SHORT TALKS

ST01_Opposing Nodal and Wnt signalling activities govern the emergence of the mammalian body plan

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Please find below the abstract, which relates to this work we have on bioRxiv (https://www.biorxiv.org/content/10.1101/2025.01.11.632562v2)

Text: Nodal and Wnt signalling play an important role in the emergence of the mammalian body plan, primarily by orchestrating gastrulation. While the literature suggests they cooperate to build the primitive streak, their individual contributions remain poorly understood. Using gastruloids, we found that Wnt/β-catenin drives a genetic program characteristic of the late primitive streak, promoting the development of posterior body structures in a time and dose-dependent manner. Conversely, Nodal activates a distinct transcriptional module resembling the early streak. By engineering gastruloids with varying levels of Nodal signalling, we demonstrate that a decreasing temporal gradient of Nodal activity is critical for establishing the anterior body, with higher Nodal levels producing more anterior structures in a concentration-dependent manner. Our findings suggest that Nodal and Wnt act antagonistically, initiating distinct developmental modules within the primitive streak. This antagonism is likely the core mechanism driving the early body plan in mammals. Additionally, our results also lead to the suggestion that the gastruloid system models the primitive streak and reveals its self-organizing potential. We foresee that this proposal for the nature of gastruloids and the work presented here sets the stage for researchers to use the signalling-dependent modularity of the PS and the ability to make gastruloid chimaeras in favour of the engineering of specific body structures in vitro with biomedical potential, given the ability to grow human gastruloids.

ST02_Self-organization of phenotypic heterogeneity and plasticity in colorectal cancer

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Text: Phenotypic heterogeneity and plasticity drive tumor growth, metastasis, therapy resistance, and relapse. This heterogeneity is mainly interpreted as a response to external signals from the microenvironment. However, here we show that cancer cells also follow intrinsic self-organized programs that are sufficient to coordinate the spatiotemporal patterning of tumor cell states. By combining quantitative measurements in tumors and organoids with theoretical modeling, we reveal emergent mechanical gradients that orchestrate cell state transitions during colorectal tumor growth. Compression at the tumor center induces a transition from a fetal-like state into a cancer stem cell (CSC) state. The CSC compartment exhibits a characteristic size determined by tumor rheological properties. Once this size is surpassed, a translationally arrested apoptotic core emerges, triggering a shift from homogeneous proliferation to a hierarchical cell turnover. These findings uncover stereotyped programs of self-organization that likely cooperate with the microenvironment to shape tumor heterogeneity and plasticity.

ST03_Biologically-inspired melt electrowriting for the generation of highly biomimetic functional myocardium

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Text: In the heart, the specific 3D structure of myocardial layers produces an efficient ejection of blood. When myocardial infarction strikes, this architecture is disrupted, adding a disarranged contraction to the decreased availability of pumping units (cardiomyocytes, CMs). In this work, we characterize the alignment of cardiac fibers in a large animal model (pig) and employ melt electrowriting (MEW) to fabricate a bio-inspired scaffold with diamond-shaped pores. Using human induced pluripotent stem cell-derived CMs and cardiac fibroblasts, we generate human cardiac tissues with a biomimetic in-plane contraction. MEW-diamond tissues beat macroscopically for over 1 month, with significantly faster kinetics, increased force and higher conduction velocity than those based on square or rectangular pores. Our diamond design induces a specific hiPSC-CM alignment resulting in the observed in-plane contraction. Transcriptomic analysis using bulk RNA-seq reveals diamond-MEW tissues present features of maturation as compared to traditional 2D cultures. Finally, we employ the bio-inspired cardiac tissues to treat an infarction model in athymic rats, showing a significant benefit on systolic function and remodeling, tied to the presence of large grafts of human cells remuscularizing the ventricular wall. All in all, we demonstrate that the new design generates superior human cardiac tissues with therapeutic capacity.

ST04_Smed-nlk-1 controls eye size during planarian regeneration and homeostasis

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Text: During embryonic development and regeneration, the growth of any organ must be tightly regulated in order to achieve their optimal final size and become fully functional. Freshwater planarians, with their remarkable ability to regenerate any part of their body based upon the presence of adult pluripotent stem cells, provide an ideal model to study how the final organ size is regulated, in a regeneration context. Also, the fact that they are constantly growing and degrowing depending on culture conditions, allows us to study how the size of the different organs is determined under those homeostatic conditions. Here, we investigate the role of Smed-nlk-1, a nemo-like kinase, known to play a role in eye development in other organisms. Planarian photoreceptors consist in two cell types: sensory photoreceptors and a pigmented eye-cup. Functional analyses show that Smed-nlk-1 silencing results in bigger eyes both in intact and regenerating planarians. This increase in eye size is associated to an increase of the number of both progenitor and differentiated eye cell types. In addition, the absence of nlk-1 disrupts the normal proportions of the cephalic ganglia. Phototaxis behavioral assays reveal that Smed-nlk-1 RNAi planarians exhibit an increased sensitivity to light. Overall, these findings highlight Smed-nlk-1 as a key regulator of eye size and neural patterning in planarians.

ST05_The role of vascular endothelial stem cells in the regeneration of the bone marrow vascular niche

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Text: Bone marrow (BM) vascular endothelial cells serve a critical role in regulating and supporting the function and regenerative capacity of hematopoietic stem and progenitor cells. Moreover, the BM vascular network has been shown to couple angiogenesis and osteogenesis. Despite its importance in several physiological and pathophysiological conditions, the understanding of the underlying mechanisms of vascular regeneration in the BM is extremely limited. Recently, a cell population with vascular stem cell potential was identified in the liver tissue, termed vascular endothelial stem cells (VESCs). VESCs were characterized by the expression of CD157/BST1. Here we performed functional assays to measure the self-renewal and differentiation capacity of CD157+ BM endothelial cells (BMECs). By label retaining assays, we identified endothelial LRCs, mainly in the CD157+ cell compartment. Upon transplantation, CD157+ BMECs could actively contribute to the formation of new vessels in the bone marrow, indicative of their stem cell potential. Taken together, our findings support the existence of an endothelial cell population with stem cell properties within the BM vasculature, that significantly contributes to its regeneration after injury and over time.

ST06_STAU2 Coordinates Metabolism and RNA Regulation in Early Human Neurogenesis

Authors: **Sandra María Fernández-Moya**[1]*, Akshay Jaya Ganesh[2], Ana Gutiérrez-Franco[2], Natalie C. Ferreira[2], Rafael Tur-Guasch[2], Damià Romero-Moya[3], Loris Mularoni[3], Alessandra Giorgetti[3], Monika Piwecka[4], Agnieszka Rybak-Wolf[5], and Mireya Plass[2].

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Text: RNA-binding proteins (RBPs) are central to post-transcriptional gene regulation during neurogenesis. Here, we dissect the role of the double-stranded RBP STAU2 in early human cortical development using single-cell transcriptomics, gene regulatory network (GRN) inference, and cerebral organoids derived from STAU2 knockout (KO) iPSCs. STAU2 loss profoundly affected neuroepithelial cells, accelerating neuronal differentiation. STAU2 coordinated downregulation of RNA-processing and translation pathways, alongside upregulation of glycolysis, oxidative phosphorylation, and cholesterol biosynthesis, indicating premature metabolic activation. GRN analysis identified CHD2 and ARID3A as downstream mediators of STAU2 function, linking its loss to altered chromatin and transcriptional regulation towards a neurogenic fate. In parallel, STAU2 deficiency disrupted miRNA host gene expression (including MIR9-1HG and SLIT2), reinforcing combined transcriptional and post-transcriptional control. Functionally, STAU2 KO organoids showed impaired progenitor expansion, disorganized architecture, and accelerated neuronal differentiation. Together, these results position STAU2 as a key integrator of metabolic, transcriptional, and post-transcriptional programs governing early human neurogenesis.

ST07_Studying the Role of Bone Marrow Niche in GATA2 Deficiency

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Text: In inherited leukemia predisposition syndromes such as GATA2 deficiency, the transition from a germline mutation to overt malignancy is a complex, process influenced by hematopoietic stem and progenitor cells (HSPCs) and the bone marrow (BM) microenvironment (BME). While most studies have focused on HSPCs, the contribution of BM niche components, mesenchymal stromal cells (MSCs) and endothelial cells (ECs), remains largely unexplored. We hypothesize that in GATA2 patients, genetically altered niche cells actively shape HSPC clonal selection and accelerate leukemogenic progression.

