CRISPR/Cas9 Gene Editing in Hematopoietic Stem Cells to Model Clonal Competition in vivo and in vitro for GATA2 Deficiency

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GATA2 deficiency has been identified as a common hereditary cause of myelodysplastic syndromes (MDS) and acute myeloid leukemia in children. Penetrance and expressivity within affected families is often variable, suggesting that cooperating factors are required to trigger the disease. Somatic mutations in MDS driver genes (SETBP1, ASXL1) have been identified GATA2-MDS. The molecular mechanism that triggers the leukemic progression in GATA2 carriers remains unknown. Particularly, these questions remain unanswered: 1) whether GATA2 germline mutation itself is sufficient to trigger MDS/AML 2) whether SETBP1 and ASXL1 mutations induce the malignant transformation. Addressing these questions has been difficult due to the lack of faithful human disease models. Here I studied in vitro/in vivo engineered cord blood CD34+ cells carrying GATA2 mutation alone or in combination with SETBP1/ASXL1 mutations in NSG mice to evaluate the engraftment capacity and the clonal evolution. Specifically, CRISPR/Cas9/rAAV6 was used to introduce the R398W mutation in CD34+ cells alone (Single) or in combination with SETBP1 and ASXL1 mutations (Multiplex). Primary and secondary transplantation showed similar multilineage constitution in all the conditions. Interestingly, genetic studies indicated that the predominant expanded clones in the multiplex condition carried SETBP1+ASXL1 mutations, while clones with only GATA2 mutation were lost. To study the impact of the mutations at transcriptomic level, scRNAseq is underway. In vitro data confirmed that cells carrying the GATA2 R398W mutation have an impaired clonogenic capacity and proliferation, recapitulating MDS patient phenotype. In summary, we developed a human model of clonal competition by CRISPR/Cas9 targeting CD34+ cells. Our findings strongly suggest that GATA2 R398W mutation is not sufficient to increase cell fitness, suggesting that co-operating of genetic, epigenetic, niche and stressor factors are necessary to trigger the disease.

Heritable states influence stem cell fates upon acquisition of cancer mutations

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Tumors with the same driver mutations can display a striking variation in their progression and treatment response, but the origins of this variation are still unclear. In this study, we unveil that heritable stem cell states can have a profound influence on how individual cells respond upon acquisition of the exact same cancer mutation. Here we develop a new method, scTRAACK, for single-cell Tracking of Recombinase Activation And Clonal Kinetics and apply it to hematopoietic stem cells carrying Cre-conditional leukemia alleles. Tracking the expansion kinetics of a common set of stem clones, with and without the same leukemia mutations, we unveil a striking heterogeneity in the malignant fates of diverse stem cell clones. First, we define that heritable clonal states persist in expansion cultures and affect their clonal composition. Then, using mouse models of the most frequent initiating AML mutation in Dnmt3a, we define that these pre-existent heritable stem cell states influence clonal expansion, differentiation properties, and unique malignant gene expression programs that arise from each individual cell upon acquisition of mutation. Whereas high-fitness slow-cycling clones expanded regardless of mutational state, cancer mutations significantly increased the survival probability in stem cell clones with higher cycling and lower fitness, which are the most abundant clones within the stem cell compartment. Moreover, mutations reprogrammed the stem cell states of low-fitness clones to maintain a pool of stem cell-like cells. These findings suggest that whereas both high and low-fitness stem cells can be at the origin of AML, the more abundant low-fitness pool likely represents the cell of origin for the majority of AML.

Generation of a bank of clinical-grade, HLA homozygous iPSC lines with high coverage of the Spanish population

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Stem Cell Bank

Induced Pluripotent Stem Cells (iPSC) derived cell therapies are an interesting new area in the field of regenerative medicine. One of the approaches to decrease costs of iPSC derived therapies is the use of allogenic homozygous human leukocyte antigen (HLA) matched donors to generate iPSC lines and to build up a clinical grade iPSC bank covering high percentage of the Spanish population.
The Spanish Stem Cell Transplantation Registry was screened for cord blood units (CBUs) homozygous for the most common, HLA-A, -B and DRB1 haplotypes. 7 donors were selected with haplotypes covering 21.37% of the haplotypes of the Spanish population.
CD34 positive hematopoietic progenitors were isolated from the mononuclear cell fraction of frozen cord blood units from each donor by density gradient centrifugation and further by immune magnetic labelling and separation using purification columns. Purified CD34+ cells were reprogrammed to iPSCs by transduction with CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit. After their expansion, clones were characterized, banked, and registered. Master Cell Banks (MCB) and Working Cell Banks (WCB) from the iPSCs of each donor were produced under GMP conditions in qualified clean rooms. These clinical grade iPSC lines will serve as starting materials for advanced therapy medicinal product (ATMP) development.

Vitamin B12 is a limiting factor for induced cellular plasticity and tissue repair

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Transient reprogramming by the expression of OCT4, SOX2, KLF4 and MYC (OSKM) is an attractive therapeutic strategy for tissue regeneration and rejuvenation, but little is known about its metabolic requirements. By analyzing the intestinal microbiota in mice expressing OSKM, we found that reprogramming causes a global depletion of vitamin B12 and molecular hallmarks of methionine starvation. Interestingly, B12 supplementation increases the efficiency of reprogramming both in mice and in cultured cells, the latter suggesting a cell-intrinsic effect. We found that H3K36me3, an epigenetic mark present in active gene bodies that prevents illegitimate initiation of transcription outside of promoters (cryptic transcription), is sensitive to B12 levels. We provide evidence for a link between B12 levels, H3K36 methylation, transcriptional fidelity and efficient reprogramming. We show that B12 supplementation also accelerates tissue repair in a model of ulcerative colitis, in association with enhancement of an embryonic-like repair program. We conclude that vitamin B12, through its key role in one-carbon metabolism and epigenetic dynamics, improves the efficiency of in vivo reprogramming and tissue repair.

The Interferon γ Pathway Enhances Pluripotency and X-Chromosome Reactivation in iPSC Reprogramming

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During reprogramming of somatic cells into induced pluripotent stem cells (iPSCs), the epigenome needs to be reset. A prime example of this is the reversal of the silent state of the inactive X chromosome in female cells, which is achieved in a process called X-chromosome reactivation (XCR). Few players have been described to be involved in XCR so far, and a comprehensive understanding of the regulatory networks has been lacking. Therefore, in this project we aimed to shed light onto the mechanism of XCR during somatic cell reprogramming into iPSCs, by performing a genome-wide CRISPR-screen during this process. Using this approach, we identified a number of previously known and unknown pathways which are involved in reprogramming and/or XCR. Of these, we focused on the interferon γ (IFNγ) pathway, the activation of which during the early phase of reprogramming accelerated pluripotency acquisition and XCR. In this study, we sought to uncover the mechanism by which IFNγ enhances XCR, and found a dependency on TET-enzyme activity and DNA demethylation. These findings will contribute to the mechanistic understanding of the process of XCR and could have a potential impact on improving iPSC generation.

Endothelial-Jag2 preserves HSC function upon aging by maintaining HSC epigenetic polarity through Notch signaling activation

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In aged mice the hematopoietic stem cells (HSCs) with the highest regenerative capacity have divided the less over time, are mostly polar for the H4K16ac distribution (epipolarity) and are exclusively located next to BM sinusoidal endothelial cells (SEC) expressing the Notch ligand Jag2. Notch signaling has been extensively investigated during development and also in young mice however, its involvement during aging is still not completely understood. Here we show that endothelial-Jag2 regulates HSC function upon aging through Notch signaling modulation. Notch signaling activation is not homogeneous within the HSC population, depends on HSC localization and correlates with H4K16ac polarity. In young mice most HSCs have active Notch signaling and a specific localization in proximity to the endosteum, arteries and sinusoids. Of note, Notch active HSCs of both young and aged mice are mainly epipolar. Importantly, sinusoidal and arteriolar specific deletion of Jag2 in young mice strongly reduces the frequency of epipolar HSCs in association with a loss of their localization at sinusoids and arteries respectively, an increase of HSC proliferation and an alteration in the symmetry of the HSC division. Upon aging, Notch inactive HSCs markedly expands, lose their epigenetic polarity, localize further away from the endosteum, arterioles and sinusoids and are often in cluster. Interestingly, SEC-specific Jag2 deletion in young mice recapitulates HSC aged phenotype by increasing Jag2 expression in HSCs and promoting HSC clustering, suggesting that Jag2 expression might induce Notch signaling in cis-inhibition in HSCs. Collectively, we show that Jag2 trans-Notch signaling is dysregulated in HSCs upon aging, inducing Notch signaling cis-inhibition and an epigenetic remodeling which impairs HSC function and regenerative potential.

Self-protection by epithelial cells in the early embryo

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Immune responses protect adult organs against perturbations. How the early embryo responds to such events before the formation of the immune system is unclear. The embryo is exposed to internal defects in pluripotent cells, as well as to external physical or biological perturbations. One example are the frequent mitotic errors, considered the main cause of human preimplantation failures. We recently identified a phagocytic program present in the embryonic surface epithelium, the first functional tissue formed during development. This tissue removes defective pluripotent cells with high efficiency, using similar mechanisms to the ones present in professional phagocytes of the immune system. However, the epithelial character of these cells imposes specific constraints to their phagocytic capacity. We are exploring the ability of this tissue to provide autonomous protection to early embryos upon different types of perturbations. Here we will present our recent advances in understanding epithelial protection using quantitative live imaging on various embryonic models. In addition to reveal embryonic self-protection mechanisms, our dynamic analysis across scales serves as a platform to capture the dynamics of epithelial phagocytosis, also operating in adult tissues.

