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ABSTRACTS BOOK

Poster number 1

Pre-existing stem cell heterogeneity dictates clonal responses to the acquisition of cancer driver mutations

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Cancer cells display wide phenotypic variation even across patients with the same mutations. Differences in the cell of origin provide a potential explanation, but these assays have traditionally relied on surface markers, lacking the clonal resolution to distinguish heterogeneous subsets of stem and progenitor cells. To address this challenge, we developed STRACK, an unbiased framework to longitudinally trace clonal gene expression and expansion dynamics before and after the acquisition of cancer mutations. We studied two different leukemia driver mutations, Dnmt3a-R882H and Npm1cA, and found that the response to both mutations was highly variable across different stem cell states. Specifically, a subset of differentiation-biased stem cells, which normally become outcompeted with time, can efficiently expand with both mutations. Npm1c mutations surprisingly reversed the intrinsic bias of the clone-of-origin, with stem-biased clones giving rise to more mature malignant states. We propose a clonal “reaction norm”, in which pre-existing clonal states show different cancer phenotypic potential.

Poster number 2

Investigating the tissue injury sensing and regenerative response in bladder urothelium

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Upon injury, most natural regenerative strategies include the formation of a blastema, i.e. a bulk of undifferentiated cells formed in the proximity of the wound site, that will subsequently proliferate and give rise to the missing organ. The similarities between blastema and tumours are striking, both deriving from differentiated cell types by loss of cell identity and being characterized by high proliferation. In regenerative organisms, the initiation of the reprogramming mechanisms is relatively lax while its progression is stringently regulated, while higher vertebrates exhibit an opposite regulation where cell reprogramming is robustly prevented, while the mechanisms controlling the cells escaping this blockade are much less efficient, probably because of the molecular players repurposing. This conclusion highlights the importance of preventing cells from escaping cell-identity mechanisms and suggests the usefulness of exploiting the limited number of signalling pathways governing the mammalian regeneration breaks for regulating the cancer initial onset and progression. Our pilot global proteome study on Non-Muscle Invasive Bladder tumor samples identified molecular cancer signatures in several of the apparently healthy biopsies. These patients relapsed faster, presenting a far more aggressive form of bladder cancer. The outlier samples and the tumour biopsies displayed the deregulation of several signalling pathways involved in cell identity maintenance, indicating that analysing apparently healthy tissue of a cancer-invaded organ may indicate disease progression. We designed a pilot study for mapping of the dynamic molecular signature characterizing the immediate stages of cell transformation in a mouse model of bladder cancer. We uncovered a transient regenerative response in the bladder, coupled with changes in the immune and metabolic signature, suggesting regeneration as an initial, however inneficient, response to stress directed at rescuing homeostasis.

Poster number 3

Exploring the role of HIF-2α in stem cell regulation and tissue renewal in an oxygen-rich environment in invertebrates and vertebrates

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The Hypoxia-Inducible Factor (HIF) pathway may be essential for tissue renewal and stem cell maintenance across animal species. In this study, we explore how HIF-2α regulates stem cell activity and tissue renewal under varying oxygen conditions in vertebrates.

First, we examine HIF-1α in invertebrates, focusing on how oxygen levels affect stem cell maintenance and lifespan in Drosophila melanogaster. We hypothesize that the absence of HIF-2α in invertebrates, which in vertebrates contributes to pseudohypoxic phenotypes, may limit tissue renewal under high oxygen conditions. Our findings show that flies exposed to 40% oxygen have reduced stemness and shorter lifespans, highlighting potential mechanisms of tissue renewal failure in oxygen-rich environments.

Next, we investigate HIF-2α's role in tissue renewal under oxygen-rich conditions using mouse intestinal organoids. We find that organoids cultured at physiologically relevant oxygen levels (3% O2) upregulate hypoxia-responsive genes like Plod2 and Adm, and shift toward developmental, stem cell-like states, as indicated by enriched fetal intestinal and spheroid signatures.

Lastly, we explore the in vivo role of HIF-2α in mice, specifically its impact on intestinal stem cell maintenance and regeneration. Using a HIF-2α knockout model, we assess the effects of HIF-2α loss on tissue renewal and wound healing following radiation-induced damage, uncovering potential compensatory mechanisms in the absence of HIF-2α.

Together, these projects deepen our understanding of how oxygen levels and HIF influence stem cell maintenance, tissue renewal, and lifespan, with important implications for regenerative medicine and developmental biology.

Poster number 4

Studying cellular memory in response to intestinal injury

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Radiotherapy plays a major role in the treatment of abdominal malignancies; however, it leads to the development of unintended side effects like radiation-induced enteritis. Radiation injury inflames the mucosa and depletes the proliferative intestinal stem cells (ISCs) that reemerge within a few days. The regenerative process occurs via proliferation of surviving ISCs and via dedifferentiation of progenitors and even fully differentiated cells to de novo produce stem cells. Here, we demonstrate that de novo formed ISCs retain a memory of their past identity and/or inflammatory memory. We demonstrate with organoid cultures and in vivo lineage tracing that ISCs after recovery from an injury are essentially different at functional level to their unperturbed counterpart. Moreover, using scRNAseq and scATACseq multiome of the intestinal epithelium, we characterize the transcriptional and epigenetic memory imposed to the different epithelial cell lineages in response to inflammation and injury.

Poster number 5

Epigenetic Regulation of Neural Stem Cells: The Role of Tet2 and Genomic Imprinting in Adult Neurogenesis

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In the mammalian brain, adult neurogenesis is primarily driven by neural stem cells (NSCs) located in the subventricular zone (SVZ) of the lateral ventricles. The self-renewal and multipotential capacities of these stem cells give rise to the three neuronal lineages: neurons, astrocytes and oligodendrocytes in the adult brain. The regulation of this complex process involves several extracellular and intracellular factors, including genomic imprinting (GI). GI is an epigenetic phenomenon that results in the monoallelic expression of specific genes, known as imprinted genes, expressed according to their parental origin. This regulation is mediated by epigenetic modifications in imprinting control regions (ICRs), which are differentially methylated regions (DMRs) on parental chromosomes. Ten-eleven translocation (TET) enzymes carry out DNA demethylation by converting 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). Although 5hmC levels are remarkably high in mouse brain, the role of TET proteins in regulating GI through the methylation status of ICRs in NSC behavior remains unclear. In this study, we focused on elucidating the role of Tet2 in adult neurogenesis by utilizing a conditional mouse model with a specific Tet2 deficiency in NSCs. Furthermore, we performed genome-wide transcriptional and DNA hydroxymethylation analyzes to elucidate the TET2-regulated gene expression landscape in NSCs to clarify the mechanisms underlying adult NSC differentiation and how imprinted genes are affecting this process.

