Developing complex human liver organoids to investigate the cellular crosstalk between the epithelia and stromal niche

Huch Lab

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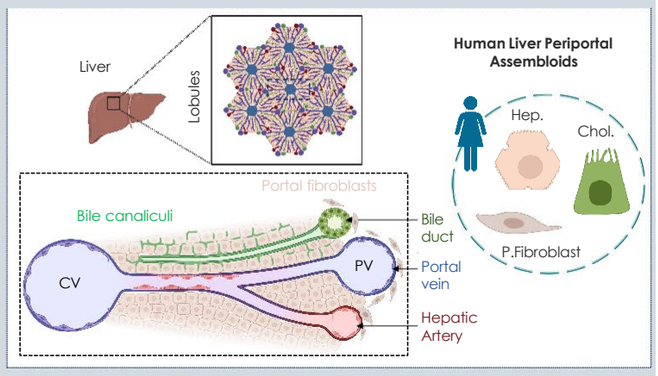
Abstract

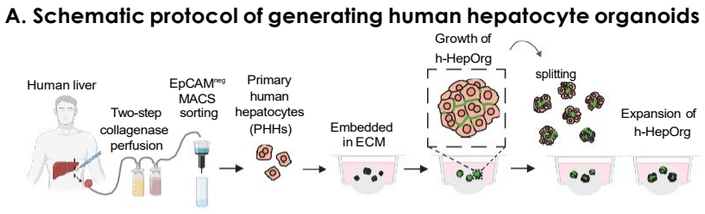
The mammalian liver is a highly complex organ, responsible for crucial functions including drug detoxification, metabolic regulation, and bile drainage. It has a unique tissue architecture, consisting of functional units called lobules, and is composed of multiple cell-types for optimal function. The development of the three-dimensional liver organoid model system has allowed the study of this complex organ *in vitro*, in the healthy as well as diseased-state. **However, they require further improvements to gain a closer representation of the native tissue. Additionally, the current liver organoid models lack the representation of non-parenchymal cell-types that play a vital role in working in concert to maintain homeostasis *in vivo*. This project aims to overcome this issue by developing an enhanced liver organoid model that can recapitulate the heterogeneous cell population present in the mammalian liver.** For that, we first developed human hepatocyte organoids from healthy tissue and then combined them with human periportal fibroblasts and cholangiocytes from the same donor to generate the next generation of human liver organoids, termed periportal assembloids, containing stromal and epithelial cell populations. This enables the examination of cell-cell interactions among the different cell-types to improve our understanding of their significance in the healthy-state and diseased-states of the liver.



Results

1. Patient-derived human hepatocyte organoids (h-HepOrgs) expand long term when cultured under high WNT and high YAP activity

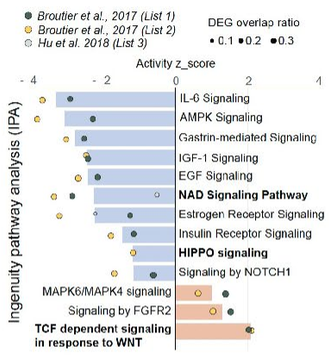
A. Schematic protocol of generating human hepatocyte organoids (h-HepOrgs)



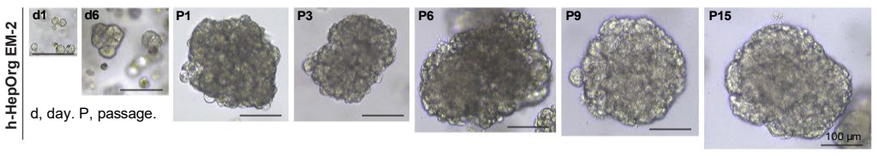
Hepatoblast Medium (MM) *(Prior et al., 2019)*

+ WNT activation
+ YAP activation (TRULI)