Epigenetic modifications driving ground state pluripotency exit require an NF-κB-independent chromatin IκBα function

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Inflammatory signals are key in development and cell differentiation but their orchestration with pluripotency and stemness signals is poorly understood. Our previous work identified a chromatin function of IκBα, the NF-κB inhibitor, that is crucial for differentiation in different types of somatic stem cells. Here we demonstrate that deficiency of IκBα imposes a profound chromatin rewiring defect that impacts on DNA methylation, histone post-translational modifications and transcriptional regulation, stabilizing mouse embryonic stem cells (ESCs) in a ground state of pluripotency while preventing them from pluripotency exit and differentiation. By engineering separation-of-function mutants of IκBα with specific bindingto either NF-κB or histones, we demonstrate that regulation of pluripotency state by IκBα independent of NF-κB but requires the chromatin-related IκBα function.

A dynamic gradient of extracellular matrix stiffness orchestrates zebrafish heart regeneration

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Cardiovascular diseases are the leading cause of death worldwide, due largely to the inability of the adult heart to regenerate after damage. In sharp contrast with humans, zebrafish efficiently regenerate large portions of the heart after experimental damage or amputation. This process has been extensively studied and the key underlying cellular and molecular mechanisms begin to be understood. However, how cardiomyocyte proliferation and migration are coordinated, or how the overall regenerative process is terminated once the final organ size is reached are still a mystery. Here, using a combination of genetic ablation, micro-mechanical characterization, live cell imaging, and single-cell transcriptomic analyses with subcellular spatial resolution, we characterized a mechanism that, at the same time, provides cues for directional migration of cardiomyocytes and for ceasing cell proliferation as the heart recovers its initial size. Specifically, increased expression of postnb (encoding a collagen crosslinking enzyme) by fibroblasts at the leading edge of the regenerating tissue results in a proximo-distal gradient of extracellular matrix (ECM) stiffness. Cardiomyocytes at the base of this gradient (soft ECM) undergo cell proliferation and polarize the expression of the chemokine receptor cxcr4, thus informing proximo-distal cell migration to replenish the amputated region. The distal part of the ECM stiffness gradient (stiffer ECM) then becomes non permissive for cardiomyocyte proliferation, hence effectively terminating myocardial regeneration. Exploiting this novel mechanism in the human context could open new therapeutic approaches to promote myocardial regeneration and prevent the development of heart failure.

Midbrain organoids with LRRK2 G2019S mutation recapitulate key aspects of PD pathology and reveal early glia dysfunction

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Three-dimensional (3D) brain organoids provide a platform to study brain development and disease using tissue and human-derived cells. Here, we report that human induced pluripotent stem cells (hiPSCs) midbrain dopaminergic organoids (hMOs) obtained from patients harboring LRRK2 PD-causing mutation and from CRISPR/Cas9 corrected isogenic control (Ctrl) hiPSCs show a preferred differentiation toward functional dopaminergic neurons (Dan), which express markers of Substantia Nigra brain identity. Moreover, single-cell RNA sequencing reveals cellular heterogeneity in both hMOs lines. However, compared to Ctrl hMOs, PD hMOs show evidence for molecular dysfunctions in apoptosis and mitochondrial pathways in Dan, and marked dysregulation in macroautophagy and proteolysis in astrocytes. To study the consequences of these dysregulations, we conducted analysis at a late stage of hMOs maturation and found accumulation of aggregated phosphorylated synuclein (αSyn), functional alterations in neuronal networks, as well as increased Dan death only in PD hMOs compared to Ctrl hMOs. Since astrocytes were the cell type with the most dysregulated transcriptome in PD hMOs, we hypothesised that they might at least partially drive aspects of PD pathology that we found at later timepoints. To test our hypothesis, a chimera organoid model consisting of Ctrl hMOs and αSyn flag-tagged LRRK2-PD astrocytes was generated. We found a spread of PD astrocytes-derived αSyn to Ctrl neurons that trigger abnormal network dynamics (similar to those found in PD hMOs), thus indicating that glia dysfunction plays a critical role in causing LRRK2-linked PD pathogenesis. In summary, our study provides the first in-depth, single-cell analysis of LRRK2-G2019S mutation using hMOs and propose a powerful platform to study PD pathology and non-cell autonomous contribution to early PD onset.

A 3D atlas of the human embryonic and fetal pancreas to study proliferation and differentiation of multipotent pancreatic progenitors

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The study of human fetal pancreas development offers valuable insights into the intricate processes governing pancreatic cell proliferation and differentiation. In this research, we employ an innovative approach integrating tissue clearing, in toto labeling, and light-sheet fluorescence microscopy to construct a three-dimensional atlas of the human embryonic and fetal pancreas during the first trimester of pregnancy. We show that first INS+ cells appear around 5 post-conception weeks, much before it was previously determined by conventional immunostaining. They are restricted to the inner part of the organ as well as other endocrine subsets like GCG+ and SST+ cells. Conversely, proliferating pancreatic progenitors are located in the periphery of the epithelium, suggesting the existence of two separated pancreatic niches for differentiation and proliferation. We also found that acinar CPA1+ cells are preferentially found in the peripheral region of the embryonic and fetal pancreas, even these differentiated cells do not display proliferation ability. Additionally, the existence of extra pancreatic INS+ cells adjacent to the gut was confirmed. On top of that, an explant culture system was developed enabling in vitro proliferation of pancreatic progenitors. This unveiled a mitogenic effect of PDGFAA in progenitors acting through the pancreatic mesenchyme. Overall, this works presents the most complete atlas of the human developing pancreas charting both endocrine and proliferating cells across early development. These novel insights contribute to a deeper comprehension of developmental biology and hold promise for advancing regenerative medicine and therapeutic interventions.

Post-transcriptional regulation in iPSC derived neural and glial cells from Alzheimer disease patients

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Alzheimer's disease (AD) is the most common cause of dementia but its pathogenesis still remains poorly understood. Post-transcriptional regulation, e.g. via RNA-binding proteins (RBPs) or alternative cleavage and poly-adenylation (APA) has been shown to be implicated in AD. However, whether the corresponding alterations play a causative role or rather represent consequential symptoms has not yet been shown. To address this question, and ideally identify potential new therapeutic targets, we investigate the transcriptional landscape of single cells progressing from iPSCs derived from AD patients and healthy controls throughout their differentiation to neural and glial cells. We detect up to 8000 genes per cell across more than 80000 cells over five developmental time points. Clustering and transcriptome profile analyses identified all major cell types (iPSCs, NECs, NPCs, Neurons and Astroctyes) including various precursor and mature cell populations in both conditions (AD and control). Differential gene expression analyses identified several differentially expressed genes that have been previously linked to AD. Amongst the differentially expressed genes were several RBPs, implicating the corresponding regulatory networks as potential targets for further investigation. Differential 3' UTR usage analyses using SCALPEL revealed that several genes previously implicated in AD pathogenesis display changes in APA between AD and control cells. Here we present those results and our efforts to validate individual findings, and integrate these lines of evidence towards a better understanding of post-transcriptional regulation in pre-neurodegeneration AD.

MACROPHAGES CROSSTALK WITH INTESTINAL EPITHELIAL CELLS TO REGULATE REGENERATION

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Cancer patients treated with radiotherapy in the abdomen develop radiation-induced enteritis and suffer from bleeding and malabsorption that impairs their quality of life. Radiation injury depletes the proliferative intestinal stem cells and progenitors, in response to this the intestine is reprogrammed into a fetal-like primitive state where committed cells de-differentiate giving rise de novo to the intestinal stem cells.
Immune cells are master regulators of inflammation and injury. Among these immune cells, macrophages have garnered significant attention due to their multifaceted functions. While their role in inflammation and injury is well-established the putative contribution of macrophages to the intricate process of intestinal regeneration remains elusive. Using a plethora of groundbreaking approaches such as in vivo ablation of specific myeloid cells, bulk RNA-seq, single-cell RNA-seq, lineage tracing, 3D imaging, mouse and human organoid cultures we demonstrate that macrophages crosstalk with the intestinal epithelium to lead the process of regeneration. Upon injury macrophages are massively recruited near the intestinal stem cell compartment and secrete various growth factors, and extracellular matrix proteins to induce the fetal-like reprogramming that actively drives the process of regeneration. These factors collectively contribute to the regulation of cellular proliferation and de-differentiation during intestinal regeneration. Consequently, we have identified and characterized a novel role of macrophages beyond the innate immune response that can be exploited to boost the process of intestinal regeneration to avoid anti-cancer treatments side effects.

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Decoding pancreatic endocrine cell differentiation and beta-cell regeneration in zebrafish

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Zebrafish possess an exceptional yet elusive ability to replenish lost beta-cells in adulthood. Understanding this framework would provide mechanistic insights for beta-cell regeneration, which may be extrapolated to human. Here, we characterize a krt4-expressing ductal cell type, which is distinct from the putative Notch-responsive cells, showing neogenic competence and giving rise to the majority of endocrine cells during post-embryonic development. Furthermore, we demonstrate a dramatic ductal remodeling process featuring a Notch-responsive to krt4+ luminal duct transformation during late development, indicating several origins of krt4+ ductal cells displaying similar transcriptional patterns. Single-cell transcriptomics upon a series of timepoints during beta-cell regeneration unveil a previously unrecognized dlb+ transitional endocrine precursor cell, distinct regulons, and a differentiation trajectory involving cellular shuffling through differentiation and de-differentiation dynamics. These results establish a new model of zebrafish pancreatic endocrinogenesis and highlight key values of zebrafish for translational studies of beta-cell regeneration.