To address this, we investigated how GATA2 mutations affect the BME, focusing on cellular states and stromal support of hematopoiesis. Primary MSCs from GATA2-deficient patients and healthy donors were isolated and functionally characterized. Although proliferation and differentiation capacities were comparable, GATA2-MSCs displayed reduced clonogenic potential, suggesting impaired self-renewal. Transcriptomic profiling revealed distinct clustering between GATA2 and healthy MSCs, with increased GATA2 expression and enrichment of aging-associated pathways, including DNA damage response, inflammation, and chromosome segregation defects. Functional assays confirmed elevated cytokine secretion and increased mitotic abnormalities in GATA2 MSCs. To overcome primary cell senescence and variability, we generated immortalized MSC and EC lines with GATA2 mutations, providing stable, renewable models for mechanistic studies. Finally, integration of $CD34^+$ HSPCs into 3D co-culture systems demonstrated that mutant MSCs induced greater matrix contraction and enhanced vessel formation than healthy cells, suggesting a niche remodeling effect that may influence HSPC behavior. This study establishes a 3D co-culture platform to dissect HSC-niche interactions and elucidate how genetically altered stromal cells contribute to leukemic progression in GATA2 deficiency, offering insights with therapeutic potential.

ST08_Human HSC self-renewal is maintained by fine-tuning RNA polymerase II activity

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Text: 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 for generating 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 modulates Pol II activity, we analyzed genome-wide Pol II activity by precision run-on sequencing (PRO-seq) 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 suggests that flavopiridol regulates HSC expansion by modulating Pol II activity rather than maintaining the HSC pool by inhibiting cell proliferation. Flavopiridol treatment leads to an improved engraftment of cultured-HSC long-term self-renewal upon transplantation, thus preserving HSC transcriptional program in culture.
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.

POSTERS

P01_Are Peribiliary Glands (PBG) and Pancreatic Ductal Glands (PDG) the same entity?

Authors: **Holguín-Horcajo, A.**, Fernández, A., Camats-Gangonells,L., Van Bulk, M., Thys, S., Bermúdez, M., Moro, J., Casamitjana, J., Chambers, C., Mularoni, L., Rooman, I., Jacquemin, P. , and Rovira, M.

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Text: 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, 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. 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 (such as AGR2 and OLFM4). These cells display transcriptional 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.

P02_Single-cell transcriptomics identifies necroptotic MEG3+ excitatory neurons in an iPSC model of Alzheimer's Disease

Authors: **Akshay Jaya Ganesh***, Marcel Schilling, Ana Gutiérrez-Franco, Maria Varea Martinez, Franz Ake, Loris Mularoni, Mireya Plass.

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Text: Alzheimer's disease (AD) is the most common form of dementia. While most studies of AD rely on post-mortem brain samples and/or mouse models, they have key limitations: post-mortem samples cannot capture early changes in AD, while mouse models often have limited translatability. In this context, patient-derived induced pluripotent stem cells (iPSCs) offer a powerful platform to identify early AD-linked alterations that may be better targets for therapies tied to the patient's genetic background.

In our study, we used iPSCs from multiple AD donors that were differentiated into neural fates alongside healthy controls. We used single-cell RNA-seq to study their transcriptomic profiles at multiple stages, revealing the expected cell types such as neuroepithelial cells, progenitors, astrocytes, interneurons, and excitatory neurons (EN). Gene set enrichment analysis showed AD-associated changes across multiple cell types. These include cell division, RNA processing, and metabolic pathways such as oxidative phosphorylation and cholesterol metabolism. Moreover, we found an AD-specific cluster of excitatory neurons marked by the lncRNA MEG3 (MEG3+ EN), previously shown to trigger necroptosis in human neuron xenografts modelling AD. MEG3+ EN also showed significant upregulation of multiple necroptosis-related genes compared to other EN clusters. However, the molecular mechanism of how MEG3 expression triggers necroptosis needs further exploration.

In summary, we found alterations related to AD including cell division, RNA processing, and metabolic shifts. We also found AD-specific excitatory neurons that may be undergoing necroptosis. Importantly, capturing these changes in an in vitro human iPSC model, despite partial loss of epigenetic and aging signatures during reprogramming, allows for diverse approaches to investigate their underlying mechanisms and reveal novel targets to prevent or slow down neurodegeneration.

P03_ Dissecting the interactions between leukemic cells and the aging bone marrow niche

Authors: **Alba Ferrer-Pérez*** 1; Rebecca Andersson 1; Polina Zjablovskaja 2; Francesca Matteini 1; Eva Mejía-Ramírez 1; Javier Lozano 1; Sara Montserrat-Vazquez 1; Miltiadis Tsesmelis 2; G Marka 2; Medhanie Mulaw 2; Maria Carolina Florian 1.

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Text: Acute myeloid leukaemia (AML) is a malignancy primarily affecting older adults, with TP53 mutations detected in over 30% of elderly patients, where they associate with chemoresistance and dismal prognosis. Leukemic stem cells (LSCs), which sustain AML progression and relapse, rely on signals from the bone marrow (BM) microenvironment to ensure survival and therapy evasion. However, how TP53-mutant LSCs remodel or depend on the aged BM niche remains poorly understood. To address this, we established an inducible Tp53 knockout mouse model (CreERT2 x acRFP x Tp53flox) allowing temporal control of p53 deletion. RFP+ p53KO LSCs and p53WT HSCs were transplanted into young and aged non-irradiated NBSGW mice, and BM architecture and cellular distribution were analyzed using FASTi3D whole-mount histology. To characterize niche cells interacting with transplanted LSCs, we adapted a modRNA-based sLPmCherry neighbour-labelling approach, enabling fluorescent tagging of proximal niche cells for subsequent histological validation, flow cytometric isolation, and single-cell RNA and ATAC sequencing. Imaging revealed that donor-derived cells in young recipients localized preferentially near the endosteal region irrespective of genotype, whereas p53KO LSCs in aged mice occupied distinct niches, residing further from the vasculature. The neighbour-labelling strategy successfully identified mCherry+ niche cells throughout the BM 24 hours post-transplantation, from which high-quality single-cell multiomic libraries were generated. Preliminary analyses highlight a heterogeneous population of niche-associated cells interacting with p53KO LSCs. Together, these results demonstrate that aging reshapes the BM microenvironment to alter LSC localization and niche composition, providing a foundation for identifying molecular and cellular targets to disrupt aged niche support of Tp53-driven leukemogenesis and improve therapeutic strategies in elderly AML patients.

P04_Targeting Ribosome Biogenesis as a Novel Differentiation Therapy in Colorectal Cancer

Authors: **Alessia Toccaceli,** Flavia Iannizzotto, Carme Cortina, Eduard Battle, Joffrey Pelletier.

Afiliation: IDIBELL

Text: Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related death. Hallmarks of CRC include genetic and transcriptional intercelular heterogeneity as well as cellular plasticity, which together contribute to resistance to chemotherapy. CRC relapse has been associated with LGR5+ cancer stem cells (CSCs). Preliminary data from our laboratory indicate that ribosome biogenesis and protein synthesis are main determinant of the stemness properties of the CSCs and contribute to their plasticity. In contrast, differentiating cells undergo an irreversible loss of their biosynthetic activity. Understanding the molecular mechanism underlying how protein synthesis controls the stem/differentiated switch of CSCs will be crucial to identify new possible targets for CRC therapies.

METHODS: For all our experiments, we used LS174 adenocarcinoma cell-lines, Patient-Derived Organoids (PDOs) and Patient-Derived Xenografts. Two approaches were used to inhibit ribosome biogenesis: i. Pharmacological inhibitors of RNA Pol I elongation; and ii. CRISPRi-based engineering to specifically silence rDNA gene transcription.
RESULTS: We showed that inhibiting ribosome biogenesis induces the Differentiation of CSCs across different models. We hypothesized that differentiation is not triggered by rDNA transcription itself— or nucleolar stress — but rather by a reduction in ribosome number and consequent alterions in protein synthesis, either global or specific. To test this hypothesis, we depleted ribosomal proteins or inhibited protein synthesis downstream of ribosome biogenesis. In both cases, differentiation was induced, suggesting a central role for translation in this process. Further characterization showed that inhibition of ribosome biogenesis leads to decreased levels of nascent and mature ribosomes, a reduction in the cellular biosynthetic activity and significant changes in chromatin accessibility that may be involved in cell plasticity

P05_LRRK2-G2019S Mutation Shifts Microglial Function from Degradation to Secretion via Endo-Lysosomal Dysregulation

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Text: Microglia act as central brain sentinels, maintaining homeostasis by clearing extracellular material through phagocytosis and degradation, a process that depends on a functional endo-lysosomal system, which is partly regulated by the kinase LRRK2. Our group and others have shown that the G2019S mutation in LRRK2, the most common genetic cause of familial Parkinson's disease (PD), enhances microglial phagocytic activity. However, how phagocytosed cargo is processed in mutant microglia remains poorly understood.