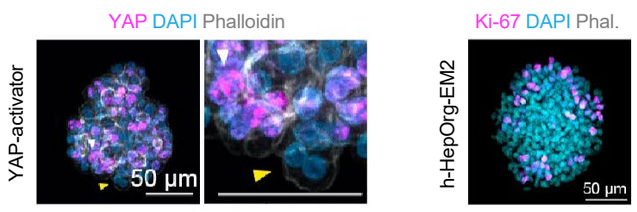
B. Signalling pathways involved in hepatocyte proliferation



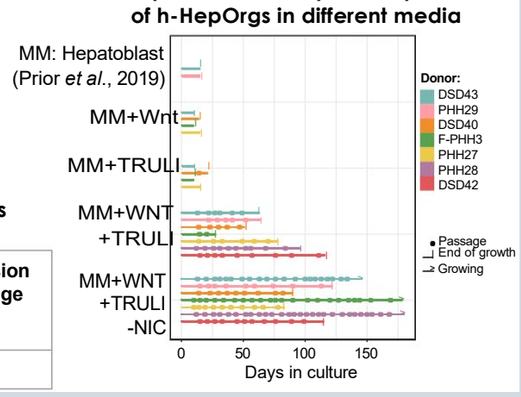
C. BF images showing the long-term passaging of human hepatocyte organoids



D. IF confirms presence of nuclear YAP and the proliferation marker Ki-67



E. Graph shows the expansion potential of h-HepOrgs in different media

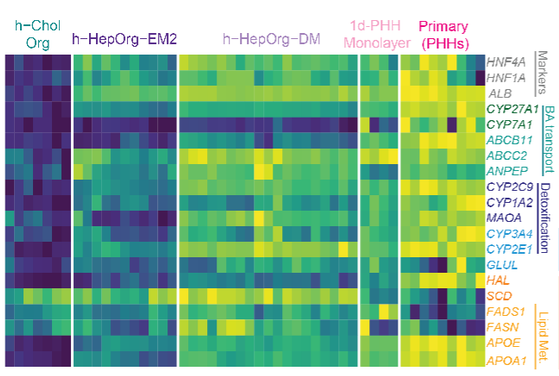


F. Table summarizing the generation of primary hepatocyte organoids from human liver patient samples

Healthy Donors	Males (%)	Age Range (mean)	Organoid derivation rate (%)	Long-term expansion In EM2 > 5 Passage (%)
28	65	11-85 (50)	100	100

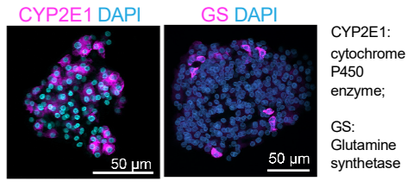
2. Differentiated human hepatocyte organoids (h-HepOrg-DM) retain gene expression and metabolic function of human liver tissue *in vitro*

A. RNAseq analysis shows h-HepOrg-DM retain gene expression similar to primary hepatocytes from tissue (PHHs)



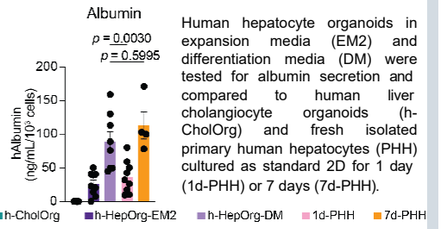
RNAseq analysis of multiple donor samples (each column one donor, each row one gene) from hepatocyte organoids in expansion media (h-HepOrg-EM2), differentiation media (h-HepOrg-DM), primary human hepatocytes (PHHs) from fresh tissue, 1-day PHH monolayer culture, and human cholangiocyte organoids (h-Chol-Org).

