Bilirubin induces the maturation of liver sinusoidal endothelial cells and human liver organoids

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Background

The development of induced human liver organoids (HLOs) is a complex and important issue as it provides an avenue for liver disease treatment and research. HLO induction from induced pluripotent stem cells (iPSCs) has been modeled by fetal development. In vivo, liver development happens in three general phases: specification, budding, and growth and maturation.

Aims / Hypothesis

Hypothesis:
- Physiological levels of bilirubin promote the formation of LSECs from endothelial cell (EC) differentiation and promote differentiation of mature hepatocytes.
Aims:
- Induce ECs, LSECs, and HLOs from stem cell precursors
- Analyze the effect of bilirubin as a metabolite for the maturation of LSECs and HLOs

Methods

iPSCs were differentiated into human liver organoids, endothelial cells, and liver sinusoidal endothelial cells. Bilirubin was added at varying concentrations to the endothelial cells for 3 days. After three days bilirubin treated endothelial cells were placed in a co-culture with the induced human liver organoids for an additional 7 days. iPSC derived LSECs were used as a control to compare morphology to bilirubin treated endothelial cells. Changes to bilirubin treated ECs were observed using immunofluorescence, RT-qPCR, and flow cytometry. HLO maturation was analyzed via an albumin ELISA.

Results 1. Bilirubin treated endothelial cell morphology

Endothelial cells treated with bilirubin did not exhibit morphological changes. Endothelial cell morphology resembles that of LSEC morphology.

Results 2. Endothelial cells express LSEC markers at physiological levels of bilirubin

Results 3. Flow cytometry analysis reveals an increase in LVE1 population in bilirubin treated endothelial cells

Results 4. HLOs-Endothelial Cell co-cultures show an increase in maturation

Conclusions

At physiological levels of bilirubin, there is an increased in LSEC characteristics as exhibited by PCR and Flow Cytometry. Additionally, PCR indicates that at physiological levels of bilirubin could activate the nitric oxide pathway allowing for the induction of LSEC and hepatocyte differentiation. The maturation of HLOs was also indicated by an increase in production of albumin with physiological levels of bilirubin treated endothelial cells that were cocultured with HLOs. This study shows that physiological levels of bilirubin induce LSEC characterization of endothelial cells as well as increase the maturation of HLOs.

References


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