embryonic stem cell cycle arrest via Tcf1

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Unraveling the mechanisms regulating the cell cycle, potency and differentiation of pluripotent stem cells is of high interest for their clinical application potential. Mouse embryonic stem cells (mESCs) are pluripotent and have the capacity to self-renew. They show a fast cell cycle with very short G1 phase due to the lack of expression of cell cycle inhibitors. In mESCs the canonical Wnt/β-catenin signaling pathway is known to play a crucial role in pluripotency maintenance through Tcf3, a well-studied downstream transcription factor of the Tcf/Lef family. However, the regulatory role of the Wnt/β-catenin pathway in proliferation remains unknown. We found that upon Wnt/β-catenin pathway activation, Tcf1 binds to a new binding motif. Thereby Tcf1 regulates the transcription of genes involved in cell cycle arrest, but without affecting pluripotency. In presence of Wnt signaling, the G1 phase is prolonged resulting in a drastic reduction of the proliferation. Thus, we observe that distinct binding of Tcf1 and Tcf3 results in the regulation of distinct target genes and functions in mESCs. Altogether, we show that canonical Wnt/β-catenin signaling controls mESC pluripotency and cell cycle through non-overlapping functions of distinct Tcf/Lef family members.

[14] Inflammation modifies the secretome and immunosuppressive properties of human skin-derived precursor cells

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Human skin-derived precursors (SKP) are somatic stem cells that reside in dermal skin throughout life harboring clinical potential. SKP have a high self-renewal capacity, the ability to differentiate into multiple cell types and low immunogenicity rendering them key candidates for allogeneic cell-based off-the-shelf therapy. However, potential clinical application of allogeneic SKP requires that these cells remain low immunogenic under all circumstances and in particular in the presence of an inflammation state. Therefore, in this study, we investigated the impact of pro-inflammatory stimulation on the immunological properties of SKP. We found that pro-inflammatory stimulation of SKP (SKP+INFL) does not increase their immunogenicity in vivo, but evokes changes in their cytokine and growth factor secretion and immune suppressive properties. More specifically, the secretion of the chemokine ligands CCL2, 5, 7, 8 and 20, CXCL1, 5, 6 and 10, colony stimulating factor (CSF) 2 and 3, hepatocyte growth factor (HGF), interleukin (IL) 6 and 8, leukemia inhibitory factor (LIF) and vascular endothelial growth factor (VEGF) was significantly increased. HGF is a soluble molecule that is mandatory for SKP to perform immunosuppressive functions. In-depth pathway analyses show that the increase in HGF secretion is linked to the combined activation of CCL2, CSF2, IL6 and LIF. Co-transplantation of SKP+INFL and human peripheral blood leukocytes into severe combined immune deficient mice showed that SKP+INFL maintain their ability to impair the graft-versus-host response, but to a lesser extent as indicated by increased plasma hIgG levels compared to co-transplanted SKP. In conclusion, we believe that SKP are a valuable cell source for cell therapeutic strategies requiring immunosuppression and as a poorly immunogenic multipotent stem cell population for cellular replacement therapy. However, our data suggest that the presence of an inflammation state in the target disease can have a significant impact on the therapeutic performance of SKP.

[15] CRISPRia genome engineering to improve differentiation of PSC-derived hepatocytes, LSECs and HSCs

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PRESENTING AUTHOR: JONATHAN DE SMEDT

To assess drug safety, efficacy and toxicity, the primary organ of interest is the liver. The liver is responsible for biotransformation and drug metabolism and hence provides the fundament of toxicity test models. Within the liver, hepatocytes are the main target for drug toxicity and metabolisation, but their homeostasis, function, and toxin response depends also on the hepatic stellate cells (HSCs), and liver sinusoidal endothelial cells (LSECs). To develop suitable drug toxicity test systems, the Verfaillie laboratory has developed differentiation protocols for embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) into cells with hepatocyte, HSC and endothelial properties. However, these cell populations do not fully reflect primary cells isolated from human liver, as shown by transcriptome studies. The overal aim is to modify the expression of transcription factors (TFs) combining dCas9-KRAB to repress expression of incorrectly activated TF, and dCas9-VPR to activate expression of insufficiently expressed TFs. To identify the TFs to be modulated, we performed a gene co-expression analysis (WGCNA) on a wide set of published microarray data on PSC derived progeny and primary human liver cells (hepatocytes, HSCs and LSECs).We identified gene co-expression clusters, associated with distinct developmental or homeostatic functions and identified the TF hierarchy within these abberrantly expressed gene clusters. Currently, we are testing the inhibitions or overexpressions of the incorrectly expressed TFs using CRISPR-dCas9 constructs RMCE-integrated in the AAVS1 locus. In conclusion, we propose an novel integrated methodology of both gene co-expression analyses and CRISPR activation and repression screenings to correct the gene expression profiles of cultured hepatocytes, LSECs, and HSCs. Once key TFs show to improve the phenotypes of these cell types, co-cultures and drug toxicity assays will be performed to demonstrate this system's augmented value as a predictive drug toxicity model.

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

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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 proportionnal to the whole tissue expansion demonstrating that the labeled population recapitulates the tissue growth. Mathematical analysis performed on clonal data suggest that basal cells present a bias toward symmetrical self-renewal leading to an increase in the number of basal cells over time. Proliferation experiments using EdU/BrdU double pulse showed that IFE basal cells slow down their cell division time progressively after birth both in scale and interscale regions which together with the high proportion of symmetrical division explain the linear growth. In sharp contrast with the homeostatic model in adult, interscale clone size distribution rather fit with a single population of cells. Whether the second population appears later on or whether they both co-exist but proliferate at the same rate until adulthood is still unknown. More experiments coupled with single cell RNA sequencing are still needed to understand the heterogeneity of the IFE during postnatal growth.

[17] Analysis of stem cell markers in the adult Octopus vulgaris brain

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Octopus vulgaris, a mollusk, has a highly centralized and lifelong growing brain. Its nervous system contains about 500 million neurons which is ten times more compared to a mouse. The octopus also bears extensive regenerative capabilities and shows complex behaviors such as problem solving and social learning. In vertebrates, an increase in number of neurons linked to complex behavior is associated with the appearance of different types of neurogenic stem cells. Furthermore, neurogenesis is continued postnatally in specific regions of the brain. This process allows a continuous supply of neurons that integrate into existing networks, supporting brain plasticity leading to adaptive behavior. It is unknown whether complex invertebrate neural systems such as the Octopus vulgaris brain also renew neurons at the adult stage. Here, we attempt to visualize postnatal neurogenesis in the adult Octopus vulgaris brain using immunohistochemical stainings for markers of cell cycle and proliferation. Using the recently published Octopus bimaculoides genome, we identified orthologues of genes involved in vertebrate postnatal neurogenesis. We use in situ hybridization to map the expression of these orthologues on different regions of Octopus vulgaris nervous tissue aiming to identify progenitor cells that support neural diversity. Our immunohistochemical stainings show a strikingly flexible presence of the mitosis marker phospho-histone H3 in the octopus vertical lobe, a region that shares both morphological and functional similarities to the vertebrate hippocampus. We also found cyclinA positive cells scattered in the vertical lobe, indicative for cells in S/G2 phase. Next to a minor population of vertical lobe neurons that is positive for the GABA transporter Slc6a1, we found abundant expression of the orthologous stem cell marker Sox2 in this region. By mapping more markers of cell cycle and proliferation, we aim to determine evolutionary convergent mechanisms that lie at the basis of neurogenesis and regeneration.