B. h-HepOrg-DM express metabolic enzymes and secrete albumin as mature hepatocytes



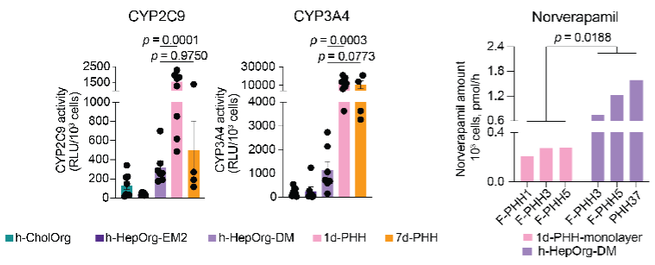
CYP2E1: cytochrome P450 enzyme;

GS: Glutamine synthetase

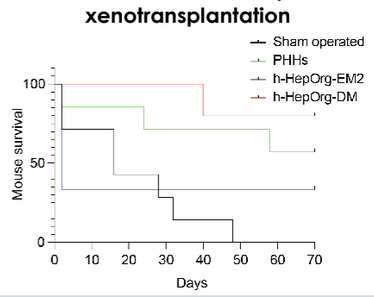


Human hepatocyte organoids in expansion media (EM2) and differentiation media (DM) were tested for albumin secretion and compared to human liver cholangiocyte organoids (h-CholOrg) and fresh isolated primary human hepatocytes (PHH) cultured as standard 2D for 1 day (1d-PHH) or 7 days (7d-PHH).

C. h-HepOrg-DM present detoxifying functions (cytochrome activity) and outperform gold standard 2D-hepatocytes in metabolizing the drug – verapamil into its active metabolite - norverapamil

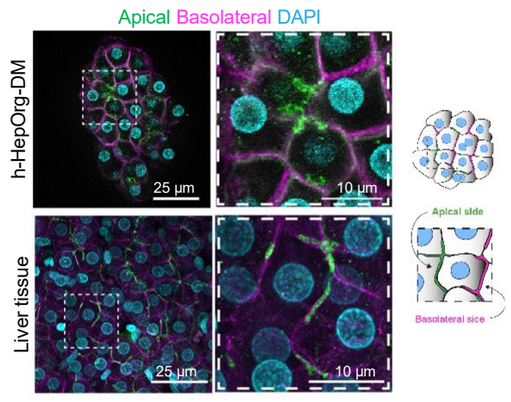


D. h-HepOrg-DM rescue the phenotype of FRG mutant mice upon xenotransplantation

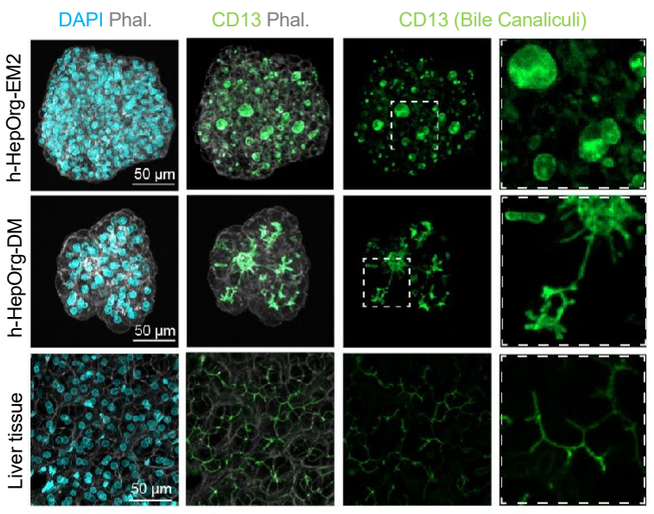


3. Differentiated human hepatocyte organoids (h-HepOrg-DM) recapitulate the tissue architecture of *in vivo* human liver tissue

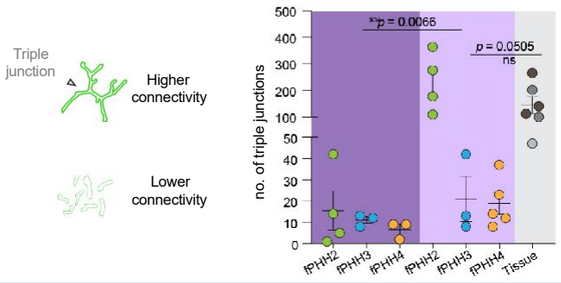
A. IF shows h-HepOrg-DM recapitulate the unique multiaxial apical-basal polarity of hepatocytes as in liver tissue



