Mechanistic insight of SARS-CoV-2 infection using human hepatobiliary organoids

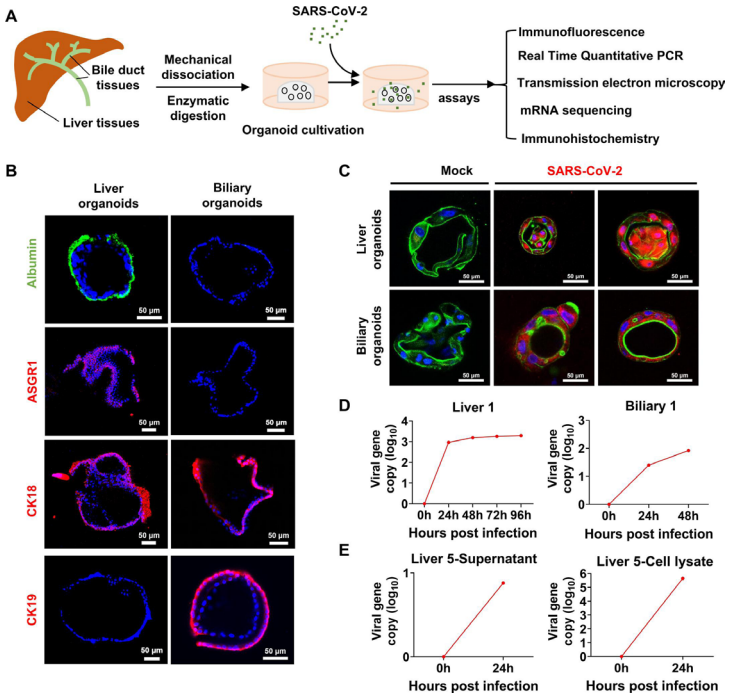

Figure 1 (A) Scheme of liver and biliary organoid culture establishment and performed assays. The liver and biliary organoids were generated from adjacent normal tissues of patients with liver cancer and coculture with SARS-CoV-2. (B) Differentiated liver and biliary organoids were immunostained using antibodies against human homologues to show albumin (green), ASGR1 (red), CK18 (red) and CK19 (red) protein, respectively. Nuclei are stained with DAPI (blue). Scale bar, 50 µm. (C) Immunofluorescent staining of SARS-CoV-2 infected liver organoids and biliary organoids at 24-hours. Virus are identified by SARS-CoV-2 spike (S) glycoprotein protein (red), nuclei and actin filaments are stained with DAPI (blue) and phalloidin (green), respectively. Scale bar, 50 µm. (D) Real time quantitative PCR analysis for viral sequences shows virus can productively replicate in the liver organoids at 0, 24, 48, 96 hours and in the biliary organoids at 0, 24, 48 hours after infection with SARS-CoV-2. (E) Live virus can be detected by RT-qPCR in supernatant and lysed organoids at 0 and 24 hours after infection with SARS-CoV-2.

We have read with interest in a recent study published in Gut by Weber *et al*,[1] reporting abnormal elevation of gamma-glutamyltransferase (37%) and total bilirubin (5%) in patients, that was associated with higher rates of COVID-19-related deaths. However, whether SARS-CoV-2 could infect human hepatocytes and cholangiocytes thus causing local damage has not been reported. Here, human organoids were used to investigate SARS-CoV-2 infection and its induced liver damage at cellular and molecular levels (figure 1A).

We first established five lines of liver organoids and two lines of biliary organoids from adjacent normal tissues of seven patients with liver cancer (figure 1B; online supplemental figure 1A,B; online supplemental methods). We examined the susceptibility of human liver and biliary organoids to SARS-CoV-2 (Guangdong/20SF014/2020; EPI_ISL_403934; 19A) directly isolated from a patient with moderate COVID-19. The expression of SARS-CoV-2 spike (S) glycoprotein protein was readily detected in patchy areas of human liver and biliary organoids, but not in uninfected control by immunofluorescence staining (figure 1C; online supplemental figure 1C). Viral load of SARS-CoV-2 was dramatically increased in liver (liver 1 to liver 4) and biliary organoids (biliary 1 and biliary 2) at 24 hours postinfection with SARS-CoV-2, which remained stable for 96 hours in liver organoids and 48 hours in biliary organoids (figure 1D; online supplemental figure 2A; online supplemental table S1). Meanwhile, we found that live SARS-CoV-2 could amplify in lysed organoids (liver 5) and its culture supernatant (figure 1E; online supplemental table S1), inferring human liver and biliary organoids are susceptible to SARS-CoV-2 and support robust viral replication.

SARS-CoV-2 enters target cells mainly through the ACE2 receptor.[2] Given that ACE2 receptor was more highly expressed in cholangiocytes (59.7%) than in hepatocytes (2.6%),[3] we investigated the ultrastructure of SARS-CoV-2-infected biliary organoids under transmission electron microscopes (TEM) and found that viral particles were present in the lumen and at basolateral and apical sides of the organoid, as well as in membrane-bound vesicles (figure 2A). Therefore, TEM captured the critical location of SARS-CoV-2 in infected biliary organoids, indicating potential dissemination route of how SARS-CoV-2 enters the cholangiocytes.