Poster number 6

Role of the Prader-Willi Syndrome critical region in the biology of adult neural stem cells

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In adult mammals, new neurons are produced along the whole life due to the presence of neural stem cells (NSCs) located in two main niches: the subventricular zone (SVZ) in the walls of the lateral ventricles and the subgranular zone (SGZ) in the dentate gyrus of the hippocampus. NSCs are multipotent stem cells with the capacity to self-renew and give rise to astrocytes, neurons and oligodendrocytes. On the other hand, genomic imprinting is an epigenetic phenomenon that causes the monoallelic expression of a subset of genes known as 'imprinted genes' in a parent-of-origin specific way. Prader-Willi Syndrome (PWS) is a neurodevelopmental imprinting disorder caused by the loss of the expression of the paternally expressed genes contained at PWS locus, located at chromosome 15 in humans. The most common molecular cause of the syndrome is a deletion of some of the genes located in this cluster, and specifically, a critical region (PWScr) has been postulated as the minimal deletion capable of giving rise to a PWS-like phenotype. This region contains several tandem-repeated copies of snoRNA-coding gene SNORD116 and the non-coding gene IPW. Here, we use a mouse model of PWS lacking the paternal copy of the PWScr to characterise the effect of the loss of expression of this region in NSCs biology. To do that, the proliferation and differentiation potential of NSCs and their lineage has been studied at both neurogenic niches. The results show an increased number of doublecortin (DCX)+ cells in the dentate gyrus of PWScr-lacking mice compared to their wildtype littermates, indicating that the neurogenic process in this niche is altered.

Poster number 7

Role of TET3 in glioblastoma: dysregulation of genomic imprinting

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Glioblastoma multiforme (GBM) is the brain tumour associated with the poorest survival in humans after its diagnostic. However, molecular mechanisms by which it originates remain unknown. The cancer stem cell theory suggests that tumor-initiating cells originate from the malignant transformation of resident stem cells in the normal tissue. In this case, it is proposed that the etiology of GBM may involve the transformation of neural stem cells (NSCs) located in the brain into glioblastoma stem cells (GSCs), which are responsible for the initiation, expansion, and resistance to treatments of GBM. On the other hand, the dioxygenase TET3 plays a role in DNA demethylation through conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcitosine (5hmC), what relates with global genomic hypomethylation characteristic of GBM. Moreover, genomic imprinting is an epigenetic process regulated by DNA methylation that causes the monoallelic expression of genes depending on its parental origin. Dysregulation of the expression of these imprinted genes or loss of imprinting (LOI) has been described in many tumours, including GBM. Therefore, in this work we propose a role of TET3 during GBM development through regulation of genomic imprinting. Our results in a GBM cell line generated from NSCs containing multiple mutations observed in human patients of glioblastoma, suggest that TET3 maintains stem cell properties of GBM cells by regulating their cell cycle. Additionally, we show that TET3 overexpression in vivo reduces animal survival promoting tumoral growth in a DNA methylation-dependent manner. These changes agree with the great variation in the transcriptomic profile detected with TET3 increase, being able to identify the imprinted gene Peg3 as a candidate through which it would be causing its effects on the tumour.

Poster number 8

Genome-wide transcriptome and methylome profiling during adult neural stem cells reprogramming reveals genomic imprinting alterations

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Genomic imprinting is an epigenetic mechanism resulting in monoallelic expression of genes according to parental origin. This mechanism is far from being fully understood, but has been implicated in crucial biological processes such as foetal development, metabolism and cognition. Moreover, loss of imprinting (LOI) has been linked to cancer progression and other diseases known as imprinting disorders (IDs). Genomic imprinting relies on DNA methylation at imprint control regions (ICRs) which are parent-of-origin differentially methylated regions (DMR) between both chromosome copies. Somatic cell reprogramming into induced pluripotent stem cells (iPSCs) is a valuable tool to understand the mechanisms associated with pluripotency and holds promise for generating patient-specific stem cells for therapeutical applications to treat different pathologies such as IDs. In the present study, we have both profiled the transcriptome (RNA-seq) and methylome (MEDIP-seq) on mouse iPSCs derived from adult neural stem cells (NSCs), to investigate the imprinting-related regulation of pluripotency. We show an extensive hypomethylation in iPSCs, which correlates with genome-wide alterations of the transcriptomic profile. These transcriptomic and epigenetic changes also hold true for imprinted genes, identifying genuine epigenetic modifications that are inherently linked to pluripotency, thus ensuring a clearer understanding of the factors influencing iPSC quality and pluripotent potential.

Poster number 9

Notch trans-activation to cis-inhibition switch promotes hematopoietic stem cells aging

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Aged hematopoietic stem cells (HSC) expand in clusters over time, while reducing their regenerative capacity and their ability to preserve the homeostasis of the hematopoietic system. Notch signaling plays a key role in priming HSC fate during embryonic development but, upon maturation HSCs gradually reduce Notch activation, hindering its involvement during adulthood and aging. The expression of Notch ligands in the bone marrow (BM) niche is necessary for adult hematopoiesis however, the impact of Notch signaling for adult and aged HSC function remains controversial.

Here we show that in the BM of young mice Notch activation is not homogeneous within the HSC pool and depends on the sinusoidal expression of the Notch ligand Jagged2 (Jag2). Upon sinusoidal Jag2 deletion or physiological aging Notch activity in HSCs decreases, their perisinusoidal localization is lost and the divisional symmetry and fate priming is altered. Strikingly, this reduction in Notch activation upregulates Jag2 expression in HSCs which induce HSC clustering by Notch cis-inhibition overall resulting in HSC expansion over blood regeneration.

Collectively, these findings highlight a critical role for sinusoidal Jag2 in preserving fate priming and regenerative potential of adult HSCs and disclose an extrinsic Notch trans-activation to intrinsic cis-inhibition switch underlying HSC aging.

