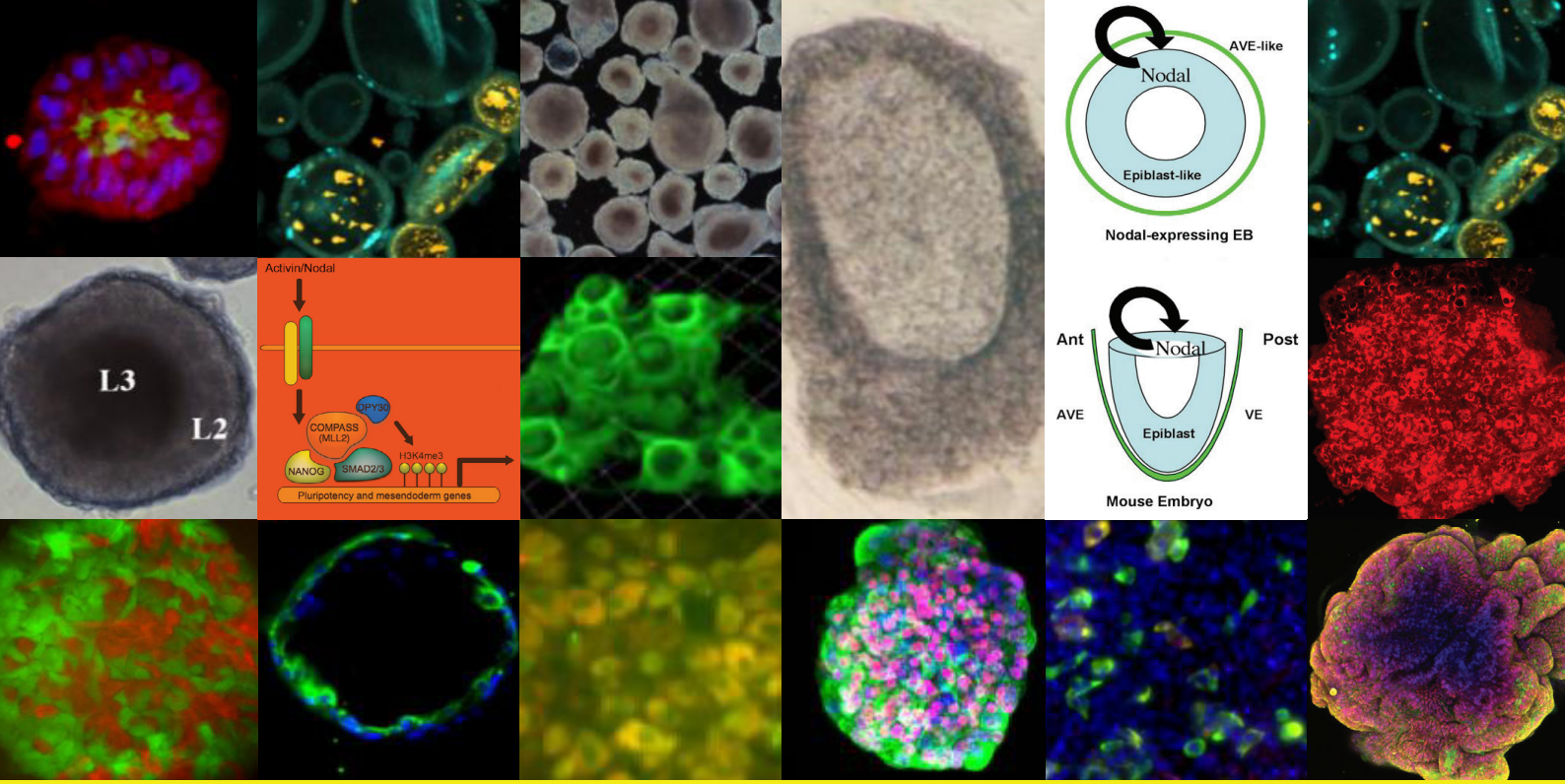

SYMPOSIUM

Human Pluripotent Stem Cells

PAST, PRESENT & FUTURE

The Vallier lab is organising a symposium to celebrate the past 25 years of research on human pluripotent stem cells. The symposium will explore all the different aspects of research on human pluripotent stem cells from basic understanding of pluripotency to clinical applications in cell based therapy and disease modelling.

The event will focus on recent progress but also on the challenges which remain to be addressed for delivering the clinical promises of hPSCs. Experts from diverse fields will share their experience and the symposium will aim to facilitate interactions between participants.