[18] Towards a bird's eye view of the histone code in stem cells using mass spectrometry

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Epigenetic histone posttranslational modifications (hPTM) regulate a plethora of fundamental biological processes by mediating activation and transcription of genes. Especially stem cell biology seems to be tightly regulated by these hPTM. With the image of the epigenetic landscape now gradually sharpening a growing sense arises that it is the interplay between different biochemical changes that dictates the final biological outcome. However, studying the proverbial grammar emerging from the combination of hPTMs, the so-called "histone code", requires a methodology that can create a bird's eye view. Mass spectrometry (MS) currently is the only technique holding the promise of simultaneously annotating and quantifying enough different hPTM combinations to considerably mine the histone code to a depth that is required for interpreting the underlying biology. Because a dedicated workflow is required during every step of the experiment, from sample preparation over data acquisition to data analysis, only a few groups world-wide dedicate their research to this specific application of MS to date. Our group is currently aiming to use "data-independent acquisition MS" for mining the histones code, which will shed a new light on histone epigenetics in stem cells the near future. Here, we will reflect on the current state of the art and the promises of this young field, with a particular focus on our work on human and mouse embryonic stem cells (ESC). It is indeed clear that even the slightest perturbation of ESC causes detectable and complex changes in the histone code. While a considerable amount of these changes have been studied individually, we will argue that the bird's eye view of the dynamics of the histone code is an essential next step in understanding stem cell biology. We thus feel confident that the potential of this field is only now starting to unfold.

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

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Ischaemic stroke is a severe condition which is defined by loss of brain function due to impaired blood flow to the brain. Current therapies are only available for a small subset of patients and are unable to sufficiently improve the functional outcome following stroke. Cell-based therapy is considered 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. Since the migration of endogenous neural stem cells (NSC) to the stroke lesion is an important step in the neuroregenerative response, 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, while the CM of human dermal fibroblasts and bone marrow-derived mesenchymal stem cells failed to attract these neuronal precursors (n = 8). To identify the key paracrine factors present in the CM of DPSC responsible for the chemotactic effect, a growth factor antibody array was performed. BDNF, NGF, GDNF, NT-3 and IGF-II were shown to be present and their 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. Future experiments will focus on investigating the IGF-II pathway in neuroregenerative processes including NSC differentiation, neurite outgrowth and angiogenesis which is also an essential process for tissue regeneration. In conclusion, we hope to shed a light on the contribution of IGF-II in the potential of DPSC as a novel treatment strategy against ischemic stroke.

[20] Human embryonic stem cell differentiation bias during early lineage specification

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Human embryonic stem cell (hESC) lines often manifest a differentiation bias towards one of the embryonic germ layers. As this phenomenon can significantly hamper the efficiency of hESC-based biomedical applications, it is important to deepen our knowledge about its molecular mechanisms. In this study, we first quantified differentiation propensity of five karyotypically normal hESC lines cultured on laminin-521 using standardized differentiation protocols for early lineage specification. Specifically, cells were induced towards mesendoderm for 1 day, definitive endoderm (DE) for 3 days and ectoderm for 4 days. Based on qRT-PCR and immunofluorescence analysis of lineage-specific markers, all cell lines showed similar differentiation efficiency towards the ectodermal lineage. However, differentiation efficiencies varied for mesendodermal derivatives. Specifically, VUB04 had a low expression level of BRACHURY after mesendoderm induction, and the lowest expression of SOX17, FOXA2 and GATA4 after DE induction. Also, quantitative immunostaining analysis showed that the VUB04-derived DE population had the lowest percentage of SOX17-positive cells and the highest number of POU5F1-positive cells. Next, we performed mRNA-sequencing of the five VUB hESC lines at the undifferentiated stage. Analysis showed that VUB04 expressed pluripotency markers (POU5F1, SOX2, LIN28A and ZFP42) at similar levels in comparison to the other four cell lines, but had elevated NANOG expression (fold change = 1.76, FDR < 0.00001). Also, comparing the expression profile of VUB04 to the hESC lines with normal DE differentiation efficiency (VUB01, VUB02 and VUB03) indicated that 693 genes were differentially expressed (fold change > 2, FDR < 0.05). Pathway involvement analysis using Ingenuity Pathway Analysis software and the DAVID functional annotation tool identified MAPK/ERK signaling as significantly deregulated in VUB04. Based on these transcriptomic data, we will further select and genetically modify candidate molecular targets and subsequently investigate possible effects on differentiation bias towards mesendodermal derivatives.

[21] Defining the cell of origin of brest cancer

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PRESENTING AUTHOR: MARCO FIORAMONTI

Breast cancer consists of heterogeneous types of tumours that are classified into different histological and molecular subtypes. The cellular origin and the mechanisms leading to tumor heterogeneity remain unknown in breast cancer. We recently used a genetic approach in mice to define the cellular origin of Pik3ca-derived tumours and the impact of mutations in this gene on tumor heterogeneity. Oncogenic Pik3ca(H1047R) mutant expression at physiological levels in mammary basal cells (BCs) induced the formation of luminal oestrogen receptor (ER)-positive/progesterone receptor (PR)-positive tumours, while its expression in luminal cells (LCs) gave rise to luminal ER(+)PR(+) tumours or basal-like ER(-)PR(-) tumours. LCs compartment is an heterogeneus population composed by both ER+ and ER- cells. Lineage tracing experiments showed that Notch1-labeled ER- LCs, which have long-term self-renewing potential, give rise to ductal and alveolar ER- cells only, demonstrating that a fraction of ER- LCs are maintained by a distinct pool of stem cells (SCs). We used a doxycycline-inducible ER-rtTA in order to perform genetic lineage tracing of ER+ LCs and study their fate and long-term maintenance. Our results show that ER+ cells are maintained by lineage-restricted SCs that exclusively contribute to the expansion of the ER+ lineage during puberty and their maintenance during adult life. The presence of the different independent subpopulations of LCs, sustained by distinct SCs, suggests that they could act as cell of origin of different types of breast cancer explaining the existing tumour heterogeneity. We will use Notch1, ER and K8 promoters to specifically target ER-, ER+ and all LCs and will introduce combinations of oncogenic mutations to study 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.