Poster number 10

Mucosal macrophages govern intestinal regeneration in response to injury

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Cancer patients treated with radiotherapy in the abdominal and pelvic cavity develop radiation-induced enteritis, a condition that impairs their quality of life. Radiation injury depletes proliferative intestinal stem cells (ISCs); in response to this, the epithelium activates a regenerative program that facilitates the healing of the intestine. However, the mechanisms that induce the activation of the intestinal regenerative program are poorly characterized.

In this study, we induced radiation-induced enteritis in mice through abdominal irradiation, mimicking clinical scenarios. Through imaging and flow cytometric analysis, we investigated the recruitment of macrophages to the small intestine during injury and healing. Additionally, we developed a co-culture system for mouse and human intestinal organoids and macrophages to explore the crosstalk between these cells. Then combining in vivo ablation of macrophages, fluorescent lineage tracing, imaging, bulk RNA-sequencing (RNA-seq), single-cell RNA sequencing (scRNA-seq), human intestinal organoids and cell trajectory analysis, we study at cellular and molecular level the macrophage induction of intestinal regeneration.

Our findings revealed that macrophages are recruited around the intestinal stem cell compartment upon radiation injury, promoting a fetal-like reprogramming and proliferation of epithelial cells that drives the regeneration process.

This study identifies macrophages as essential contributors to intestinal regeneration beyond their innate immune response. Targeting macrophages therapeutically may hold promise in enhancing regeneration and improving the quality of life for cancer survivors.

Poster number 11

Mechanisms of Intestinal Regeneration in human 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 number 12

Single-cell transcriptomics reveal broad alterations in RNA processing in iPSC-derived neurons and glial cells from Alzheimer's Disease patients

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Alzheimer's Disease (AD) is the most common cause of dementia. Alterations in RNA-binding protein (RBP) abundance and mRNA isoform have been implicated in AD. However, whether these changes are consequential or potentially causative of the onset of AD remains unknown.

To identify potential early AD-linked alterations, we screened for changes in gene and isoform expression in scRNA-seq data of cells derived from iPSCs from AD patients and controls. The isoform-level quantification and downstream statistical analyses we performed using SCALPEL, a new tool developed for that purpose in our lab.

We analyzed more than 60,000 cells from six donors at five time points of neural differentiation. We identified all expected major cell types, including stem cells, neural progenitor cells, neurons, and astrocytes amongst the 24 broad cell types identified based on the isoform-resolution data obtained with SCALPEL. Differential expression analyses comparing AD and control derived cells revealed multiple cell-type specific alterations including several genes previously associated to AD, such as TTR, ERBB4 and FAM162A. Furthermore, we found RBPs like HNRNPA2B1 and ELAVL4 to change expression between AD and control cells. Comparing the isoform-usage of individual genes, we identified cell-type specific changes in hundreds of genes.

In conclusion, we identified cell-type specific transcriptomic alterations in iPSC-derived cells from sporadic AD patients. The affected genes are linked to RNA metabolisms and neuronal projection morphogenesis, suggesting that these alterations may contribute to early molecular changes associated to AD. Together, our work highlights the importance of RBPs and RNA metabolism in AD and suggests a more prominent role of RNA processing in early AD pathogenesis.

Poster number 13

Establishing human iPSC-derived brain organoids to model Tyrosine Hydroxylase Deficiency (THD) disorder

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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 some patients experience symptom improvement in response to L-DOPA (mild form, THDA), a significant percentage is currently without effective treatment options (very severe form, THDB). New treatments and a more understanding of the cellular and molecular mechanisms of THD are therefore needed. To develop a patient tailored medication therapy to increase dopamine synthesis in THDB patients, here we generated control (age-matched and isogenic) and patient-derived ventral midbrain organoids (vmO). We confirmed the capacity of THDB vmO to reproduce disease-specific phenotypes, such as a reduction in TH-expressing cells and DA release. Moreover, we found that both THDB and control vmO present spontaneous neuronal activity, with elevated bursting and increased neuronal excitability detected only in THDB vmO. Importantly, the administration of TH cofactor tetrahydrobiopterin (BH4) at early stage of differentiation reverts the disease phenotypes (most likely through the stabilization of the enzyme). Thus, our study reveals that BH4 treatment can offer an option for THDB and other disorders associated with neurotransmitters affecting the central nervous system.

Poster number 14

iPSC-derived NF1-CDKN2A-PRC2 deficient neural crest acquire a neuro-mesenchymal identity and form MPNST-like tumors in vivo

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Malignant Peripheral Nerve Sheath Tumor (MPNST) is a highly aggressive soft tissue sarcoma that appears in rare sporadic cases in the general population or with a much greater prevalence in the context of Neurofibromatosis type 1 (NF1). Currently, there exist limited therapeutic options for this tumor type beyond a timely surgery. In NF1 patients, MPNSTs start developing through the complete inactivation of different tumor suppressor genes (TSGs), NF1, CDKN2A, and SUZ12/EED (PRC2), and frequently also TP53, that characterizes the progression from a benign plexiform neurofibroma to an MPNST.

This study aims to elucidate the functional roles of these TSGs and the biological consequences of their loss in the progression towards an MPNST. For that, we employed genome editing to sequentially knockout NF1 (1KO), CDKN2A (2KO) and SUZ12 (3KO) genes in induced pluripotent stem cells (iPSCs) to generate a progressive series of cellular models representing the PNF-MPNST progression. We have characterized these models genomically, evaluated the functional impact of each TSG inactivation and tested their tumor formation capacity in vivo.

In our system, we found that the order of TSG loss (NF1-CDKN2A-PRC2) is biologically constrained, requiring the complete inactivation of p14 and p16 CDKN2A genes. Loss of PRC2 function in the absence of NF1 and CDKN2A (3KO) induced loss of iPSC pluripotency. In addition, derived neural crest (NC) lose the capacity to differentiate towards glia and acquire a neuro-mesenchymal phenotype, both being epigenetically determined. Analysis of 3KO NC transcriptome and epigenome allowed the identification of characteristic biological processes related to neurogenesis and wound-healing in MPNST cell lines. Although expression differences exist between 3KO NCs and MPNST cell lines, engrafted 3KO NC spheroids can form MPNST-like tumors in mice, making it an excellent model for studying MPNST progression and treatment both in vitro and in vivo.

