Supporting Information

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SI Materials and Methods

Antibodies and Reagents. Antibodies of α-smooth muscle actin (α-SMA), p-STAT3 (Tyr705), STAT3, cyclin D1, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), p-Smad2 (Ser465/467), p-Smad3 (Ser423/425), Smad2, Smad3, PARP, PDGF receptor-β (PDGFR), p-PDGF receptor-β (p-PDGFR) (Tyr857), and P-Akt (Ser473) were purchased from Cell Signaling. Myeloid cell leukemia sequence 1 (Mcl-1) and Akt were purchased from Santa Cruz Biotechnology. WP-1066, a specific STAT3 pathway inhibitor, was obtained from Calbiotech. Sodium vanadate (a nonspecific phosphatase inhibitor) was purchased from Cayman Chemical. SHP-1 inhibitor (PTP inhibitor III) was purchased from Calbiochem. Thioacetamide was purchased from Sigma.

Histological Analysis for Liver Fibrosis. Mouse liver tissues were fixed by 10% (vol/vol) formalin in water and thin-sliced for pathological examination and Sirius Red staining. The severity of liver fibrosis was graded according to the Metavir fibrosis scoring system. The collagen-positive areas were quantified by a TissueFAXs Plus image cytometer with TissueFAXS acquisition software (version 3.0.5120.119), and analyzed by HistoQuest software (version 3.0.3.0158; TissueGnostics).

Immunohistochemical Staining. The immunohistochemical staining was performed by Leica Microsystems BONDMAX autostainer according to the manufacturer's protocols.

Cell Culture. The HSC-T6 cells were cultured in Waymouth's medium and the LX2 cells and mouse primary HSC in DMEM (Invitrogen), and all were supplemented with 10% (vol/vol) FBS, 100 units/mL penicillin G, 100 μg/mL streptomycin sulfate, and 2 mM L-glutamine. All cells were cultured in a 37 °C humidified incubator under an atmosphere of 5% (vol/vol) CO_2 in air. Quiescent HSC cells were activated after FBS supplementation.

Primary Mouse HSC Isolation. After we anesthetized the mouse with isoflurane according to our institution's approved animal protocol, the mouse was fixed, disinfected, and a laparotomy was performed to expose the liver and portal vein. The portal vein was cannulated for continuous perfusion with Leffert's buffer/EGTA solution. The infrahepatic inferior vena cava was open and the suprahepatic inferior vena cava was clamped to ensure antegrade perfusion of the liver. The liver was perfused with Leffert's buffer/EGTA for 5–10 min and then Leffert's buffer for 3–5 min. Then the liver was perfused with collagenase (type IV; Sigma) for 15 min until the liver was well digested. After removing the catheters from the portal vein, the liver was explanted into a Petri dish containing Leffert's buffer/$CaCl_2$. The liver was gently minced with scrapers within a sterile cell-culture hood. The digested mouse liver was filtered through a 70-μm cell strainer into a 50-mL tube. The cell mixture was centrifuged (Kubota, Japan, Model 2800, Rotor RS-240) at 600 rpm for 5 min (4 °C). The supernatant was centrifuged at 2,000 rpm for 10 min (4 °C). Three milliliters of DMEM was added with the pellet, and then the cell suspension was added into Histodenz (Sigma) gradient [containing 3 mL of 20% (wt/vol) Histodenz in the bottom and 3 mL of 8.2% (wt/vol) Histodenz at the top] and then followed by a centrifugation at 3,000 rpm for 20 min (4 °C) without break. At the end of centrifugation, the HSCs were visible as a thin white layer in the interface of the Histodenz gradient and the HSC was collected and transferred into new 10-mL tube. The DMEM was filled up to 10 mL and followed by centrifugation at 1500 rpm for 8 min (4 °C). The HSC-containing pellet was resuspended with DMEM with 10% (vol/vol) FBS and seeded in the culture dish for cell culture.

Western Blotting. Whole-cell extracts were obtained after RIPA buffer (Millipore), and the protein concentrations were quantified by using a BCA protein assay kit (Thermo). Twenty-five micrograms of protein were loaded onto SDS-acrylamide gels of various percentages and blotted onto PVDF membranes. Membranes were then incubated with primary antibodies. After extensive washing, membranes were incubated with blocking buffer containing horseradish-peroxidase conjugated secondary antibody. Proteins were detected by Immobilon Western Chemiluminescent HRP Substrate (Millipore).