[22] Leukocyte- and platelet rich fibrin priming does not enhance the neuroregenerative properties of human dental pulp stem cells

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Neurodegenerative disorders are characterized by loss in neuronal function which cannot be restored by the endogenous repair response. This stresses the need of novel treatment options such as cell-based therapies that can salvage the lost tissue or stimulate repair. The regenerative potential of stem cell-based therapies is believed to be mediated by their secreted factors. Therefore, this study aimed to investigate the neuroregenerative potential of the conditioned medium of human dental pulp stem cells (CM-hDPSCs) on neural stem cell (NSC) proliferation and migration in addition to their effect on neuroprotection and neurite outgrowth of primary cortical neurons (pCNs). Moreover, the effect of Leukocyte- and Platelet-Rich Fibrin (L-PRF) priming on the neuroregenerative potential of CM-hDPSC on NSCs and pCNs was evaluated. L-PRF contains factors that were shown to enhance stem cell-induced regeneration, but its effect on hDPSC-mediated neuroregeneration is unknown. This study demonstrated a neuritogenic effect of CM-hDPSCs on pCNs whereas the neuroprotective effect in an oxygen-glucose deprivation model remains to be clarified. Moreover, CM-hDPSCs had a chemoattractant effect on NSCs in a transwell assay. Although priming hDPSCs with L-PRF increased brain-derived neurotrophic factor secretion, no additional effects on the investigated repair mechanisms were observed. These data support the neuroregenerative potential of hDPSCs and although L-PRF priming of hDPSCs did not enhance these properties, the potential of L-PRF-primed hDPSCs on distinct regenerative mechanisms remains to be clarified

[23] Motor neurons derived from ALS patients with FUS mutations mimic disease-related phenotypes in vitro

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder due to selective loss of motor neurons (MNs). Mutations in the 'fused in sarcoma' (FUS) gene can cause both juvenile and late onset ALS. We generated and characterized induced pluripotent stem cells (iPSCs) from ALS patients with different FUS mutations, as well as from healthy controls. Patient-derived MNs show typical cytoplasmic FUS pathology, hypo-excitability as well as progressive axonal transport defects. Axonal transport defects are rescued by CRISPR/Cas9-mediated genetic correction of the FUS mutation in patient-derived iPSCs. Moreover, these defects are reproduced by expressing mutant FUS in human embryonic stem cells (hESCs) while knockdown of endogenous FUS has no effect, confirming that these pathological changes are mutant FUS dependent. Pharmacological inhibition as well as genetic silencing of histone deacetylase 6 (HDAC6) increases aa-tubulin acetylation and restores the axonal transport defects in patient-derived MNs.

[24] Using the Fluidigm C1 whole genome amplification system for high throughput single cell shallow sequencing

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Human pluripotent stem cells (hPSC) have significant levels of low-grade genetic mosaicism, which commonly used karyotyping techniques of bulk DNA fail to detect. These abnormal copy number variations (CNVs) remain a hurdle for the clinical translation of hPSC, and the ability to detect them will be necessary as the field advances. Single cell shallow sequencing can be used to identify such CNVs, but is less accessible for the analysis of a large number of cells due to the high cost of whole genome amplification (WGA) and the time required for single cell isolation. We sought to reduce the cost per sample and time input by utilizing the Fluidigm C1 for the WGA on 96 cells simultaneously. Utilizing an integrated fluidic circuit, the C1 was able to capture and amplify single cells on 71 of the 96 available sites, using only the equivalent WGA reagents typically used for a single cell (74% capture and amplification success rate). We used a mosaic subline of the human embryonic stem cell line VUB02, carrying an isochromosome 20, with a loss of the p-arm and gain of the q-arm. This served as an internal control that could validate the sensitivity of the method. The isochromosome was found in 80% of the cells, with 2/40 failing to call either the loss or gain of the p or q arms respectively, leading to an estimated false negative rate of 5%. Furthermore, 30% of the cells carried additional unique CNVs, larger than 26Mb and not observed in the bulk analysis. This highlights the methods ability to detect large mosaic CNVs as well as to establish the fraction of cells that carry a given CNV. To our knowledge, this is the first use of the Fluidigm C1 combined with single cell shallow sequencing for CNV analysis in hPSC.

[25] Finding the winning combination: Combinatorial screening of Cell responsive 3D niches to Improve PSC derived hepatocytes

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Pluripotent stem cell (PSC) derived hepatocytes might be an attractive alternative for primary hepatocytes for drug toxicity, metabolisation and hepatitis virus studies. However, currently published protocols to generate PSC-hepatic progeny create cells lacking functional maturity. One cause herefore, is that differentiations are done in very stiff culture plates, whereas the natural environment of hepatocytes and their precursors is the soft 3D liver. In this study, we created hundreds of cell responsive 3D hydrogel-based microenvironments functionalized with ECM and cell adhesion peptides, with the aim to improve the maturation of hepatic progeny from hPSCs. Studies were performed with a PSC cell line created in the lab, expression 3 doxycycline inducible transcription factors, recombined in the AAVS1 locus. The culture system was composed of a hydrogel of 4-arm-polyethylene-glycol (PEG) blocks linked to different adhesion ligand peptides and cross-linked with MMP cleavable peptides, and varying mechanical properties. All microenviroments had stiffness ranges from 500-2000Pa, resulting in a decrease in the activity of the Hippo pathway by 8-30 fold. A system level analysis allowed us to select 3 unique microenvironments that improved hepatocyte phenotype and function by 50-fold: the top 3 hydrogels induced a ±50-fold increased ALB secretion and CYP3A4 activity. In addition, a significant increase in mature hepatocyte genes, including HNF6, PROX1, PXR, PGC1α and G6PC was observed. This strongly suggests that changes in mechanotransduction/specific adhesion receptor engagement, akin to what is found in primary liver, may enhance further hepatocyte maturation from hPSCs. Ongoing studies are aimed to further validate the hydrogel-based cultures for improved hepatocyte maturation, and to identify the molecular mechanisms underlying these effects. Additionally, PSC-hepatic progeny matured in these hydrogels will be tested for the efficiency of drug toxicity and metabolisation, to determine its ultilsation by the pharmaceutical industry in their drug development pipelines.

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

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Osteoarthritis is a degenerative and inflammatory condition of synovial joints with loss of supportive cartilage matrix. Durable restoration of damaged articular cartilage remains an unachieved goal. Adult autologous chondrocyte implantation could be efficient for treating chondral lesions but has several drawbacks. Alternatively, bone marrow-derived mesenchymal stem cells (BMMSCs) have been used to treat chondral lesions in preclinical settings. However, we used dental pulp stem cells (DPSCs) because of their less invasive isolation. Furthermore, 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 as a promising strategy in regenerative medicine due to its growth factor content and supportive fibrin matrix. We hypothesize that combining DPSCs with L-PRF will enhance their chondrogenic differentiation potential. The effect of L-PRF on the chondrogenic differentiation of DPSCs (n=2) was determined and compared to BMMSCs (n=1) in vitro. DPSCs and BMMSCs were subjected to a 3D differentiation system, with or without L-PRF conditioned medium (5% and 25%) and exudate (3%). Chondrogenic differentiation was determined via (immuno)histology and transmission electron microscopy (TEM). Differentiated BMMSCs form more densely organized 3D cell spheres compared to differentiated DPSCs. Preliminary data suggest that L-PRF does not increase the expression of chondrogenic markers in differentiated BMMSCs and DPSCs. Differentiated BMMSCs express aggrecan and collagen II, while differentiated DPSCs only express collagen II. Cartilage-like fragments could be detected in both cell types by TEM. Despite the preliminary and inconclusive nature of these data, our ultimate goal is to improve and investigate the mechanisms of DPSC-mediated cartilage regeneration. Future studies will determine the optimal concentration of L-PRF components on chondrocyte differentiation of DPSCs. Moreover, the effect of the secreted factors of DPSCs and L-PRF on primary chondrocyte matrix production and de- and redifferentiation will be evaluated.