Poster number 15

Unraveling the contribution of LRRK2 mutated astrocytes and microglia in PD pathology using hiPSC modelling and scRNAseq

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Parkinson's disease (PD) is a prevalent, incurable neurodegenerative disorder characterised by the progressive loss of dopamine-producing neurons (DAn) in the substantia nigra and the accumulation of misfolded α-synuclein deposits within surviving neurons. Cumulative evidence points to neuroinflammation as mediator of the initiation and/or progression of PD, with brain astrogliosis and microgliosis present in post mortem PD patients. However, the precise mechanisms through which these changes influence PD pathological course remain unclear.

This project investigates the role of astrocytes and microglia (MG) in PD pathogenesis and examines how their dysregulated pathways may drive or exacerbate neurodegeneration, focusing on the LRRK2 G2019S mutation, the most common cause of familial PD. To elucidate the contribution of LRRK2-mutated glial cells to neurodegeneration in PD, we employed a hiPSC-derived triple culture model, where LRRK2-PD astrocytes and MG, or their respective isogenic controls, are co-cultured with healthy DAn.

After analysing our model with a combination of in vitro and in silico techniques, we found that LRRK2-PD astrocytes are the primary contributors to DAn degeneration. When mutated, they fail to provide adequate support to DAn, increase their own reactivity, and induce MG activation. scRNAseq analyses identified dysregulated genes in LRRK2-PD astrocytes, which may ultimately be responsible for the downstream effects we observed. A follow-up with in vitro analyses confirmed these impairments at the protein level. Importantly, we demonstrated that restoring the levels of some of the dysregulated proteins in LRRK2-PD glial cell can rescue neuronal death.

Ongoing analyses aim to further characterise the pathways underlying degeneration and drug-induced neuronal rescue, to propose a mechanism for the non-autonomous contribution of astrocytes and MG to PD, potentially opening new therapeutic avenues for treatment.

Poster number 16

Understanding the role of the RNA-binding protein Staufen 2 during neurogenesis using single cell transcriptomics

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Neurogenesis is a crucial process involving the formation of new neurons in the embryonic cortex, regulated by multiple factors including RNA-binding proteins (RBPs). Staufen 2 (STAU2) is an RBP implicated in the asymmetric distribution of mRNAs in radial glial cells, thereby dysregulating the balance between neural stem cell maintenance and differentiation. However, the molecular mechanisms of STAU2-mediated regulation in human neurogenesis remain largely unknown. To investigate the role of STAU2, we first generated STAU2 KO iPSC lines using CRISPR-Cas9. We differentiated the iPSCs to neural cell types and profiled the impact of STAU2 KO at multiple timepoints during the differentiation (day 0, 11, 25, 55, 70) using scRNA-seq. We identified cell types characteristic of the different timepoints including iPSCs, progenitor cells, and mature neurons and astrocytes. Using differential expression analysis, we found most differentially expressed genes between STAU2 KO and control cells were in the neuroepithelial cell cluster (NEC). Gene Set Enrichment Analysis of NECs, mostly found at day 11, showed significant upregulation of oxidative phosphorylation and glycolysis in STAU2 KO. These metabolic shifts have been linked to the transition between progenitor and differentiated cells, suggesting faster differentiation of NECs upon STAU2 KO. We confirmed the accelerated differentiation using 2D cell cultures, which have more cells with mature neuronal marker expression, and cortical organoids, which present altered neuroepithelial cell organization and smaller size. Gene Regulatory Network (GRN) analysis showed significant downregulation of the GRNs controlled by CHD2 and ARID3A in STAU2 KO NECs, potentially driving the accelerated differentiation observed. Given that CHD2 and ARID3A are STAU2 targets and have been shown to regulate stem cell differentiation, we propose that STAU2 controls human neurogenesis at the NEC stage by altering the expression of CHD2 and ARID3A.

Poster number 17

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 number 18

The genomic imprinting landscape of human glioblastoma

Jennifer Díaz-Moncho* and Sacri R. Ferrón

Glioblastoma (GBM) is considered the most malignant tumor of the central nervous system (CNS). A characteristic of this type of tumor is the deregulation of its epigenomes, particularly DNA methylation, which acts as a mechanism promoting carcinogenesis. The methylation status at the imprinting control regions (ICRs) is crucial for regulating the allele-specific expression of imprinted genes within the clusters they regulate. Mutations or alterations in DNA methylation in these regions can result in a loss of imprinting (LOI), as described in numerous cancers, leading to bi-allelic or null expression of these genes, thereby altering gene dosage. This study aimed to evaluate the status of genomic imprinting in human glioblastoma, with a focus on the clusters of the most relevant and studied imprinted genes in this disease. In this work, we demonstrate the existence of global deregulation of genomic imprinting based on differentially expressed gene analysis of TCGA and GTEx RNA-Seq data from human GBM. The results are validated in both glioblastoma stem cells (GSCs) and patient solid tumors, where the ICRs methylation, studied by pyrosequencing, appears to be widely hypomethylated. We identify alterations in methylation and gene expression primarily in the imprinted gene clusters H19/IGF2, PEG3, PWS IC, and DLK1-DIO3, leading to a loss of imprinting (LOI) in most of them.

Poster number 19

Are 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 (CBD), 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, especially the Main Pancreatic Duct (MPD), there are similar structures, although smaller in size, than those located 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 and share a common embryonic progenitor. In order to do that, we performed Single Nuclei RNA-Seq from the CBD and the MPD, identifying a similar population in PBGs and PDGs that shared some common markers (such as AGR2 and OLFM4). These cells display transcriptional similarities with other adult stem cell populations, such as intestinal stem cells. In situ characterization of population markers confirmed the presence of these population in PDGs and PBGs. Moreover, our omics studies identified surface markers that allow us to isolate the newly identified populations (Stem cell population_1 & 2) to perform in vitro organoid cultures to further assess the capacity of these cells to differentiate into endocrine and exocrine pancreatic lineages. Finally, novel lineage tracing mouse lines have been generated to assess in vivo the role of these populations in regeneration and pathogenesis by lineage tracing.