Characterization of the murine pancreatic ductal tree identifies novel cell populations with potential implications in pancreas regeneration

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Pancreatic ducts form an intricate network of tubules that secrete bicarbonate and drive acinar secretions into the duodenum. This network is formed by centroacinar cells, terminal, intercalated, intracalated ducts, and the main pancreatic duct. Ductal heterogeneity at the single-cell level has been poorly characterized; therefore, our understanding of the role of ductal cells in pancreas regeneration and exocrine pathogenesis has been hampered by the limited knowledge and unexplained diversity within the ductal network.

We used scRNA-seq to comprehensively characterize mouse ductal heterogeneity at single-cell resolution of the entire ductal epithelium from centroacinar cells to the main duct. Moreover, we used organoid cultures, injury models and pancreatic tumor samples to interrogate the role of novel ductal populations in pancreas regeneration and exocrine pathogenesis.

We have identified the coexistence of 15 ductal populations within the healthy pancreas and characterized their organoid formation capacity and endocrine differentiation potential. Cluster isolation and subsequent culturing let us identify ductal cell populations with high organoid formation capacity and endocrine and exocrine differentiation potential in vitro, including Wnt-responsive-population, ciliated-population and FLRT3+ cells. Moreover, we have characterized the location of these novel ductal populations in healthy pancreas, chronic pancreatitis, and tumor samples, highlighting a putative role of WNT-responsive, IFN-responsive and EMT-populations in pancreatic exocrine pathogenesis as their expression increases in chronic pancreatitis and PanIN lesions.

Poster 1

Differentiation of human pancreatic organoids towards the endocrine lineage and characterization of human pancreatic ductal heterogeneity.

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Background: Restablishment of β-cell mass by replacement with exogenous cells or by regeneration of the endogenous ones is a promising approach for the cure of diabetes. Although the presence of progenitor cells in the adult pancreas is a controversial issue, the fact that ductal and endocrine cells share a common progenitor during pancreatic development suggest that ductal cells could be used to obtain a pool of β-cell. Using sc-RNAseq, Rovira et al have described 15 novel ductal populations in adult mouse pancreas, some of them with progenitor characteristics. Cells with this progenitor signature are predominantly located in medium and big ducts.
Aims: In order to translate the above-mentioned results into the human scenario, we isolated different size ducts from human pancreas of cadaveric donors with the aim of: 1) to test their organoid formation capacity, and 2) to evaluate their potential for endocrine differentiation.
Methods: For organoid cultures, after human islet isolation, small and medium size ducts were handpicked and cultured in Matrigel. Organoids were expanded and induced to differentiate towards insulin-producing cells using a modification of a previously described protocol to differentiate stem cells into β-cells (Rezania et al. Nat Biotechnol 2014;32:1121).
Results and conclusions: At the end of differentiation period, organoid cultures expressed endocrine progenitor markers (NGN3 and NEUROD1). Organoids from medium size ducts display a higher potential for differentiation into endocrine progenitor cells when compared to small ducts. Therefore, human pancreatic organoid differentiation towards endocrine cells could reproduce the mouse results. Further studies to investigate human ductal heterogeneity are needed to evaluate species differences/similarities and isolate ductal novel populations to investigate their endocrine differentiation potential.

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Poster 2

A novel medium-throughput platform for target validation of regulators of cardiac regeneration in zebrafish larvae

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Cardiovascular diseases remain the leading cause of death worldwide, and few effective treatment options are available. Myocardial injury, such as myocardial infarction, causes irreversible damage of the heart muscle and its replacement by scar, leading to a chronic decrease in heart function. In contrast to humans, the injured zebrafish heart muscle regenerates efficiently through robust proliferation of myocardial cells. Thus, the zebrafish presents a beneficial vertebrate model for studying genetic programs behind cardiac regeneration, which may be present, albeit dormant, in the adult human heart.
To this end, we established a novel platform for studying heart regeneration after cardiomyocyte ablation in zebrafish larvae. The specific ablation of cardiomyocytes is achieved through a transgenic construct inducing the expression of nitroreductase, a bacterial enzyme, in a pool of ventricular cardiomyocytes. Subsequent treatment with antibiotics induces cell death specifically in nitroreductase-expressing cells. In combination with automated 3D heart imaging, this platform can be used for medium-throughput screening of genes and compounds with presumed effects on regeneration. Our results confirm that we induce a loss of >90% of the targeted cardiomyocytes, which are replaced through the proliferation of remaining cardiomyocytes within 4 days post injury. Our results further show that treatment with known anti-regenerative molecules causes a significant delay in regeneration kinetics, providing a proof of principle for this platform in identifying anti-regenerative effects of genes and drugs. Using this platform, we aim to discover therapeutic targets and drugs that will allow us to activate the dormant regenerative potential of the human heart."

Poster 3

UNRAVELING THE MOLECULAR BASIS OF PEDIATRIC MYELODYSPLASTIC SYNDROME: INSIGHTS FROM CRISPR/CAS9-EDITED IPSCS BEARING THE SAMD9 P.I1567M MUTATION

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Pediatric Myelodysplastic Syndromes (pMDS) are rare hematological disorders, characterized by ineffective hematopoiesis with a high risk to progress to acute myeloid leukemia (AML). While most of the adult MDS/AML cases are associated with random somatic mutations, an increasing number of pediatric cases are associated to germline variants.
Nowadays, we know more than 20 predisposing MDS genes, with SAMD9 as one of the most common mutated. However, understanding the molecular basis that leads to pMDS development by SAMD9 mutations remains unexplored. To understand the effect of germline SAMD9 mutations on hematopoiesis, CRISPR/Cas9 system was used to introduced heterozygous and homozygous p.I1567M mutation in a healthy human induced pluripotent stem cell (iPSC) line.
To assess whether SAMD9 mutation affects hematopoiesis, both iPSC-SAMD9mut lines were differentiated toward blood progenitors. FACS analysis revealed that SAMD9 mutation blocks the maturation of early hematopoietic progenitors (CD34+CD43+CD45+). The increased hematopoietic output of hiPSC-SAMD9mut lines could be related to the higher proliferation/survival of early HPCs. However, cell-cycle analysis of CD34+CD43+ cells revealed no significant differences among hiPSC-SAMD9mut and control. These data suggest a specific effect of SAMD9 mutation on blood specification rather than proliferation. In line with this finding, differentiation of HSPCs to myeloid terminal lineages leads to a significant decrease in monocytes (CD14+CD45+) formation when compared to their isogenic control. These data were also validated by CFU assay. Finally, given the role of SAMD9 in inflammation, we stressed the system culturing hiPSC-SAMD9-mut in the presence of proinflammatory cytokines TNFα. Interestingly FACS analysis suggests that SAMD9mut in a stressed hematopoiesis leads to a significant decrease of HSPCs differentiation. Overall, our study provides new insights into the role of inherited hematological disorders.

Poster 4

Generation of blood outgrowth endothelial cells from human peripheral blood and their reprogramming to induced Pluripotent Stem Cells by non-modified

Marta Perez Franco*, Silvia Selvitella, Begoña Aran, Anna Veiga, Bernd Kuebler

Stem Cell Bank

Since the publication of Yamanaka and colleagues 2007 demonstrating the successful reprogramming of human skin fibroblasts into induced pluripotent stem cells (iPSCs) by the retroviral infection of cells with the transcription factors Oct4, Sox2, Klf4, and C-myc, researchers have been working towards achieving footprint-free reprogramming, where the generated iPSCs are free from exogenous factors. Reprogramming by RNA transfection is currently considered to be one of the most advanced techniques, as it avoids any genomic traces in the iPSCs, due to the short lifespan of RNA. However, this technique requires adherent cells to be transfected, such as human fibroblasts or endothelial progenitor cells (EPCs).
The aim of the study was to reprogram blood derived cells by RNA transfection to set up a new reprogramming methodology at the Barcelona Stem Cell Bank.
We generated EPCs from human whole blood and from human umbilical cord blood. Mononuclear cells from both sources were isolated by density gradient centrifugation and mononuclear cells from cord blood were further purified by immune magnetic separation using CD34 specific antibodies. Both populations were differentiated to EPCs and characterized by immunocytochemistry.
EPCs were reprogrammed to iPSCs by 8 daily RNA transfections using the StemRNA 3rd Gen Reprogramming Kit. The resulting iPSC clones were expanded and characterized by evaluating their self-renewal capacity. Immunocytochemistry was performed to show positive staining of nuclear pluripotency marker, NANOG, OCT-4, and SOX2, and surface pluripotency marker, SSEA-3 and SSEA-4.
Further experiments will be carried out to completely characterize the iPSC clones, including analysis of microsatellites and karyotype, embryoid body formation and differentiation to the three germ layers.