Gene expression changes induced by SARS-CoV-2-infection in liver and biliary organoids was identified by RNA sequencing. Heatmap and volcano plot of differentially expressed genes revealed robust induction of proinflammatory chemokines/cytokines including CXCL1 in SARS-CoV-2-infected liver and biliary organoids. Consistently, KEGG Orthology Based Annotation System revealed multiple upregulated proinflammatory pathways in infected organoids, including NF-κB signalling pathway etc. Of note, the significant upregulation of TNF signalling pathway and apoptosis pathway in infection group indicated that SARS-CoV-2 infection may induce cell death of hepatocytes and cholangiocytes. Besides, UGT1A1 was upregulated in liver and biliary SARS-CoV-2-infected organoids, which was associated with elevation of bilirubin. While, bile acid transporter gene SLCO4C1 was significantly decreased in SARS-CoV-2-infected biliary organoid, and the steroid hormone biosynthesis and bile secretion pathways were upregulated in SARS-CoV-2-infected organoids, which could cause bile acid accumulation. Moreover, the upregulation of IL1R2 and IL1RL1 could be associated with the albumin production inhibition, thereby impairing liver functions.[4] It was

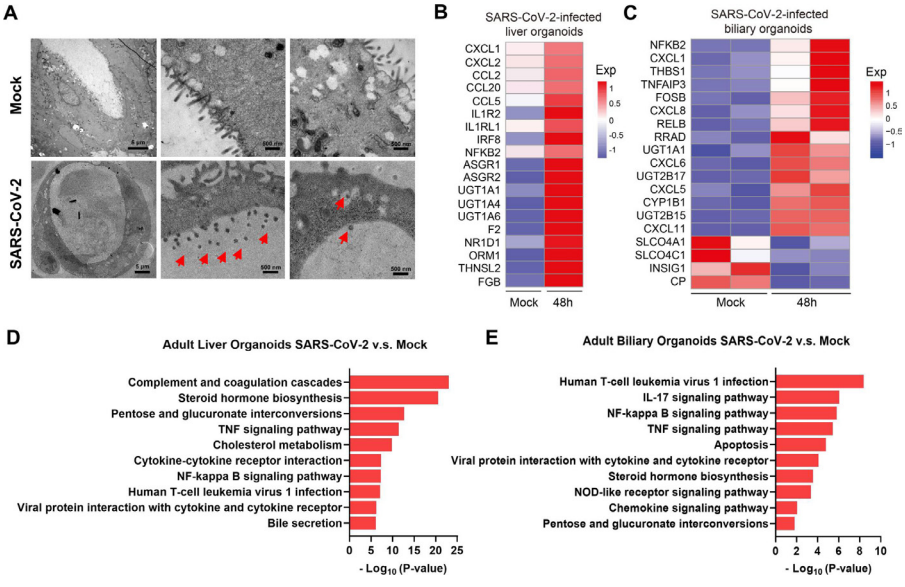

Figure 2 (A) Representative TEM imaging of biliary organoids at 0 and 24 hours of SARS-CoV-2 virus infection. Coronaviruses were observed in the lumen of the organoid (arrows) and are found at the membrane-bound vesicles (arrows). Scale bar 500 nm to 5 µm. (B, C) Heatmaps depicting the 19 most significantly enriched genes related to viral infection and immune system on SARS-CoV-2 infection in liver and biliary organoids. Coloured bar represents the log2-transformed values. (D, E) KEGG orthology-based annotation system of differential gene expression profiles from SARS-CoV-2-infected liver and biliary organoids compared with mock infection. TEM, transmission electron microscope.

reported an interacting host receptome of SARS-CoV-2 and demonstrated ASGR1 as alternative functional receptors, providing insight into SARS-CoV-2 tropism and pathogenesis.[5] We found that ASGR1 was upregulated in infected liver organoids, which may explain that SARS-CoV-2 viral load was higher in liver organoids compared with biliary organoids, though expression of ACE2 receptor was upregulated in cholangiocytes than hepatocytes (figure 2B–E; online supplemental figure 2B; online supplemental table S1). Consistently, bilirubin levels were reported to be significantly higher in non-survivors than survivors with SARS-CoV-2 infection.[1 6] Immunohistochemistry further validated the upregulated expressions of inflammatory factor CXCL8 and CXCL11 in SARS-CoV-2-infected biliary organoids compared with mock (online supplemental figure 2C).

In conclusion, SARS-CoV-2 can effectively infect human liver and biliary organoids. SARS-CoV-2 viruses are rapidly replicated in infected organoids to induce the production of proinflammatory cytokines/chemokines, local hepatocytes and cholangiocytes damage and consequent bile acid accumulation, which may contribute to liver injury.

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