Human iPSC-derived hepatic models for the study and therapeutic targeting of liver disorders

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Abstract

Liver diseases are a growing global health problem, creating the need for *in vitro* models that better reproduce the complexity of the human liver for disease research and therapeutic development. An important factor in developing realistic liver models is the presence of multiple interacting cell types. The liver contains hepatocytes along with non-parenchymal cells, such as hepatic stellate cells and Kupffer cells, and these heterotypic interactions are essential to maintain hepatocyte function. Induced pluripotent stem cells (iPSCs) offer a promising approach because they can be differentiated into various liver cell types that closely resemble their *in vivo* counterparts. From a single iPSC line, we can obtain hepatocyte-like cells (HLCs) and hepatic stellate-like cells (HSCs), allowing the creation of co-culture systems that better mimic the hepatic microenvironment. The aim of this study was to generate HLCs and HSCs from iPSCs, to characterize their phenotype and function, and to optimize their co-culture conditions. The HLCs produced albumin, exhibited ureogenic capacity, and showed cytochrome P450 activity, confirming hepatocyte-like functionality. The HSCs expressed typical stellate cell markers and responded to TGF-β stimulation by increasing the expression of fibrosis-related genes. When co-cultured as 3D spheroids, both cell types maintained their key characteristics. Overall, these results show that iPSC-derived liver cells reproduce essential aspects of liver physiology and pathology, supporting their use as a relevant platform for studying liver disease mechanisms and developing new therapeutic strategies.

Outcomes

iPSCs can be successfully differentiated into functional hepatocyte-like cells (HLCs)

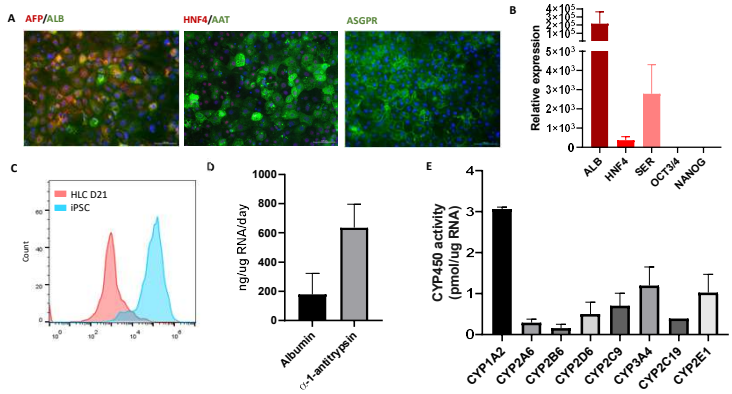


Figure 1. Characterization of HLCs derived from iPSCs. A) Immunofluorescence of hepatocyte markers in HLCs (ALB: albumin; AFP: α-fetoprotein; HNF4: hepatic nuclear factor 4 alpha; AAT: α-1-antitrypsin; ASGPR: asialoglycoprotein receptor). Hoechst33342: nuclei. B) mRNA expression of hepatocyte markers (ALB, HNF4, and SER: SERPINA1) and pluripotency markers (OCT3/4 and NANOG) in HLCs, normalized to iPSCs. C) Flow cytometry analysis of the pluripotency marker SSEA-4 in HLCs and iPSCs. D) Albumin and α-1-antitrypsin production over 24 hours in HLCs. E) Activity of different CYP450 isoenzymes after 24-hour incubation with specific substrates.

iPSCs can be efficiently differentiated into stellate cells (HSCs) capable of responding to pro-fibrotic stimuli

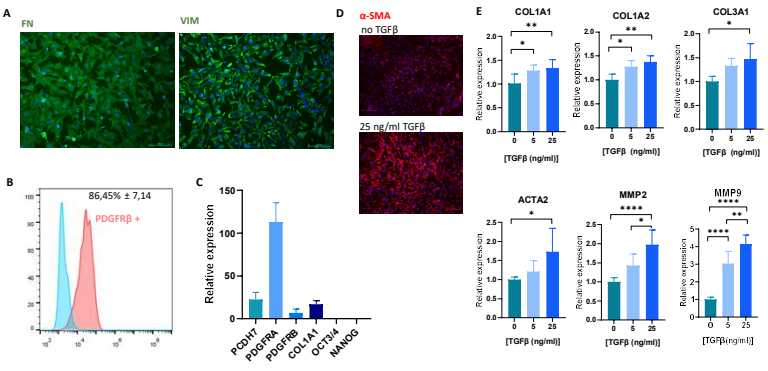


Figure 2. Characterization of HSCs differentiated from iPSCs. A) Immunofluorescence analysis of hepatic stellate cell markers in HSCs (FN: fibronectin; VIM: vimentin). Hoechst33342: nuclei. B) Flow cytometry analysis of PDGFRβ in HSCs at day 12. C) mRNA expression of hepatic stellate cell markers (PCDH7, PDGFRA, PDGFRB, and COL1A1) and pluripotency markers (OCT3/4 and NANOG) in HSCs, normalized to iPSCs. D) Immunofluorescence of α-SMA after 24 hours of treatment with 25 ng/mL TGFβ. E) mRNA expression of profibrotic markers following exposure to 5 ng/mL and 25 ng/mL TGFβ for 24 hours, normalized to untreated HSCs. * p ≤0.01 (compared to no treated cells)(ANOVA followed by Tuckey's multiple comparisons test).