Poster number 20

Metabolic heterogeneity as a driver of stem cell fate and tumorigenesis in the intestine

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Metabolic reprogramming is a hallmark of cancer that allows cancer cells to obtain biosynthetic precursors and energy for cell proliferation and tumour growth. Despite the efforts to elucidate the metabolic adaptations of cancer cells, little is known about the metabolic rewiring during tumour initiation. Furthermore, most of the studies addressing metabolic reprogramming have considered tumours as metabolic homogeneous entities, however, it is known that tumours are heterogeneous and, therefore, different cancer cell populations may rely on distinct metabolic pathways. Although adult stem cells have been described as key elements in cancer development and progression, the metabolic control of stem cell self-renewal, differentiation and lineage commitment has been poorly addressed. In this context, identification of metabolic pathways controlling the fate of these cells will help in designing new therapeutic approaches.

Using the intestine as a model tissue, our lab has unmasked a highly glycolytic cell population that expresses high levels of pyruvate dehydrogenase kinase 1 (Pdk1). These cells are also present in intestinal adenomas and play a key role during tumour initiation. In order to study the role of glucose metabolism on the fate and function of Pdk1+ cells, we have generated several mouse models carrying genetically-encoded metabolic reporters that enable the visualisation, tracing and functional characterisation of these cells during homeostasis and tumorigenesis. Preliminary results show that Pdk1+ cells possess stem cell potential in vitro and in vivo and drive tumour initiation. Importantly, inhibition of Pdk1 using dichloroacetate (DCA) reduces stem cell activity in these cells. Our data suggests that glucose metabolism regulates stem cell activity and tumour initiation in the intestine and that metabolism can contribute to cancer heterogeneity.

Poster number 21

Deciphering Intestinal Epithelial Cells Role during Inflammation and Regeneration

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Inflammatory Bowel Disease (IBD) disrupts gastrointestinal tract mucosal homeostasis. Inflammatory episodes impair the intestinal epithelium, leading to barrier dysfunction and dysregulation of immune and homeostatic pathways, altering cell composition. Recent research highlights the crosstalk between epithelial cells and pro-inflammatory proteins in IBD pathogenesis. Intestinal crypt cells contain stem cells, progenitors and Paneth cells (PC) that are crucial for intestinal homeostasis, offering anti-microbial defense and supporting the intestinal stem cell (ISC) niche. While PC are primarily in the small intestine, Paneth-like cells also exist in the colon.

Our aim is to understand the cell-type specific responses and mechanisms during inflammatory stimuli that drive epithelial repair in IBD, focusing on the role of PC and stem cells. To that end, we use computational analysis of scRNA-seq and RNA-seq datasets and in vitro 3D intestinal organoid models derived from healthy mouse and from IBD patient biopsies. Computational analysis from public datasets revealed an increase in PC during inflammation, which was confirmed by qPCR in our organoid patients' models. We then enriched PC content in mouse organoids subjected to inflammation stimuli and performed RNA-seq. Transcriptome analysis revealed that organoids enriched with PC content during experimentally induced inflammation showed a specific increase in the activity of genes related to the suppression of cytokine production. Furthermore, our results also unveiled a specific downregulation of lipid and fatty acid metabolism in PC-enriched samples subjected to induced inflammation compared to the control.

In conclusion, our findings suggest an increase in PC during inflammation, potentially associated with the need for regulation of cytokine production and change in lipid metabolism, which might influence intestinal regeneration. These results may help to understand how treatments can be more effective in IBD.

Poster number 22

Disruption of Lysosomal Degradation and Endosomal Trafficking in LRRK2 G2019S Microglia: Insights from a Patient-Specific Induced Pluripotent Stem Cells

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The endolysosomal system is essential for cellular degradation and secretion, and its disruption is linked to neurodegenerative diseases like Parkinson's disease (PD).Mutations in the LRRK2 gene, especially G2019S, are associated with late-onset autosomal dominant PD and can occur in sporadic cases. LRRK2, a large protein with GTPase and kinase domains, plays a key role among others in autophagy and vesicle trafficking, and is highly expressed in immune cells like microglia. Although the role of LRRK2 in endolysosomal system is well known, it remains unclear whether the G2019S mutation affects the rerouting of materials for secretion. This project aims to fill this gap by investigating the relationship between degradation and secretion in microglia cell.

Using our previously established iPSC-based model of PD, we generated microglia from LRRK2G2019S PD patients and their isogenic iPSC controls, solely differing in the correction of the mutation. We confirmed a decrease of lysosomes biogenesis in LRRK2-PD microglia compared to controls, leading to impaired degradation. Moreover, only in microglia carrying the LRRK2 G2019S mutation we found an increased biogenesis and greater recruitment of endosomes to lysosomes, most likely in an attempt to compensate for the reduced degradative capacity. Our results suggest that this process involves Rab7, which is a direct substrate of LRRK2. We also found more late endosomes at the plasma membrane of LRRK2 PD microglia, likely for secretion. These results demonstrate that the G2019S-LRRK2 mutation in microglial cells enhance endosomal biogenesis and trafficking, likely as a compensatory mechanism for the lysosomal defect. The increased rerouting of endosomes toward the plasma membrane suggests the possibility of transient fusions, or "kiss-and-run" events, facilitating material transfer and secretion. These insights enhance our understanding of the G2019S-LRRK2 mutation and may lead to new therapeutic strategies for PD.

Poster number 23

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

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While the majority of MDS and AML are sporadic, rare germline predisposition syndromes have been delineated recently. GATA2 deficiency is one of the most common hereditary causes of MDS/AML in children, with a very high prevalence in those with monosomy 7. There remain important unanswered questions in GATA2 deficiency, including: (i) Which are the epigenetic/transcriptomic changes cooperating with monosomy 7 in leukemic progression of GATA2 deficiency? (ii) 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.

In this study we aim to identify chr7 genes cooperating with GATA2 haploinsufficiency in malignant transformation using a CRISPRi library targeting chr7 genes in GATA2 deficiency background. First, we generated K562 cell lines harboring GATA2 R398W mutation, creating both heterozygous and homozygous clones, by CRISPR/Cas9. Both mutant lines showed significant decrease in proliferation and megakaryocytic differentiation compared to isogenic controls, with homozygous mutants manifesting a more severe phenotype. These results are in line with hematological alterations observed in GATA2 deficiency, validating our model.

