Drug-Induced Perturbations of Non-Parenchymal Cellular Communications in Human Liver Organoids

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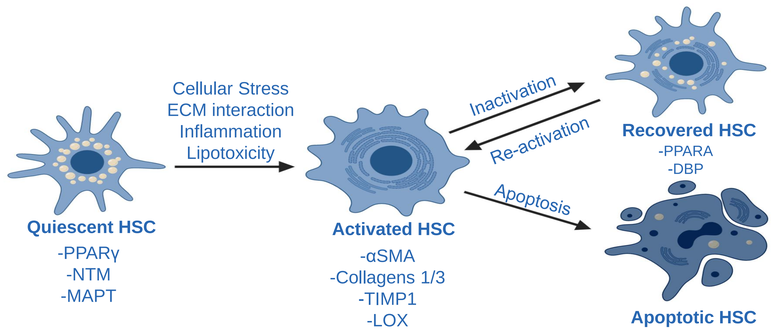
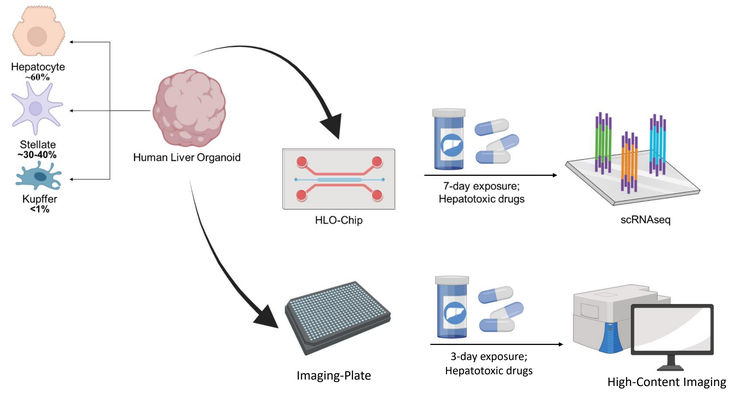
Introduction

In efforts to shift *in vitro* models of hepatotoxicity towards recapitulation of human metabolism and spatial specificity, directed differentiation of stem cells towards human liver cells and multicellular 3D models such as human liver organoids (HLOs) have been in development and practice in preclinical research. In this study, we employ a multicellular human liver organoid[1] microfluidic chip platform as a model of drug-induced liver injury that is composed of ~60% hepatocytes, ~20-30% hepatic stellate cells, and <1% tissue resident macrophages. We hypothesize that the hepatic stellate cell population of our multicellular HLO model is competent of fibrotic signaling and intercellular communications that are unique to each drug treatment. Here, we characterize fibrotic signaling of the HLO hepatic stellate population through single-cell RNA sequencing and morphological profiling.