Poster 5

Interrogating the importance of X-chromosome inactivation and reactivation for meiotic potential

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X-chromosome dosage compensation is achieved by X-chromosome inactivation, a process where one X chromosome is epigenetically silenced in female mammals in order to attain X-dosage parity with male cells. Subsequently, the silenced X chromosome uniquely reactivates during development of the germline, before the cells ultimately initiate meiosis. Using a dual X-chromosome reporter mouse embryonic cell line, we observed a subpopulation of primordial germ cells with two active X chromosomes, rather than the expected single active X, which were found to exhibit abnormal behaviour and a low efficiency of meiotic entry. This data suggested that X-chromosome kinetics may have an important role in correct female germline development. In order to functionally test this, we developed a knockout model of Xist, a long non-coding RNA that mediates X-chromosome inactivation. Our results demonstrate that the majority of cells cannot maintain two active X chromosomes after differentiation, resulting in X-chromosome loss or cell death. However, primordial germ cells that survive this bottleneck event while maintaining two active X chromosomes can progress efficiently to meiosis prophase I, indicating that X-chromosome inactivation and reactivation may not be strictly required for female germ cell development. These findings provide insight into the prerequisites of X-chromosome epigenetic states for proper meiotic entry in female germ cells.

Poster 6

Comprehensive CRISPRi screen to study the functional consequence of monosomy 7 in GATA2 deficiency.

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While the majority of myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are sporadic, rare germline predisposition syndromes have been delineated recently. GATA2 deficiency is one of the most common hereditary cause of MDS/AML in children, with a very high prevalence in those with monosomy 7. There remain important unanswered questions in GATA2 deficiency: Which are the epigenetic/transcriptomic changes cooperating with monosomy 7 in leukemic progression of GATA2 deficiency? Which are the genes lost in chromosome 7 (chr7) aneuploidy of GATA2-deficient related to clonal transformation? Answering these questions has been thus far hampered by the absence of trustable human disease models. Here we attempt to identify chr7 genes cooperating with GATA2 haploinsufficiency to promote malignant transformation, combining CRISPR/Cas9 technology and hematopoietic stem progenitor cells (HSPC).
We generated two stable K562 pro-myelocytic lines carrying GATA2 R396Q and R398W mutations, creating both heterozygous and homozygous clones. The differentiation assays towards megakaryocytes revealed that GATA2 deficiency impairs differentiation upon PMA.
We developed and introduced by lentivirus the pLVX_TRE_dCas9_KRAB_Puro and the rtTA-Neo plasmids into K562 GATA WT/R396Q, creating stable lines through antibiotic selection.
Following line establishment, we infected it with a CRISPRi library targeting 300 selected chr7 genes, with 5-6 sgRNAs each. A multiplicity of infection (MOI) of 2 enabled simultaneous knockdown to investigate the cooperative effect of the GATA2WT/R396Q mutation with chr7 gene inhibitions. Proliferation assays and genomic analyses are underway.
Lastly, we will utilize human induced pluripotent stem cells with the GATA2 R396Q mutation, engineering them to express Dox-dCas9-KRAB. This will silence 5-10 chr7 candidate genes post-screening in the K562 line, aiding our investigation into the effect of chr7 loss in a GATA2 deficiency background.

Poster 7

Constructing an iPSC-based Malignant Peripheral Nerve Sheath Tumor model system

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Malignant Peripheral Nerve Sheath Tumor (MPNST) is an aggressive soft tissue sarcoma that can develop both sporadically and in the context of the genetic disease Neurofibromatosis type 1 (NF1). They account for 3-10% of all soft tissue sarcomas. Currently, there are no therapeutic options for MPNSTs and only a timely surgery constitutes an effective curative option. MPNSTs are the main cause of mortality of children and adults with NF1. There is a clear need for the development of additional strategies that greatly rely on the generation of genuine in vitro and in vivo model systems.

MPNST cells bear hyperploid and highly rearranged genomes. Most recurrent discrete genetic alterations identified in MPNSTs are the complete inactivation of the NF1, CDKN2A and SUZ12/EED (PRC2) tumor suppressor genes (TSGs). In our lab, we have developed an in vitro/in vivo iPSC-based 3D model system of plexiform neurofibromas, an NF1(-/-) benign Schwann cell tumor, precursor of an MPNST. In this work, we aimed to further develop this neurofibroma model towards an MPNST model by CRISPR-Cas9 editing the three recurrently inactivated TSGs, in a step-wise manner. By generating 1, 2 and 3 KO iPSC-lines, we investigated fundamental aspects of MPNST biology such as the temporal order of TSG inactivation; the progression towards hyperploidy and the switch from a Schwann cell fate towards a mesenchymal tumor cell identity.

Poster 8

Understanding the cellular memory to develop treatment for cancer survivors

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IDIBELL

The increasing number of cancer survivors is leading to more patients suffering from unintended side effects, including radiation-induced enteritis from abdominal radiotherapy. Radiation injury depletes the proliferative intestinal stem cells (ISCs) and progenitors. Interestingly, the ISCs reemerge within a few days, suggesting an unchartered cellular plasticity allowing committed cells to dedifferentiate to de novo produce stem cells. We hypothesize that committed cells that dedifferentiate into stem cells upon irradiation retain a memory of their past identity and/or inflammatory memory. Using in vivo lineage tracing, multiome sequencing (scRNAseq and scATACseq) and organoid cultures we demonstrate that de novo formed ISCs have different functional properties compared to the original ISCs. This condition affects their response to subsequent radiation injuries; the progeny of de novo formed ISCs harbor increased capacity to dedifferentiate upon a second insult. However, this enhanced regenerative potential is at expenses of stemness.

Poster 9

Unraveling the role of HIF-2α in multicellularity within an oxygen-rich environment

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The emergence and proliferation of animal life on Earth have long intrigued scientists. During this essential event, diverse animal species successfully colonized a vast spectrum of ecological niches on the surface of Earth. While conventional wisdom attributes this event to the rise in atmospheric oxygen levels, we propose a novel perspective: the development of advanced control mechanisms for cellular responses to hypoxia played a pivotal role, potentially preserving stem cell characteristics.
Specifically, in vertebrates, we turn our attention to HIF-2α, a key factor known to induce (cancer) stem cell properties under physiologically oxygen-rich (physoxic) conditions. We postulate that HIF-2α is central to the emergence and maintenance of multicellularity in oxygen-rich tissue environments. To explore this hypothesis, we employ human and mice intestinal organoids as a versatile experimental model.
We hypothesize that HIF-2α expression plays a crucial role in sustaining adt stem cells in regions where tissue oxygenation reaches physoxic concentrations. In contrast, we anticipate that HIF-1α expression will be associated with sub-physoxic conditions, defined as oxygen concentrations below 1-2%. To investigate these dynamics, we attempt to map the intricate oxygen gradients, and the role that HIF-2a plays for crypt morphologies, within our organoid models.
Our research is specifically focused on elucidating the influence of HIF-2α on the morphology of intestinal organoids and the extent of its expression under physoxic conditions. This study aims to deepen our understanding of the cell stemness at physoxic (oxygen-rich) tissue environments. Our findings shed light on the critical role that oxygen concentrations play in shaping the development and renewal of multicellular life, with a particular emphasis on the context of human intestinal organoid morphology.

Poster 10

GENERATION OF EQUINE INDUCED PLURIPOTENT STEM CELLS: A KEY STEP TOWARDS 'ONE MEDICINE' STRATEGIES

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Animals share many diseases with human, like osteoarthritis, asthma or diabetes. Veterinary patients, like horses, can benefit from advanced regenerative therapies while providing key preclinical knowledge in conditions much closer to human disease. This One Medicine approach is already being pursued using adult stem cells (e.g. mesenchymal stromal cells), but induced pluripotent stem cells (iPSCs) technology remains critically underdeveloped in veterinary. The generation of animal iPSCs is very challenging owed to differences in their reprogramming requirements, which makes it necessary to develop specific protocols for equine cells. We compared the reprogramming capacity of equine cells from three origins (embryonic, perinatal and adult). After preliminary optimisation, equine cells were lentivirally transduced with OSKM factors and plated onto feeder cells (inactivated mouse embryonic fibroblasts) with serum-free media supplemented with fibroblast growth factor and leukaemia inhibitory factor. Perinatal and adult cells showed morphological changes and formed alkaline phosphatase (AP)+ iPSC-like colonies, but the lines were not stable and started differentiating after a few passages. Only embryonic-derived cells formed characteristic iPSC colonies with well defined borders. Interestingly, embryo-derived cells had higher proliferation potential and expressed SOX-2 in basal conditions. A total of 16 iPSC lines could be expanded from embryo-derived cells and all expressed SOX2, OCT4, NANOG and REX1 (qPCR and immunofluorescence), and were able to differentiate into cells of the three germ layers in vitro. However, these lines retained transgene expression so are considered as putative iPSCs. While transgene-independent maintenance of pluripotency is still a goal to pursue in animal iPSCs, obtaining putative lines is critical to advance our understanding of iPSC biology in veterinary species."