Next, K562 GATA2-mutant cell lines were engineered to express idCas9-KRAB and used to screen 604 selected chr7 genes. Interestingly the downregulation of chr7 genes resulted in a a rescue of the GATA2 mutant phenotype. Notably, heterozygous and homozygous GATA2 R398W mutant lines showed a 5.04- and 20.59-fold increase in proliferation, respectively, compared to the isogenic control. To identify chr7 genes responsible for this increased proliferation, deep sequencing analysis of these cells are underway. In conclusion, our CRISPRi idCas9-KRAB GATA2-mutant cell lines provide a robust model for identifying potential chr7 driver genes involved in malignant transformation in GATA2 deficiency.

Poster number 25

Transcriptomic changes induced by organoid media composition could influence pancreatic ductal organoid formation and differentiation

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β-cell replacement therapies have been developed for the last decades as a new strategy for the treatment of diabetes mellitus. Pancreatic ductal cells appear as one of the best candidates as a β-cell source for various reasons, highlighting the plasticity these cells acquire in vitro to form organoids, a specific attribute of adult progenitors. Organoids are a 3-dimensional culture with self-renewal and self-organization capacity that mimic the structure of the organ they come from. Pancreatic ductal organoids are cultured in Matrigel with a medium based on the one used in intestinal crypts organoids, with minor modifications. This initial medium contains Epithelial Growth Factor (EGF), Noggin (BMPs inhibitor) and Rspondin1 (Wnt pathway agonist). During the last years, this medium has been supplemented with various molecules involved in pancreatic development. However, there is no consensus on a specific culture medium for pancreatic organoids, and it has not been studied whether these factors are indeed necessary and what impact they have on cellular growth and identity of pancreatic ductal derived organoids. Thus, this study aims to optimize the minimal cell culture media requirements for ductal derived organoids formation and to evaluate how media cytokine/growth factor composition influences plasticity, cell fate and differentiation capacities towards pancreatic endocrine lineages of pancreatic organoids at the transcriptional level.

Poster number 26

Generation of human iPSCs at the Stem Cell Bank Barcelona: Statistical evaluation of reprogramming efficiencies from over 100 human samples

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The Stem Cell Bank of Barcelona (BLC-B) is a functional unit of the Regenerative Medicine Program, at the Institut d´Investigació Biomedica de Bellvitge (IDIBELL) dedicated to the derivation, maintenance, characterisation and preservation of human pluripotent stem cells (embryonic & induced pluripotent stem cells (iPSCs), with the aim of developing research activities in the area of regenerative medicine.

The BLC-B offers services of generation and characterization of hiPSC lines, including administrative support for the authorisation of projects involving the use and /or the generation of hiPSC. hiPSC can be generated from different cell types such as fibroblasts, keratinocytes, peripheral blood cells, umbilical cord blood, tumor and urine cells among others. We offer integrative and non- integrative reprogramming methodologies including retrovirus, lentivirus, Sendai virus, episomal plasmids and mRNA techniques. The BLC-B has cell lines of patients affected by more than 35 diseases, which can be used as models of disease.

During the last years, over 120 different samples of human somatic cells from healthy donors and from patients suffering more than 30 diseases were reprogrammed to hiPSCs, characterized and banked. A statistical evaluation of the reprogramming efficiency was carried out on this large pool of generated iPSC lines, including comparison of somatic cell types, passage number of cells, age and gender of the donor used for reprogramming, the reprogramming methodology and growth conditions.

Poster number 27

Macrophage impact on cellular plasticity in pancreatic basal cells

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Tissues employ conserved cellular plasticity to adapt and maintain homeostasis, which is crucial for resilience against disease-related challenges. Whilst plasticity of the pancreatic acinar cells has been widely studied, the duct cell compartment has been neglected. Our lab discovered pancreatic basal cells (PBCs) within this ductal compartment in both healthy and in diseased context. The prevalence of PBCs was increased in pancreatic ductal adenocarcinoma, the most prevalent and most deadly pancreatic cancer, as well as in chronic pancreatitis (CP), which is a known risk factor for developing this cancer. These cells could play a role in cancer development, transitioning into different cell states in response to environmental stressors, much like basal cells in other organs. Indeed, in human airway, macrophages stimulate the differentiation of basal cells and aid in the restoration of the epithelial barrier after injury. We hypothesize a similar process occurs in the pancreas.

We use spatial transcriptomics (STX), IHC and in vitro co-culture systems to study how immune cells influence PBC differentiation, focusing on CP. The HPDE cell line is used as an experimental model of PBC. THP-1 cells can be polarized into distinct macrophage subsets upon treatment with cytokines.

Using STX, we demonstrate that macrophages infiltrate the basal layer of pancreatic ducts and confirmed this on protein level. Additionally, our preliminary co-culture experiments hint that macrophages modulate the phenotype of HPDE cells, causing them to lose basal characteristics and adopt a more duct-like phenotype. To more closely represent the human condition, we are working to isolate basal cells and primary macrophages directly from the pancreas.

Above mentioned findings implicate that macrophages impact on the basal cell state. Later on, insights gained from this non-cancer context will be applied to cancer models to examine how these cell interactions are altered during tumorigenesis.

Poster number 28

Rapid and efficient differentiation of neural progenitor cells to mature neurons and astrocytes using lentiviral transduction

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and the main cause of dementia worldwide. AD is characterized by the accumulation of beta amyloid peptides and the formation of tau aggregates leading to neuronal degeneration and death. However, the molecular mechanisms responsible for neuronal death remain poorly understood. The aim of this study is to investigate the contribution of astrocytes to neurodegeneration. For this purpose, we have established a new protocol to generate mature neurons and astrocytes from neural precursor cells (NPCs) in a period of 7 days using lentiviral transduction. We obtained NPCs from iPSCs by dual SMAD inhibition. These NPCs are further transduced with lentiviruses expressing human Neurogenin 2 (NGN2) and nuclear factor I B (Nfib) to obtain mature neurons and astrocytes respectively. We adjusted experimental conditions for an optimal lentivirus transduction with minimal cell death. Individual differentiation of neurons and astrocytes could be maintained up to 14 days, although neuronal cell cultures were not optimal. After that, we optimized co-culture conditions. Passaging of neurons and astrocytes simultaneously lead to poor neuronal attachment and high cellular death. In contrast, differentiation of NPCs into neurons and astrocytes in parallel for 7 days and seeding of neurons one day later on top of the astrocytes resulted in optimal culture conditions. This co-culture could be kept at least for additional 7 days and contained neurons with a mature morphology. Subsequent optimization of culture media by adding Ara-C, an antimitotic agent, lead to an increase in the number of mature neurons and a rapid maturation of astrocytes compared to co-cultures without Ara-C. Together, our work shows that lentiviral transduction of NPCs is a fast and efficient method to generate co-cultures of neurons and astrocytes. We are now using this approach to study cell-cell communication using scRNA-seq.