HLCs and HSCs can be successfully co-cultured while maintaining their functionality

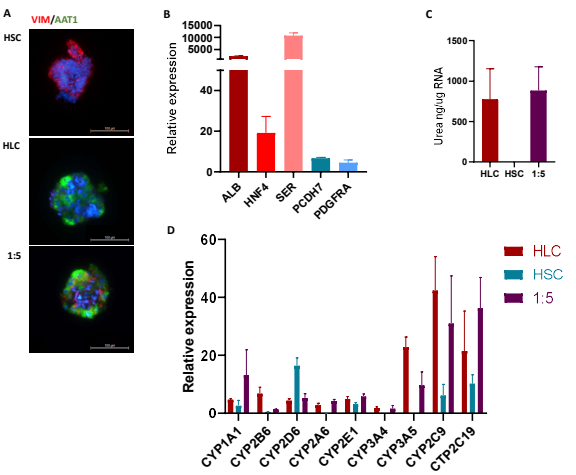


Figure 3: Characterization of HSC:HLC 1:5 co-culture. A) Immunofluorescence of HLC and HSC markers (AAT: α-1-antitrypsin; VIM: vimentin) in spheroids. B) mRNA expression of HLC and HSC markers in 1:5 spheroids, normalized to iPSCs. C) Albumin and urea production over 24 hours in HLCs, HSC and 1:5 spheroids. D) mRNA expression of different CYP450 isoenzymes in HLCs, HSCs, and 1:5 spheroids, normalized to iPSCs.

Co-cultures of HLCs and HSCs provide a platform to study fibrosis *in vitro* and assess drug responses

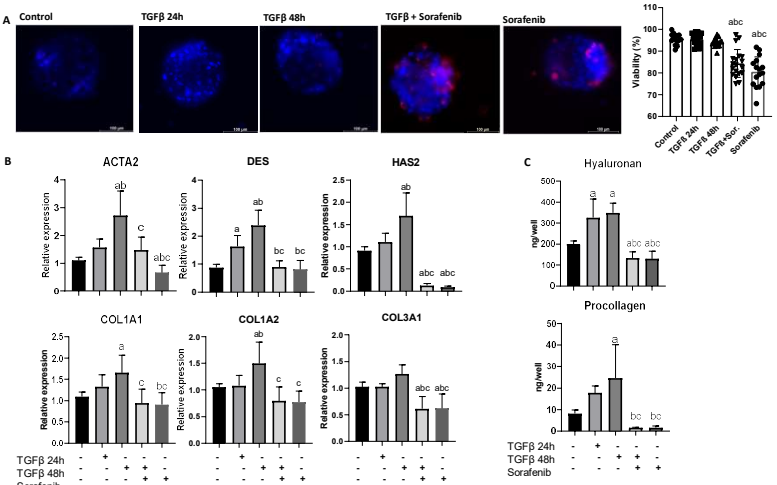


Figure 4: TGFβ stimulation and sorafenib treatment. A) Live/Death assay of HSC:HLC 1:5 co-culture spheroids under the following conditions: no stimulation, 24-hour TGFβ stimulation, 48-hour TGFβ stimulation, combined TGFβ and sorafenib treatment, and sorafenib alone. B) mRNA expression of fibrotic markers, normalized to no stimulated condition. C) Hyaluronan and procollagen production in 24 hours. Mean ± SD. One-way ANOVA with Tukey's post hoc test. Different letters indicate significant differences ($p < 0.05$): a vs. control, b vs. 24 h TGFβ, c vs. 48 h TGFβ.

CONCLUSIONS

1. HLCs derived from iPSCs recapitulate key hepatocyte features and functions, including albumin and α-1-antitrypsin production and CYP450 metabolic activity. Additionally, they lose the expression of pluripotency markers after differentiation.
2. HSCs differentiated from iPSCs express specific hepatic stellate cell markers, lose the expression of pluripotency markers, and respond to TGFβ stimulation by increasing the expression of myofibroblast markers.
3. Co-culture of HLCs and HSCs in spheroids maintain the expression of HLC and HSC markers and hepatocyte functions such as albumin and urea production.
4. HLC:HSC co-culture respond to TGF-β stimulation by upregulating profibrotic markers and increasing the production of extracellular matrix proteins. Treatment with the antifibrotic agent sorafenib attenuates this TGFβ induced response, leading to reduced expression of profibrotic markers at both mRNA and protein levels.

ACKNOWLEDGEMENTS

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