Here, we used human iPSC-derived microglia carrying the LRRK2 G2019S mutation and their isogenic gene-corrected controls to test the hypothesis that impaired lysosomal degradation leads to intracellular accumulation of phagocytic cargo in mutant cells, and that this dysfunction drives compensatory remodelling of the endosomal system. We found that although G2019S microglia internalize extracellular substrates such as synaptosomes effectively, they fail to degrade them, resulting in marked intracellular accumulation, consistent with autophagy–lysosomal pathway disruption.

Strikingly, mutant microglia showed a robust increase in endosomal vesicle biogenesis and trafficking toward the plasma membrane, culminating in vesicle fusion and active secretion. As a result, we observed elevated extracellular levels of synaptosomes that had previously been engulfed. Mechanistically, this shift from degradation to secretion was linked to increased levels of Rab35, a known LRRK2 substrate involved in endosomal trafficking.

Together, our findings demonstrate that the LRRK2-G2019S mutation reprograms microglial vesicle trafficking from degradation to secretion, highlighting endo-lysosomal dysfunction as a candidate therapeutic target in LRRK2-linked Parkinson's disease.

P06_Inflammatory memory primes the intestine for enhanced regenerative potential

Authors: **Borja Arozamena** *, Ilias Moraitis, Loris Mularoni, Olga Wienskowska, Jasin Taelman, Mònica Díaz, Jordi Guiu.

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Text: How do inflammation and activation of the regenerative genetic program affect intestinal epithelial cell behavior and shape responses to future injuries? Following injury, which typically induces intestinal stem cell (ISC) dead, regeneration occurs via the proliferation of surviving ISCs and via cellular plasticity through dedifferentiation of progenitors and committed cells. While inflammation and cellular plasticity are hallmarks of regeneration, its long-term effects on epithelial cell behavior are not well understood. Using single cell multiomics, we demonstrate that inflammatory exposure and cellular plasticity imprint a lasting transcriptional and epigenetic memory on ISCs and their progeny, priming them for enhanced repair after subsequent injuries. Mechanistically, we identify IFN-γ as a critical driver of this primed regenerative state. These findings uncover how prior inflammation reprograms regenerative capacity, with implications for tissue resilience and disease susceptibility.

P07_Using CRISPR barcoding to explore DNA damage responses in neurodevelopment

Authors: **Carla El Khouri González***, Paula España Bonilla, Murielle Saade, Irene Hernando Herráez.

Text: 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.

P08_Induced senescence drives regeneration in planarians

Authors: ***Daniel Moreno-Blas,** Cristina González-Estévez and Teresa Adell.

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Text: Cellular senescence, traditionally viewed as a hallmark of aging and disease, is now recognized as a beneficial process that contributes to tissue repair and regeneration in several organisms. Planarians, flatworms with remarkable regenerative capacity driven by pluripotent stem cells (neoblasts), exhibit negligible aging and are considered biologically immortal. Whether senescence occurs in planarians and contributes to their regenerative success remains unknown. Here, we investigated the presence and role of senescent cells during regeneration in Schmidtea mediterranea. Following amputation, regenerating tissues displayed induction of senescence-associated markers, including SA-β-gal activity and DNA damage signals. Functional inhibition of senescent cells using senolytic compounds impaired regeneration, leading to morphological defects and reduced tissue restoration. These findings reveal that senescence is activated upon injury and is required for efficient regeneration in planarians. Our study establishes planarians as a unique model to explore the conserved and beneficial roles of senescence in regeneration and its interplay with stem cell dynamics.

P09_Modeling severe tyrosine hydroxylase deficiency using patient-derived midbrain

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Text: Biallelic mutations in the tyrosine hydroxylase (TH) gene cause tyrosine hydroxylase deficiency (THD), a rare neurodevelopmental disorder characterized by impaired dopamine (DA) synthesis in the brain. Affected patients typically present in early infancy with motor symptoms such as hypokinesia, dystonia, tremor, and postural instability, together with neurological and behavioral issues. Currently, there are no disease-modifying treatments or clinical trials for patients with the severe form of THD who are unresponsive to L-Dopa (THDB).
To characterize disease-specific cellular phenotypes and explore potential therapeutic avenues, we generated ventral midbrain organoids (vmOs) from iPSCs derived from a THDB patient. These organoids faithfully expressed midbrain-specific factors and recapitulated hallmark features of the human midbrain. Protein-level, morphological, and functional analyses revealed multiple cellular defects, including reduced TH+ cells, impaired DA release, decreased neurite outgrowth and branching, and reduced expression of synaptic proteins. GCaMP-based calcium imaging further demonstrated diminished spontaneous neuronal activity in mature THDB vmOs and neuronal cultures, characterized by desynchronized network activations and hypoexcitable behavior. Notably, some of these phenotypes were rescued by correcting a single deleterious allele via introduction of a functional TH gene. Moreover, early treatment with the TH cofactor tetrahydrobiopterin (BH4)—a clinically used therapy—normalized neuronal morphology, enhanced synaptic integrity, and restored neuronal activity. These findings establish THDB vmOs as robust models of severe THD and highlight their value as a platform for therapeutic discovery in dopamine-deficiency disorders.

P10_Bulk Profiling Enhances Detection of Subtle Regulatory Signals in GATA2 Deficiency

Authors: **Eric Torralba-Sales*,** Joan Pera, Damia Romero-Moya, Sara Montserrat-Vazquez, Rebecca Andersson, Oskar Marin-Bejar, M Carolina Florian, Alessandra Giorgetti.

Text: Patients with GATA2 (G) deficiency are predisposed to developing myelodysplastic syndrome (MDS), which can progress to acute myeloid leukemia (AML). This evolution is often driven by additional somatic or cytogenetic alterations. Mutations in SETBP1 (S) and ASXL1 (A) are recurrently observed in pediatric GATA2 patients, yet their functional impact remains unclear. To investigate their regulatory effects, we generated a human induced pluripotent stem cell (hiPSC) model carrying combinations of G, S, and A mutations and differentiated them toward hematopoietic stem progenitor cells (HSPCs). To gain insights into the consequences of these mutations on the chromatin landscape we applied bulk ATAC-seq or SHARE-seq analysis on sorted HSPCs derived from mutant iPSCs (Parental, G, GS, GA, and GSA). Dimensionality reduction revealed a single unified cluster with no evidence of major substructures within our populations. To validate this result, we quantified the Shannon entropy of each cell, which showed that all conditions exhibited comparable chromatin accessibility profiles. We further assessed cluster robustness using the Silhouette width; the consistently low values across all conditions indicate the absence of clear structures or sub-clustering. Differential accessibility analysis comparing all conditions versus P identified 986 DAPs and 474 associated genes. To increase sensitivity, we aggregated single-cell profiles into pseudo-bulk datasets, revealing differential regulatory signatures between conditions, particularly affecting transcriptional programs linked to myeloid differentiation and chromatin remodeling. These findings were validated through bulk assays, which uncovered broader and more statistically significant changes across genotypes. Our results demonstrate that in a homogeneous population, bulk and pseudo-bulk analyses outperform single-cell resolution for detecting fine-grained regulatory effects in comparison with standard scATAC-seq approaches.

P011_ Unlocking the Haematopoietic Potential of Amniotic Fluid Stem Cells for Prenatal Therapy

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Text: The amniotic fluid is a unique source of accessible foetal stem cells with therapeutic potential. Freshly-isolated human CD117+ Amniotic Fluid Stem Cells (AFSCs) can differentiate into all haematopoietic lineages in vitro and are able to repopulate the haematopoietic system in vivo in mouse xenografts. However, AFSCs are a heterogeneous population and those with haematopoietic potential are found in small numbers within the amniotic fluid. To obtain adequate cells for therapy, AFSCs would need to be expanded. This work investigates the isolation, culture, and expansion of AFSCs to clinically relevant cell numbers whilst maintaining their haematopoietic potential. Single cell transcriptomic (scRNAseq) and proteomic (scCITEseq) characterisation of the amniotic fluid has revealed a population of cells expressing genes characteristic of foetal haematopoietic stem cells (HSCs) (HLF, SPINK2), with low levels of CD117 protein expression. Unique cell surface markers of AFSCs with haematopoeitic potential have been identified and are currently being validated. We have established a protocol to isolate and expand CD117low AFSCs in vitro and have demonstrated these cells maintain an HSC-like gene expression profile (MLLT3, SPINK2, HOXA9, RUNX1) and cell surface phenotype (CD43/45+, CD38-, CD34+, CD90+) post expansion. Whilst these results confirm AFSCs maintain some level of haematopoietic potential in culture, future work will determine this by assessing the in vivo engraftment abilities of these expanded AFSCs in murine xenograft models. If AFSCs can be isolated and expanded while retaining haematopoietic engraftment capabilities, they may serve as a prenatally accessible stem cell source for treating congenital haematological disorders before birth. This provides a new platform that shifts the established concept of autologous gene-corrected stem cell transplantation to the foetus.