B. IF shows h-HepOrg-DM form narrower and inter-connected bile canaliculi (BC) that resemble human liver tissue



C. Connectivity within the bile canaliculi (BC) network



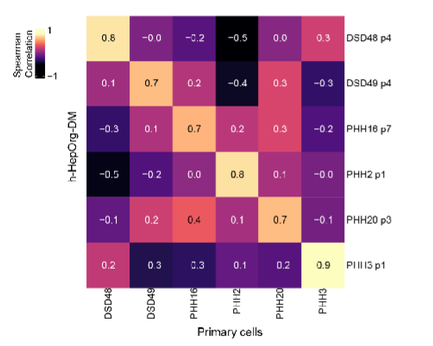
EM2
DM
Tissue

The graph shows the total number of triple junctions as a proxy for connectivity within the bile canaliculi (BC) network. For tissue, dot represents one field of view and colour a different donor (n=3). For organoids, dot represents one structure (organoid) in the indicated donors.

Connectivity within the bile canaliculi (BC) networks increased in differentiated organoids (DM) compared to organoids from the same donor in expansion media (EM2).

4. Hepatocyte organoids retain patient-to-patient variation at both gene expression levels and tissue architecture

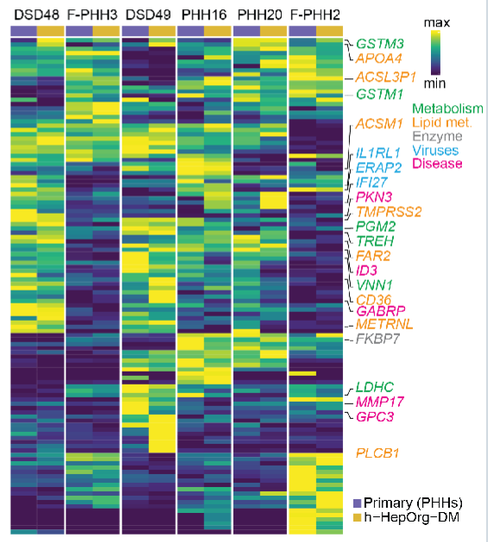
A. RNAseq analsyis of h-HepOrgs and matching patient liver tissue indicates that h–HepOrgs retain patient-to-patient variation at the gene expression level



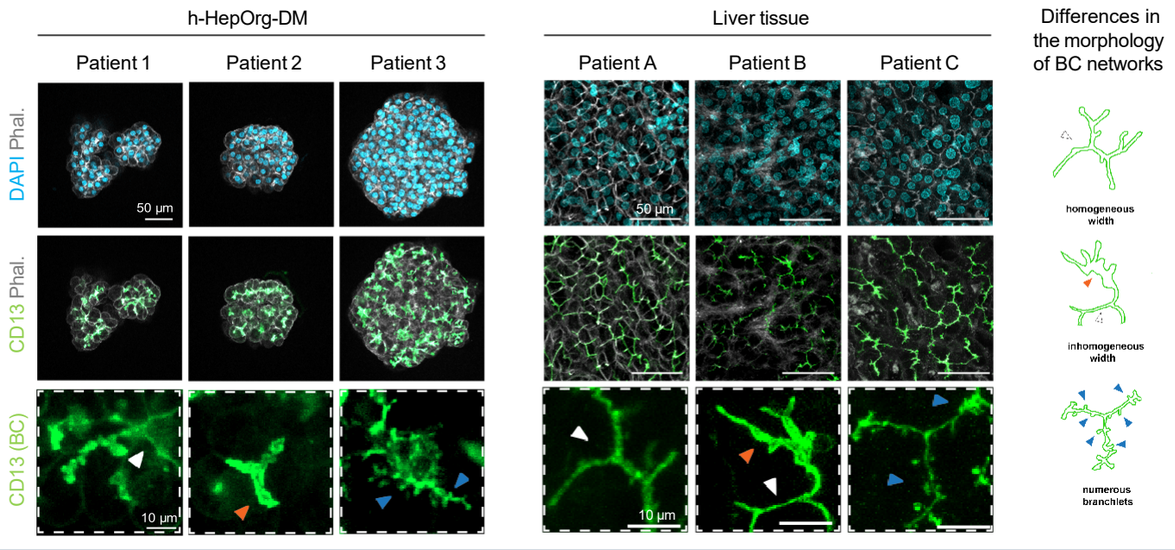
Spearman correlation heatmap from RNAseq analysis showing pairwise similarities between primary hepatocyte samples (PHHs) from tissue and differentiated hepatocyte organoids (h-HepOrg-DM). Warmer colours indicate higher correlation coefficients, representing stronger transcriptomic similarity. p, passage.
A strong correlation (R^2=0.7-0.9) between the organoids and the original tissue was observed.