Poster 11

Abrogation of Notch signaling induces a quiescent state in hematopoietic stem cells during fetal development

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Hematopoietic stem cells (HSCs) are generated in the embryonic aorta and subsequently migrate to the fetal liver (FL), the major site for embryonic HSCs expansion and maturation, and later colonise the bone marrow (BM). In the adult BM, the most immature form of HSCs (about 30%) are predominantly dormant, harbor the highest long-term reconstitution potential and are activated only when there is a serious demand. How the dormancy state is established, initiated or controlled is mainly unknown.
The Notch signaling pathway is an important regulator of different embryonic HSCs and other types of stem cells, but it does not regulate adult HSCs. Hematopoietic deletion of RBPj in the fetal liver stage (with vav1-cre) has an important reconstitution defect. We have analysed the RBPjvav1:cre fetally deleted HSCs in the adult BM and found that are more quiescent than Notch-competent HSCs, displaying a transcriptome that is compatible with a dormant phenotype along with a defect in repopulation ability. In contrast, they have a self-renewal defect when serially transplanted. These results prompt us to further investigate the effect of Notch signalling in the fetal liver and on the cell cycle status of HSCs. Our results show that Inhibition of Notch signalling in fetal liver LSK cells using gamma-secretase inhibitors or specific Notch receptor blocking antibodies induce quiescence in fetal HSCs in vitro. Similarly, deletion of Notch2 (N2 vav1:cre) has a similar effect on the cell cycle of HSCs. Conversely, when Notch1 is overactivated (lox-stop-loxNICvav1:cre), fetal HSCs are more active, but the overall percentage of fetal HSCs decreases significantly.
In conclusion, the Notch signalling pathway may be regulating the acquisition of the quiescent/dormant state of fetal HSCs and determining their functionality in the adult BM.

Poster 12

Human 3D bioprinted tubular cardiac tissues as a tool for cardiac therapies

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Heart failure remains a global health concern, requiring advances in regenerative strategies. Tubular engineered cardiac tissues (tECT) integrated into ventricular assist devices, represent a significant advancement in heart failure treatment. This study aims to generate tECTs that closely mimic ventricular wall organization and functionality.
The myocardium is emulated by 3D bioprinting (BIO XTM, Cellink), forming a tubular construct and using a bioink containing iPSC-derived cardiomyocytes (iPSC-CM), iPSC-derived cardiac fibroblasts (iPSC-CF), and a composite of biomaterials mainly comprising gelatin and fibrinogen. The selected bioink was optimized to ensure its suitability (biocompatibility and printability), resulting in tECTs that maintained their morphology for several days post-bioprinting, and that showed significant improvements in microarchitecture, particularly regarding extracellular matrix (ECM) stability and cellular organization, although tissue retraction is still a challenge.
Subsequently, the epicardial layer is incorporated by rolling a collagen membrane, pre-seeded with iPSC-CF, around the 3D bioprinted construct. In addition, we are currently addressing the incorporation of iPSC-derived endothelial cells (iPSC-EC) to cover the inner wall and mimic the endocardial layer.
To enhance tissue functionality, tECTs are then cultured under continuous flow perfusion and subject to mechanical stimulation using a dedicated bioreactor (model TC-3F, EBERS).
Immunohistochemical analysis reveals the expression of key cardiac markers, such as troponin T, confirming cell maturation. Spontaneous contractions at macroscopical level demonstrate proper cardiomyocytes adaptation, even 30 days post-bioprinting.
This work represents a significant advancement in creating physiologically accurate cardiac tissues for therapeutic applications, disease modelling and drug testing in the cardiology field.

Poster 13

The role of the RNA-binding protein Staufen 2 during neurogenesis

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Neurogenesis is a crucial process in which new neurons are formed during cortical neurogenesis in the embryo. However, the brain has a limited capacity to regenerate after birth and throughout life. In humans, this regenerative capacity is linked to adult neurogenesis, and alterations in this process are a common hallmark of neurodegenerative diseases such as Alzheimer's disease (AD). Recent studies indicate that post-transcriptional regulation of gene expression by RNA binding proteins (RBPs) is a critical step in fine-tuning neurogenesis and mediating cortical development. However, little is known about the identity and role of these RNA regulatory networks during this process. Staufen 2 (Stau2) is a double-stranded RBP that has been implicated in the asymmetric distribution of mRNAs in radial glial cells (RGCs), thereby dysregulating the balance between neural stem cell maintenance and differentiation during mouse brain development (Kusek, Cell Stem Cell 2012, Vessey, Cell Stem Cell 2012). To unravel the RNA regulatory networks controlled by Stau2 at different stages of human neurogenesis, we established a protocol to differentiate human induced pluripotent stem cells (hiPSCs) into different neurogenic populations. Using CRISPR-Cas9, we obtained several heterozygous clones showing a strong reduction in Stau2 mRNA and protein levels. After 11 days of differentiation of hiPSCs into neuroepithelial cells, we observed a clear enrichment of Map2+ and Tuj1+ neuronal populations compared to the control cell line. Furthermore, the mRNA levels of Sox2, Foxg1 and Map2 are altered in the knock-down compared to control at the neuroepithelial stage of differentiation. Taken together, these data provide new insights into the functional role of Stau2 in early neurogenesis and confirm our Stau2 knock-down cell line as a valid model to further identify and analyse Stau2 protein and RNA regulatory networks during human neurogenesis.

Poster 14

Developing an iPSC-based in vitro double inducible Cre/LoxP conditional model as a platform for the study of mendelian diseases.

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Introduction: The generation of induced pluripotent stem cells (iPSC) in conjunction with the establishment of protocols for differentiation into different cell types is increasingly used for disease modeling. Cellular disease models based on single inducible vectors of Cas9 nuclease or Cre recombinase have been developed. Still, a major drawback relies on the inherent leakiness of these transgene expression systems. Therefore, double inducible expression systems take special importance.
Objective: Establishment of a doxycycline/tamoxifen double inducible Cre recombinase-iPSC line that allows the study of the impact of mono/biallelic gene inactivation in a common genetic background.
Methods: An iPSC line was carefully chosen after discarding the presence of mutations in cancer-predisposing genes by multigene test panel analysis. CRISPR/Cas9 editing system with an in-house generated ERT2-Cre-ERT2 donor plasmid, containing mOrange reporter gene, targeting the AAVS1 locus was used to nucleofect the selected iPSC line. Neomycin resistance was used to select positive clones. Proper insertion of the Cre vector was confirmed through PCR and Sanger sequencing. RT-qPCR, flow cytometry and fluorescence microscopy were used to characterize Cre recombinase expression both over time and doxycycline doses.
Results: Two clones were selected, showing monoallelic and biallelic Cre insertion. Both clones showed similar Cre expression levels, being 5 ug/mL for 48h the doxycycline combination that achieves the highest Cre expression levels with limited cytotoxicity. A round of cell sorting increased the Cre-mOrange positive cell percentage. Reduced Cre mRNA levels were observed in the absence of doxycycline, thereby proving minimum leakiness, which will presumably be controlled by the ERT2 domain.
Conclusion: The generation of a stably expressed inducible Cre recombinase in iPSC comprises the first step for the successful development of a platform for the study of mendelian diseases.

Poster 15

Generation of human induced Pluripotent Stem Cells from Haplo-selected cord blood samples (HAPLO-iPS) CA21151

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COST (European Cooperation in Science and Technology) is a funding organisation for research and innovation networks. COST connects research initiatives across Europe and enables researchers and innovators to grow their ideas in any science and technology field by sharing them with their peers. HAPLO-iPS is a COST Action that aims to create a collaborative network to provide a framework for hiPSC generation of hiPSC homozygous for frequent HLA haplotypes, compatible with a significant percentage of the population to be used for cell therapy clinical trials.
HAPLO-iPS will establish a European-based excellence network on hiPSC-derived cell-based medicines. This network includes all the relevant stakeholders: hiPSC generation/banks centres, CB banks that will supply cord blood units; manufacturing centres (GMP complying), immunology experts, regulatory bodies, National Agencies, and ethics experts. The challenge will be approached essentially by networking with all the stakeholders involved sharing knowledge, standardizing methodology and developing an educational training programme for researchers.
HAPLO-iPS also promotes the participation of researchers from less research-intensive countries, as a significant percentage of the members come from these countries. Participants from these countries will have access to research facilities, training courses, and mentoring programs for young researchers, contributing to spreading excellence and widening participation. Furthermore, key leadership positions in the Action Management are reserved for COST ITC members. The project is structured in 7 independent working groups interacting among them.
HAPLO-iPS offers new approaches that will foster the progress of haplo-selected hiPS generation through the development, implementation, and exploitation of a registry with all the information for the benefit of patients.

Poster 16

Mobilization of hematopoietic stem cells from the bone marrow into the bloodstream by phytonaturals in patients

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Several active principles from plants could trigger the release of stem cells from the bone marrow into the peripheral blood. Stem cell mobilizers have shown side effects in patients and the use of natural active principles from plants (curcuminoids, glycosinolate of sulforaphane, blue green algae AF) could enhance the hematopoietic (CD34+) stem cell mobilization into the bloosdstream without adverse effects in patients. The number of circulating HSC were measured by flow cytometry in peripheral blood of healthy patients (22 healthy subject). We have evaluated whether short-term (AFA-Aphanizomenon flos aquae-algae or curcuminoids: powder or liquid formulation) consumption over 48 hours could increase HSC mobilization (n = 22, n = 5-7 subjects/group). The total number of circulating CD34+ cells were quantified by flow cytometry (CD34 levels) after short-term and long-term nutritional supplementation with these phytonaturals (n = 5-7 by group), Peripheral blood was taking before and after nutritional supplementation (n = 7) and we evaluated whether long-term nutritional supplementation could differentially mobilizate HSC at 7 or 38 days of long-term supplementation (curcuminoids: 2000 mg/day, equivalent to 120 mg of curcuminoids/day), glycosinolate of sulforaphane: 6 mg/day), plus AFA algae extract (400 mg/day)]. On the last day of treatment, (10 A.M.), blood samples were collected six hours after taking these supplements; these findings suggest that AFA plus curcumine increase HSC mobilization into peripheral blood of patients.