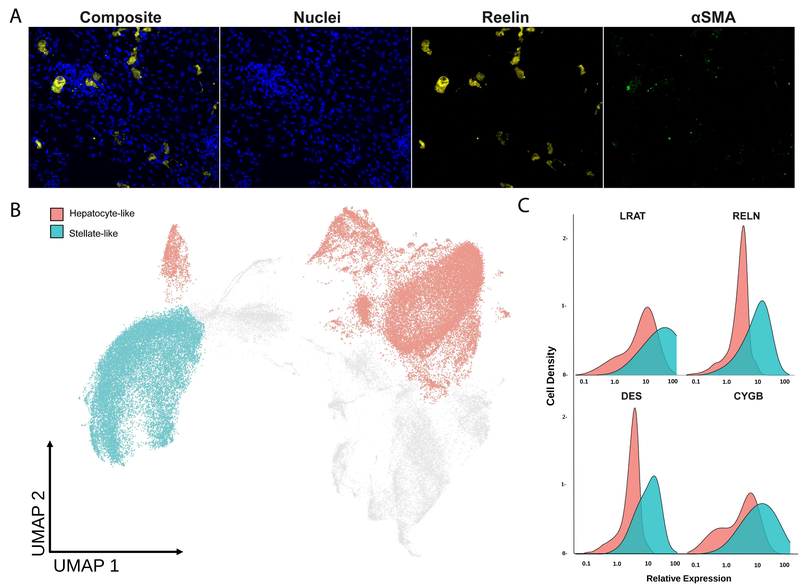
Methods



HSC Phenotype Population Statistics

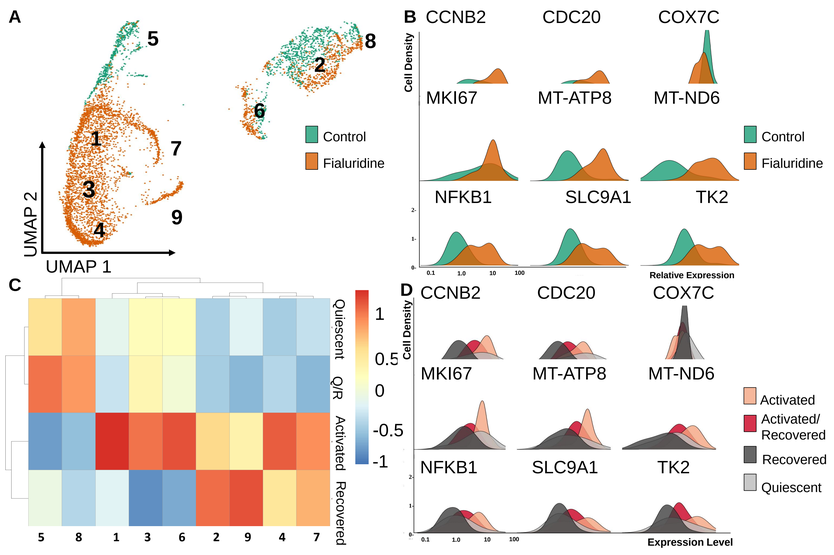
HSC Phenotype (scRNA-seq Markers)	Day 7 Control (DMSO)	1μM Fialuridine x 7 Days	100μM Acetaminophen x 7 Days
Quiescent (NTM, MAPT, NGFR, GFAP)	80%	40%	20%
Activated (ACTA2, COL1A1, LOX)	2%	35%	38%
Inactivated (PPARA, DBP, NGFR, GFAP)	18%	25%	18%

Hepatic Stellate Cells Comprise a Large and Identifiable Population of HLO-Chip Cultures



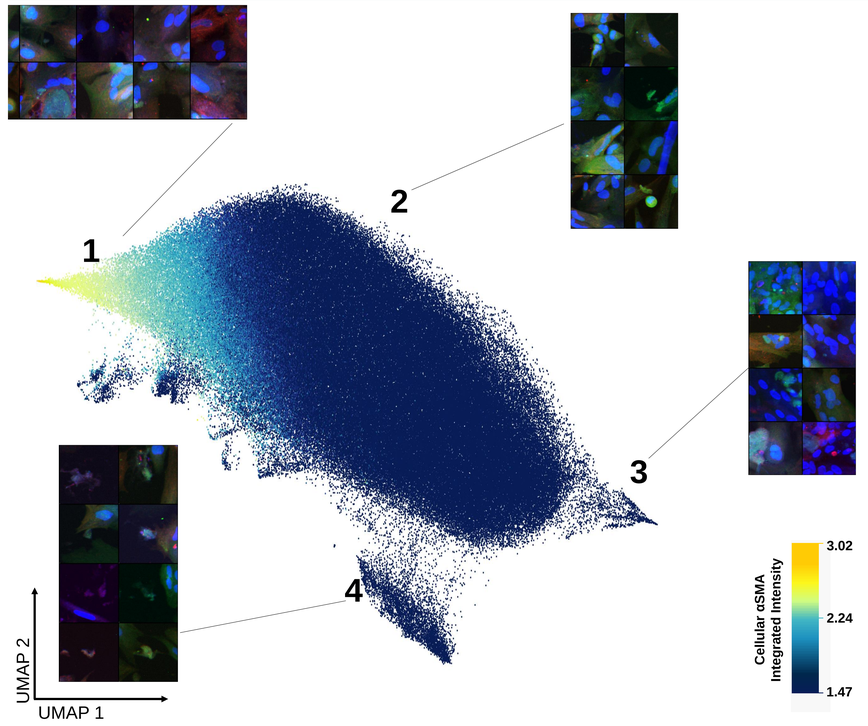
HLO cultures on chip are a physiologically relevant model of metabolic liver function and injury. (**A**) Immunofluorescent images of HLO 2D dispersed static culture staining for nuclei (Hoechst), hepatic stellate cells (Reelin), and activated hepatic stellate cells (αSMA). (**B**) UMAP embedding of HLO-chip single-cell RNA sequencing data highlighting hepatocyte-like clusters and stellate-like clusters. (**C**) Densities of hepatic-stellate markers between hepatocyte-like clusters and stellate-like clusters.