Transcriptome analysis of primary hepatocytes at the time of isolation (PHHs) and their matching h-HepOrgs under differentiation medium (h-HepOrg-DM) identified specific gene signatures of each patient.

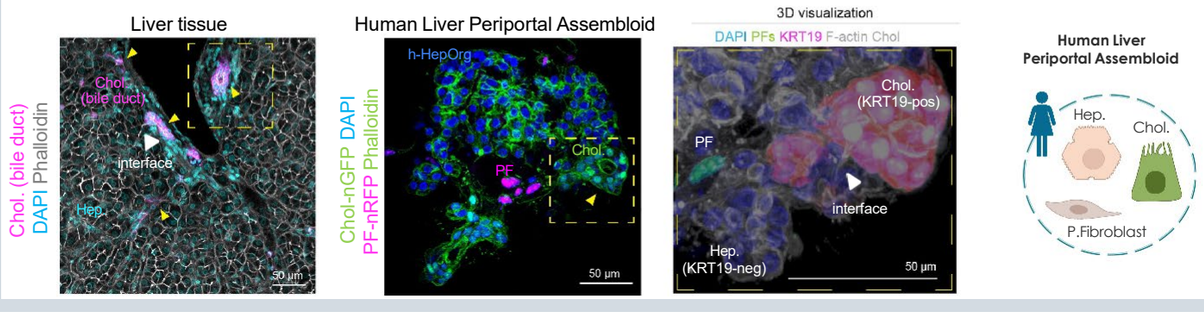
Some of the patient-specific genes found expressed in the organoids and their source cells included associations with susceptibility to hepatitis virus infection (*IL1RL1* or *ERAP2*), liver cancer (*GPC3*), cholestasis during pregnancy (*GABRP*). Some genes were involved in metabolic pathways like the glutathione-related genes *GSTM3* and *GSTM1*, the lactate dehydrogenase *LDHC* and the lipid metabolic related genes *APOA4*, the fatty acyl-CoA reductase *FAR2* or the Acyl-CoA synthetase *ACSM1*. These findings indicate that h-HepOrgs could preserve patient-to-patient specific signatures with significant implications for modelling human liver diseases.



B. Differentiated h-HepOrgs capture the variations in bile canaliculi (BC) morphology observed in liver tissue among different human patients



5. Novel human hepatocyte organoids when combined with human cholangiocytes and portal mesenchyme enable formation of human periportal assembloids that recapitulate human liver tissue architecture



Conclusions and Significance

- Optimized the growth and development of human hepatocyte organoids, which exhibit a closer representation of the liver tissue and also capture differences observed among patient cohorts.
- This enabled the development of complex liver organoids consisting of three different cell-types to generate human liver periportal assembloids, representing the homeostatic-state of the human liver.

Acknowledgements

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