P012_A Notch trans-activation to cis-inhibition switch underlies hematopoietic stem cell aging

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Text: Aged hematopoietic stem cells (HSCs) expand in clusters over time, while reducing their regenerative capacity and their ability to preserve the homeostasis of the hematopoietic system. The expression of Notch ligands in the bone marrow (BM) niche is essential for hematopoiesis. However, the impact of Notch signaling for adult HSC function and its involvement in HSC aging remains controversial. Here we show that Notch activation in young HSCs is not homogeneous, and it is triggered by sinusoidal expression of the Notch ligand Jagged2 (Jag2). Sinusoidal Jag2 deletion in young mice recapitulates the decrease in Notch activity observed in aged HSCs and alters HSC divisional symmetry and fate priming, promoting myeloid-biased HSCs (My-HSCs) expansion. Mechanistically, our data reveals that upon decreasing sinusoidal Jag2 expression, HSCs themselves up-regulate Jag2, which cis-inhibits Notch signaling, resulting in the expansion of My-HSCs and in reduced hematopoietic regeneration. Collectively, these findings identify the crosstalk between BM niche-driven and HSC intrinsic features in regulating HSC fate priming and regenerative potential and reveal an extrinsic Notch trans-activation to intrinsic cis-inhibition switch underlying HSC aging.

P013_A new hypomorphic NF2 isoform induced by antisense gene therapy is able to partially recover NF2 deficiency on NF2-related Schwannomatosis iPSC-based

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Text: NF2-related Schwannomatosis (NF2-related SWN) is an inherited autosomal dominant disorder caused by loss of function (LOF) variants in the NF2 gene and, currently, with no effective treatment. Previously, we have shown that a Phosphorodiamidate Morpholino Oligomers (PMOs) antisense can induce the skipping of exon 11 of the NF2 gene harbouring a nonsense variant, and partially rescue the NF2 phenotype in fibroblasts (1). To evaluate the potential of this therapy approach in a more appropriate cellular model, we developed an induced pluripotent stem cell-based model to study NF2 deficiency and its capacity to restore merlin function.

Using CRISPR/Cas9 genome editing, we generated NF2(+/-) and NF2(-/-) iPSC lines with truncating variants on exon 11 of NF2 gene to achieve an inexhaustible cell source that can be differentiated toward the Neural Crest - Schwann Cell axis (NC-SC), called iPSCmut11. We differentiated the obtained cell lines into SC-spheroids and treated them with PMOs after 7 days of differentiation. After 3 days of treatment, we observed a restoration of merlin levels in both NF2 +/- and NF2 -/- lines. RNA sequencing analysis revealed a recovery of key pathways associated with NF2-deficiency in schwannomas and Schwann cells, such as the mTORC pathway and cell cycle progression. Upon further analysis, we noted a trend toward the normalization of expression levels for several key genes, including ANKRD1, CCND1, and COL1A2, and a slight recovery in ITGA6 and PDGFRB as well. We confirmed the recovery of Cyclin D1 protein level, which suggests a reduction in cell cycle progression, as well as a restoration of the pS6/S6 and pAkt/Akt ratios, indicating mTORC pathway inhibition, both relevant pathways upregulated in NF2-deficient Schwann cells. We are currently investigating additional aspects as toxicity, cell proliferation and cell adhesion, among others.

P014_Deciphering the role of extracellular matrix towards amyotrophic lateral sclerosis pathogenesis

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Text: Amyotrophic lateral sclerosis (ALS) is an untreatable and progressive degenerative disease that affects motor neurons (MNs) in the brain and the spinal cord, further leading to muscle weakness, atrophy, and paralysis. Although multiple studies have also identified extracellular matrix (ECM)-related alterations among different ALS cases and experimental models at the RNA level, little is known about their contribution to the disease. We utilized detergent-based decellularization methods to isolate the ECM (dECM) from the spinal cord of wild type (WT) and an ALS animal model, SOD1G93A mice, to determine their biophysical properties and to be used as natural coatings for induced pluripotent stem cells-derived motor neurons (iPSC-MN). The isolated dECM from SOD1G93A from SOD1G93A generated alterations in morphology and electrophysiological properties of iPSC-MN. We also performed biochemical decellularization coupled to mass spectrometry (MS) that provides a high coverage of ECM protein profiles, also known as matrisome. We were able to identify biochemical changes in the matrisome of SOD1G93A animals, validated through multiple methods. We performed experiments with iPSC-MN to investigate the relevance of ALS-specific components detected in the matrisome. One of these ECM proteins, the Cathepsin D (CTSD), was found to be produced by microglia phenotypes. The overexpression of CTSD impaired the locomotion of mutant flies and the microglial CTSD promoted alterations in iPSC-MN functioning. These alterations may be attributed to PNN disassembling, which increased the susceptibility of MN to oxidative stress. Collectively, our findings not only represent the first deep ALS matrisome map but allow us to elucidate both new biomarkers, and novel cell extrinsic effectors for human ALS-associated degeneration.

P015_Using iPSC Modeling and Single-Cell Transcriptomics to Dissect Glial Roles in LRRK2 Parkinson's Disease

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Text: Parkinson's disease (PD) is a prevalent, incurable neurodegenerative disorder characterized by the progressive loss of dopamine-producing neurons (DAn) in the substantia nigra and the accumulation of misfolded α-synuclein in surviving neurons. Growing evidence implicates neuroinflammation as a critical driver of PD onset and progression, yet the precise cellular crosstalk by which reactive glia influence and modulate neurodegeneration remains poorly understood. To address this, we used a human iPSC-derived co-culture model that faithfully recapitulates key neuropathological features of PD. To incorporate PD-related pathology, we used glial cells carrying the LRRK2-G2019S mutation, a common familial PD variant, or their isogenic corrected counterparts, and co-cultured them with healthy DAn. Using this platform, we identified LRRK2-PD astrocytes as initiators of a neurotoxic cascade that drives microglial activation and DAn degeneration. We performed a cytokine multiplex array and found that the chemokine CCL2, among other proinflammatory cues, was upregulated in the co-culture when astrocytes were mutant. To address which cell type was playing a role in the CCL2 secretion we performed a single-cell transcriptomic profiling and identified that LRRK2-PD astrocytes not only express CCL2 but induce its expression in isogenic microglia. Importantly, CCL2 inhibition rescued DAn degeneration in co-culture, reinforcing the crucial role of glia-associated inflammation in PD. To unveil the consequences of the proinflammatory environment on neurodegeneration we performed gene ontology analysis that revealed dysregulated genes related to metabolism and protein translation. We now aim to integrate transcriptomic, proteomic, and metabolomic analyses to elucidate how inflammatory pathways, particularly those involving CCL2, intersect with metabolic changes to drive neurodegeneration.

P016_Cdc42 activity in Skeletal Muscle Stem Cell aging and Rejuvenation

Authors: **Javier Lozano-Bartolomé*,** Roberta Patulia, Francesca Matteini, Carolina Florian.

Afiliation: IDIBELL

Text: Aging of somatic stem cells, such as muscle stem cells (MuSCs), reduces tissue regeneration and contributes to age-related conditions like sarcopenia. MuSCs, located between muscle fibers and the basal lamina, are essential for skeletal muscle maintenance and repair. With age, their number and function decline, leading to loss of muscle mass and strength, reduced mobility, and increased mortality.
In homeostasis, MuSCs remain quiescent and activate upon injury, undergoing asymmetric and symmetric divisions to regenerate muscle fibers and maintain the stem cell pool. This balance depends on cell polarity, regulated by mechanisms including Cdc42, a small RhoGTPase. Cdc42 controls stem cell self-renewal and differentiation, but its activity increases with age, disrupting polarity and impairing function, as observed in the hematopoietic system.

Mouse models with elevated Cdc42 activity show premature aging, while systemic inhibition of Cdc42 inhibition via CASIN in aged mice improves fitness, lifespan, and MuSC function. This study examines whether these effects act directly on MuSCs or are mediated by the hematopoietic system, through the approaches below: We isolated MuSCs from aged CASIN-treated mice to assess polarity and regenerative capacity via transplantation, alongside young and aged controls. Additionally, we used an inducible Cdc42 gain-of-function mouse model to evaluate muscle regeneration and polarity after injury.

Future experiments will assess muscle function in aged mice after transplantation of rejuvenated hematopoietic stem cells, based on evidence of a potential systemic contribution to muscle rejuvenation. We will also perform scRNA-seq and SHARE-seq on MuSCs to uncover molecular mechanisms under these conditions. These insights could provide valuable information for identifying novel therapeutic targets to combat age-related sarcopenia, with the potential to improve healthspan and quality of life in the elderly population experiencing frailty.