Cell Viability and Apoptosis Analysis. The HSC-T6 and LX2 cells were seeded in 96-well, flat-bottomed plates with 5,000 cells per well with 10% (vol/vol) FBS for 24 h. The cells were exposed to sorafenib or SC-1 at various concentrations for 24 and 48 h. The effects of the individual agent on cell viability were evaluated by using a CellTiter 96 AQueous one solution cell proliferation assay containing 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS; Promega) in triplicate according to the manufacturer's protocol.

After sorafenib and SC-1 treatment for 24 h, the percentage of apoptotic cells were determined by Annexin V and propidium iodide (PI) double-staining after summation of both the early (Annexin V+/PI-) and late (Annexin V+/PI+) apoptotic cells, on a BD FACS Verse flow cytometer (BD).

Ectopic Expression of STAT3. Rat STAT3 (Open Biosystem) was constructed into pLVX-AcGFP-N1 expression vector (Clontech), which was later cotransfected into 293FT cells along with the lentiviral packaging and expression vectors (P8.91 and VSV-G) by using Lipofectamine 2000 transfection reagent (Invitrogen). The lentiviral supernatant was harvested 48 h after transfection and infected 5×10^5 HSC-T6 cells, which were seeded on 6-cm dish.

Colony Formation Assay. HSC-T6 cells were plated in 10-cm dishes (1,500 cells per dish) and cultured in Waymouth's medium for 2 wk. Then the cells were fixed with 4% (vol/vol) formaldehyde and stained with 0.1% crystal violet.

SHP-1 Phosphatase Activity. The RediPlate 96 EnzChek tyrosine phosphatase assay kit was used for SHP-1 activity assay (Molecular Probes) according to the manufacturer's protocol.

Interleukin-6 Stimulation. For interleukin-6 stimulation, HSCs were serum deprived for 4 h, and then treated with sorafenib or SC-1 10 μM for 4 h, followed by induction with interleukin-6 100 ng/mL (R&D Systems) for 30 min.

PDGF-BB Induction. For PDGF-BB induction, HSCs were serum deprived for 4 h, and then treated with sorafenib or SC-1 10 μM for 4 h, followed by induction with recombinant human or rat PDGF-BB 50 ng/mL (R&D Systems) for 10 min, on LX2 or HSC-T6 cells, respectively.

TGF-β Induction. For TGF-β induction, HSCs were serum deprived for 4 h, and then treated with sorafenib or SC-1 10 μM for 4 h, and followed by stimulation with recombinant human TGF-β1 10 ng/mL (R&D Systems) for 20 min.

Plasma IL-6 Quantification. The plasma IL-6 levels were quantified by Quantikine Colorimetric Sandwich ELISA kit (R&D Systems) as a noninvasive marker for hepatic p-STAT3.

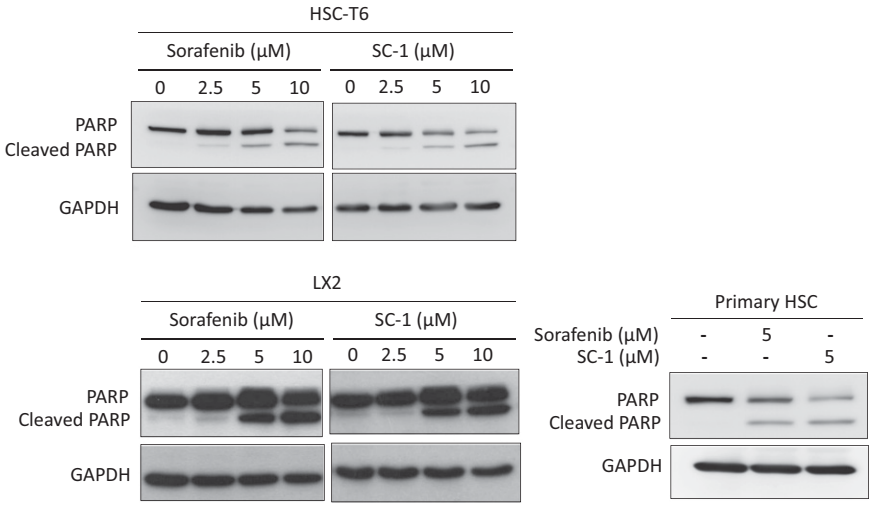

Fig. S1. Sorafenib and SC-1 induced apoptosis on LX2, HSC-T6, and primary mouse HSCs. The increasing cleaved PARP fragments indicate cell apoptosis.