Poster 17

Dissecting the interactions between leukemic cells and the aging bone marrow niche

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AML is a hematological disorder with a median age at onset of 70 years. TP53 mutations are observed in 5–10% of young adult AML cases, while TP53 mutation frequency increases significantly to more than 30% in older patients. Mutations result in inhibition of p53 function and are a strong predictor of inferior response to induction chemotherapy. Leukemic Stem Cells (LSCs) present similar properties to normal adult hematopoietic stem cells (HSCs) and depend on the bone marrow (BM) niche, which is modified by the LSCs to their advantage for propagation and treatment survival. This suggests that specifically targeting interactions between LSCs and the niche represents a possibility to improve therapy outcome and reduce the rate of relapse.
The aim of this work is to characterize a mouse model of Tp53 AML and investigate changes in the old compared to the young leukemic BM niche.
To observe the development of AML in an aged niche compared to a young one, we use CreERT2-Rosa26 knock-in mice crossed with Tp53flox mice. Hematological analyses were performed measuring percentages of different blood populations to determine the appearance of a hematological disorder. The resulting survival curve shows that old p53KO mice die faster compared with young p53KO mice.
To study the behaviour of LSCs in BM and the role of the niche in the disease development, we sorted young p53KO and p53WT HSC, and we transplanted them into young and old recipients. We observed that old mice transplanted with young p53KO cells were still dying faster compared with both the old control and the young p53KO recipients.
Therefore, our data support that old mice that that carry p53 mutation die faster compared with young ones and that this outcome is largely driven by the old microenvironment. An in-depth functional characterization with histology and scRNAseq experiments are currently ongoing to mechanistically unravel how TP53 LSCs are interacting with the nearby niche in young and aged mice.

Poster 18

A custom-made exocrine monoclonal antibody panel for understanding pancreatic disease and targeted regenerative therapies

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The human pancreas is composed of an exocrine compartment generating and transporting digestive enzymes to the duodenum and an endocrine compartment responsible for hormone secretion and regulation of glucose metabolism. Pathophysiologies of the different compartments of the human pancreas can lead to diabetes, cancer and pancreatitis. Monoclonal antibodies (mAbs) are a valuable resource to study pancreatic disease, develop new immune-based therapies and target delivery of drugs to the affected cellular compartments. To this end, our laboratory has generated a custom-made pancreas-specific mAb panel by immunizing rats with human islets. In this project, we focused on the characterization of a set of 46 affinity-purified mAbs that preferentially target the exocrine compartment of the pancreas. We first validated their expression pattern and subcellular compartment targeting in human pancreatic sections and human islet extraction byproducts using immunofluorescence and FACS. To narrow down the most promising mAbs in terms of studying disease pathogenesis, targeted drug delivery and tissue regeneration, we generated human pancreatic ductal organoids as an in vitro model of exocrine tissue. Most of our mAbs recognized their cognate antigens in human organoid cultures generated from healthy, obese and type 2 diabetic donors, with some mAbs showing varying expression patterns depending on disease status. Further, we have shown that several of our antibodies were uptaken from the ductal organoids in vitro suggesting that they can be utilized as drug delivery carriers for regenerative (diabetes) and cytotoxic (pancreatic adenocarcinoma) purposes. Finally, we are currently assessing the potential of the mAbs in terms of inflammatory, proliferative and functional capabilities in human pancreatic ductal organoids. Overall, our work has characterized a novel exocrine mAb panel with future implications for studying and treating pancreatic disease.

Poster 19

Cell-laden biomimetic tissue-engineered vascular grafts with Human Induced Pluripotent Stem Cells-derivatives

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Cardiovascular diseases, responsible for 17.9 million fatalities annually, are the leading cause of death worldwide. The standard approach involves graft transplantation, which utilizes autologous/synthetic grafts to bypass and replace diseased vascular segments. However, these grafts cannot grow and remodel, may lead to thrombogenicity and are not suitable for patients with small target vessels or insufficient autologous tissue[1].
Vascular tissue engineering offers a promising approach for constructing blood vessels but requires a large amount of endothelial cells (EC). Induced Pluripotent Stem Cells can solve this challenge as they can be derived from a person's cells and differentiated into vessel cell types. 3D bioprinting – a versatile tool of tissue engineering capable of generating biocompatible vessel grafts with growth potential – is particularly powerful in developing anatomically structured grafts for individuals with arterial tortuosity. Developing a bioink with extracellular matrix proteins could provide a solution to the challenging task of creating 3D cell-laden vascular grafts[2].
Here, we present a unique approach that combines the hierarchical architecture of elastin-gelatin-based composites with nano-/micro-patterned cues for luminal surface patterning to enhance EC adhesion and migration. Remarkably, we achieved patterned tubes with critical dimensions of 300 nm – a persisting challenge in fabrication community – by a combination of nanoimprint and soft lithography. We then utilized cell-laden hydrogels for the fabrication of tissue-engineered conduits. We discuss the accuracy of 3D bioprinting, cell-material interactions and the effect of topography, particularly nano/micro patterns on EC behavior, as these cues size-dependently influence cell growth[3].

Acknowledgement
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Poster 20

Transcriptomic characterization of the interplay between Noggin and the RNA-binding protein Staufen 2 during hiPSCs neuronal differentiation.

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Efficient neural stem cells and functional neurons can be produced from human induced pluripotent stem cells (hiPSCs), providing a new tool to study human neurogenesis and neurodegenerative diseases. This differentiation is achieved through the dual SMAD-inhibition of the BMP and the TGF-β pathways by Noggin, Dorsomorphin and SB431542, added during the first 10-12 days of culture. Reports have suggested that due to a redundant inhibition of the BMP pathway by Noggin and Dorsomorphin, using either of these inhibitors would be sufficient for neural differentiation. Indeed, when control hiPSCs were differentiated in the presence or absence of Noggin in the lab, no significant phenotypic variation could be observed and we obtained full cortical culture differentiation upon 70 days of induction. By depleting Staufen 2 (STAU2), an RNA-Binding Protein (RBP) crucial for neurogenesis, in hiPSCs, we found an important effect during neural differentiation that depends specifically on Noggin addition. To characterize the interplay between STAU2 and Noggin during neural differentiation, we performed bulk RNASeq of Day 11 STAU2KO cultures with (NOG) or without (NO NOG) Noggin addition. We found multiple STAU2 targets dysregulated including BMP4.The transcription factor Neurogenin 2 (NEUROG2), which is crucial to neuronal differentiation, was also significantly upregulated in NO NOG condition. Moreover, GSEA showed significant enrichment of TGF-β signaling and cell cycle pathways in NO NOG cultures. Experimental validation with qPCR showed an increased expression of NEUROG2 and neuronal markers such as MAP2 in NO NOG. Interestingly, NEUROG2 and MAP2 show similar expression levels in both conditions at the neural stem cell stage (Day 25). Taken together, we hypothesize that when exogenous Noggin is not added to hiPSC STAU2KO cultures, unregulated STAU2 gene targets may activate non-canonical BMP signaling that accelerates neuronal maturation at earlier stages of differentiation.

Poster 21

The impact of ER-stress on β-cell development and function in Akita pigs.

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Endoplasmic Reticulum (ER)-stress plays a crucial role in insulin processing and β-cell dysfunction, affecting β-cell development and disease progression in all diabetes types. Patients carrying an insulin mutation develop Mutant INS-gene-Induced Diabetes of the Youth (MIDY), an ER storage disease. In this neonatal form of diabetes, misfolded proinsulin aggregates in the ER, leading to severe and chronic ER-stress in β-cells. However, the exact mechanisms driven by ER-stress to trigger β-cell failure and diabetes onset are poorly understood. Given the similarities in pancreas physiology between pigs and humans, the Akita pig, carrying the INSC94Y MIDY mutation, is an ideal model to timely resolve the mechanisms of ER-stress-induced loss of functional β-cells. We characterized phenotypic and transcriptomic features of pig pancreata from four stages (embryonic, pre-weaning, post-weaning and adult) and three groups of study (Healthy, Akita and Akita under insulin treatment). To establish a timeline of islet-like cluster formation and destruction in healthy and Akita pigs, we are comparing development of islets of Langerhans regarding size, composition, proliferation and apoptosis levels. Histological profiling from healthy pigs showed that newly formed islets prevailed and increased in size over time. Whereas in Akita, endocrine cell clustering is impaired with disrupted islet cluster formation upon the early post-weaning stage. In contrast to the healthy, Akita β-cells do not show an increase in granularity with age, suggesting impaired insulin granule formation in the MIDY model. Transcriptional profiling of healthy and Akita β-cells confirmed ER-stress imbalance, with differential expression of genes involved in the ER-quality control system, such as the Unfolded Protein Response. Overall, this study will allow us to uncover the mechanisms of ER-stress-induced β-cell dysfunction during diabetes and identify the critical time points for therapeutical intervention.

Poster 22

Modelling intestinal regeneration through dedifferentiation in response to irradiation using human intestinal organoids.