P017_Modelling aging-associated neurodegeneration in Alzheimer's Disease using iPSC-derived co-cultures

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Text: Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and the leading cause of dementia worldwide. AD is characterized by the accumulation of beta amyloid peptides and the formation of tau aggregates, which ultimately result in neuronal dysfunction and cell death. Although AD is highly heritable, 95% of AD cases are sporadic and have late onset, implicating aging as a major risk factor. However, the fact that not all aged people develop AD suggests a complex interplay between aging and genetic susceptibility.
In this study, we present a novel in vitro model to investigate the contribution of aging to neurodegeneration in AD. We developed a protocol to generate aged, mature neurons and astrocytes from human induced pluripotent stem cell (iPSC)-derived neural precursor cells (NPCs), enabling both monoculture and co-culture systems. Neuronal differentiation was achieved via lentiviral transduction of NPCs with human Neurogenin 2 (NGN2), while astrocytes were generated by transducing Nuclear Factor I B (NFIB) and Sox9. After eight days of differentiation, cellular aging was induced using a neddylation inhibitor (NEDDi), which promotes the accumulation of misfolded proteins, impairs stress responses, and triggers mitochondrial dysfunction and senescence.

Phenotypic analysis revealed that NEDDi treatment had a more pronounced effect on AD-derived cells compared to controls. Notably, astrocytes from AD patients exhibited significant morphological alterations in both monocultures and co-cultures, whereas neurons showed subtler changes. These findings suggest that astrocytes are particularly sensitive to aging-related stress and may play a critical role in AD pathogenesis.

Ongoing work includes single-cell RNA sequencing (scRNA-seq) to dissect cell-cell communication and molecular responses to aging in this model. Our approach provides a scalable platform for studying aging-associated mechanisms in AD and identifying potential therapeutic targets.

P018_Insights into trophoblast phagocytosis in the preimplantation mammalian embryo

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Text;Preimplantation embryos develop in a dynamic environment where they may be exposed to external perturbations, including commensal or pathogenic bacteria present in the uterine cavity where they develop and implant. In our previous study, we demonstrated that trophoblast cells of the mammalian blastocyst can internalize and destroy bacteria invading the blastocoelic cavity, revealing an unexpected immunological role in the early embryo.
Here, we extend these observations by investigating the mechanistic basis of bacterial uptake by trophoblast cells in both mouse and human embryos. We explore the molecular pathways that enable epithelial cells to form phagocytic cups and mediate bacterial internalization, as well as additional properties of this tissue that facilitate microbial engulfment beyond those previously described.
This work positions the trophoblast as an active immune interface with distinctive phagocytic features and provides new insight into the cellular machinery that safeguards the preimplantation embryo in potentially non-sterile environaments

P019_Temporal profiling of the murine endometrium during postpartum regeneration

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Text: The endometrium–epithelial lining of the uterus, presents a unique model to study regeneration, as it undergoes cyclical breakdown and intensive physiological injury such as childbirth. Postpartum uterine repair depends on rapid re-epithelialization of the endometrium to re-establish homeostasis and reproductive capacity. However, the mechanisms orchestrating this process are unresolved, with conflicting evidence supporting roles for epithelial progenitors or mesenchymal-to-epithelial transition (MET). Therefore, an unbiased systematic and temporal exploration is important to define how progenitor cells, stromal remodeling, and immune interactions coordinate postpartum regeneration.

To begin addressing this gap, we characterized the structural and cellular dynamics of murine postpartum regeneration across defined stages: non-pregnant control, late pregnancy (D16.5), and postpartum days (PPD) 1, 7, and 30. Hematoxylin and eosin (H&E) staining revealed temporal changes in endometrial thickness, glandular organization, and luminal architecture. Immunostaining for EPCAM and FOXA2 further distinguished luminal versus glandular epithelial dynamics. In parallel, flow cytometry profiling quantified shifts in major populations such as epithelial, stromal, endothelial, and immune cells, highlighting dynamics in tissue composition across the postpartum regeneration axis. Finally, preliminary qPCR analysis suggests dynamic activation of candidate regenerative pathways during the early postpartum phase.

Building on these findings, our next step is to generate the first single-cell temporal atlas of postpartum uterine regeneration. This approach will delineate the transcriptional hierarchy of epithelial restoration, identify progenitor-like states, and map the signaling pathways coordinating epithelial interactions. Together, this work will provide an unbiased framework for understanding postpartum tissue repair and offer insights for regenerative biology and reproductive health.

P020_Investigating the initiation of distinct types of NF1-associated MPNSTs using an iPSC-derived neural crest-based model system

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Text: Malignant peripheral nerve sheath tumors (MPNSTs) are a heterogeneous group of aggressive soft tissue sarcomas that arise both sporadically and, with a high prevalence, in individuals with neurofibromatosis type 1 (NF1). Currently there is no effective therapy for MPNSTs beyond surgery.

MPNST initiation is highly marked by the combined inactivation of tumor suppressor genes (TSGs) like NF1, CDKN2A, PRC2 (SUZ12 or EED), and, at a lesser frequency, TP53. In our group, we performed a genomic characterization of NF1-associated MPNSTs and identified different groups bearing distinct TSG inactivation signatures. To better understand the molecular mechanisms driving the initiation of the different MPNSTs and their impact on cell identity, we used an induced pluripotent stem cell (iPSC)-derived neural crest (NC)-based model to reproduce the combinations of TSG inactivation identified.

We previously established iPSC-derived NC cell lines with NF1, CDKN2A, and SUZ12 inactivation (“3KO”) and showed that this model genuinely mimics early-stage MPNST development (Uriarte-Arrazola I. et al. 2025). Since some MPNSTs also lose TP53, we used CRISPR/Cas9 technology to deplete TP53 in 3KO cells. TP53 loss was confirmed by genetic analysis, and selected “4KO” clones underwent physiological and functional characterization. Interestingly the proliferation capacity of 4KO cells was significantly increased as compared to 3KO cells. Currently, cell identity markers and genetic stability are being tested. Furthermore, to evaluate the tumorigenic potential of 4KO cells, we generated luciferase reporter cells for real-time monitoring. These engineered cells will be engrafted in 3D into nude mice for in vivo tracking of tumor growth.
These NC-based cell lines will help define the impact of different TSG losses on tumorigenicity and cell identity and provide a preclinical platform for testing therapies and comparing treatment responses in distinct genetic backgrounds.

P021_CDK8 Inhibition for rejuvenating aged hematopoietic Stem Cells

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Aging is associated with a decline in the regenerative capacity of long-term hematopoietic stem cells (LT-HSCs), leading to various hematological disorders. The activity of HSCs is vital for the regeneration of the hematopoietic tissue, and we recently showed that improving the function of aged HSCs could represent a promising strategy to rejuvenate tissues and organs, thereby extending health span and delaying the aging process. This study focuses on the role of Cyclin- Dependent Kinase 8 (CDK8) in the process of HSC aging and rejuvenation. CDK8 is a cell cycle kinase associated with the Mediator complex and acts as a negative regulator of RNA pol II recruitment, contributing to chromatin remodeling. Using high resolution confocal 3D analysis of CDK8 in young and aged LT-HSCs, we observed significant changes in CDK8 expression and localization upon aging, showing a 96,27% increase in CDK8 expression in aged HSCs compared to young ones. Interestingly, CDK8 expression was restored to youthful levels in aged HSCs treated with a CDK8 inhibitor (CDK8i), implying a link between CDK8 activty and expression levels in HSCs. We profiled young, aged and aged+CDK8i HSCs by SHAREseq to characterize the transcriptional and chromatin accessibility remodelling associated with changes in CDK8 activity in aged HSCs. Preliminary transplantation studies of young, aged, and aged HSCs treated with CDK8i into NSGW recipient mice demonstrate restored B cell lineage differentiation and increased overall peripheral blood engraftment in mice receiving CDK8i aged HSCs compared to aged control cells. Additional transplantation assays of HSCs infected with CDK8 overexpression, CDK8 knockdown, and CDK8 death-kinase vectors are ongoing. Our findings suggest that CDK8 might play a crucial role in driving aging of HSCs and that CDK8i could be a promising therapeutic agent for enhancing HSC function and combating age-related hematopoietic decline.

P022_3D bioprinted hiPSC-derived cardiac tissues with vascular and mechanical stimulation for enhanced survival and structural Organization

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The generation of functional, clinically relevant cardiac tissue remains a major challenge in regenerative medicine. In this work, we present two complementary strategies, both aimed at enhancing the survival and functionality of engineered cardiac constructs.

Using 3D bioprinting with optimized bioinks containing hiPSC-derived cardiomyocytes and hiPSC-derived cardiac fibroblasts, we fabricated constructs that mimic the native ventricular wall.

On one hand, we developed tubular engineered cardiac tissues as a proof-of-concept for ventricular assist device substitutes. These maintained structural integrity and exhibited spontaneous contractions for up to 30 days. Immunohistochemical analysis and 3D reconstructions confirmed that perfusion is essential for homogeneous cell survival throughout the construct thickness, and that mechanical forces significantly influence hiPSC-CM organization and overall tissue function.

On the other hand, to improve tissue survival post-implantation, we implemented a vascularization strategy for large-scale cardiac constructs by incorporating adipose-derived microvascular fragments into bioprinted tissues. This approach enabled rapid vascular integration and significantly improved graft survival following transplantation into immunodeficient mice.

Knowing that cell orientation and organization can be regulated using our approach and that our strategy enables tissue survival, future work will focus on integrating these approaches to define optimal pre-implantation conditions and advance the clinical translation of engineered myocardial constructs.

P023_Single-Cell RNA sequencing Analysis Reveals Functional Heterogeneity in iPSC-Derived Hepatic Stellate Cells

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Text: Development of liver fibrosis is associated with the activation of quiescent hepatic stellate cells (HSCs) into myofibroblasts. However, little is known about the cellular heterogeneity of HSCs in their quiescent state. In this study, we employed differentiated HSCs from human induced pluripotent stem cells (diHSCs) to uncover distinct cell states and subpopulations, and to identify signaling pathways and regulatory elements involved in their specialization and heterogeneity. We applied a 12-day in vitro differentiation protocol combined with single-cell RNA sequencing (scRNA-seq), evaluated at five time points, to characterize the transcriptional and phenotypic progression of diHSCs. CellChat and SIMIC were used to analyze intercellular communication and transcription factor activity, respectively.

scRNA-seq revealed a progressive increase in cell heterogeneity and interactions over time, indicating that diversity increases as differentiation progresses. Clustering identified 13 clusters, from undifferentiated cells at day 0, through mesothelial precursors at day 4, to clusters related to mature HSCs at the end of differentiation. During the maturation phase, five distinct HSC subpopulations emerged: Stellate 1, with remodeling features; Stellate 2, proliferative; Stellate 3, profibrogenic; Stellate 4, myofibroblastic; and Stellate 5, enriched in ribosomal genes. Comparison with healthy human liver datasets confirmed that our model recapitulates in vivo-like stellate cell phenotypes. Pathway analysis of remodeling HSCs (Stellate 1) identified roles in extracellular matrix (ECM) organization and cell migration. Together with Stellate 2, they showed high communication activity. We identified MEIS1 as a predicted key transcription factor associated with specialization of the remodeling phenotype.

Our diHSC model recapitulates stellate cell heterogeneity and provides a powerful tool to investigate acquisition of a remodeling profile in the context of liver fibrosis.

P024_Modelling PCARE-Associated Retinopathy in Human RetinalOrganoids and in a Mouse Model

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Text: Retinitis pigmentosa (RP) is a common and severe retinal dystrophy characterised by the progressive loss of rod photoreceptor cells, which are responsible for night vision. The C2orf71 gene, encoding the photoreceptor cilium actin regulator protein (PCARE), is essential for the formation of photoreceptor outer segment discs. Pathogenic variants in C2orf71 have been identified in approximately 40 families or cases, mainly linked to RP type 54 (RP54), for which no cure exists. Currently, available therapies only slow the progression of the disease. To understand PCARE-related RP54 mechanisms, a human induced pluripotent stem cell (hiPSC) line was obtained from a patient with a homozygous 1 bp deletion (c.946delA; p.Asn316MetfsX5) in C2orf71 diagnosed with RP54, and the CBiPS30-4F-5 hiPSC line was used as a control. RP54-hiPSCs expressed pluripotency markers and successfully differentiated into the three germ layers. Patient and control hiPSC lines were differentiated into retinal organoids (ROs) and retinal pigment epithelial (RPE) cells to model the disease in vitro. Interestingly, patient-derived RPE cells exhibited altered RPE-specific gene expression but retained normal morphology and cilia length, suggesting PCARE mutations may impair RPE cell functionality. Early mutant ROs showed no major differentiation defects; however, later stages revealed disrupted retinal organisation, mislocalised PCARE expression, and deficient outer segment formation compared to controls.Additionally, the BC-/- mouse model, a knockout of the C2orf71 ortholog BC027072, displayed early photoreceptor layer loss at one month and severe retinal degeneration by three months, accompanied by reduced visual responses. Overall, these results demonstrate that PCARE mutations affect photoreceptor development, maturation, and survival in both in vitro and in vivo models. In future experiments, AAV-PCARE gene therapy will be used to correct pathological mechanisms in ROs and the BC-/- mouse model.

P025_Cell type proportion and gene expression perturbations in cbp-3 RNAi in S. mediterránea

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Text: Their regenerative capabilities have made planarians a well established model organism for stem cell research: Leveraging an abundant population of adult pluripotent stem cells, the so-called neoblasts, pla- narians can re-generate any part of their body upon injury. Recent findings have shown that the neoblast population does not constitute a heterogeneous group of pluripotent stem cells, but rather a heteroge- neous population of stem cells ranging from pluripotent to more specialized cells closer, and potentially pre-committed, to certain differentiated cell types. The exact mechanisms regulating maintenance and differentiation of neoblasts, however, remain poorly understood.

Here, we present our findings from RNAi perturbation experiments to knock down, in regenerating and intact planarians, Smed-cpb-3, a transcriptional co-activator that regulates gene expression by several means, including acetylating histones and other proteins. This perturbation results in the formation of a normal-sized blastema, but fails the regeneration of eyes, neurons and other terminal differentiated cell types. Instead, these blastemas are enriched in neoblasts. Thus, we postulate that cbp-3 is essential for terminal differentiation of progenitor cells.

To elucidate which cell types are affected and through what cbp-3 target genes, we performed RNA- seq (bulk and single-cell) in cbp-3 RNAi animals. Here, we present our findings regarding over- and under-represented cell types/populations and (cell type specific) differential gene expression analyses.

P026_Cardiac organoids: novel regenerative therapy for myocardial infarction

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Text: -Cell therapy for acute myocardial infarction (AMI) faces two challenges: cellular integration and arrhythmic risk. The project aims to produce human cardiac organoids (COs) at a scale and validate their safety and efficacy in a translational swine model of AMI.

-COs were derived from human induced pluripotent stem cells in a single, scalable workflow using stirred-tank bioreactors. Twelve immunosuppressed animals underwent AMI induction and were randomized into: Short-term CO (8-day follow-up, n=2), Long-term Ctrl (30-day follow-up, n=4), and Long-term CO (30-day follow-up, n=6) receiving 5-6 intramyocardial injections of 3500 COs (CO groups) or Plasmalyte (control group) 30 minutes post-AMI. CO integration into host tissue was analyzed by immunohistofluorescence (IF) at 8 and 30 days. Heart rhythm and arrhythmia were monitored for 15 days using an ECG Holter recorder. In long-term groups, cardiac function and scar size at 2 and 29 days post-AMI, and arrhythmic inducibility at 30 days were assessed via magnetic resonance imaging (MRI) and electroanatomic high-density mapping (HDM), respectively.
-IF confirmed the presence of CO cells at 8 and 30 days, indicating the survival of transplanted COs. All animals maintained a sinus rhythm without severe arrhythmias. Paired t-student test of Long-term CO-treated animals demonstrated significant improvements in left ventricular (LV) stroke volume, LV ejection fraction, scar size, cardiac output, and cardiac index. HDM revealed smaller low-voltage areas in CO-treated animals compared to ctrls (0.5 ± 0.8 cm2 vs 1.3 ± 0.3 cm2, respectively), although statistical significance was not reached.
-The scalable production of mature COs using this novel, single workflow is feasible. COs integrate rapidly into host tissue, remain viable for up to 30 days, and do not induce severe arrhythmias, demonstrating their safety. Although improvements, a larger sample is needed to confirm their full impact on cardiac function.