SPEAKERS:

Fabian Bachinger - Berlin Institute of Health at Charité (BIH)
Alessandro Bertero - University of Turin
Giovanni Canu - University College London
Carla Frau - Berlin Institute of Health at Charité (BIH)
Charlotte Grey-Wilson - University of Cambridge
Nick Hannan - University of Nottingham
Sasha Mendjan - IMBA
Carola Morell - IRCCS Humanitas Research Hospital
Anna Osnato - KU Leuven
Tamir Rashid - Imperial College London
Floris Roos - University of Cambridge
Foad Rouhani - The Francis Crick Institute
Irene Talon - Max Planck Institute for Molecular Genetics
Adrian Teo Kee Keong - National University of Singapore and IMCB, A*STAR
Charis Segeritz-Walko - Novo Nordisk
Marta Vila Gonzalez - University of Balearic Islands
Loukia Yiangou - Leiden University Medical Center
Dylan Liabeuf - Universitätsklinikum Carl Gustav Carus Dresden

WHERE?

Harnack-Haus
Tagungsstätte der Max-Planck-Gesellschaft
Ihnestr. 16-20, 14195 Berlin

Tuesday, 16th of April 2024

From 8:30 am

BIH Berlin Institute of Health @Charité

EINSTEIN Foundation.de

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SYMPOSIUM

Human Pluripotent Stem Cells

PAST, PRESENT & FUTURE

PROGRAM

Tuesday, 16th of April 2024

8h30 – 9.15 **Welcome**

9.15 – 9.30 **Introduction** by Ludovic Vallier

Session 1 9.30 – 10.30: *Cardiac cells with heart.*
Speaker 1: Sasha Mendjan
Speaker 2: Alessandro Bertero
Speaker 3: Loukia Yiangou

10.30 – 11.00 **Coffee break / poster session**

Session 2 11.00 – 12.00: *Pancreas, lung, gut, and more.*
Speaker 2: Adrian Teo
Speaker 3: Marta Vilà González
Speaker 4: Nick Hannan

12.00 pm – 1.30 pm **Lunch / poster session**

Session 3 1.30 – 2.30 pm: *Modelling human development in vitro. Maybe.*
Speaker 1: Anna Osnato
Speaker 2: Giovanni Canu
Speaker 3: Carola Morell

Session 4 2.30 – 3.30pm: *Liver diseases. What's new?*
Speaker 1: Dylan Liabeuf
Speaker 2: Foad Rouhani
Speaker 4: Carla Frau

3.30 - 4 pm **Coffee break / poster session**

Session 5 4.00 – 5.00 pm : *Modelling human liver development in vitro. We can do it.*
Speaker 1: Charlotte Grey-Wilson
Speaker 2: Floris Roos
Speaker 3: Irene Talon

Session 6 5.00 – 6.00 pm: *Cell based therapy in liver disease. When and how?*
Speaker 4: Charis Segeritz-Walko
Speaker 5: Tamir Rashid
Speaker 6: Fabian Bachinger

6.30 pm **Dinner**

SYMPOSIUM

Human Pluripotent Stem Cells

PAST, PRESENT & FUTURE

ABSTRACTS | TALKS

Human beta cell genes and diabetes predisposition
|Adrian Teo

Diabetes is a debilitating chronic disease that is spiralling out of control. Fundamentally, the progressive failure of pancreatic beta cells results in decreased insulin secretion, ultimately giving rise to hyperglycaemia and overt diabetes. Unfortunately, the lack of access to developing human pancreatic cells impedes a thorough understanding of the processes that account for human beta cell failure. The availability of patient-derived human induced pluripotent stem cells (hiPSCs) and the ability to differentiate them into pancreatic islet-like organoids in fully-suspension cultures now provide an invaluable opportunity to study these important processes in vitro. Here, I will highlight our years of efforts in using diabetes patient-specific hiPSC-derived pancreatic islet-like organoids harbouring defined gene mutations or variants to study human diabetes disease mechanisms. In addition, these pancreatic islet-like organoids are also increasingly transplanted in vivo for the purpose of beta cell replacement therapy in diabetes patients. Collectively, these approaches exemplify the increasing value of human organoid research with immense potential to model human diseases and even for regenerative medicine.