[27] The role of DNA hydroxylases Tet1 in cell fate decision

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TET (ten-eleven-translocation) genes are DNA hydroxylases that convert 5-methylcytosine to 5-hydroxymethyl-cytosine, an intermediate form involved in DNA demethylation. Previous studies have been showed that Tet1 and Tet2 can regulate 5hmC production and cell lineage specification in mouse embryonic stem cells (ESCs). Here, we showed that Tet1 null ESCs displayed increased upregulation of mesendoderm marker genes during embryoid body (EB) differentiation, accompanied by activation of Smad2 and impaired downregulation of Oct4. Moreover, Tet1–deficient ESCs showed abrogated expression of neuroectoderm related markers and defective formation of neurons during in vitro differentiation; these defects can be partially rescued by restoring the expression of Tet1 or its catalytic domain mutant, suggesting that TET1 uses both catalytic and non-catalytic mechanisms to regulate lineage genes. We further defined cell-state specific genomic regions bound by Tet1 in both primed epiblast-like cells (EpiLCs) and naive ESCs. Gene ontology (GO) analysis indicated that the primed state specific regions, but not the naive state specific regions are enriched in terms associated with lineage determination. In addition, further ChIP-seq analysis showed that Tet1 and Oct4 co-bind at inter-genetic regions in EpiLCs. In contrast, they strongly co-bind at promoter regions in naive ESCs. Collectively, these results suggest the importance of Tet1 in the regulation of pluripotency state transition and germ-layer specification. Further studies will provide insights into the physiological functions of Tet1 in cell fate choice and development.

[28] Gain of 20q11.21 negatively affects ectoderm differentiation potential in human embryonic stem cells

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Human pluripotent stem cells frequently acquire chromosomal abnormalities during in vitro culture, one of the most common being the amplification of 20q11.21, present in more than 20% of stem cell lines worldwide. Up to now, there is an important gap in our knowledge, as there are no studies describing in detail the effects of the same aberration in multiple carrier lines. Therefore, we aimed at establishing how the gain of 20q11.21 affects the differentiation process. For this study, we used 10 hESC lines: 4 wild type, 5 with a gain of 20q11.21 and 1 transgenic line overexpressing Bcl-xL, the driver gene of the 20q11.21 cell culture take-over. Gene-expression microarray analysis showed that the mutant lines and the Bcl-xL-overexpressing line had a significantly lower gene expression of CHCHD2. CHCHD2 up-regulation has been associated to enhanced ectodermal differentiation by modulation of the TGFB1 signaling (Zhu et al., 2016). Therefore, we hypothesized that, if the opposite effect was also true, the hESC with a gain of 20q11.21 should present with decreased ectodermal differentiation. Experiments on all 10 lines (3-6 biological replicates) confirmed the above hypothesis, as lines with a 20q gain as well as the transgenic line consistently express lower levels of ectodermal markers after a 4-day ectoderm differentiation. Overall, the mesendoderm differentiation did not appear to be affected, but we identified one carrier subline of VUB03 that is endoderm differentiation deficient. To address this phenomenon in more detail we are currently performing DNA massive parallel sequencing on the line. Moreover, two of the carrier lines tested were sublines of VUB02 with two different variants, one of which carried an isochromosome 20. This line, next to the decreased ectoderm differentiation, showed high levels of residual undifferentiated cells.

[29] Integrative proteomics identifies H3K27me3 as an epigenetic gatekeeper of ground state pluripotency

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The ground state of pluripotency is postulated to be a basal proliferative state that is free of epigenetic restriction, as present in early blastocyst cells or captured in vitro by mouse embryonic stem cells (ESCs) cultured in minimal media supplemented with two kinase inhibitors (so-called "2i"). Thus far, it has remained enigmatic what defines this unique naive epigenome. In this study, we identify epigenomic features characteristic for ground state pluripotency by global quantitative profiling of post-translational histone modifications (hPTMs) and chromatin-associated proteins in 2i ESCs. As compared to conventional serum-grown ESCs ("serum"), which represent a more primed developmental state, unique features of 2i ESCs include largely facultative heterochromatin, histone clipping, H4 hypoacetylation and, surprisingly, high levels of H3K27me3 and its epigenetic writer PRC2, associated with gene silencing. By selective chromatin purification using MNase and PICh, as well as ChIP-seq, we show that H3K27me3 and PRC2 are significantly enriched on both euchromatin and heterochromatin. Interestingly, the reorganization of H3K27me3 and PRC2 between 2i and serum ESCs does not necessarily affect transcription. hPTM and chromatin-associated protein profiling of PRC2-deficient ESCs, in which H3K27me3 is ablated, identifies extensive crosstalk between H3K27me3 and other epigenetic marks. Most notably, removal of H3K27me3 in 2i ESCs leads to augmented levels of marks associated with early lineage specification, including H4 acetylation and DNA hypermethylation. Together, these results indicate that H3K27me3 protects the epigenome from acquisition of primed-like features such as DNA methylation and hence we propose that H3K27me3 acts as an epigenetic gatekeeper of ground state pluripotency.

[30] NR2F2 is an essential regulator of skin 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 has revealed that NR2F2, a transcription factor of the family of the orphan nuclear receptors, is among the rare genes upregulated specifically in cancer cells from invasive SCC, implicating a role in malignant progression. We have shown that NR2F2 is absent in normal epidermis and benign tumors and begins to be expressed in a heterogeneous manner in SCC. To determine whether NR2F2 is playing a role in the malignant transition of SCC we used state-of-the-art genetic approaches to modulate its expression in chemically or genetically induced skin tumors. The deletion of NR2F2 in the mouse epidermis inhibits the progression of tumors induced by chemical carcinogenesis. In addition, NR2F2 deletion is able as well to arrest tumor progression in a genetic model that normally leads to the formation of SCC exclusively. Moreover, the conditional ablation of NR2F2 is able to induce the regression of existing SCC or prevent the formation of secondary tumors upon grafting, indicating an essential role in tumor maintenance and propagating capacity. In particular, histological analysis and transcriptional profiling after NR2F2 ablation suggests that the tumors are driven towards differentiation, and that this process is accompanied by modifications of the tumor microenvironment, including the recruitment of immune cells. We are currently defining the downstream targets mediating NR2F2 effect on tumorigenesis. Altogether, our results demonstrate the critical role of NR2F2 during tumor progression. Since NR2F2 belongs to the family of nuclear receptor transcription factors, it could represent the ideal target for the development of novel targeted therapies in SCC treatment.

