

Supplementary methods

Patient samples

This study was approved by Ethics Committee of the First Affiliated Hospital of Sun Yat-Sen University. Liver and biliary organoids were derived from adjacent normal tissues in primary liver cancer patients who underwent surgical resection. Five lines of liver organoids and two lines of biliary organoids were collected from seven patients.

Cell culture of human liver and biliary organoids

Liver tissues of adult livers were dissected to $\sim 1\text{cm}^3$ sections and cut into pieces. Liver pieces were dissociated into single cells in Advanced DMEM/F12 (GIBCO) with collagenase, type IV (GIBCO) and dispase (Corning) for 1 hour in 37°C with gentle shaking. After filtering, hepatocytes were resuspended in 200 μL of growth factor reduced Matrigel (Corning) and plated in $\sim 30\ \mu\text{L}$ droplets in a 48-well tissue culture plate. The expansion medium was used for 5 days, then replaced by the medium with fresh differentiation medium for 15 days to differentiate organoids into hepatocytes. The expansion medium contained Advanced DMEM/F12 supplemented with 1% penicillin/streptomycin, 1% Glutamax, 10 mM HEPES, 1:50 B27 supplement (without vitamin A), 1:100 N2 supplement, 1.25 mM N-acetyl-L-cysteine, 10% (vol/vol) Rspo-1 conditioned medium, 10% (vol/vol) WNT3a-conditioned medium, 10 mM nicotinamide, 10 nM gastrin I, 50 ng/ml recombinant human EGF, 100 ng/ml recombinant human FGF10, 25 ng/ml recombinant human HGF, 10 μM forskolin, 5 μM A8301, 25 ng/ml Noggin and 10 μM Y27632. The human liver differentiation medium is the expansion medium supplemented with 10 μM DAPT, 3 μM dexamethasone, 25 ng/ml BMP7 and 100 ng/ml recombinant human FGF19, as described previously [1]. The medium was changed every 5 days.

Biliary tract tissues were received intact and the wall of the ducts was incised with a scalpel to expose the lumen. The mucosal surface was then abraded with a scalpel to

mechanically dissociate the epithelium. For organoid derivation, epithelium was resuspended in GFR Matrigel. Droplets (30 μ L) were plated per well into a 48-well plate. The medium contained Advanced DMEM/F12 supplemented with 1% penicillin/streptomycin, 1% Glutamax, 10 mM HEPES, 1:50 B27 supplement (without vitamin A), 1:100 N2 supplement, 1.25 mM N-acetyl-L-cysteine, 10% (vol/vol) Rspo-1 conditioned medium, 10 mM nicotinamide, 10 nM gastrin I, 50 ng/ml recombinant human EGF, 10 μ M forskolin, 5 μ M A8301 and 10 μ M Y27632, as described previously [2].

For passaging, organoids were dissociated into single cells using TrypLE express (GIBCO). After 3 passages, cultures were used for infection experiments.

Virus stock preparation

SARS-CoV-2 (isolate Guangdong/20SF014/2020; EPI_ISL_403934) was propagated on Vero E6 cells in MEM (Gibco), supplemented with 2% FBS, penicillin (10,000 IU/mL) and streptomycin (10,000 IU/mL). The culture supernatant was collected when CPE (cytopathic effect) was observed on ~80% cells. The supernatant was cleared by centrifugation and stored in aliquots at -80°C . Stock titers were determined according to the method of Spearman & Kärber. SARS-CoV-2 virus propagation and infection was performed in the BSL-3 laboratory of Guangdong Provincial Center for Diseases Control and Prevention.

SARS-CoV-2 infection

Organoids were infected at MOI of 1 in organoid culture medium. Time was set as zero when organoids were initially incubated with viruses. After 1 hour adsorption, culture media were removed and organoids were washed twice with ice cold PBS to remove unattached virus, then re-embedded in Matrigel and maintained in the culture medium. Supernatant and organoid samples were collected at different timepoints after inoculation.

Determination of virus titers using RT-qPCR

Viral RNA in supernatant and organoid samples was extracted by using Viral RNA minikit (Qiagen, Germany). Relative viral loads were calculated by using SARS-CoV-2 quantitative reverse-transcription PCR (RT-qPCR) kit (Daan Gene, Guangzhou, China). RT-qPCR assays were set up in triplicate. All protocols were performed according to the manufactures' instructions. Results were analyzed with the $\Delta\Delta CT$ method.