Cardioids unravel human heart development and defects
| Sasha Mendjan

The number one cause of human fetal death are defects in heart development. Because the human embryonic heart is inaccessible and the impacts of mutations, drugs, and environmental factors on the specialized functions of different heart compartments are not captured by in vitro models, determining the underlying causes is difficult. Here, we established a human cardioid platform that recapitulates the development of all major embryonic heart compartments, including right and left ventricles, atria, outflow tract, and atrioventricular canal. By leveraging 2D and 3D differentiation, we efficiently generated progenitor subsets with distinct first, anterior, and posterior second heart field identities. This advance enabled the reproducible generation of cardioids with compartment-specific in vivo-like gene expression profiles, morphologies, and functions. We used this platform to unravel the ontogeny of signal and contraction propagation between interacting heart chambers and dissect how mutations, teratogens, and drugs cause compartment-specific defects in the developing human heart.

Unraveling the role of Wnt signaling in liver organogenesis using human hepatoblast organoids
| Irene Talon

In this work we took advantage of human hepatoblast organoids (HBOs) to investigate the importance of Wnt in self-renewal and cell fate decisions. Briefly, our results showed that Wnt signaling acts to preserve the proliferative capacity of hepatoblasts without being sufficient to maintain their bipotent state. These results could help to further understand the role of Wnt in the adult liver homeostasis and in disease.

SYMPOSIUM

Human Pluripotent Stem Cells

PAST, PRESENT & FUTURE

ABSTRACTS | TALKS

A novel organoid model of human liver bud development and hepatoblast specification
| Charlotte Grey-Wilson

Understanding the liver in health and disease is vital for the development of new therapies against liver diseases, such as the production of hiPSC-derived hepatocytes for regenerative medicine. For that, understanding early liver organogenesis is pivotal since it will provide essential information about liver cells specification and their functional maturation. However, early human organogenesis remains difficult to study due to technical and ethical challenges. To address these major questions, we have developed a novel 3D culture system allowing for differentiation of human induced Pluripotent Stem Cells (hiPSCs) into self-organising human Hepatic Bud organoids or HepBuds. This method relies on a heterogenous differentiation containing anterior/posterior foregut cells, cardiomyocytes and endothelial cells as shown by immunostaining and singe cell transcriptomic analyses. After 3D seeding, these populations self-organise into complex budding structures which recapitulate the initial organogenesis of the liver bud. This includes a “bud” containing hepatoblast (ALB+AFP+HNF4A+) and a “trunk” containing hepato-pancreato-biliary progenitors (KRT19+CDX2+PDX1+HNF1B+) and extra-hepatic cholangiocytes (ACE2+MUC13+). Thus, our HepBud organoids are composed of the multiple cell types produced during the early organogenesis of the hepato-biliary system. Finally, we decided to demonstrate the functional relevance of these organoids by deriving hepatoblast organoids (iHBOs). For that, HepBuds underwent serial passaging in culture conditions supporting hepatoblast self-renewal. We observed the formation of homogenous self-renewing organoids expressing hepatoblast markers. These iHBOs were able to self-renew for an additional 8 passages while maintaining their morphological characteristics and displayed a capacity to differentiate into intra-hepatic ductal plate cholangiocytes and hepatocytes. Critically, we also show that hiPSC-derived hepatoblast share many features with their primary counterparts. Taken together, these results show that our model of liver bud development provides a new platform not only to study hepatobiliary lineage specification in vitro but also to produce cell types with a clinical interest.

Insights into liver regeneration using spatial genomics
| Foad Rouhani

Many epithelial tissues share properties of cell turnover in the healthy state and the ability to enact programs of repair and regeneration following injury. Indeed, it is now known that skin and oesophagus, amongst others, are a patchwork mosaic of epithelial clones, each with distinct genetic profiles. Certain clones of cells acquire driver mutations which are positively selected for, which enable better survival and to out-compete their neighbours. The liver is well known for its regenerative capabilities and therefore we explored the clonal competition which results in the complex genetic architecture of nodules in the cirrhotic liver. We discovered novel driver genes with evidence of convergent evolution whereby there are independent mutational events in the same gene and occasionally in the same genomic position, across disparately located hepatocytes and even between patients.

Matching cell therapy to different diseases in hepatology
| Tamir Rashid

SYMPOSIUM

Human Pluripotent Stem Cells

PAST, PRESENT & FUTURE

ABSTRACTS | TALKS

A single-cell RNAsequencing organoid atlas for studying human foetal development | Floris Roos