[31] The angiogenic potential of extracellular vesicles originating from human dental pulp stem cells

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Tooth loss remains a major health problem since current therapies cannot regenerate damaged dental tissues including pulp and enamel. To induce successful pulp regeneration, angiogenesis is required for the supply of nutrients and oxygen. Proangiogenic properties have already been assigned to mesenchymal stem cells (MSCs) present in the dental pulp. Paracrine factors, including VEGF, have been so far identified as responsible mediators for the angiogenic effect. However, more recent studies indicate that extracellular vesicles (EVs) produced by bone marrow-derived MSCs (BM-MSCs) also have the potential to induce neovascularisation. Therefore, we investigated the angiogenic properties of EVs derived from dental pulp stem cells (DPSCs) in comparison with those of BM-MSCs. EVs were isolated from serum-free conditioned medium of DPSCs and BM-MSCs 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 TSG101 and absence of non-exosomal marker mitochondrial complex V. The functional effect of EVs on the migration of human umbilical vein endothelial cells (HUVEC), as a key step in angiogenesis, was studied in a transwell system. Preliminary data suggest that EVs from DPSCs induce HUVEC migration (n=3). However, this effect was less in comparison with BM-MSC EVs (n=2), which is possibly caused by the lower EV yield from DPSCs as measured by NTA. In addition, the uptake of DPSC EVs by HUVEC was confirmed with confocal microscopy. To conclude, our preliminary data show promising in vitro proangiogenic effects of DPSC EVs. In the future, we will compare the angiogenic factors present in DPSC and BM-MSC EVs and analyse their potential to induce blood vessel formation in ovo. Acquired insights have positive implications for pulp tissue regeneration and diseases associated with insufficient angiogenesis, including stroke and myocardial infarction.

[32] Derivation of otic progenitors from human iPSCs with a mutation on CDK5RAP2 gene for modeling hereditary hearing loss

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Hearing impairment (HI) affects almost 250 million people worldwide and is mainly caused by damage to cochlear hair cells (HCs) that convert mechanical stimuli of sound into electrical signals. At least 60% of the people with early-onset HI have hereditary hearing loss (HHL) due to genetic mutations. In this context, we are studying the Cyclin-dependent kinase 5 regulatory subunit-associated protein 2 (CDK5RAP2). Mutations in CDK5RAP2 gene are associated to autosomal recessive primary microcephaly 3 (MCPH3) and recent observations showed that they can also be associated to congenital hearing loss. In order to study the mechanisms leading to HHL in MCPH3 patients, we are using the technology of induced pluripotent stem cells (iPSCs). Patients with mutations in CDK5RAP2 gene and showing a neuro-sensory deafness are recruited at the Neuropaediatry Service of the Robert Debré Hospital. Fibroblasts from these patients and healthy individuals are then reprogrammed into iPSCs. These iPSCs are differentiated into otic progenitors (OSCs) which can be differentiated into HCs. These progenitors can be maintained for several passages, allowing us to study the expression and location of CDK5RAP2 in the wild type (WT) and mutated progenitors, as well as their proliferation, survival and the expression of otic markers. In WT OSCs, we observed that CDK5RAP2 is located at the centrosome, an organelle involved in cell division. However, CDK5RAP2 is not detected at the centrosome in the mutated OSCs, suggesting that the mutated protein is degraded and that the mutation could affect the cell division in the OSCs. Indeed, OSCs from mutated iPSCs show a lower proliferation rate and a lower expression of OSC markers when compared with WT OSCs. In parallel, isogenic iPSCs cell lines are generated by correcting the mutations with the CRISPR-Cas9 technology.

[33] Hepatic cells derived from human skin progenitors show a typical phospholipidotic response upon exposure to amiodarone

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Phospholipidosis is a metabolic disorder characterized by an excessive intracellular accumulation of phospholipids (PL). It can be caused by short-term or chronic exposure to cationic amphiphilic drugs (CAD). These compounds share a high affinity to bind to PL, leading to inhibition of their degradation and consequently to their accumulation in lysosomes. Hereby, characteristic intracellular structures, described as lamellar inclusions or whorls, are formed. Drug-induced phospholipidosis (DIPL) is frequently at the basis of discontinuation of drug development and post-market drug withdrawal. Therefore, reliable human-relevant in vitro models must be developed to speed up the identification of compounds that are potential inducers of phospholipidosis. In this study, hepatic cells derived from human skin (hSKP-HPC) were evaluated as an in vitro model for drug-induced phospholipidosis. These cells were exposed for 24, 48 and 72 hours to amiodarone, a CAD known to induce phospholipidosis in humans. Transmission electron microscopy revealed the formation of the typical lamellar inclusions in the cytoplasm of the cells. In addition, increased accumulation of PL and neutral lipids was also observed in amiodarone-exposed cells. An increase of phospholipids was already detected after 24 hours exposure to amiodarone, whereas a significant increase of neutral lipid vesicles could be observed after 72 hours. At the transcriptional level, the modulation of genes involved in DIPL was detected. These results provide a valuable indication of the applicability of hSKP-HPC for the quick assessment of drug-induced phospholipidosis in vitro. Consequently, these cells represent a useful tool to screen phospholipidogenic compounds, early in the drug development process.

[34] RMCE applications in mESCs: from structure-function studies to mouse models for leukemia and lymphoma

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Capturing pluripotency in vitro paved the way for regenerative medicine and gene targeting in mice. Gene targeting traditionally relied on homologous recombination, which is inefficient. A more reliable technique to enable gene targeting is recombination mediated cassette exchange (RMCE), which is based on site-specific recombination systems, such as Cre/loxP. RMCE takes advantage of heterospecific recombination sites that are embedded in a genomic locus and in the incoming targeting vector. The recombinase will insert the incoming DNA fragment into the RMCE-compatible genomic locus and only correctly RMCE-targeted clones can render drug resistance. This results in a very high targeting efficiency, often close to 100%. We have used RMCE for both basic and applied research. First, we used RMCE to perform structure-function studies in KO mESCs. We investigated the role of mouse p120ctn in early embryogenesis, mESC pluripotency and early fate determination. To pinpoint the underlying mechanism, we introduced various rescue constructs into the R26 locus of p120ctn KO mESCs via RMCE. Rescue of p120ctn-null mESCs with different p120ctn mutant expression constructs revealed that the interaction between p120ctn and E-cadherin is crucial for primitive endoderm formation. Secondly, we introduced conditional overexpression (OE) cassettes into the R26 of mESCs via RMCE. Targeted mESCs were used to generated R26-based conditional OE mice, such as R26-Ccdn2 and R26-Myb. Overexpression of cell cycle regulator cyclin D2 (encoded by Ccnd2) has been observed in mantle cell lymphoma (MCL), an aggressive and incurable B-cell malignancy. We found that hematopoietic expression (using VaviCre) of cyclin D2 recapitulates MCL in mice. Furthermore, we found that R26-driven Myb expression can significantly accelerate the formation of T-cell acute lymphoblastic leukemia in mice. Tumor cells from both the leukemia and lymphoma model contain a luciferase reporter and were used for preclinical drug testing.

[35] Roles of Nedd4.1/2 in the auditory portion of inner ear

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The development of the inner ear requires close coordination between numerous cellular processes such as proliferation, migration and differentiation. These processes depend on transcriptional programs and post-translational modifications that must be coordinated with precision in time and space. Ubiquitination is a reversible modification involved in the degradation, localization, and activity of proteins, which plays a crucial role during development. The enzymes at the center of the ubiquitination mechanism are the E3 ligases that control substrate specificity. Among the more than 700 E3 ligases identified, we have focused our research on Nedd4.1 and Nedd4.2 since mutations of the gene coding for NEDD4.2 are responsible for deafness in humans. In order to understand the functions of Nedd4.1/2 during the development of the inner ear, we first determined their expression profile. Our in situ hybridization results indicate that these ligases are expressed throughout the cochlear epithelium as well as in the spiral ganglion as early as the 14th embryonic day. After birth, the expression of Nedd4.1 persists but that of Nedd4.2 decreases sharply. Our immunohistochemistry analyses suggest that Nedd4.1/2 are localized at the intercellular junctions of the otic epithelium and that they are enriched at the tricellular junctions. We analyzed the integrity of the cochlear epithelium in mice invalidated for the Nedd4.2 gene within the inner ear. Our data suggests that Nedd4.2 contributes to the establishment of planar cell polarity since the orientation of the hair cells and their hair bundle is disrupted in conditional mutants. Our research emphasizes the importance of Nedd4.1/2 ligases during the development of the auditory portion of the inner ear.

