Supplementary information

A novel ameliorated rat model of reversible obstructive jaundice

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Materials and methods

Animals

Male Sprague-Dawley rats (8-10 weeks and 200–250 g) were provided by Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). All animal procedures were performed in accordance with the Experimental Animal Management Ordinance of Wuhan University. This research was approved by the Institutional Animal Care and Use Committee of Wuhan University Center for Animal Experiments.

Experimental design

Twenty-four rats were randomly divided into four groups of six each. The groups were as follows: The sham operation (SH) group, the common bile duct ligation (BDL) group, the ameliorated bile duct ligation (ABDL) group, and the reversal of ameliorated bile duct ligation (R-ABDL) group. Another 12 rats were used for survival analysis.

Surgical approaches

Bile duct ligation: Rats were anesthetized with 1% (w/v) pentobarbital sodium (0.005 mL/g). Skin preparation, sterilization, and draping were done prior to surgery. The abdominal cavity was entered through a median abdominal incision about 1.5 to 2.0 cm, in the linea alba. Two sterile

cotton balls about 0.5 cm in diameter were placed in the left and right subhepatic spaces to provide excellent exposure. The bile duct was completely exposed and gently separated by a sterile cotton swab near the porta hepatis. Two ligatures were placed in the bile duct with 4-0 silk suture. The bile duct was then bisected between the two ligatures. The proximal end of the bile duct was then ligated again to avoid slipping. Strict asepsis was followed and hence antibiotics were not required. In the SH group, only laparotomy and exploration of the bile duct were performed.

Ameliorated bile duct ligation (ABDL): A rubber catheter (Yangzhou Jiangyang Special Rubber and Plastic Products Co., Ltd.) and a platinum-cured silicone tube (8600-3015, Thermo Fisher Scientific) were used in this model. The rubber catheter was used as a spacer with two punctures to avoid direct injury during suture ligation. In brief, the bile duct was exposed by the procedure described above. The bile duct was then separated, and a spacer was placed behind it using a 4-0 non-absorbable suture (Mersilk, Johnson Medical Ltd., Shanghai, China). A second spacer was placed in front of the bile duct through the punctures on both sides of spacer. Two sutures were crossed over the silicone tube and tightly knotted onto a third spacer which was embedded subcutaneously on the right of median abdominal incision. The incision was then sutured in layers with a 4-0 thread. The whole procedure took approximately 15 minutes.

Reversal of biliary obstruction based ABDL: The reversal of ABDL (R-ABDL) was performed seven days after the obstruction by ABDL. Preoperative preparation was as described above. A small incision was made to the right of the median abdominal incision and the spacer in the subcutaneous tissue was exposed. The suture securing the third spacer was divided, the silicone tube was removed and the non-absorbable suture was gently removed. The whole procedure took less than 5 minutes.

Biochemical analysis

Samples of whole blood were collected from the inferior vena cava and immediately centrifuged at 3000 rpm for 10 minutes to obtain the serum. The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL) and albumin (ALB) were measured at the biochemistry laboratory of the Zhongnan Hospital of Wuhan University.

Histological analysis and Masson's trichrome staining of the liver tissue

Liver tissue was removed and fixed in 10% formalin. After embedding in paraffin, the liver tissue was cut into 5-μm-thick sections. The sections were stained with hematoxylin and eosin (H&E), Masson's trichrome and Sirius red using standard techniques. Each section was assessed under light microscopy. Quantification of the area of fibrotic lesions and collagen was done by analysis of five randomly selected fields in each liver sample, using Image J software as described in literature (Sundaram et al., 2019). For immunohistochemical detection, liver sections were incubated with primary antibodies of CK19 (1:600, Servicebio, China), subsequently with

Horseradish Peroxidase labeled IgG (IgG-HRP) and diaminobenzidine to develop color. All images were collected using an optical microscope (Olympus, Japan).

Western blot analysis

Samples containing an equal amount of protein (40 μg) were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane. The membranes were blocked with 5% nonfat milk for 2 h at room temperature. After washing three times with TBST, the membranes were then incubated overnight at 4 °C with primary antibodies against p16 (1:1000; Proteintech), p21 (1:1000; Proteintech), or β-actin (1:5000; Proteintech). Subsequently, a secondary horseradish peroxidase-conjugated anti-rabbit antibody (1:5000; Proteintech) was applied. Finally, the blots were scanned by unfiltered rays with an imaging system (Automatic Gel Imaging Analysis System, China), and the bands were quantified by ImageJ software.

Survival analysis

Survival time was compared between the biliary obstruction group (BDL) and the reversal of biliary obstruction group (R-ABDL).

Statistical analysis

Data were expressed as mean ± standard deviation. Statistical analysis was performed using the statistical package SPSS Statistics for Windows, Version 17.0. (SPSS Inc., Chicago, IL, USA). The differences in serum levels of ALT, AST, TBIL and ALB between the two groups were analyzed by the one-way analysis of variance. A $p < 0.05$ was considered significant.

Reference

Sundaram B, Behnke K, Belancic A, et al., 2019. iRhom2 inhibits bile duct obstruction-induced liver fibrosis. *Sci Signal*, 12(605):eaax1194.
https://doi.org/10.1126/scisignal.aax1194

Supplementary Figure

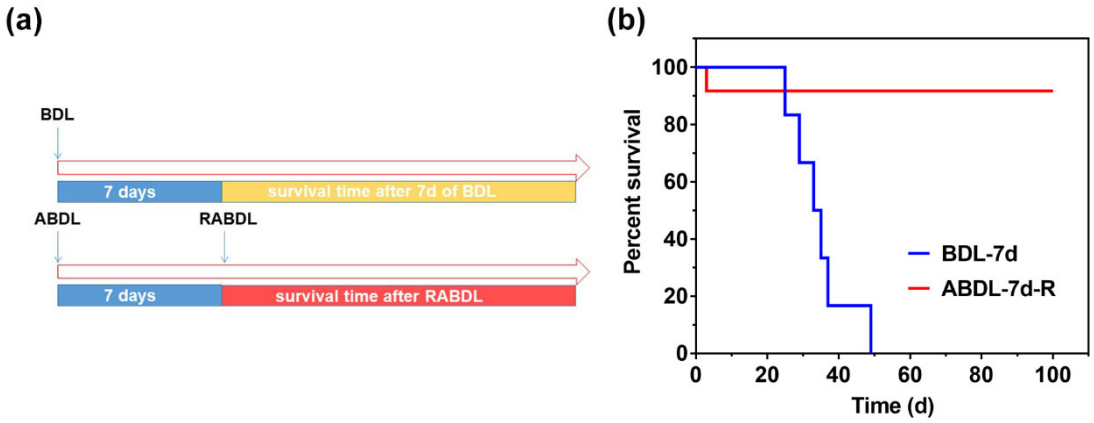

Fig. S1 Biliary reversal after biliary obstruction rescue death in rats. (a) Experimental design; (b) Survival time was compared between groups of BDL and R-ABDL.