Discrete Populations of Hepatic Stellate-like Cells are Identified by Transcriptomic Profile



Hepatic stellate-like cells cluster in UMAP by identifiable transcriptomic perturbation profiles. (**A**) UMAP of stellate cells treated with Fialuridine for 7 days and control with cluster labels. (**B**) Gene expression of inflammatory, metabolic, and fialuridine toxicity specific markers from fialuridine treatment and control. (**C**) Heatmap of hepatic stellate cell phenotype markers amongst clusters labeled in panel (A). Clusters are colored by expression of 9 total genes that describe the phenotype as either quiescent, Q/R (quiescent or recovered), activated, or rescued). Clusters with highest expression of Q/R genes are deciphered based on their expression of exclusive quiescent or rescue genes. (**D**) Gene expression of inflammatory, metabolic, and fialuridine toxicity specific markers between hepatic stellate cell phenotypes.

Morphological Characterization of Stellate Populations



UMAP projection of cellular objects analyzed from Immunofluorescent images stained for **Nuclei**, **Reelin**, and **α-smooth muscle actin**. UMAP is numbered by morphological region that correlates to Reelin and α-smooth muscle actin intensities and colored by log-scale α-smooth muscle actin intensity. Representative immunofluorescent image thumbnails are displayed next to each morphological region.

Future Directions

Due to resource limitations, we were unable to use the HLO-Chip model for morphological characterization. Though this morphological analysis does not consider the mature phenotype of HLO cell types that we have characterized previously[2], it is appropriate for future consideration when using this model in a 2D, high throughput format. Moving forward, we will use HLO-Chip to characterize stellate fibrotic phenotypes in the same methodology as we had done previously for scRNA-seq studies and, using markers informed by pathway enrichment analysis, confirm appropriate markers of drug-induced hepatic stellate signaling.

Conclusions

- Hepatic stellate cells are 20-30% of the HLO cell population
- When cultured on a microphysiological system, the naïve HLO transcriptomic profile suggest that most hepatic stellate cells are quiescent and can thus be used as a model for physiologic hepatic stellate readouts
- Drug-treated HLO stellate cells populations are majorly comprised of the activated phenotype and the activated and proliferating stellates have increased relative expression of the identified transporter (SLC9A1) and kinase (TK2) responsible for transport of the drug and toxic post-translational modifications
- Stellate morphology is quantifiable by IF and correlating features to stellate activation can be deconvoluted by this method (cell perimeter, size, texture, neighboring/recruiting cells)

References and Acknowledgement

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1. Ouchi, R. et al. Modeling Steatohepatitis in Humans with Pluripotent Stem Cell Derived Organoids. Cell Metab. (2019) doi:10.1016/j.cmet.2019.05.007.
2. Zhang, C. et al. A Human Liver Organoid Screening Platform for DILI Risk Prediction. J. Hepatol. (2023) https://doi.org/10.1016/j.jhep.2023.01.019

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