[36] Embryonic DNA damage as a potential inducer of premature differentiation during corticogenesis

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The developing brain is particularly sensitive to DNA damage. For example, microcephaly is often seen in DNA repair deficiency syndromes as well as after in utero exposure to ionizing radiation. A previous study suggested that radiation-induced DNA damage in the embryonic mouse brain results in the activation of a p53-dependent neuron differentiation program. Since premature neuronal differentiation can result in microcephaly, we aim to investigate whether this process also occurs in prenatally irradiated brains. In this study, we investigated brain development in mice irradiated at embryonic day 11, which display a significant reduction in brain size. A transient, induction of DNA damage was accompanied by a G2/M cell cycle block followed by massive apoptosis. PAX6 and DCX immunostainings unveiled a decrease of radial glia cells, while ectopic neurons emerged in the subventricular zone, indicative for premature neuronal differentiation. To study the underlying cause in more detail, we performed stainings for ZO-1, which is essential for attachment of glial stem cells to the apical membrane and for modification of their cleavage plane orientation, to initiate neuronal differentiation. We found a reduction of overall ZO-1 expression and a clear disruption of ZO-1 expression at the apical surface in irradiated brains as compared to sham-irradiated controls, indicating that radiation affects glial-to-neuronal transition. A possible involvement of p53 in this process is exemplified by a partial rescue of the brain size reduction in Emx1-cre+/-;Trp53fl/fl mice. Finally, irradiation of Neuro2a neuroblastoma cells reduced their proliferative capacity and induced neurite outgrowth, which could be prevented by prior treatment with a p53 inhibitor. Our results suggest that prenatal radiation-induced DNA damage indeed leads to premature differentiation of cortical neuron progenitors, which may contribute to the observed microcephalic phenotype. Whether this mechanism is mediated by p53, is currently being investigated.

[37] Major depletion of SOX2+ stem cells in the adult pituitary is not restored which does not affect hormonal cell homeostasis and remodelling

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The pituitary gland contains SOX2-expressing stem cells. However, their functional significance remains largely unmapped. We investigated their importance by depleting SOX2+ cells through diphtheria toxin (DT)-mediated ablation. DT treatment of adult Sox2CreERT2/+;R26iDTR/+ mice (after tamoxifen-induced expression of DT receptor in SOX2+ cells) resulted in 80% obliteration of SOX2+ cells in the endocrine pituitary, coinciding with reduced pituisphere-forming activity. Counterintuitively for a stem cell population, the SOX2+ cell compartment did not repopulate. Considering the more active phenotype of the stem cells during early-postnatal pituitary maturation, SOX2+ cell ablation was also performed in 4- and 1-week-old animals. Ablation grade diminished with decreasing age and was accompanied by a proliferative reaction of the SOX2+ cells, suggesting a rescue attempt. Despite this activation, SOX2+ cells did also not recover. Finally, the major SOX2+ cell depletion in adult mice did not affect the homeostatic maintenance of pituitary hormonal cell populations, neither the corticotrope remodelling response to adrenalectomy challenge. Taken together, our study shows that pituitary SOX2+ fail to regenerate after major depletion which does not affect adult endocrine cell homeostasis and remodelling. Thus, pituitary SOX2+ cells may constitute a copious stem cell reserve or may have other critical role(s) still to be clearly defined.

[38] Loss of transfer RNA U34 modifying enzymes impairs hematopoietic stem and progenitor cell differentiation and function

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Hematopoietic stem and progenitor cells (HSPCs) require fine-tuned protein translation for their normal maintenance and function. Conserved modifications of the wobble uridine base (U34) in transfer RNAs catalyzed by the Elongator complex are required for optimal protein translation efficacy and fidelity, but their biological importance in mammalian stem and progenitor cells remains largely unexplored. Here, we studied the impact of loss of activity of the catalytic subunit Elp3 of Elongator on HSPC differentiation and function. Hematopoietic-cell-specific depletion of Elp3 in conditional knockout mice resulted in shortened lifespan associated with hematopoietic failure and lymphoma development. Elp3 deletion caused apoptosis of specific bone marrow multipotent progenitors and blocked differentiation of committed progenitors, resulting in blood and bone marrow pancytopenia. In contrast, Elp3-deficient hematopoietic stem cells (HSCs) expanded with age and did not exhaust throughout life, although they were defective in reconstituting hematopoiesis in competitive transplantation assays. Mechanistically, loss of Elp3 did not result in detectable alterations in global protein synthesis rates in any HSPC subset. Rather, Elp3-deficient HSPCs displayed enhanced activity of the stress integrator and apoptosis and cell cycle regulator p53. Thus, this study supports the notion that Elongator activity is required in distinct HSPC subsets to avoid aberrant p53 activation, which otherwise results in discrete loss of function phenotypes in HSCs and downstream progenitors.

[39] Automated stem cell culture platform in KU Leuven Bio-Incubator

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As part of the NextGenQBio (Next Generation Quantitative Biology) high-throughput functional genomic and drug discovery screening platform funded by the Hercules Foundation and EWI (Departement Economie, Wetenschap & Innovatie), an automated stem cell culture platform is under construction in the KU Leuven Bio-Incubator in Leuven. The building of the NextGenQBio platform is a joint project of KU Leuven (prof. Catherine Verfaillie), and VIB (profs. Frederic Rousseau and Joost Schymkowitz from the Switch laboratory), and the platform will be a combination of an automated stem cell culture unit (KUL) and an automated high throughput / high content imaging unit (VIB). The automated stem cell culture unit will contain a robotic arm, two liquid handlers, automated incubator, centrifuge, microscope, cell counter, and freezing unit within an enclosure providing sterile environment. The high content imaging unit will contain a Opera Phoenix HT automated microscope with plate handler and liquid handler robot. This rare combination will provide the opportunity to perform phenotypic compound screens, target identification/validation, secondary screens, lead discovery, and toxicology application using patient-derived induced pluripotent stem cell disease-in-a-dish models or normal pluripotent stem cells in 384-well plate format.

[40] A doycycline-inducible system for differentiation of human pluripotent stem cells to sensory neurons

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Three types of sensory neurons are distinguished based on the based on their perceptual modalities: proprioceptors, mechanoceptors, and nociceptors. Easy access to the latter type could be a great advantage for understanding the physiology of pain in humans, since traditional methods for studying sensory neurons have largely relied on animal models. The difference in the mechanisms of pain and itch perception between humans and rodents propelled the effort to develop protocols for growth factor-directed differentiation of nociceptors from human pluripotent stem cells in several laboratories. Our laboratory has taken a different approach. We have generated gene-engineered human pluripotent stem cell lines with doxycycline-inducible over-expression of one or three transcription factors important in the development of nociceptors. We compared the response to various noxious agents of the cell population obtained with a very simple and inexpensive differentiation protocol (neural crest induction by dual-SMAD inhibition using two small molecules, followed by doxycycline treatment) with the response to the same agents of the cell population obtained using a published growth factor-directed differentiation protocol optimized in our laboratory. Since the ion channels responsible for nociception are good molecular targets for developing antagonists as analgesics, our model may be useful for drug discovery.