Immunofluorescence and immunohistochemistry

For whole mounting liver and biliary organoids staining, organoids were fixed in 4% paraformaldehyde and permeabilized with 0.3% Triton X-100 (biosharp, X100) in PBS, then blocked by 10% goat serum in PBS. Organoids were then incubated with mouse anti-SARS-CoV-2 S protein (prepared by Guangdong Provincial Center for Diseases Control and Prevention, 1:200), Albumin (Proteintech, 66051-1-Ig, 1:50), CK18 (Invitrogen, PA5-14263, 1:25), ASGR1 (Abcam, ab254261, 1:50), CK19 (Abcam, ab76539, 1:1000) at 4 °C overnight, and then incubated with mouse IgG (H+L) cross-adsorbed secondary antibody (Invitrogen, A11003, 1:500) and rabbit IgG (H+L) cross-adsorbed secondary antibody (Invitrogen, A32723, 1:1000), and phalloidin (Sigma, P5282, 1:300) at room temperature. Organoids were eventually incubated with DAPI. Imaging was performed on Multiphoton Microscope STELLARIS 8 DIVE (Leica, Germany).

Immunohistochemistry was performed on 4 μm sections of paraffin-embedded organoids. Immunostaining for CXCL8 (Proteintech, Cat No. 27095-1-AP, 1:500) and CXCL11 (Abcam, ab9955, 1:1000) proteins were performed for patient-derived biliary organoids at 0 hour and 24 hours after SARS-CoV-2 infection. Images were acquired on OLYMPUS BX63F microscope.

Transmission electron microscopy

Organoids were fixed with 2.5% glutaraldehyde in 0.1M PBS to pH 7.4 and 2% paraformaldehyde at 4 °C overnight. Organoids were then dehydrated at room temperature in a graded ethanol series (30, 50, 70, 80, 90, and 100%) and embedded in epon812. Epon was polymerized for 48h at 60°C. Ultrathin sections at 70nm were cut using a diamond knife (Diatome) on a Leica UC7 ultramicrotome, and transferred onto 200 Mesh copper grids covered with a formvar and carbon film. Sections were post-stained with uranyl acetate and lead citrate. All TEM data were collected by transmission electron microscope (FEI Tecnai G2 Spirit Twin, USA)

Bulk RNA sequencing

Total RNA was extracted from biliary organoids by RNeasy Mini Kit (Qiagen, Germany). Beads (Invitrogen) with oligo (dT) were used to isolate poly (A) mRNA after total RNA was collected. Fragmentation buffer was added for interrupting mRNA to obtain short fragments. Using these short fragments as templates, random hexamer-primer was used to synthesize the first-strand cDNA. The second-strand cDNA was synthesized using buffer, dNTPs, RNase H, and DNA polymerase I. Short fragments were purified with QIAQuick PCR Extraction Kit (Qiagen) and resolved in EB buffer for end reparation and adding poly (A). After that, the short fragments were connected to sequencing adaptors. For PCR amplification, suitable fragments were selected as templates based on agarose gel electrophoresis. The library was sequenced using HiSeq X TEN platform and 150-bp paired-end reads were generated.

mRNA sequencing data analysis

Gene differential expression analysis between normal controls and 48h group was performed by the R DESeq package using count data of high-throughput sequencing. DESeq result was filtered by p value (< 0.05) and fold change (> 2). Ensembl ID of differentially expressed genes was then converted to Entrez ID by the R org.Hs.eg.db package and submitted to the R clusterProfiler package to perform KEGG pathway enrichment. From the KEGG pathway enrichment result, significant KEGG pathways (p value < 0.05) related to viral infection and immune system were selected for further

investigation. The heatmap plot of gene expressions, the volcano plot of result of gene differential expression analysis, and the bar plot of pathways were generated using the R ggplot2 package.

Statistical analysis

Error bars in these figures indicate S.D. (for RT-qPCR). Student's t-test or ANOVA test was employed to compare between two groups of $n = 3$ or more samples. $P < 0.05$ was defined as statistical significance. All of the statistical analyses in this study were done with GraphPad Prism 8 software.

References

- [1] Broutier L, Andersson-Rolf A, Hindley CJ, et al. Culture and establishment of self-renewing human and mouse adult liver and pancreas 3D organoids and their genetic manipulation. *Nat Protoc.* 2016;11:1724-43.
- [2] Tysoe OC, Justin AW, Brevini T, et al. Isolation and propagation of primary human cholangiocyte organoids for the generation of bioengineered biliary tissue. *Nat Protoc.* 2019;14:1884-1925.