Studying human organogenesis is a formidable challenge due to the absence of suitable in vitro systems. While organoids have emerged as a promising option, the majority of existing models are derived from adult-, rather than foetal tissue. In this study, we sought to overcome this limitation by developing a novel protocol to culture organoids derived from foetal organs at various stages of development. Specifically, organoids were successfully generated from the lungs, heart, intestine, gallbladder, liver, and pancreas at gestational age 8 and 12 weeks, thereby establishing the first organoid atlas of human developing organs. Subsequently, all organoids were characterised using single-cell mRNA sequencing and key markers were confirmed in vivo and in vitro using immunofluorescence. By comparing lung organoids with available data from primary cells of developing lungs, we reconfirm the ability of our organoids to faithfully recapitulate their tissue-of-origin. Interestingly, we show that foetal lung development, and liver-cell differentiation processes can be recapitulated in vitro, and that foetal lung progenitor cells can be differentiated to mature cell-types. Moreover, by focussing on the biliary tree, we show that biliary epithelial cells (cholangiocytes) from the gallbladder quickly acquire mature functionality and transcriptome, while intrahepatic cholangiocytes exhibited significant immaturity even up to 21 weeks after gestation. In conclusion, our findings highlight the unique capabilities of organoids for investigating human organogenesis. Organoids can serve as a comprehensive platform for studying developmental processes and exploring functional aspects. Therefore, our results provide the basis to incorporate organoids as a valuable addition to primary tissue in human cell atlases.

HiPSC-derived airway epithelial cells as a tool to study pulmonary ionocytes: unveiling their function, disease implications and potential for cell-based therapies | Marta Vilà González

Pulmonary ionocytes are a rare population of cells in the airway epithelium whose function is not fully understood. As their name suggests, they express a high number of ion transport channels and they have been reported to play a role in regulating airway surface liquid homeostasis. However, whether they interact with other cell types or have other functions in the epithelium is still unknown. In addition, their potential role in cystic fibrosis (suggested by their high expression of the protein mutated in the disease) is still under investigation. The study of airway epithelium is challenging due to the limited availability of primary tissue, and most studies on ionocytes have been based on animal models and modified primary cell lines, both of which have limitations in terms of representativeness and appropriate controls. We have developed a hiPSC-based model of the airway epithelium that includes abundant cells such as basal, secretory and ciliated cells, as well as the rarer pulmonary neuroendocrine cells and ionocytes. We have used this model to study the role of ionocytes in the airway epithelium through loss-of-function assays, to investigate their potential implications in cystic fibrosis and to explore their engraftment capacity for future cell therapy applications.

SYMPOSIUM

Human Pluripotent Stem Cells

PAST, PRESENT & FUTURE

ABSTRACTS | TALKS

Hepatoblasts commitment during liver organogenesis
| Carola Morell

Background: The global incidence of chronic liver disease is on a steep rise. Hepatic injuries are often life threatening, with liver transplantation being the only option for end stage forms. Understanding how human liver develops will help to produce alternative treatments, by providing new knowledge on the mechanisms regulating disease onset and liver regeneration. However, human organogenesis studies have been profoundly impacted by technical and ethical challenges. We propose to address this gap by combining state-of-the-art single cell analyses methods and in vitro validations to define the developmental mechanisms directing generation and maturation of liver epithelial cells.
Methods: Human hepatic tissues were collected at various developmental time points, ranging from 5 to 21 post conceptional weeks (pcw). Organoids cultures were derived at multiple stages, and characterized for their maturation degree and differentiation potential. In parallel, we developed a method to perform single nuclei transcriptomics on HNF4a+ve enriched population, to investigate the mechanisms by which hepatocytes progressively acquire metabolic functions in organogenesis.
Results: Using our previously established hepatoblasts culture conditions, we successfully derived organoids from all samples, up to 21pcw. Interestingly, organoids survival and differentiation potential were reduced in cultures generated from older stages (>12pcw). These observations suggest that organoids derived at different stages of organogenesis could represent different functional states. Accordingly, single nuclei transcriptomics in the developing liver revealed a dynamic signature in hepatoblasts, with age emerging as the main driver. Indeed, the increase of specific maturation and functional markers (ABCB11/ALB/CYP3A5/CYP3A7) was reported in older samples. Taken together these results suggest that hepatoblasts lose their plasticity while becoming more specialised.
Conclusion: Throughout foetal development, hepatoblasts progressively differentiate into hepatocytes. This process can be captured through organoids derivation, while single cell transcriptomic analyses could reveal the molecular mechanisms driving this functional maturation. This knowledge represents an important step toward developing new strategies to control liver regeneration following a natural path of development especially in the context of chronic disorders.

| Charis Segeritz-Walko

For over a century, Novo Nordisk has successfully leveraged its core capabilities in developing leading biologics to provide treatments for those living with serious chronic diseases. Novo Nordisk continuously evolves and strengthens its capabilities through investments also in advanced technology platforms, such as cell therapy. To that end, Novo Nordisk aims to develop and deliver transformative cell therapy treatments with sustainability as a guiding force and is partnering with global innovators to accelerate the development of cell therapy breakthroughs at scale. By partnering with academia, biotechs, and device experts, Novo Nordisk can share knowledge, data, and capabilities in ways that bring us closer to developing potential cell therapies for people living with serious chronic disease. This talk will provide an overview of Novo Nordisk's strategic focus on developing cell therapies for patient living with type 1 diabetes, chronic heart failure and Parkinson's disease.