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Radiotherapy is widely used to treat abdominopelvic cancers. However, proliferating intestinal stem cells (ISCs) are highly sensitive to irradiation. Consequently, these cells largely degenerate in response to radiotherapy, causing acute and potentially lasting perturbations in intestinal function. In mouse, it is known that ISCs can be formed from more differentiated cells through a dedifferentiation process. This leads to the formation of new ISCs and intestinal regeneration. In human, it remains poorly understood to what extent dedifferentiation operates as a driving force of intestinal regeneration. Human intestinal organoids (hIOs) provide a useful in vitro model of the human intestinal epithelium, both in homeostasis and injury conditions. We have established hIO lines to study dedifferentiation and intestinal regeneration in response to irradiation. We first observed an initial radiation-induced reduction in the expression of ISC-marker gene LGR5 by 8 hours (h) and a subsequent recovery by 24h. We then performed single cell RNA-sequencing on hIOs before and at 8 and 24 hours after irradiation. We identified a major shift in the directionality of RNA velocity, following irradiation. Cell fates at 8h post irradiation demonstrated a reversion in the cell fate trajectory towards ISCs and progenitors. A population of ribosome-high progenitor cells was identified as a new origin state post-irradiation, showing RNA velocity directionality towards both ISCs and differentiated cell types. Furthermore, during the reversion at 8h, we have identified upregulated gene expression patterns related to alternative splicing. Our findings demonstrate that hIOs can be used as a model to study dedifferentiation in the human intestinal epithelium, without the presence of a complex surrounding microenvironment.

Poster 23

Towards a hereditary spastic paraplegia type 52 (SPG52) AAV-based gene therapy strategy

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Hereditary spastic paraplegia type 52 (SPG52) is an ultra-rare inherited neurological disorder caused by biallelic mutations in the AP4s1 gene, which encodes a subunit of the adaptor protein complex 4 (AP-4), whose function is believed to be key in the biogenesis of the autophagosome. The disease leads to lower-limb spasticity, weakness, global developmental delay, intellectual disability, and seizures and no effective treatment is available. Gene therapy aimed at restoring AP4s1 expression represents a rational therapeutic approach for SPG52. As a proof-of-concept, we successfully treated patient-derived fibroblasts with AAV vectors carrying a correct copy of the AP4s1 gene. We report the restoration of AP-4 complex expression, confirming the efficacy of this AAV gene therapy approach. To further study the effects of our treatment on human target cells, we generated induced pluripotent stem cells (iPSCs) from an SPG52 patient that were differentiated into cortical-like neurons and compared with control neurons derived from healthy individuals. Consistent with patients, SPG52 iPSC-derived neurons recapitulate aspects of the disease's pathophysiology, making them a great tool to test the efficacy of our novel gene therapy strategy. In parallel to our *in vitro* studies, we have developed the first knock-out (KO) mouse model of SPG52. Upon characterization, KO mice show decreased motor coordination and muscle strength by grip strength, inverted grid, RotaRod, and clasping tests, as well as hippocampal-related learning impairments with no signs of anxiety. Distinctive hallmarks described in patients have been observed in these animalsby histology, magnetic resonance imaging, and electrophysiology tests. Currently, we are treating AP4s1 KO animals systemically with a neurotropic AAV vector carrying a correct copy of the AP4s1 gene at different ages and assessing their phenotype rescue. The generation of the hiPSC model together with the mouse model will be crucial to elucidate SPG52 pathogenesis and to validate our gene therapy approach, key milestones on the road to a successful treatment for SPG52.

Poster 24

Multiocular organoids from human induced pluripotent stem cells displayed retinal, corneal, and retinal pigment epithelium lineages

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Recently, great efforts have been made to design protocols for obtaining ocular cells from human stem cells to model diseases or for regenerative purposes. Current protocols generally focus on isolating retinal cells, retinal pigment epithelium (RPE), or corneal cells and fail to recapitulate the complexity of the tissue during eye development. Here, the generation of more advanced in vitro multiocular organoids from human induced pluripotent stem cells (hiPSCs) is demonstrated. A 2-step method was established to first obtain self-organized multizone ocular progenitor cells (mzOPCs) from 2D hiPSC cultures within three weeks. Then, after the cells were manually isolated and grown in suspension, 3D multiocular organoids were generated to model important cellular features of developing eyes. In the 2D culture, self-formed mzOPCs spanned the neuroectoderm, surface ectoderm, neural crest, and RPE, mimicking early stages of eye development. After lifting, mzOPCs developed into different 3D multiocular organoids composed of multiple cell lineages including RPE, retina, and cornea, and interactions between the different cell types and regions of the eye system were observed. Within these organoids, the retinal regions exhibited correct layering and contained all major retinal cell subtypes as well as retinal morphological cues, whereas the corneal regions closely resembled the transparent ocular-surface epithelium and contained of corneal, limbal, and conjunctival epithelial cells. The arrangement of RPE cells also formed organoids composed of polarized pigmented epithelial cells at the surface that were completely filled with collagen matrix. This approach clearly demonstrated the advantages of the combined 2D-3D construction tissue model as it provided a more ocular native-like cellular environment than that of previous models. In this complex preparations, multiocular organoids may be used to model the crosstalk between different cell types in eye development and disease

Poster 25

Inflammation-induced aging characteristics in Hematopoietic Stem Cells.

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Hematopoietic Stem Cells (HSCs) are specialized master cells that give rise to all the blood cells in the body. During steady state, they give rise to an unbiased lineage output but upon infection leading to inflammation, stress hematopoiesis is activated. Inevitably HSCs are exposed to such stressors throughout life. Hence, it is important to study these events in order to prevent possible immune-related dysfunctions during organismal aging. To address the effects of inflammation on HSCs we used Glucose-6-phosphate isomerase (G6pi)-in-duced murine rheumatoid arthritis model. Previously, we dentified Clca3a1, a calcium-activated chloride channel accessory membrane protein as a novel true aged HSC (aHSC) marker by distinguishing the aHSCs to 2 sub-populations: Clca3a1hi having true aged transcriptome with functional defects and Clca3a1lo resembling young-like HSCs. 56 days post arthritic induction, interestingly, around 50% of HSCs from young arthritic mice were Clca3a1hi, a phenotype that is native b to aged HSC compartment. Principal component analysis from RNA-Seq data revealed that arthritic and control HSCs are transcriptionally distinct. Moreover, the upregulated genes of arthritic inflammation-exposed HSCs (iHSCs) significantly overlapped with aHSCs despite their young age. However, the statistical significance in both upregulated and downregulated genes of iHSCs to aHSCs was more significant when the iHSC population was phenotypically separated to Clca3a1hi and Clca3a1lo. Hallmark aHSC and myeloid-biased genes are among the significantly upregulated and genes related to differentiation are downregulated in young Clca3a1hi iHSCs. Overall, this shows that rheumatoid arthritic inflammation induces an aging phenotype and transcriptome in young HSCs. Further analysis on related signaling involved in the induction of young HSC to aging would help design preventive approaches that in turn can protect HSCs and hematopoiesis.

Poster 26

Implementation of proximity labeling by APEX2 to study STAU2 interactome in neural progenitor cells

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IDIBELL

Staufen2 (STAU2) is a double stranded-RNA Binding Protein (RBP) whose expression is enriched in the brain. Previous studies have shown that STAU2 has a major role in neurogenesis, where it oversees the asymmetric division of radial glial cells by regulating the asymmetric localization of Prox1 mRNA during brain development in mouse. Yet, despite its importance it is not know which are the molecular mechanisms coordinated by STAU2 that regulate these processes.
To answer this question, in this project we propose to study the protein-protein interactions of STAU2 in neural progenitor cells (NPCs) using APEX2 proximity labelling technique. APEX2 is a soybean mutant ascorbate peroxidase that can biotinylate molecules located in a range of 10 nm by attaching to them a biotin phenoxy group. The labeled proteins can be purified by pulldown using magnetic beads covered by streptavidin. By creating a fusion protein containing APEX2 and STAU2, we will be able to identify all the proteins directly or indirectly interacting with STAU2.
We tested the APEX2-STAU2 labelling activity in HEK-293T cells. APEX2-STAU2 fusion proteins shows a distinct distribution within cells that resembles that of endogenous STAU2. Moreover, we confirmed the biotinylation capacity of APEX2-STAU2 within 2 minutes after the addition of hydrogen peroxidase.
Using western blot, we have seen that the protein labelling pattern is different from that of control cells only transfected with APEX2. Among the purified proteins after the pulldown, we can identify the APEX2-STAU2 fusion protein itself. Preliminary data on NPCs shows a different distribution of APEX2-STAU2 protein within cells and a lower labelling activity compared to HEK293T which could be related to a lower transfection efficiency. We will direct our future work towards improving the transfection rate and identifying the protein interactors of STAU2 in NPCs by mass spectrometry analysis.

Poster 27

Targeting H3K9me2 reduces self-renewal and proliferation of leukemic cells.

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Acute myeloid leukaemia (AML) is a heterogeneous disease with a median age at onset of 70 years and is characterized by high rate of relapse and mortality in aged patients. Currently, chemotherapy is the main treatment, which disregards epigenetic alterations acquired during aging. Heterochromatin modifications such as histone 3 lysine 9 dimethylation (H3K9me2) are involved in gene regulation and cell fate decisions. Recently, we showed that H3K9me2 is altered upon aging of murine hematopoietic stem cells (HSCs). However, very little is known about a possible role of H3K9me2 in leukemic transformation and whether it contributes to increased disease aggressiveness and relapse in the elderly. Here, we used a combination of microscopy, immunoassays, and in vivo/vitro approaches for characterizing the role of H3K9me2 in aged murine and human leukemic cells. A reduction of H3K9me2 was observed in healthy old murine progenitors compared to young cells, while leukemic cells generally showed low levels of H3K9me2 regardless of cell age. Pharmacological inhibition of histone demethylases specific for H3K9 by treatment with IOX1 (8-hydroxyquinoline-5-carboxylic acid) resulted in the reduction of the self-renewal capacity of both mouse and human leukemic cells in vitro, while healthy hematopoietic progenitors remained unaffected. Human cell lines receiving the same treatment showed an upregulation of di- and trimethylation on H3K9 with simultaneously decreasing cell proliferation in vitro. Altogether, our data suggests that the reduction of H3K9me2 upon aging might support leukemogenesis in aged patients and targeting leukemic cells by increasing H3K9me2 could represent a novel epigenetic strategy to treat AML in the elderly.

