Supplemental Methods & Materials

Isolation and culture of primary hepatic stellate cells

Primary murine HSCs were isolated from WT, JCAD-KO or HSC-JCAD-KO mice, purified by Nycodenz gradient centrifugation (D2158, Sigma-Aldrich Chemical Co.), and seeded on collagen-type I-precoated plastic dishes according to the method as we reported previously,[1-3] which was modified according to the methods reported.[4,5] Fivedays after isolation, they were cultured in a complete medium or medium with TGF-β (100-21C, Peprotech) at 10 ng/mL for 24 and 48 hours. Total RNA or protein was extractedthereafter. For mouse bile (mBAs) stimulation, mBAs collected 14 days post-BDL surgery underwent 0.22 μm filtration and 1:100 dilution for stimulation of primary mouse HSCs. After 24 hours of exposure to diluted bile, total RNA was extracted. Isolation of *in situ*-activated stellate cells from BDL-induced fibrotic livers were undertaken 5 or 14 days after BDL surgery. In these experiments, HSCs from 3 livers were pooled to increase the yield. Total RNA was extracted from pooled HSCs withoutculture. *In situ* activation of HSCs was confirmed by expression of α-SMA, TGF-β, TIMP1, etc. with quantitative reverse transcriptase polymerase chain reaction (qRT- PCR) analysis.

Ultrastructural analysis by electron microscope

Immediately after the mice were sacrificed, the distal part of the left lobe was resected.Liver tissue was cut into proper pieces in size of 0.5–1.0 mm^3 and fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide (OsO_4), and prepared for routine transmission electron microscopy. Tissue blocks containing areas with ductular structures were selected on semi-thin sections by light microscopy, and further selectedfor cutting ultra-thin sections. Four mice from each test group were used for ultrastructural examination as we reported previously.[6]

Hepatic hydroxyproline content measurement

Hepatic hydroxyproline content was evaluated in liver tissue snap-frozen in liquid N2 and stored at –80°C until analysis. Tissue was weighed (100 mg), and treated with 1,000 microliters of HCl (6 mol/L), and hydrolyzed at 100°C for 5 hours. After the homoge natewas filtered through activated charcoal, detection buffer was added to the hydrolysate and mixed, followed by incubation at 60°C for 15 minutes. Optical density (OD) value at 550 nm was recorded after the samples were cooled to room temperature. Hydroxyproline content was determined by acid hydrolysis method as reported[1,7,8] witha kit from Jiancheng Bioengineering, China, and expressed as μg hydroxyproline/mg liver.

Second harmonic generation/two-photon excitation fluorescence (SHG/TPEF)microscopy and AI analyses

Unstained liver sections from WT and HSC-JCAD-KO mice were examined using SHG/TPEF microscopy with computer-assisted analyses. These liver tissues on glass slides, prepared by the formalin-fixed and paraffin-embedded (FFPE) procedure, were first deparaffinized using a standard protocol, then scanned by Genesis®200 (a fully- automated, staining-free multiphoton fluorescence imaging microscope manufactured by Histoindex Pte. Ltd). Samples were laser-excited at 780 nm, SHG signals were recorded at 390 nm, and TPEF signals were recorded at 550 nm. Images were acquiredat 20X magnification with a resolution of 512×512 pixels for each image tile of 200×200 μm^2. Multiple adjacent tiles were captured to encompass the whole tissue area.As described previously,[9,10] an AI-based image analysis system recognizes and segregates the whole biopsy area into five histologic zones, namely, central vein (CV),pericentral (PeriCV), portal tract (PT), periportal (PeriPT) and peri-sinusoid (PS) zones (Supplementary Fig. 5A). Fibrosis features from the SHG channel, and the surroundingcellular structures including steatosis features from the TPEF channel, in each zone andthe overall region were analyzed, and the data created an objective, unbiased

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and repeatable assessment of collagen deposition in the tissue.

Dual luciferase activity assays

LX2 cells were co-transfected with empty vector or expression plasmids at 0.5 μg encoding YAP, JCAD or siRNAs against JCAD or YAP together along with 8xGTIICluciferase reporter plasmid at 0.5 μg and renilla luciferase plasmid pRL-SV40 at 100 ng (Promega) as an internal control in 12 well plates. Dual luciferase assays of firefly and renilla luciferase activity were carried out (Promega) according to themanufacturer's instructions. Firefly luciferase activity was expressed as a ratio in relative light units and normalized to the renilla luciferase activity (firefly luciferase over renilla luciferase) as previously reported.[11,12]

In situ EdU labeling of mouse tissues

To *in situ* determine cholangiocyte proliferation, 5-ethynyl-2-deoxyuridine (EdU) wasinjected at a dosage of 100 mg/kg body weight intraperitoneally (i.p.) four hours beforesacrifice.[13] EdU staining was performed on paraffin-embedded tissue sections following the BeyoClick™ EdU proliferation kit instruction (C0071S). Positive areasfrom five fields (magnification×100)/section were quantified with ImageJ. N=Six animals per group.