ABSTRACTS | TALKS

Molecular mechanisms of human ductular reaction during progression of chronic liver disease and liver cancer

| Carla Frau

Introduction/Background: Ductular Reaction (DR) is a hallmark of chronic liver diseases and represents a dynamic process characterized by the hyperplasia of the intra-hepatic biliary tree following hepatic injury. Increasing evidence associates DR with liver fibrosis, liver regeneration, and liver cancer. However, the molecular mechanisms involved and the consequences for disease progression remain enigmatic. The dearth of knowledge is especially notable in humans, as most insights have been derived from animal models which replicate only in part the pathophysiology of human disease.
In this study, our primary objective is to unravel the mechanisms governing DR during Metabolic dysfunction-associated fatty liver disease (MAFLD). More precisely, we want to elucidate the interplays between cholangiocytes, the main cell type of the biliary epithelium, and their surrounding niche in the context of disease progression and cancer development.
Materials and Methods: To study DR in humans, we have generated the largest single-cell map of the human liver during Fatty Liver Disease progression (n=110 patients), unraveling new markers for DR. Then, we explored the significance of these markers using an in-vitro human model for DR by establishing primary cultures of human intrahepatic cholangiocyte organoids.
Results: Our single-nucleus RNA Sequencing unraveled that active cholangiocytes in cirrhotic livers exhibit markedly elevated levels of genes encoding for ECM protein receptors which prompted us to hypothesize that the interaction of cholangiocytes with their surrounding ECM could be a pivotal mechanism for their activation. By culturing patient-derived cholangiocyte organoids in hydrogels containing defined matrix proteins we were able to mimic DR in vitro and elucidate downstream mechanisms. In addition, we found that inhibiting specific ECM receptors leads to the impairment of cholangiocyte growth and reverses DR in vitro, thereby confirming the functional importance of these interactions. Finally, we found that a specific ECM-rich matrix can accelerate the growth of liver cancer organoids reinforcing the possibility that activation of cholangiocytes through DR could contribute to tumorigenesis.
Conclusions: Our results validate that ECM modification during fibrosis and cancer serves as a permissive component for cholangiocyte activation and thus DR. These findings reveal key biology of DR in humans, providing valuable scientific and clinical insight, and potential avenues for therapeutic interventions.

ABSTRACTS | TALKS

Modelling early human development with blastoids |Anna Osnato

The first steps of embryo development are when most pregnancies fail: 70% of human conceptions end before reaching full term, the majority before implantation. Despite recent efforts in precisely identifying the series of events driving one single cell to form a full embryo, which genes and processes coordinating human embryogenesis remains largely unclear. Unravelling human specific mechanisms has important implications, including gaining insights in pregnancy failures and improving in vitro fertilisation. Recently, an ethical opportunity to complement human embryo research has been developed and named blastoids. Blastoids closely resemble the spatial organisation of the human pre-implantation embryo and represent a scalable model to functionally investigate early human development. Leveraging this, we are currently investigating how chromosomal instabilities impact development, in a time window where genomic abnormalities are frequently acquired and tolerated in the embryo. Taking advantage of the scalability of the system, we are also exploring how transcription factors regulate cell fate decisions by setting up a perturbation screening platform. Taken together, we show that stem cell derived embryo models represent an ethical alternative to complement human embryo research and can offer a valuable platform to investigate early development, despite their limitation.

Haematopoietic development: from the dish to the embryo, and back | Giovanni Canu