P027_Uncovering novel molecular drivers of zebrafish heart regeneration

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Text: Heart regeneration in adult humans is limited, making it a major challenge for the treatment of cardiovascular disease. Some species, such as zebrafish, retain the ability to fully regenerate their hearts. Understanding these mechanisms may provide insights for developing novel human cardiac therapies.
We have previously performed transcriptomic analyses of heart regeneration using single-cell and spatial resolution approaches and identified six cardiomyocyte subpopulations with distinct transcriptional states. Pairwise comparisons of differentially expressed genes between control and regenerating hearts at 7 days post-amputation (dpa) and 30 dpa focused on genes altered at 7 dpa but returning to baseline by 30 dpa, identifying 55 genes across all clusters. Four genes were selected for further study: anxa2a, mustn1b, fhl1a, and casq2. These genes were upregulated at 7 dpa and had not been previously associated with heart regeneration. Preliminary loss-of-function tests suggested they are essential for the regenerative process, as adult F0 biallelic knockout fish failed to regenerate their hearts. Based on these findings, our current goal is to characterize the role that these four genes play during zebrafish heart regeneration. For this, we are generating stable biallelic knockout zebrafish using CRISPR/Cas9 technology. Currently, we have obtained anxa2a and fhl1a mosaic fish, mustn1b heterozygous fish, and casq2 homozygous fish. Adult homozygous will be thoroughly studied for their ability to regenerate their hearts. After amputation of ~20% of their ventricles, fish will be sacrificed at 7, 14, and 30 dpa and their hearts harvested. The extent of regeneration will be evaluated by standard histological analyses, cardiomyocyte proliferation assays, and expression of early regeneration-associated transcripts by in situ hybridization. These results will advance our knowledge on heart regeneration and support the development of new approaches for human cardiac repair.

P028_Modelling human epithelial repair dynamics using 3D Epithelioids

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Text: 3D epithelioids offer an advanced platform to study human physiology and repair mechanisms in vitro during extended time periods. This model reproduces the complex cellular architecture and microenvironment of native human tissue, providing a more physiologically relevant context for regenerative medicine applications.

We established a 3D epithelioid model using primary human skin tissue. Growth and differentiation were optimised through supplementation with different factors to assess their effects on model establishment.

To evaluate the model´s potential for wound-healing study, standardised injuries were generated on mature skin epithelioids. The closure dynamics were monitored over time using live imaging phase-contrast microscopy and immunofluorescence analysis, enabling visualisation and characterisation of re-epithelisation processes in real time.

P029_ Multi-Target Modulation of Rheumatoid Arthritis via a Computationally Engineered MSC-Derived Proteins Cocktail

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Text: Mesenchymal stem cells (MSCs) have shown excellent potential in ameliorating autoimmune conditions including Rheumatoid Arthritis (RA), yet their use is limited due to issues related to cell engraftment. It is now known that the MSCs perform their activity by paracrine mechanism of the factors that they secrete, referred to as the secretome. This secretome ubiquitously secrete some proteins to exert their regenerative effect. These proteins provide a potential of replacing the use of entire secretome as a therapeutic agent. Therefore, in this study, proteome of different MSCs sources have been investigated and analyzed computationally to propose a protein cocktail against RA. Briefly, Secretome from primary five MSCs samples was collected for proteomic analysis using label free tandem mass spectrometry. 205 proteins were found to be common in all 5 analyzed samples, out of which 68 were found to interact directly with 1571 RA related therapeutic targets. After examining the involvement of these proteins with RA through functional annotation studies, 06 proteins were found to be highly involved with RA treatment. Finally, STRING and KEGG pathway analysis confirmed that these proteins target RA from different avenues, indicating the potential of these proteins in being developed into a therapeutic cocktail to ameliorate RA. This computationally proven cocktail is currently under wet lab validations to confirm its therapeutic potential. Through this bioinformatics analysis, we can streamline the development of an off-the-shelf therapeutic cocktail directed to impact the key signaling pathways associated with RA.

P30_Dormant Hematopoietic Stem Cells are established During Embryonic Development: a process regulated by Notch

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Text: Hematopoietic stem cells (HSCs) arise from the embryonic aorta, migrate to the fetal liver (FL) for maturation and expansion, and later colonize the bone marrow (BM). In the adult BM, the most immature HSCs (about 30%) remain dormant, harbor the highest long-term reconstitution potential and are activated only under stress. However, when and how HSC dormancy is established remains unknown. The Notch signaling pathway is a key regulator of HSC generation but is dispensable in the adult BM. Moreover, Notch regulates dormancy in various stem cell types.

Hematopoietic deletion of the Notch effector RBPj after HSC generation using vav1-Cre results in increased quiescence with a dormancy signature in HSCs. However, both BM and FL RBPj-deficient HSCs show impaired competitive reconstitution capacity. Conversely, Notch1 overactivation (lox-stop-lox;NICDvav1:cre) induces increased proliferation and differentiation of FL HSCs, reducing their overall frequency. Transient Notch inhibition in HSCs using gamma-secretase inhibitors or Notch receptor blocking antibodies induces quiescence in FL HSCs in vitro but not in BM HSCs. This temporal inhibition preserves FL HSC numbers in vitro, and upon competitive transplantation, these cells exhibit enhanced reconstitution potential.

These results suggested the hypothesis that dormancy could be acquired during embryonic development in HSCs and that Notch activation would prevent HSCs from acquiring dormancy.

A previous study detected quiescent HSCs in the BM at 4 weeks after birth, with some of them acquiring dormancy. Our results using label retention assays (BrdU, TTA;H2B-GFP) revealed that a subset of HSCs acquires a dormant state during development and persists into adulthood.

In conclusion, dormancy is acquired during development and Notch regulates this decision in fetal HSCs, thereby influencing their functionality in the adult BM.

P031_Modeling the Bone Marrow Niche in GATA2 deficiency

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Text: GATA2 deficiency is a rare hematological disorder characterized by immunodeficiency, bone marrow (BM) failure, and predisposition to myeloid malignancies. While most studies have focused on hematopoietic intrinsic defects, increasing evidence highlights a key role of the BM microenvironment. We aim to investigate how the GATA2 R396Q mutation affects BM niche formation and function. We established a protocol to generate BM-like organoids from wild-type (WT) and GATA2-mutant human induced pluripotent stem cells (hiPSCs). The differentiation relies on embedding cells in a collagen I/Matrigel 3D matrix mimicking the BM extracellular environment, enabling the generation of hematopoietic progenitor cells (HPCs), endothelial progenitor cells (EPCs), and mesenchymal stromal cells (MSCs). Flow cytometry showed efficient hematopoietic differentiation, with ~60% CD45+ cells in both genotypes. HPCs (CD34+CD45+) represented ~17% in both, indicating comparable potential. In contrast, EPCs (CD31+CD45−) were significantly reduced in GATA2 organoids (~4.5% vs. ~10%, p = 0.0015). MSCs (CD271+CD31−CD45−) differentiated similarly (~13% in GATA2, ~10% in WT). Immunofluorescence confirmed the presence and spatial organization of hematopoietic, endothelial, and stromal populations. This BM organoid model provides a platform to study subtle BM niche alterations driven by GATA2 mutations and the stromal–hematopoietic crosstalk in a controlled 3D context. Future work will employ high-resolution techniques to dissect transcriptional and architectural changes in greater detail.

P032_Single-cell profiling of the bone marrow niche in TP53-AML patients reveals age-associated cell-cell interaction remodelling

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Text: Acute Myeloid Leukemia (AML) incidence increases drastically after 60 years of age, and the prognosis of elderly patients is very poor, with less than 20 % of patients 65 years or older surviving for more than 1 year. Advanced age increases the incidence of TP53 mutations, a genetic lesion associated with dismal outcomes. TP53-mutated AML (TP53-AML) induces distinct biological changes, both in the leukemic stem cells (LSCs) and the bone marrow niche (BMN) that supports hematopoietic stem/progenitor cells (HSPCs). The BMN is also altered upon ageing. These age-related changes are unexplored in the context of TP53-AML, and thus, the aim of this study is to identify changes in the cell-cell interaction landscape between the BMN and LSCs in young vs. elderly TP53-AML patients. HSPCs/LSCs (CD34+) and BMN cells (CD45/CD235a/CD71-depleted) from diagnostic BM aspirates of 4 young (≥18 - ≤61 yo, avg. 58.5 yo) and 4 elderly (≥62 yo, avg. 75 yo) TP53-AML patients were profiled using SHARE-seq, to analyse the transcriptome and chromatin accessibility at single-cell resolution. After processing the sequencing data using our in-house SHARE-seq pipeline, we could annotate 37922 HSPC/LSCs and 11260 BMN cells, spanning all major tissue types in the BM. Further annotation with a mixed manual and Random Forest prediction model for LSC annotation identified a cluster of probable LSCs stemming from Multipotent progenitors (MPP). Using CellChatDB v. 1.6.1 to analyse the differential interactions between MPPs and LSCs vs. BMN cells from aged patients, as well as between LSCs from young vs. elderly patients, revealed a remodelling of the transcriptional networks involved in cell-cell interactions in the BM of elderly TP53-AML patients. While LSCs from both young and elderly patients interact with the vascular niche, the interactions between LSCs and osteo-linage MSCs increase with age, highlighting an age-related change in the BMN that may offer a new way to treat older TP53-AML patients.