Culture of HSCs

Human immortalized hepatic stellate cells (HSCs) provided by Prof. David Brenner[14]and LX2 cells from Prof. Scott Freidman[2,15] were incubated in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, 1% L-glutamine and 1% sodium pyruvate. All cells were cultured in a humidified incubator at 37°C with 5% CO_2 in air. To genetically suppress JCAD-Hippo-YAP signaling, LX2 cells or HSC cells were transfected with small interfering RNAs (siRNA) with RNAi MAX reagent (InvitrogenCat. No. 13778150). siRNAs against JCAD (JCAD-siRNA), siRNAs against YAP (YAP-siRNA) and siRNA negative control were purchased from Shanghai Gene Chem(Shanghai, China) (see Supplementary Table 2). On the day of transfection, LX2 or HSC cells in a logarithmic growth stage were uniformly inoculated into six-well platesat 2×10^6 cells in each well. The knockdown efficiency was validated by qRT-PCR and Western blotting analysis of JCAD or YAP at mRNA and protein levels. Hippo-YAP signaling activity was inhibited pharmacologically by treating cells with either Lats- IN-1, a potent and ATP-competitive inhibitor of Lats1 and Lats2 kinases that promotesYap-dependent proliferation (HY-138489, MedChemExpress), or verteporfin, an inhibitor of YAP activity (HY-B0146, MedChemExpress). For TGF-β1 treatment, cellswere starved for 24 hours with FBS-free DMEM medium overnight, and then treated withTGF-β1 at 10 ng/mL (100-21C, Peprotech). Cell growth was monitored with CellTiter-Lumi™ (C0065M, Beyotime). For mBAs treatment, mBAs collected 14 days post-BDLsurgery underwent 0.22 μm filtration and 1:100 dilution for stimulation of HSCs. After24 hours of exposure to diluted bile, qRT-PCR analysis was performed on collected RNA, and proteins were extracted at 48 hours for subsequent Western blot analysis.

Protein lysate and Western blotting analysis

Tissues, primary HSCs or other cells were lysed in RIPA lysis buffer with protease inhibitor (catalog no:10925700; Roche, Mannheim, Germany) and phosphatase inhibitor (catalog no:78420; Thermo Scientific, Rockford, IL, USA). Proteins were separatedon 4–12% Bis-Tris gels (catalog no.: NP0322BOX; Invitrogen) by electrophoresis andtransferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, IPVH00010).The membranes were blocked with 5% non-fat milk or 5% BSA in Tris-buffered saline(TBS) containing 0.1% Tween 20 for 1 hour at room temperature, and incubated with primary antibodies overnight at 4℃, and then corresponding secondary antibodies for 60 min at room temperature. All experiments were performed in triplicate wells and repeated at least three times. Primary and secondary antibodies were listed in Supplementary Table 3.

RNA isolation and quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

Total RNA was isolated by TRIZOL reagent (Life Technologies, Grand Island, NY, USA) and reversely transcribed to cDNA (Takara Bio, Dalian, China). Quantitative RT- PCR was carried out with Hieff® qPCR SYBR Green Master Mix (11202ES03, Yeasen,Shanghai, China). Sequences of primer pairs used in PCR analysis are listed in Supplementary Table 4. mRNA levels were normalized to β-actin as a house-keeping gene control. Relative gene expression levels were calculated using a $2^{-\Delta\Delta CT}$ method aspreviously reported.[8]

Histology and immunohistological staining

Fresh liver tissue was fixed in formalin, paraffin-embedded, and dehydrated. H&E, Masson and Sirius red staining was conducted on paraffin-embedded liver sections to visualize liver morphology and to assess fibrotic extent. Hepatic fibrosis as visualized by immunohistochemistry was photometrically semi-quantitated by Image-J software in a blinded manner. Immunohistochemical (IHC) and immunofluorescent staining wasperformed as reported previously[8] and following the instruction provided by the manufacturers of the respective antibodies. Semi-quantitative fibrotic score was assessed in a blinded manner by a board-certified pathologist according to the well- accepted scoring system.[16] Antibodies used for immunohistochemical or fluorescent staining are listed in Supplementary Table 3.

SUPPLMENTARY REFERENCES

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