Every year more than 40,000 people in Europe require a haematopoietic stem cell (HSCs) transplant to treat end-stage diseases such as blood cancers. Nevertheless, immunologically matched donors are often unavailable, and current methods can only achieve modest expansion of donor HSCs in culture before self-renewal and engraftment ability are lost. Attempting to overcome these limitations, human pluripotent stem cells (hPSCs) have been used to generate haematopoietic progenitors in vitro and to study mechanisms that control early haematopoietic development. Using this approach, we previously analysed transcriptional changes associated to the endothelial-to-haematopoietic transition and showed that cell cycle progression is a driver of haematopoietic differentiation. Nevertheless, to date it has not been possible to differentiate cells equivalent to HSCs, i.e. capable of self-renewal and long-term engraftment. This is due to our limited understanding of how HSCs are produced and maintained in vivo. After their initial generation from the embryonic dorsal aorta, HSCs migrate to the foetal liver (FL), which constitutes the main haematopoietic organ until birth. Our knowledge of the FL as a haematopoietic niche remains rudimentary. Here, we report of a novel population of embryonic haematopoietic cells that also seed the FL. Genetic lineage-tracing in the mouse embryo suggests that this population originates from paraxial mesoderm, a tissue that was not previously shown to possess haemogenic potential. These cells have hallmarks of haematopoietic progenitors and the ability to produce erythroid and myeloid cells both in vitro and in vivo. However, their importance for supporting HSC development is unclear. Our findings highlight how the FL environment for HSCs maturation and self-renewal remains poorly understood. Investigating the cell populations composing the FL niche, along with the molecular signals and cellular crosstalks supporting foetal HSCs, should advance current efforts to recapitulate haematopoietic development in vitro using hPSCs to produce haematopoietic cells for clinical applications.

ABSTRACTS | TALKS

Development of a universal, hIPSC derived, immune competent multi-tissue organoid platform for developmental and disease modelling applications | Nick Hannan

Our understanding of human disease is constrained due to reliance non-human disease models. Animal and ex-vivo human disease models have provided important insights, however translational application remains challenging due to species and donor-to-donor variability. Stem-cell-derived tissues are important model platforms enabling fine tuning of genetic, cellular, immune and ECM parameters that mimic normal and diseased tissue, however currently available approaches often utilise animal derived and undefined components that limit their application. Here we develop a serum and xeno-free platform for production of stem-cell-derived, self-organising 3D multi-tissue organoids. To create the organoids, hIPSC derived endothelial, fibroblasts, macrophages and dendritic cells were generated using a unified differentiation platform consisting of a single media formulation and a synthetic hydrogel and combined with mature, hIPSC-derived parenchymal cells at tissue specific configurations. All cells types remain present in the organoids for more than 3 weeks and the initial cellular composition is stable over the same time period. Our platform allows precise configuration of immune competent, tissue-specific organoids, that are tuneable to a range of different disease parameters which will facilitate experimental insight into mechanisms of human disease with accuracy and precision that is difficult to achieve using current animal and ex-vivo human models.

Single cell transciptional perturbome to understand congenital heart disease pathogenesis using human induced pluripotent stem cell-derived cardiac organoids |Alessandro Bertero

Functional genomics screens in pluripotent stem cell (PSC) models offer immense potential yet are plagued by several specific challenges. These include high sensitivity to genotoxic nucleases, genetic and epigenetic clonal variability, asynchronous and heterogeneous differentiation, unstable transgene expression, and limited susceptibility to transfection and transduction after differentiation. Screens in emerging PSC-based organoids compound these challenges with increased cell type diversity and morphological constraints. To bridge this technological gap we developed iPS2-seq: iPS-optimized inducible Postranscriptional Silencing in pool deconvoluted by single cell sequencing. This method allows phenotype-agnostic screens in PSCs and their derivatives through mRNA-depleting, clonally controlled, single cell aware, isogenic engineered, and stage specific loss-of-function (LoF) perturbations. iPS2-seq is compatible with both commercial microfluidics and homebrew split-pool scRNA-seq protocols, enabling a variety of screen designs in terms of scale, cost, and input material. We demonstrate this technology by studying congenital heart disease-associated genes in both monolayer cardiomyocytes and cardiac organoids derived from human induced PSCs (hiPSCs). iPS2-seq robustly assigns unique perturbations to >75% of analyzed cells, and identifies and controls for confounding effects arising from molecular cloning inaccuracies and iPSC clonal variability. Pseudotime analyses allows ranking of LoF perturbations by their effect on cardiomyogenesis. Clone- and induction-matched enrichment analyses in organoid cell type clusters identify genes involved in developmental bifurcations and highlights 3D culture-specific functions. In all, the iPS2-seq platform promises to standardize, strengthen, and democratize access to functional single cell genomics in and beyond hPSC-derived organoids.