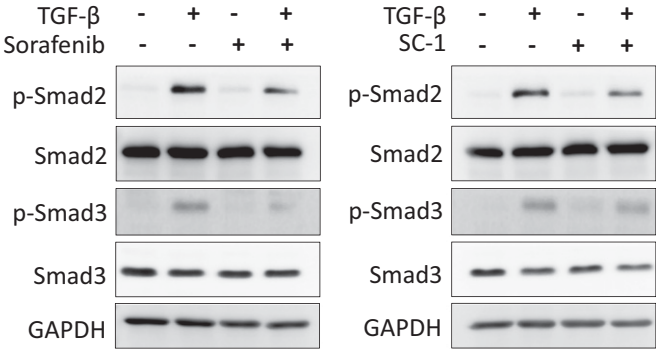

Fig. S2. The down-regulation of p-Smad2 and p-Smad3 in the TGF-β pathway by either sorafenib or SC-1 on LX2 cell by the induction of recombinant human TGF-β1.

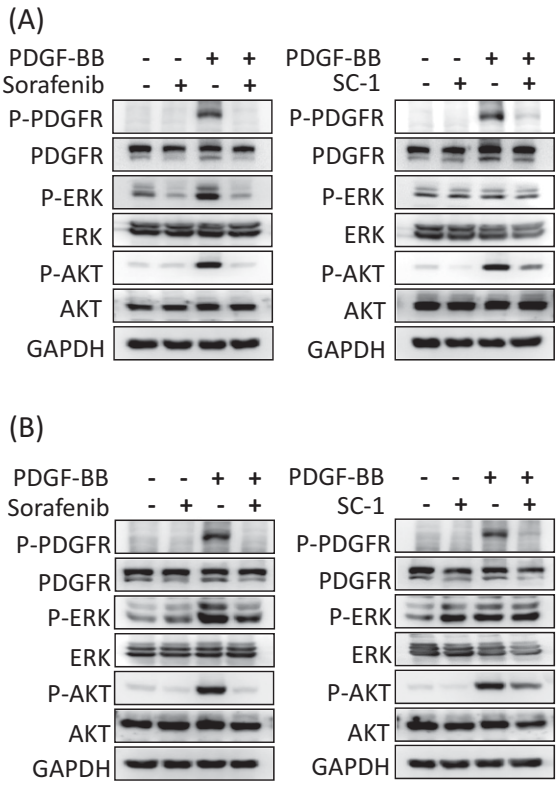

Fig. S3. The down-regulation of p-PDGFR and p-Akt in the PDGF pathway by either sorafenib or SC-1. (*A*) LX2 cell, by the induction of recombinant human PDGF-BB. (*B*) HSC-T6 cells, by the induction of recombinant rat PDGF-BB.

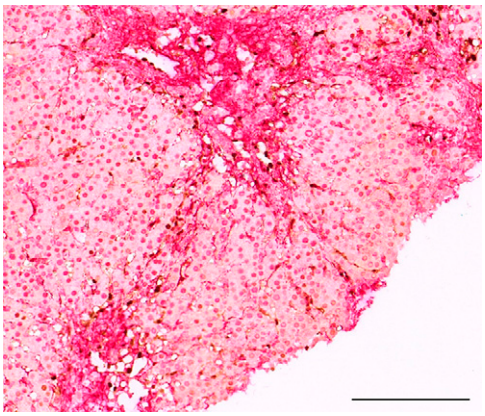

Fig. S4. The nuclear p-STAT3 (brown color)–positive cells are largely HSCs (cytosolic α-SMA–positive, red color) in the fibrotic areas in CHB cirrhotic patients. (Scale bar: 200 μm.)

Table S1. Characteristics of 40 chronic hepatitis B patients

Characteristics	Value
Age, yr	42 (12)
Male, *n*(%)	33 (83)
Genotype B, *n*(%)	26 (67)
HBeAg-positive, *n*(%)	20 (50)
BMI, kg/m^2	24.6 (3.2)
Albumin, g/dL	4.3 (0.3)
ALT, U/L	190 (211)
Bilirubin-T, mg/dL	1.1 (0.3)
HBsAg, log_{10} IU/mL	3.51 (0.66)
HBV DNA, log_{10} IU/mL	6.13 (1.52)
HAI score	7.5 (4.1)
Metavir score (0:1:2:3:4)	8:8:8:8:8

Data are expressed as mean (SD) or number (percentage) accordingly. ALT, alanine aminotransferase; BMI, body mass index; HAI, histology activity index.