[41] Wnt inhibition confers a unique transcriptional state of pluripotency to hESCs, but impedes their derivation

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Human embryonic stem cells (hESCs) and their ability to differentiate to specific cell types hold great value for future clinical applications. However, standard culture conditions maintain hESCs in a primed state, which bears heterogeneity and a tendency for spontaneous differentiation. To counter these drawbacks, hESCs have been converted to a naive state, but this has in turn restricted the efficiency of existing directed differentiation protocols. In mouse, Wnt inhibition has been found to markedly improve derivation and maintenance of primed epiblast stem cells (EpiSCs). We verified whether inhibition of Wnt signaling (Wnt-i) can similarly improve primed hESC traits. We showed that Wnt-i during maintenance of established hESCs resulted in a unique transcriptional profile with high levels of pluripotency markers, and reduced expression of lineage-specification markers, compared to primed hESCs. Moreover, unlike naive hESCs, neuronal differentiation of Wnt-i hESCs progressed in a similar manner to primed hESCs. Cardiac differentiation of Wnt-i hESCs yielded more beating cardiomyocyte clusters than primed or naive hESCs overall. Surprisingly, derivation of hESCs from the blastocyst stage under Wnt-i conditions failed, suggesting that active Wnt signaling is necessary for the transition from inner cell mass to hESCs, in contrast to mouse. Altogether, we demonstrated that Wnt signaling is critical during establishment of hESCs, but Wnt inhibition on existing hESCs results in a unique pluripotency state, which improves on the drawbacks of both primed and naive states and potentially serves as a more suitable starting point for directed differentiation.

[42] Disease model for GRN-linked FTLD using patient and engineered stem cells

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The strong genetic link in Frontotemporal lobar degeneration (FTLD) makes it an ideal starting point to unravel the mechanisms underlying neurodegeneration. We focus on FTLD linked to null mutations in the progranulin gene (GRN) and use patient derived iPSC to model the disease. The pathological events leading from PGRN haploinsufficiency to the development of the neurodegenerative disorder FTLD are currently poorly understood. To study the molecular mechanisms whereby heterozygosity of GRN affects neural cells, we created GRN IVS1+5G>C mutated and isogenic control hPSC lines. We have seamlessly inserted the GRNIVS1 +5 G>C gene mutation into a control iPSC line and embryonic stem cell line. Genome engineering was performed using TALE nucleases and the Piggybac transposon system. In addition, we have inserted a cassette suitable for FLPe recombinase-mediated cassette exchange (RMCE) in the AAVS1 locus of patient iPSC lines, using Zinc-finger nucleases. The cassette in this system is exchangeable and allows for the highly efficient generation of stable reporter and overexpression cell lines. Using RMCE, we created patient iPSC lines that conditionally overexpress a copy of the GRN gene, permitting us to correct the progranulin haploinsufficiency at any given time point. Because progranulin haploinsufficiency leads to a selective loss of cortical neurons, we differentiated control and GRN mutant lines to cortical neurons and adapted the neural differentiation protocol to High Content Imaging. Functional characterization is ongoing with the aim to identify robust disease phenotypes, prove the causal relationship with progranulin haploinsufficiency and further develop possible therapeutic approaches.

[43] Defining the cellular hierarchy that governs 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. Interestingly, the average clone expansion agreed with the average cellular expansion of the tissue. 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 of the elongating ducts. Taken together, these preliminary observations 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. Further experiments will allow us to better understand the spatiotemporal and molecular regulation of basal multipotency in the developing prostate.

[44] Regulation of the LIN28B/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. I demonstrate that the LIN28B/Let-7b axis, which is important during liver development, is aberrantly expressed in hPSC-HLCs. LIN28B, a RNA-binding protein, is highly expressed in hPSCs, fetal liver and cancer, which results in low expression levels of microRNA Let-7b. In contrast, PHHs do not express LIN28B, leading to high levels of Let-7b in PHHs. I hypothesize that correct modulation of this axis via the knockout of LIN28B or via overexpression of microRNAs that are known to target LIN28B, will induce hPSC-HLC maturation.

[45] Comparison of autologous versus allogeneic epithelial-like stem cell treatment in an in vivo equine skin wound model

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Introduction: Several studies report beneficial effects of autologous and allogeneic stem cells on wound healing. However, no comparison between autologous versus allogeneic epithelial-like stem cells (EpSCs) has been made so far. For this reason, we compared both EpSC types to vehicle treatment and untreated controls in an equine in vivo skin wound model. Based on other studies, we hypothesized that there would be no difference in wound healing response after autologous and allogeneic EpSC treatment. Material and methods: Twelve full-thickness skin wounds were created in six horses. Each horse was subjected to (i) autologous EpSCs, (ii) allogeneic EpSCs, (iii) vehicle treatment or (iv) untreated control. Wound evaluation was performed at day 3, 7 and 14 through wound exudates and at week 1, 2 and 5 through biopsies. Wound dimensions were measured at days 0, 1, 3, 7, 14, 21, 28 and 35. Results: Wound circumference and surface were significantly smaller in autologous EpSC-treated wounds. A significantly lower amount of total granulation tissue (overall) and higher vascularization (week 1) was observed after both EpSC treatments. Significantly more major histocompatibility complex II-positive and CD20-positive cells were noticed in EpSC-treated wounds at week 2. In autologous and allogeneic groups, the number of EpSCs in center biopsies was low after 1 week (11.7% and 6.1%), decreased to 7.6% and 1.7%, respectively (week 2), and became undetectable at week 5. Discussion: These results confirm the first hypothesis and partially support the second hypothesis. Besides macroscopic improvements, both autologous and allogeneic EpSCs had similar effects on granulation tissue formation, vascularization and early cellular immune response.

[46] Cdk1 is essential for long-term maintenance of neural stem cells in the postnatal hippocampus

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In contrast to the majority of neurons of the mammalian CNS, which are born during embryogenesis, hippocampal dentate granule neurons are continuously produced from mid-gestation to old age, including in humans. Postnatally born hippocampal neurons develop locally from neural stem cells (NSCs) anchored in a specialized niche of the dentate gyrus (DG), the subgranular zone (SGZ). We have previously shown that cyclin-dependent kinase (Cdk) 6, an important regulator of G1 progression, specifically drives the expansion of transit-amplifying progenitors in the postnatal hippocampus. However, it is not clear yet which Cdk is involved in the proliferation and long-term maintenance of hippocampal NSCs. Here, we investigated the role of Cdk1, an essential Cdk for M-phase, in postnatal hippocampal neurogenesis. Consistently with the crucial role of Cdk1 in the cell cycle, our results demonstrate that conditional loss of Cdk1 in hippocampal NSCs dramatically reduce their proliferation rate. Moreover, the total number of Sox2+ NSCs rapidly declines upon Cdk1 deficiency, and this is accompanied by a concomitant increase in the number of newborn neurons produced. Surprisingly, we also observed that the loss of one allele of Cdk1 was sufficient to phenocopy most of the phenotypes encountered in the complete absence of Cdk1. These results suggest a role for Cdk1 beyond cell cycle regulation, where Cdk1 dosage regulates hippocampal NSC fate choice between neuronal differentiation and stem cell maintenance. We are actually investigating the mechanisms by which Cdk1 control cell fate in hippocampal NSCs.