ABSTRACTS | TALKS

The heart of the matter: Using 2D and 3D hiPSC-derived systems to model and understand cardiac disease
| Loukia Yiangou

hiPSC-derived cardiovascular cell types are widely used for cardiac disease modelling and drug screening studies. I will give an overview of the ways through which we utilize 2D and 3D cardiac models in our lab to understand cardiac pathologies and uncover disease mechanisms. Firstly, we developed optogenetic tools for functional readouts in hiPSC-derived cardiomyocytes, specifically voltage and calcium handling properties, which we exploit for disease modelling and drug screening studies. I will then describe our efforts to compare 2D and 3D hiPSC platforms to study complex cardiac diseases such as hypertrophic cardiomyopathy. We used hiPSCs from an HCM patient carrying the sarcomeric MYBPC3-2373insG mutation and investigated the ability of several independent hiPSC-derived cardiomyocyte models to capture disease phenotypes, including 3D engineered heart tissues to measure direct force, cardiac microtissues with controlled multicell-type composition and cardioids, that capture heart development. In mutant 3D models, we observed altered contractile properties and calcium transients compared to isogenic controls, showing the hypocontractility evident in many patients. 2D cultures showed altered metabolism in mutant hiPSC-cardiomyocytes, while forced alignment revealed sarcomere disorganization in mutant hiPSC-cardiomyocytes compared to isogenic controls. Overall, our study indicates which of several options to model HCM phenotypes are best suited to study contractile dysfunction, calcium handling abnormalities and metabolic or structural changes, setting the stage to further explore HCM disease mechanisms. Lastly, we developed a cardiac fibrosis model to uncover novel therapeutic targets for this severe condition. Cardiac fibrosis manifests in the case of myocardial infarction, aging as well as in the presence of additional pathologies such as genetic cardiac diseases, increasing the risk for heart failure for these patients. I will explain how we are generating a cardiac fibrosis model to uncover mechanisms of fibrosis development.

Large scale production of hiPSC derived hepatocytes for cell based therapy
|Fabian Bachinger

In recent years, researchers have been developing a bridging therapy for Acute Liver Failure based on encapsulating hepatocytes into allogenic alginate beads which are then injected into the peritoneal space assisting liver function until either the liver can regenerate, or an organ can be found for transplantation. As primary hepatocyte availability is very limited, human induced pluripotent stem cells (hiPSCs) could provide an alternative source for the production of large quantity of hepatocytes. In this work, we have taken an advantage of a Forward Programming approach which relies on the overexpression of hepatocyte nuclear factors and culture optimizations to create a rapid, simple process for producing hepatocytes with mature characteristics. For smooth transition into an industrial setting this process has further been adapted into GMP-like conditions with no loss of quality due to change in reagents. Further the differentiation protocol has been adapted into a suspension 3D bioreactor system that allows large scale production of cells expressing key maturity markers comparable to priamry hepatocytes.

ABSTRACTS | POSTERS

Establishment of a large-scale organoids biobank from liver cancer patients | Laura Valentina Avila Rondon

Liver cancer, a prominent cause of cancer-related deaths globally, demands the creation of new treatments for hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), and mixed HCC/CCA, as there are currently no available cures. To address this major challenge, our group at the Berlin Institute of Health is establishing a unique biobank of liver tumoroids from patients at Charité – Universitätsmedizin Berlin. This involves creating paired organoids from tumoral and non-tumoral regions, that are characterized using genotyping/karyotyping, RT-qPCR, and immunofluorescence and then compared with the original tumor through single-nuclei RNA sequencing. Thus, the biobank aims to provide insights into cellular plasticity in liver cancer cells, offering a platform for studying the disease and identifying potential therapeutic agents.

| Amar Azad

There is considerable patient-patient variability within atopic diseases emphasising the need for human autologous disease models which accurately reflect the condition patients face. This is especially important as many patients with atopic dermatitis develop allergic asthma later in life. These models can be used in order to improve patient stratification and disease course management. While progress has been made in the field of atopic dermatitis, complex 3D iPSC-derived skin equivalent models are still lacking, while iPSC-derived allergic asthma models have considerable limitations. We aim to address the need for improved autologous disease models.

| Silvia Becca

Leveraging on hiPSCs-derived 3D cardioids, we investigate the mechanistic basics of topological activation of cardiac genes, to determine whether the alteration of this process affects cardiac development and result in congenital heart disease phenotypes. We are focusing on the role of GATA4, found to be mutated in CHD patients, and CTCF. We found that CTCF binding and the resulting intragenic looping poses a repressive barrier to premature upregulation of cardiomyocyte genes that must move from the inactive (B) to active (A) compartment during cardiogenesis. On the other hand, GATA4 acts as a pioneer factor promoting the unwinding of chromatin at the same loci.