Poster number 29

Autonomous defense of the early embryo

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Early embryos are exposed to various internal and external perturbations that can affect their development. How exactly these embryos respond to encountering bacteria is poorly understood. We previously showed that early zebrafish and mouse embryos have a protective phagocytic epithelium capable of clearing defective pluripotent cells from the embryo interior. Here, we present our recent results indicating that this epithelium can also resolve bacterial infiltrations. Our quantitative imaging approach across scales allows us to study the dynamic interaction of live bacteria with epithelial tissues in their natural environment. Understanding this epithelial protective function could be essential for exploring new causes of failures at the onset of human development.

Poster number 30

How do phagocytes in the early embryo recognize apoptotic cells?

Irene Lozano* and Esteban Hoijman

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Failures in early embryogenesis are caused by both internal cellular errors and environmental perturbations. We have found that the first tissue formed during vertebrate development, the surface epithelium (epithelial enveloping layer or EVL in zebrafish, trophoblast in mammals), protects the embryo by eliminating apoptotic cells. This phagocytic clearance of dead cells is evolutionary conserved and uses similar mechanisms to those of professional phagocytes of the immune system. Epithelial cells identify and phagocyte apoptotic cells through phosphatidylserine-mediated target recognition. In professional phagocytes the recognition of phosphatidylserine (PS) occurs through both direct or indirect receptors, where binding molecules known as opsonins act as a bridge. The specific receptor that recognizes PS in the embryonic epithelium is still unknown. The aim of this study is to decipher the molecular mechanisms of PS recognition. Performing specific interferences, we found that the expression of the molecule HMGB1 interferes with phagocytosis, which will help us to identify the molecules involved in the recognition process. We will present the data supporting this hypothesis. These findings could be relevant in different biological contexts in which epithelial cells operate as phagocytes.

Poster number 31

iPSC-derived ventral midbrain organoids as a model to study Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disease affecting more than 1-2% of people over 65 years old worldwide. PD is characterized by the loss of neuromelanin containing dopaminergic neurons (DAn) in the substantia nigra pars compacta and the accumulation of phosphorylated α-synuclein forming Lewy bodies. There is currently no cure for PD partly due to the lack of appropriate models that recapitulate disease features. The advance of induced pluripotent stem cells (iPSC) technology allows to model patient specific derived cells and generate 3D models such as ventral midbrain organoids (vmOs). These models resemble the structural complexity of neuronal circuits but it remains challenging to obtain mature features, in fact most vmOs protocols used in literature fail to accumulate neuromelanin (NM) in neurons. In our laboratory, we took advantage of iPSC technology to generate vmOs containing DAn. We wondered whether these vmOs were able to present mature characteristics such as the presence of NM and Lewy bodies. We demonstrated that over late timepoints vmOs carrying LRRK2-G2019S mutation, the most common form of familial PD, present Lewy body-like aggregates as seen by the presence and co-localisation of phosphorylated α-synuclein and VPS29, as it occurs in PD post-mortem brains. Additionally, treatment with L-DOPA, the precursor of NM, induced massive accumulation of the pigment, thus demonstrating that patient-derived vmOs from subjects with LRRK2-related PD represent an innovative human model to reproduce PD pathology and may be used to study the disease progression.

Poster number 32

Models to study epithelial protection in the early embryo

June Olaizola-Muñoa*, Joan Roncero-Carol, Esteban Hoijman

IDIBELL and IBMB (CSIC)

The early embryo develops exposed to both internal and environmental perturbations. We previously demonstrated that the early embryo can clear defective pluripotent cells, recognizing and eliminating apoptotic cells through phagocytosis. To explore how early embryos handle other perturbations, we developed various zebrafish models of infection. By exposing the embryo to different bacteria and employing various experimental approaches, we captured phagocytic events in the live embryo. Specifically, we analyzed the role of the cytoskeleton, discovering a significant role for Actin dynamics in this process. We will present our recent data, which improve these models and analyze the specificity of ingestion and the molecular mechanisms involved.

Poster number 33

Knocking-out Zfp800 using Crispr in murine pancreatic ductal-derived organoids reverts ductal cells to pancreatic multipotent progenitor state

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Zinc Finger 800 (Zfp800) has been recently described as a major regulator in intestinal organoids, by repressing transcription factors Ngn3 and Pax4. By knocking it out, enterocytes spontaneously differentiated into enteroendocrine cells.

Importantly, Ngn3 and Pax4 are master regulators in the normal endocrinogenesis in the pancreas, driving bipotent cells to the endocrine fate and eventually beta cell lineage. Zfp800 has never been described as a transcription factor in the pancreatic endocrine development.

Pancreatic ductal cell-derived organoids have the same potential as the embryonic bipotent cells, and are a potential source of new beta cell for diabetes treatment. Endocrine differentiation in pancreatic organoids mimics the development of beta cells in the embryo, however the efficiency of current protocols is still limited to ~15%. Therefore, we wanted to assess whether Zfp800 may have a role in improving beta cell induction in pancreatic organoids.

Here, we have knocked-out Zfp800 in murine pancreatic organoids using Crispr-Cas9 technology. While only achieving a partial knock-out, qPCR in the organoids revealed dramatic changes in gene expression, including reduction in Sox9 and Onecut1 and an increase in Pdx1, FoxA1/2 and Nkx6.1. When KO organoids underwent classical endocrine differentiation, no endocrine lineage were observed, but ductal (Sox9) and acinar (amylase) lineages were induced. These changes indicate that cells reverted from the bipotent tip-trunk stage typical of pancreatic organoids to a pancreatic multipotent stage, earlier in development.