[47] Understanding the regulation of Ror2-dependent pathway in hair follicle stem cells

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Multipotent adult stem cells (SCs) can self-renew and generate tissue-specific progenies during tissue homeostasis and upon injury. To sustain these functions, a regulation between microenvironmental cues and intracellular signaling is required to balance SC quiescence and activation. Investigating the underlying signaling pathways is fundamental to understand SC regulation and tissue regeneration. While canonical Wnt/β-catenin pathway is known as a major regulator of SCs, the function of non-canonical Wnt pathways remains unclear. To unravel the role of a non-canonical Wnt signaling, we focus on tyrosine kinase-like orphan receptor 2 (Ror2)-mediated signaling in regulation of hair follicle stem cells (HFSCs). We first examined Ror2 signaling activities, and found elevation of Ror2 expression and downstream JNK activity in activated HFSCs in comparison to quiescent HFSCs. By generating a HFSC-specific Ror2 conditional knockout (cKO) mouse line, we found deletion of Ror2 at onset of growth phase caused a delay of HFSC activation and hair cycle entry. Cultured Ror2 cKO HFSCs displayed reduced self-renewal capacity and eventually underwent differentiation. Interestingly, using a double mutant strategy, we identified the collaborative effect of Ror2 and β-catenin in hair follicle fate determination. Together, our results unravel previously unidentified function of Ror2 in regulation of HFSCs.

[48] The identification and in vivo characterization of embryonic cartilage cells

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The healing of large bone defects are 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. A major challenge is the identification of the cells and cell sources to produce such constructs efficiently. The aim of our work is to identify these cells by studying growing embryonic cartilage and establish selection and culture conditions of cells leading to successful endochondral bone formation in ectopic and orthotopic assays. We focused on 14.5dpc mouse femurs as the cell source. Femurs were isolated, dispersed into single cell suspensions and live sorted using a panel of previously published surface markers for skeletal stem cell and skeletal progenitors. Sorted populations were encapsulated in either collagen or alginate gels 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 derived from the femurs: CD51+CD105-CD90-CD200- progenitors and CD51+CD105-CD90-CD200+ skeletal stem cells. Surprisingly, both populations have the ability to form bone in both gels. Collagen implanted cells formed a complete ossicle with bone marrow, while the alginate implanted cells created small bone organs scattered across the unabsorbed alginate hydrogel. Our data indicates the ability of these cells to continue their developmental path and form bone in an ectopic environment. The findings of this study will be used as a tool to purify and select for adult stem cells from different sources with a high biological potency for bone formation.

[49] BMP signaling silences the postnatal stem cell capacities of the pulmonary neuroepithelial body microenvironment

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Epithelial cells of intrapulmonary conductive airways show a very low turn-over rate in healthy postnatal lungs. Own and literature data strongly suggest that the neuroepithelial body (NEB) microenvironment (ME), consisting of innervated groups of neuroendocrine cells covered by Clara-like cells (CLCs), may be regarded as a unique airway stem cell niche. Inhibition of the bone morphogenetic protein (BMP) pathway has been reported to induce proliferation of basal progenitor cells in the mouse trachea [1]. Transient mild acute lung injury (ALI) was induced in mice via a single intratracheal instillation of lipopolysaccharide (LPS). Life cell imaging showed that broncho-alveolar lavage fluid (BALF) of LPS-challenged mice induces a selective reversible Ca2+-mediated activation of CLCs in the NEB-ME in lung slices of control mice. Combined LPS-instillation and BrdU-injection revealed a remarkable increase in divided (BrdU-labeled) cells that specifically surround NEBs, as compared to control lungs after 48h. qPCR [2] showed a higher gene expression of BMP2, BMP7 and BMP receptor IA in the laser microdissected NEB-ME than in control airway epithelium, and immunostaining for BMP2 protein localized to vagal afferent nerve terminals in NEBs. BMP2 and BMP7 gene expression were downregulated in the NEB-ME of LPS-treated mice. Injection of mice with BrdU and LDN-193189, a small molecule inhibitor of BMP type I receptors, also revealed a potent effect on the induction of cell proliferation in the NEB-ME. In conclusion, BMP appears to play an intriguing role in the NEB-ME. Both the specific location of BMP2-expressing nerve terminals in NEBs of healthy mouse lungs and the proliferative effect of inhibiting BMP receptors, suggest the involvement of BMP signaling in silencing cell proliferation in the postnatal stem cell niche of the NEB-ME. Support:UA-grant GOA-BOF 2015 (30729 to DA). [1] Tadokoro et al. (2016) Development 143: 764-773 [2] Verckist et al. (2017) Respir Res 18: 87

[50] Unravelling the role of the CAK complex in the morphological development of cortical neurons

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Many brain diseases, such as epilepsy, autism or schizophrenia, may arise from abnormalities in neuronal morphology/connectivity occurring during brain development. However, the mechanisms responsible for these alterations are not yet well characterized. A better understanding of the molecular regulation of neuronal morphology development is therefore necessary to develop new therapeutic approaches to correct/compensate for neuronal deficits in these diseases. In this context, we focus on the role of the CDK-activating kinase (CAK) complex, an important regulator of transcription, in the morphological development of neurons. We first clarified the expression profile of Cdk7, the main CAK complex member, in the brain and observed that it is extensively expressed in postmitotic cortical neurons from brain development to adulthood. To decipher Cdk7 functions, we use a conditional knock-out mice (NexCre) in which Cdk7 is deleted from postmitotic cortical and hippocampal neurons. Our preliminary results indicate that Cdk7 promotes the dendritic development of postmitotic cortical neurons in vivo and in vitro, suggesting a role for Cdk7 in neuronal connectivity. We plan to confirm these results and to further investigate the precise requirement for Cdk7 and the CAK complex in this process.

[51] Mitochondrial DNA variants in pluripotent stem cells: origin and effect of long-term culture

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Introduction Mitochondrial DNA integrity in human pluripotent stem cells has been subject to little research so far. Our aim was to investigate the incidence and origin or mtDNA variants in human pluripotent stem cells, and to evaluate the effect of long term culture. Materials and methods Massive parallel sequencing of the mtDNA of eleven oocytes, eight inner cell masses of blastocysts, eight embryonic stem cell (hESC) lines at early and late passages, including single cells, 20 clonal induced pluripotent stem cell lines (iPSC) from two different donors, and ten cells of the source fibroblast culture. Results Early passage hESC carry few variants, with low load. They are probably inherited, since oocytes and embryos carry similar variants. Prolonged culture increases the number and load of the variants. Single cell analysis showed significant mosaicism in the cultures, and proves that the increase in variant load in late passages is caused by clonal growth of a subpopulation of cells. All iPSCs lines showed more variants and at much higher loads, which single cell analysis proved pre-existed in the source cells. Conclusions hESC and iPSC significantly differ in the type and load of variants in their mtDNA.