Determining the role of transcription factors involved in human liver cell plasticity | Marta Cagna

Although liver regeneration is well-established in the context of acute liver injury, its relevance for chronic diseases remains to be demonstrated. We mapped at the single cell level the progression of metabolic dysfunction-associated steatotic liver disease (MASLD) and then investigated potential regenerative events. Our analysis revealed that hepatocyte and cholangiocyte can transdifferentiate into each other during chronic liver injury. Our data uncover in part the molecular mechanisms controlling regeneration during chronic liver disease and thus path the way to develop new therapies promoting tissue repair during chronic injury.

ABSTRACTS | POSTERS

| Robert Opitz

We use hiPSC-derived cerebral organoids (hCOs) to study the role of thyroid hormones (TH) during early human cortex development. At several stages during a 10-week-long culture protocol (recapitulating GW5 - GW16 cortex development), hCO tissue is analyzed by IF for TH effects on cytoarchitecture and by scRNA-seq for cell type-specific TH effects on gene expression. Our studies detected > 1,600 TH-responsive genes in developing hCOs, revealed a TH-dependent up-regulation of OXPHOS activity and identified strong effects of TH on the abundance of upper layer neurons.

Generation of hepatic organoids from primary hepatocytes
| Carmen Ortuño Costela

Liver disorders account for more than 2 M deaths annually in the world. However, the only treatment available nowadays is liver transplantation, with many associated drawbacks. Cell therapy could depict an ideal alternative, employing mature hepatic cells that could recover the functionality of the damaged organ. Primary hepatocytes have classically been used for cell therapy applications, but they cannot be cultured extensively in vitro, losing their functionality and morphology. In recent years, different groups have described in vitro conditions that prompt a proliferative state of hepatocytes, enabling their culture. We have performed a screening of different conditions in 3D to identify the best settings and media to culture primary hepatocytes in vitro. To do so, we have employed human primary hepatocytes derived from three different donors, and we have identified some defined conditions that allow the maintenance of hepatic organoids up to 10 passages. They also express hepatic markers like ALB and HNF4a. The development of reproducible ways to derive hepatic organoids from primary hepatocytes can have important implications in cell therapy and disease modelling.

The role of cellular plasticity in human liver cancer
| Katja Püstow

Liver cancer, often linked with MASLD, lacks effective treatments. Mechanisms underlying tumorigenesis remain unclear, including the origin of iCCA and HCC. Our group employed snRNA-Seq on MASLD patients' liver biopsies, revealing bi-phenotypic cells from cholangiocytes transdifferentiating into hepatocytes, expressing markers of both cell types and plasticity factor candidates. Validation in cancer-patient tissues and tumoroids confirms the presence of bi-phenotypic cells and plasticity factor expression. Functional studies in tumoroids will elucidate plasticity factor roles. Our findings provide insights into liver cancer progression within chronic liver disease, aiding therapeutic development.

ABSTRACTS | POSTERS

Modeling age related macular degeneration (AMD) using bio printed iPSC derived retinal pigment epithelium and choroid
| Ruchi Sharma

| Xun Xu

Inspired by nature, we developed a blastocyst motif substrate (BMS) to physically revert primed pluripotent stem cells (PSCs) to a naïve state in vitro. BMS features diverse microstructures mimicking blastocyst geometry. Time-resolved analysis showed efficient primed-to-naïve reversion, especially with motifs representing mouse blastocyst scaled curvature range (BSCR). Apical constriction, enhanced E-cad/RAC1 signaling, and YAP activation on BSCR+ motifs regulated histone modification, boosting naivety gene expression. BMS-PSCs maintained elevated NANOG levels for over ten days, displaying enhanced potential for embryoid body, teratoma and organoid formation. Human PSC validation underscores BMS's potential for large-scale application.

Screening of endocrine disruptive chemicals using hiPSCs-derived hepatoblast organoids
| Wilson Iyare

| Junyao Zhang

The absence of hyaluronic acid (HA) in Matrigel, a key extracellular matrix component in lung, complicates the lung epithelium development, impeding efficient iPSC-derived organoid generation. To tackle this, inspired by the natural lung habitat, we develop a hybrid hydrogel combining HA and 23 % Matrigel. Besides the biochemical support, this hydrogel exhibits enhanced viscoelastic performance. Organoid development in the HA-hydrogel is accelerated compared to Matrigel, yielding homogeneous bipotential organoids containing SOX2+ bronchial and SFTPC+ alveolar cells within 8 days. Elevated levels of PIEZO1, ITGB1, RRAS, and NMHC IIA suggest an active mechanosensing initiated by altered viscoelasticity from HAhydrogel.

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FUNDING SOURCES: