Bilirubin Induces Maturation of Human iPSC-derived Liver Sinusoidal Endothelial Cells and Organoids

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Introduction: The current model for inducing human liver organoids (HLOs) from induced pluripotent stem cells (iPSCs) leads to an inadequate maturation needed for advancing the functional capacity of the organoids. Prominent vasculature characteristics essential to liver survival and function are lacking in this model. Bilirubin acts as an antioxidant whose fetal production begins slightly before liver sinusoidal endothelial cells (LSECs), potentially serving as a signaling molecule. This study analyzes the effect of bilirubin on endothelial cell maturation and the effect of co-culturing bilirubin treated endothelial cells with HLOs.

Hypothesis: We hypothesized that physiologic levels of bilirubin to the endothelial cells will induce LSEC characteristics, thereby promoting hepatocyte maturation in human organoid culture.

Methods: Endothelial cells (ECs) derived from iPSCs were treated with varying concentrations of bilirubin (0.1μM, 0.5μM, 1.0μM, 2.0μM, and 5.0μM) for 3 days, and collected to study the LSEC characteristics by RT-qPCR for gene expression and immunofluorescence, flow cytometry and enzyme-linked immunosorbent assay (ELISA) for protein production. Additionally, bilirubin treated ECs were co-cultured with HLOs and were analyzed for liver maturation by albumin production levels.

Results: RT-qPCR showed that LSEC markers, GATA4, CLEC4m, STAB2, increased when cultured with physiological levels of bilirubin indicating a shift to LSEC lineage from endothelial lineage ($p < 0.01$), and GATA4 expression was also increased by immunofluorescent staining. Additional RT-qPCR results revealed that nitric oxide pathway markers, EGLN1, HIF1a, NOS2, and NOS3, were also significantly stimulated when cells were cultured with bilirubin ($p < 0.01$). Flow cytometry analysis showed an increase in LYVE1 population with bilirubin exposure. The albumin ELISA showed a significant increase with the highest albumin production by HLOs that had been co-cultured with ECs treated with 1μM of bilirubin.

Conclusions: Our data suggest that physiological bilirubin levels induce LSEC differentiation and subsequent maturation of hepatoblasts. This indicates that physiological levels of bilirubin could activate the nitric oxide pathway to induce differentiation of LSECs and liver development via modulation of hepatocyte growth factor.

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