Poster number 34

Characterization of the transcriptional and chromatin landscapes of human pancreatic ductal cells

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Diabetes Mellitus (DM) is a group of metabolic disorders consisting in elevated blood glucose levels accompanied by loss or dysfunction of pancreatic islet beta cells. Among current strategies for the development of long-term treatments for DM, we find the generation of beta cells from endogenous progenitors. Recently, we have defined and characterised at single cell level 15 novel pancreatic ductal subpopulations in mice, some of which display superior progenitor capabilities and endocrine differentiation potential in organoid cultures. Thus, we have sought to characterise human pancreatic ductal cells as well; in this work, we have performed the first single cell multiomics analysis to date of human pancreatic ductal cells. Through single-nuclei multiome RNAseq / ATACseq of over 40.000 ductal cells from 6 human pancreas, we have characterised their transcriptomic and chromatin-accessibility profiles, revealing the presence of multiple ductal subpopulations that correlate with those found in mice. Importantly, our cells recapitulate the differential marker expression that separates smaller and bigger ducts in mice, confirmed through immunostaining, as well as a differential endocrine differentiation potential in organoid culture. Moreover, we have identified differences in chromatin states, novel enhancers and gene regulatory networks, and transcription factors regulators that tightly correlate with population-specific gene expression. Finally, we are mapping risk variants of endocrine (Type 1 and Type 2 DM) and exocrine (cystic fibrosis, pancreatitis and pancreatic cancer) pathologies to find candidate target genes. Our results will provide a resource for studying ductal enhancer function and identify genes involved in pancreatic pathogenesis risk.

Poster number 35

The central clock drives metabolic rhythms in muscle stem cells

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Circadian rhythms are essential for organismal health. Satellite cells (SCs), the muscle resident stem cells, maintain a state of quiescence yet exhibit robust circadian oscillations at the transcriptional level. Although peripheral clocks have been extensively studied in various tissues, how the intrinsic clock of stem cells interacts with the central, distal clock is largely unknown. We used SC-specific reconstitution of the essential clock gene Bmal1 to elucidate the role of the local SC clock and its interplay with the central clock in the mouse brain and found that daily transcriptional control of metabolic processes in SCs depend on central clock input, independent of the SC clock. Central clock-driven genes were involved in lipid metabolism, functionally important for SC-mediated muscle repair, and autophagy was required for their oscillation. In summary, we provide the first evidence of circadian coordination of central and local clocks for control of rhythmic gene expression in quiescent stem cells.

Poster number 36

Human HSC self-renewal is maintained by fine-tuning RNA polymerase II activity.

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Hematopoietic stem cell (HSC) transplantation represents a life-saving intervention for blood disease. However, limitations such as donor-matching and insufficient HSC supplies require new strategies forgenerating or expanding transplantable HSCs in vitro. To achieve this goal, we need better understand the molecular determinants of HSC stemness that become defective in culture. Expression of MLLT3, a key regulator of HSC self-renewal and engraftment, promotes transplantable HSC expansion in culture. MLLT3 is a component of the Super Elongation Complex, which regulates RNA polymerase II (Pol II) activity for balanced transcriptional elongation and mRNA processing. To understand if MLLT3 action on its target genes is mediated by Pol II activity, we analyzed genome-wide Pol II activity by precision run-on sequencing (PRO-seq), and Pol II CTD phosphorylation, after modulating MLLT3 levels in HSC. Sustained MLLT3 expression revealed direct regulation of transcriptional activation at the upregulated genes, while it led to an increased pausing index at a large group of downregulated genes, previously identified as aberrantly upregulated in culture. To further investigate the role of transcriptional pausing/elongation in HSC, we treated human HSC with low doses of the CDK9 inhibitor flavopiridol, which resulted in an expansion of immunophenotypic HSCs in culture comparable with MLLT3 overexpression, without altering the cell cycle. This suggest that flavopiridol regulates HSC expansion by modulating the Pol II activity rather than maintaining the HSC pool by inhibiting cell proliferation. Altogether, these results highlight the crucial role of modulating Pol II pausing/elongation to maintain HSC transcriptional signature in vitro and identify a novel layer of regulation of self-renewal, with great potential to increase the efficiency of human HSC expansion in culture.

Poster number 37

Using CRISPR barcoding as a molecular clock to capture dynamic processes at single-cell resolution

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Biological processes are fundamentally dynamic, yet existing methods for capturing these temporal changes are limited. We present scDynaBar, a novel approach that integrates CRISPR-Cas9 dynamic barcoding with single-cell sequencing to enable the recording of temporal cellular events. In this system, genetic barcodes accumulate mutations over a 4-week timeframe and then are sequenced together with the transcriptome of each single cell. We propose that this gradual accumulation of genetic diversity can be exploited to create a time-ordered record of cellular events. To demonstrate this, we apply the system to track the transition from a pluripotent state to a two-cell (2C)-like state in mouse embryonic stem cells (mESCs). The results provide compelling evidence for the transient nature of the 2C-like state. Additionally, our system shows consistent mutation rates across diverse cell types in a mouse gastruloid model, underscoring its robustness and versatility across various biological contexts. This technique not only improves our ability to study cellular dynamics but also creates exciting new opportunities for future applications based on recording temporal signals at the single-cell level, in other words, using dynamic barcoding as a molecular clock.

Poster number 38

Novel approach to explore DNA damage responses throughout neurodevelopment

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DNA double strand breaks (DSBs) are repaired through two primary mechanisms: homologous recombination (HR) and non-homologous end joining (NHEJ). The choice of repair pathway is often cell type-dependent and can vary based on numerous factors, including cell fate. In the brain, which exhibits a high degree of cellular heterogeneity, the mechanisms underlying DSB repair may differ depending on the specific cell type and developmental stage, potentially impacting cellular functions such as differentiation, survival, and response to injury. However, much remains unknown regarding the exact mechanisms involved, particularly with respect to how neural progenitors and post-mitotic neurons repair DSBs, and how these mechanisms contribute to brain development and aging. Here, we apply scDynaBar, a novel approach that repeatedly induces DNA double- strand breaks (DSBs) at a specific locus to investigate the dynamics of DSB repair across various cell types in the developing brain. By incorporating scDynaBar into human-induced pluripotent stem cells (iPSCs), we will generate brain organoids—self-organizing structures that recapitulate early stages of human brain development. Using single-cell RNA sequencing (scRNA-seq) on these organoids, we aim to capture individual cell gene expression profiles alongside locus-specific repair outcomes. This approach offers a powerful tool to explore the complexity and heterogeneity of DNA damage responses throughout human brain development.