P033_Defining the role of haematopoietic stem cell (HSC) metabolism and nutrient availability in HSC self-renewal

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Text: Cord blood-derived haematopoietic stem cells (CB-HSCs) are a promising alternative for treating haematological disorders, owing to their greater tolerance for human leukocyte antigen (HLA) mismatches. However, their clinical application in adults and adolescents is limited by the low number of HSCs. Attempts to expand CB-HSCs in vitro often result in functionally compromised cells. One contributing factor is the altered metabolic environment during culture, which includes elevated glucose levels compared to physiological conditions. This shift affects the epigenetic landscape, favouring histone acetylation over other acylations such as

histone crotonylation.

MLLT3, a key regulator of HSC self-renewal and engraftment identified by our lab, is an epigenetic reader of histone acylations with a specific affinity for crotonylated histones. We hypothesise that culture-induced metabolic changes lead to increased histone acetylation at the expense of histone crotonylation, thereby impairing MLLT3 function. To test this, we treated HSC cultures with crotonate, a short-chain fatty acid (SCFA), and observed increased histone crotonylation via western blot. Flow cytometry revealed that crotonate treatment enhanced the number of haematopoietic stem and progenitor cells (HSPCs). Colony-forming and T cell differentiation assays confirmed the functional capacity of the expanded cells. However, RNA sequencing indicated a markedly altered gene expression profile, with an evident bias towards upregulation of gene expression unrelated to HSC function. These findings suggest that histone crotonylation promotes functional HSPC expansion in vitro, although the resulting cells differ transcriptionally. Future xenograft studies in mice are necessary to evaluate their engraftment potential.

P034_Is Karyotyping Enough? Complementary qPCR-Based Detection of Recurrent CNVs Acquired During PSC Culture

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Text: Genomic alterations can arise during pluripotent stem cell (PSC) culture, potentially affecting their behavior and compromising pluripotency. Conventional karyotyping, although widely used, lacks the resolution to detect small but biologically relevant changes such as copy number variations (CNVs). To address this limitation, we applied quantitative PCR using the hPSC Genetic Analysis Kit (StemCell Technologies) to screen nine genomic regions frequently affected by CNVs (1q, 4p, 8q, 10p, 12p, 17q, 18q, 20q, Xp). We evaluated three embryonic stem cell (ESC) lines (ES2, ES3, ES6) and nine induced PSC (iPSC) lines (PB_iPSC_001–PB_iPSC_007; FB_iPSC_001 and FB_PBiPS_002), all previously reported as karyotypically normal. Each ESC line was analyzed at four different passages, ranging from p6 to p260, while iPSCs were assessed at early (p4–p6) and late passages (p14–p18). DNA was extracted from each passage and analyzed to detect CNVs that may have gone unnoticed by G-banding karyotyping. In ESCs, recurrent losses at 10p were observed across all three lines, and gains at 20q were detected with elevated copy number at passage 260 in ES6. In iPSCs, gains at 12p were the most frequent alteration, appearing in multiple lines, even at early passages. Additional CNVs were sporadically identified in other regions, suggesting dynamic genomic changes during culture. These findings highlight the limitations of karyotyping and underscore the importance of integrating complementary methods with higher resolution than karyotyping, such as CNV screening, into routine quality control to ensure the genomic integrity of PSCs for disease modeling and therapeutic applications.

P035_Modeling Microglial Contributions to Parkinson's Disease Using iPSC-Derived LRRK2-G2019S

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Text: Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons (DAn) in the substantia nigra and the accumulation of misfolded α-synuclein. Growing evidence implicates neuroinflammation as a key driver of PD progression, with reactive microglia (MG) and astroglia contributing to neuronal dysfunction and death. Non-neuronal cells are now increasingly recognized as active participants in PD pathology. We previously demonstrated that human microglia-like cells (hMG) derived from induced pluripotent stem cells (iPSC) carrying the familial PD mutation LRRK2-G2019S exhibit hyperreactivity to lipopolysaccharide (LPS), marked by increased cytokine secretion, compared to their respective isogenic controls. To further investigate this mechanism, we modeled LPS-induced microglial activation in iPSC-derived DAn–MG cocultures. Preliminar results showed that pharmacological blockade of the LPS receptor CD14 in LRRK2 hMG suppressed proinflammatory cytokine release and significantly rescued both neuronal loss and microglial hyperactivation. These findings suggest that targeting microglial receptor CD14 may provide a promising therapeutic strategy to attenuate dopaminergic neurodegeneration in PD.

P036_LRRK2 mutation influences Microglia–T Cell interactions leading to neuronal degeneration

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Text: Parkinson's disease (PD) is characterized by dopaminergic neuron (DAn) loss and intracellular α-synuclein (α-syn) aggregation. Growing evidence links immune activation, such as reactive microglia and lymphocyte infiltration observed in the brain parenchyma of PD post mortem brain, to PD pathophysiology. However, the dynamic crosstalk between brain-resident antigen-presenting cells and CNS-infiltrating T cells, and its contribution to PD progression, remains poorly understood. To address this, we developed an iPSC-based autologous in vitro model comprising iPSC-derived DAn and microglia from PD patients carrying the LRRK2 mutation, along with their gene-corrected isogenic controls, and peripheral T lymphocytes from the same donors. When co-cultured with LRRK2 microglia, T cells induced DAn degeneration, an effect absents with isogenic microglia, suggesting that the LRRK2 mutation may compromise microglial antigen presentation required to activate T cells, thereby promoting neurotoxicity. To probe this, microglia were stimulated with α-syn PFF and, after 24h, LRRK2 microglia displayed increased HLA-DR expression and elevated HLA-DR/α-syn complexes compared to isogenic controls, suggesting enhanced peptide presentation through impaired HLA-DR degradation, which was further confirmed by altered autophagic flux. To prove that microglia-T cells interaction causes neurodegeneration we are currently blocking HLA-DR with neutralizing antibodies in order to determine: i) DAn degeneration and survival; ii) T cells activation by measuring intracellular IFNg; iii) total cytokines production by performing a cytokines array. Moreover, we aim to modulate autophagy using rapamycin or threolase to determine whether restoring HLA-DR turnover can rescue antigen presentation and prevent neurodegeneration. This human autologous neuroimmune model reveals immune mechanisms in PD and supports LRRK2 mutations disrupting microglia–T cell communication, highlighting new therapeutic targets.

P037_Using CRISPR barcoding as a molecular clock to capture dynamic processes at single-cell resolution

Authors: **Yolanda Andres-Lopez,** Alice Santambrogio, Ioannis Kafetzopoulos, Christopher Todd, Celia Alda-Catalinas, Stephen J. Clark, Wolf Reik & Irene Hernando Herraez.

Afiliation:IBMB-CSIC

Text: Biological processes are fundamentally dynamic, yet existing methods for capturing these temporal changes are limited (1, 2, 3). 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.

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P038_ncreasing the cellular complexity of patient-derived liver organoids to model MASLD

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Background and Aims: Previous work have shown that patient-derived liver organoids (b-Orgs) recapitulate liver parenchymal cells fraction but lack non-parenchymal cells (NPCs).

In this study, we aim to develop a functional 3D multicellular liveroid by integrating b-Orgs with NPCs generated from induced pluripotent stem cells (iPSCs) for better mimicking the liver and studying Metabolic- associated steatotic liver disease (MASLD).

Methods: B-Orgs were generated from patients with MASLD. iPSCs-derived hepatic stellate cells (diHSCs) and macrophages (diMacs) were generated following previously described well-established protocols. A new protocol was optimized to generate iPSC-derived endothelial cells (diECs). qPCR, immunofluorescence and functional studies were used to characterize the liver cells. Liveroid were generated with 2:1 cell ratios between b-Orgs and NPCs. Oleic, palmitic acids, and TGF-β were used to for 3- or 7-days model MASLD.

Results: diECs acquired endothelial phenotype expressing CDH5, CD31 and CD34 at gene and protein level and showed the capacity to form tubes under an angiogenic assay. The coculture of b-Orgs and diHSCs— Liveroids were formed at day 2 and increased compaction over time. At days 7 and 14, the expression of ALBUMIN and CYP3A4 were upregulated, indicating that the liveroid promoted cell maturation. In MASLD model, the liveroid showed increased lipid accumulation by Nile Red, a tendency to altered lipid metabolism (PLIN2), increased inflammation (CCL2) at gene expression level. 4 cell types (b-Orgs, diHSCs, diECs, diMacs) of liveroids were generated. Compact liveroids was formed at day 4 in which

hepatocytes were distributed at the outer zone while diHSCs and diECs were located inside and diMacs were scattered throughout the structure.

Conclusions: Here we describe a new methodology to increase the cell complexity of patient-derived organoids by incorporating NPCs in 3D liveroid configuration.

Liveroid are a promising tool to bet.