Human cytomegalovirus (HCMV) infection of macrophages induced abnormal development of cholangiocytes in an organoid co-culture model for biliary atresia

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Background and Aims: Biliary atresia (BA) is a devastating biliary disease of neonates, but its pathogenesis is unclear. Human cytomegalovirus (HCMV) has been implicated to contribute to BA development from clinical association without mechanistic evidence. Macrophages mediate innate immune responses against HCMV infection. This study addresses how the interactions between HCMV, macrophages and cholangiocytes (bile duct epithelial cells) contribute to BA.

Method: Human induced pluripotent stem cell (iPSC)-derived macrophages infected by HCMV (multiplicity of infection = 1.0) and then cleansed of free viruses were co-cultured with iPSC-derived cholangiocytes in 1:1 ratio. We performed morphological examination; expression analysis of genes relevant for viral propagation/immune responses/cholangiocyte development; single cell RNA-sequencing (scRNA-seq) of infected and un-infected co-cultures at different post-infection days (PD).

Results: We detected expression of HCMV latent viral genes (UL138 and LUNA), elevated level of pro-inflammatory genes (IL-1β, TNF-α, CD86) in PD5 infected co-cultures; deformed cholangiocyte organoids in PD7 infected co-cultures. Bioinformatics analysis of the scRNA-seq data on PD5 co-cultures revealed two clusters of cholangiocytes (BCL2-expressing small cholangiocytes & BCL2-non-expressing large cholangiocytes). Viral genes were highly expressed in the large cholangiocytes, suggesting that large cholangiocytes were susceptible to HCMV infection. Moreover, we detected downregulation of cholangiocyte markers (KRT19, KRT18, KRT7, KRT8, EPCAM), pro-apoptotic genes (BAX and CYCS), and pro-apoptotic pathways (MYC-target v1, oxidative phosphorylation and reactive oxygen species pathway) in HCMV-infected cholangiocytes.

Conclusion: Our data showed that (i) HCMV-infected macrophages could transfer the virus to the large cholangiocytes; (ii) HCMV-infected cholangiocytes exhibited dysregulated cholangiocytic development; (iii) HCMV-infection induced upregulation of pro-inflammatory genes. HCMV infection could cause abnormal development of bile duct epithelium, promote inflammatory responses in the liver and contribute to disease initiation/progression of BA.

Figure:

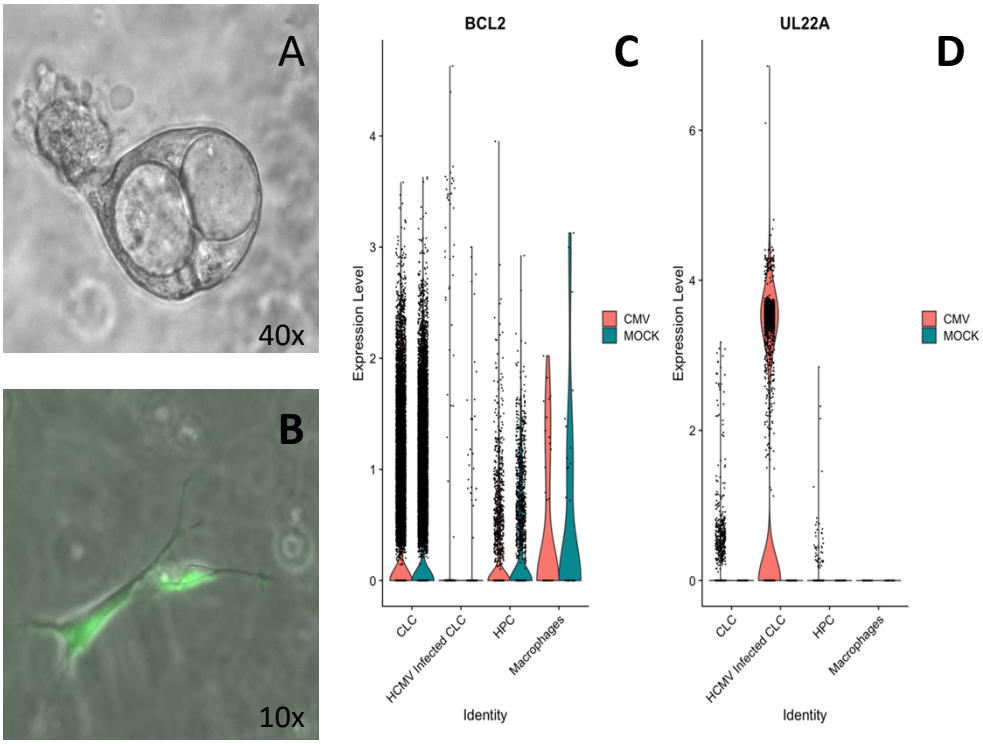

Figure: Microscopic and single cell view of the co-culture system. (A) Poorly expanded organoids with multiple vacuole morphology in the HCMV infected co-culture. (B) HCMV infected macrophages (green fluorescence) in the co-culture system. (C) Small cholangiocytes (BCL2-expressing) but not the large cholangiocytes (BCL2-non-expressing). (D) Viral gene UL22A was highly expressed in the large cholangiocytes. (CLC = Cholangiocyte like cell, HPC = Hepatic progenitor cell)