Poster 28

SCALPEL, A NEW TOOL FOR ALTERNATIVE POLYADENYLATION CHARACTERIZATION AT SINGLE CELL RESOLUTION

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IDIBELL

Alternative polyadenylation (APA) is a widespread mechanism of gene regulation that generates mRNA isoforms with distinct 3' ends. APA is well known to be regulated during cell differentiation and it is a major source of gene regulation in the brain. Proliferating cells tend to have shorter 3' UTRs while differentiated cells have longer 3'UTRs. Changes in APA patterns are not only characteristic of cellular differentiation but also have been associated with pathological processes such as cancer or neurodegenerative diseases like Alzheimer's disease.

The rapid development of 3'tag-based single-cell RNA sequencing (scRNAseq) has enabled the study of gene expression and the implementation of methods for describing isoform usage at single cell resolution. Here we present SCALPEL, a tool to quantify 3' isoform expression at single cell resolution using standard scRNA-seq data. Additionally, SCALPEL allows to identify alternative isoform usage between cell populations and experimental conditions. We used SCALPEL to study the changes in APA during the differentiation of human induced pluripotent stem cells (iPSCs) to neuroprogenitor cells (NPCs). The results from our analysis show clear changes in 3'end usage between iPSCs and NPCs. We aim to use SCALPEL to investigate the role of APA during neural differentiation and how these changes are altered in neurodegenerative diseases.

Poster 29

Targeting RhoA activity rejuvenates aged haematopoietic stem cells by reducing nuclear stretching

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Haematopoietic stem cell (HSC) function declines upon aging and biomechanical changes over time might contribute to the decreased regenerative capacity of aged stem cells. The small RhoGTPase RhoA is a key regulator of mechanosignalling and is critical for HSC cytokinesis and differentiation, but the role of RhoA-mediated mechanotransduction in HSC aging has not been investigated so far. Here, by taking advantage of inducible RhoA knock-out mice, our data show that in HSCs RhoA is necessary to survive nuclear stretching, which cell intrinsically induces RhoA activation. Interestingly, we measure a 2-fold higher RhoA activity level in aged HSCs, associated with constitutive increased nuclear stretching. We developed an imaged-based computational framework to reveal the chromatin changes produced by nuclear stretching and to demonstrate their reversibility in aged HSCs upon treatment with a selective RhoA inhibitor. Furthermore, reducing RhoA activity in aged HSCs decreases chromatin accessibility and transcription of several repetitive elements (REs), downregulating inflammation and interferon response and functionally improving aged stem cells regenerative capacity and lympho/myeloid skewing in transplantation assays.
Overall, our data support that an intrinsic mechanosignalling axis dependent on RhoA is necessary for HSCs to survive nuclear stretching and it can be pharmacologically targeted to rejuvenate HSC nuclear architecture and function upon aging.

Poster 30Are Peribiliary Glands (PBG) and Pancreatic Ductal Glands (PDG) the same entity?

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UB-IDIBELL

Peribiliary Glands (PBGs) are outpouch structures that emerge from the Common Biliary Duct, around the ampulla of Vater, the cystic duct and the common hepatic duct. Previous studies described the inside of these structures as a location of a progenitor cell population with regenerative capacities. Likewise, inside the pancreas and associated to the biggest pancreatic ducts inside the pancreas, there are similar structures as the one found in the Common Biliary Duct, called Pancreatic Ductal Glands (PDGs). These PDGs are not well defined, but it is known that this structure can be the niche of adult pancreatic progenitors. Therefore, our main goal is to study the similarities between both structures and to understand whether these compartment harbour adult progenitor cells. In order to do that, we performed Single Nuclei RNA-Seq from the CBD and the Main Pancreatic Duct (MPD), identifying a similar population in PBGs and PDGs that shared some common markers (AGR2, OLFM4). These cells display similarities with other adult stem cell populations, such as intestinal stem cells. Our omics studies identified surface markers to isolate the newly identified populations to perform in vitro organoid cultures to further assess the capacity of these cells to differentiate into endocrine (β-cells) and exocrine pancreatic lineages.

Poster 31

Using 2D and 3D hiPSC-based models of Tyrosine Hydroxylase Deficiency (THD) to investigate disease mechanisms and develop personalized treatments

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Tyrosine hydroxylase deficiency (THD) is an inherited neuropediatric disorder characterized by the lack of the enzyme tyrosine hydroxylase (TH), an important source of dopamine (DA) in the brain. There is currently no cure for THD, and while the majority of patients experience symptom improvement in response to L-DOPA (THD Type A), a significant percentage is currently without effective treatment options (THD Type B). New treatments and a more complete understanding of the cellular and molecular mechanisms of THD are therefore needed. We have recently generated human iPSC-derived dopaminergic neuron (DAn) from both THD forms and showed that they recapitulate disease-specific phenotypes and treatment response. Interestingly, early L-DOPA treatment at an early stage of development rescues disease phenotype in THDB cells, indicating the existence of a developmental window to treat severe THD cases. In this study we evaluated the therapeutic potential of BH4 in THD human neurons and found a significantly positive effect on the TH protein level and enzymatic activity as well as in the restoration of intracellular dopamine levels in THDA patient-derived neurons. Moreover, to investigate the effect of TH p.Thr399Met mutation on pathogenic processes of THD, we generated cortical organoids (COs) from THDB patient and CRISPR/Cas9 corrected isogenic control (Ctrl) hiPSCs. Interestingly, calcium dynamic studies revealed differential activity patterns between THDB and control cortical organoids, as THDB COs show higher collective activation amplitudes and hypersynchronous activation compared to Ctrl COs. These data indicate that dysfunction in dopamine signaling can cause excessive glutamate release, thereby increasing cortical excitation. Analysis of the expression of the inhibitory synapse markers such as gephryn and PSD95 are ongoing. We hope that identifying mechanisms implicated in THD pathology through our iPSC-based models will lead to the development of new treatments.

Poster 32

CELLEQUS: developing an haplo-bank of equine MSCs characterised for the MHC

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Allogeneic application of mesenchymal stem cells (MSCs) presents several advantages over autologous therapy, but the immune response to allo-MSCs needs further study. The immunogenicity of MSCs is key for safe and effective allogenic treatments and can vary upon the degree of major histocompatibility complex (MHC) matching/mismatching between donor and patient. The horse is highly valuable as both patient and translational model. Thus, establishing the most common equine MHC haplotypes provides critical information for biobanks aiming at providing allogenic MSCs for therapy in horses.
The goal of our project was to create CELLEQUS, a bank of equine MSCs derived from homozygous donors for the most common MHC haplotypes, which facilitate the use of well-characterized allogeneic cells, and matched with the recipient horses.
To facilitate the recruitment of MHC-matched donors, a validated 10 intra-MHC microsatellites panel was used to study MHC diversity in Purebred Spanish, Purebred Arabian, Hispano-Arabian and Lusitano, as the most common breeds in Spain. This proof of concept could be further extrapolated to other equine breed and even other species. After identifying the most common MHC haplotypes in the predominant breeds in Spain, we searched for horses homozygous for the most frequent haplotypes and recruited them as MSC donors. Their MSCs are isolated from bone marrow, characterized by immunophenotyping, tri-lineage differentiation, MHC-I and II surface expression, and assessed for their proliferation capacity and viability post-freezing.
CELLEQUS Bank stands out with its distinctive multiple mission: to provide optimal foundational resources for the advancement of veterinary regenerative medicine, spanning research, future commercialization, and clinical deployment. Furthermore, CELLEQUS not only pursues safety and efficacy benefits but also extends its advantages to the realm of human medicine, underscoring the pivotal concept of One Health, One Medicine.

Poster 33

Mimicking the embryonic pancreatic stromal niche for beta cell (re)generation

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Diabetes mellitus (DM) is a metabolic disorder characterised by loss or dysfunction of pancreatic β cells. Diabetes rates are dramatically increasing worldwide, and DM patients are 2-4 times more likely to die from cardiovascular diseases. Curing DM requires the restoration of β cell mass, either by replacement with exogenous cells or regeneration from endogenous ones. The presence of progenitor cells in the pancreas is however controversial. Our laboratory has been able to generate ductal derived organoids with the ability to differentiate into an endocrine progenitor state, characterised by the expression of Neurog3. However, these pancreatic organoids remain locked into a progenitor state, with only few cells becoming insulin expressing.

During the normal development, the stromal signalling is key to properly form pancreatic β cells, yet most of the signals are involved during the pancreatic morphogenesis are unknown. Understanding the embryonic developmental queues may be the key to reproduce β cell induction in pancreatic organoid cultures.

We have isolated stromal cells from different murine embryonic stages (e12.5 to e18.5) and adult pancreata and cultured them in low serum conditions. Conditioned media was collected daily for 7 days, and by means of a cytokine array (640 cytokines), we have identified 61 proteins being differentially expressed. Simultaneously, we have co-cultured pancreatic organoids with the same conditioned media in a β cell differentiation assay. Together, we have identified missing candidate molecules to generate a novel and efficient protocol to generate de novo β cells.