A Novel Small Compound That Promotes Nuclear Translocation of YB-1 Ameliorates Experimental Hepatic Fibrosis in Mice

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Transforming growth factor-β (TGF-β) is considered to be a major factor contributing to liver fibrosis. We have previously shown that nuclear translocation of YB-1 antagonizes the TGF-β/Smad3 signaling in regulating collagen gene expression. More recently, we have demonstrated that the novel small compound HSc025 promotes nuclear translocation of YB-1, resulting in the improvement of skin and pulmonary fibrosis. Here, we presented evidence as to the mechanism by which HSc025 stimulates nuclear translocation of YB-1 and the pharmacological effects of HSc025 on a murine model of hepatic fibrosis. A proteomics approach and binding assays using HSc025-immobilized resin showed that HSc025 binds to the amino acid sequence within the C-tail region of YB-1. In addition, immunoprecipitation experiments and glutathione S-transferase pulldown assays identified poly(A)-binding protein (PABP) as one of the cytoplasmic anchor proteins of YB-1. HSc025 directly binds to YB-1 and interrupts its interaction with PABP, resulting in accelerated nuclear translocation of YB-1. Transfection of cells with PABP siRNA promoted nuclear translocation of YB-1 and subsequently inhibited basal and TGF-β-stimulated collagen gene expression. Moreover, HSc025 significantly suppressed collagen gene expression in cultured activated hepatic stellate cells. Oral administration of HSc025 to mice with carbon tetrachloride-induced hepatic fibrosis improved liver injury as well as the degree of hepatic fibrosis. Altogether, the results provide a novel insight into therapy for organ fibrosis using YB-1 modulators.

Irrespective of the initial stimuli, excessive deposition of extracellular matrix is a common hallmark of fibrotic disease in various organs, including the liver (1). Hepatic fibrosis is a common response to chronic liver injury, which ultimately leads to cirrhosis and is often associated with hepatocellular carcinoma. When fibrosis progresses to cirrhosis, a number of life-threatening complications associated with portal hypertension and liver failure occur, including variceal bleeding, ascites formation, and the hepatorenal syndrome (2). Although multiple factors play a role in fibrogenesis, it is well recognized that transforming growth factor-β (TGF-β) is the key molecule accelerating hepatic fibrosis (1). TGF-β stimulates gene expression of profibrogenic molecules such as collagen and plasminogen activator inhibitor-1. Identification and characterization of Smad proteins, intracellular mediators of the signal transduction of TGF-β superfamily members, have led to a better understanding of the precise mechanisms of TGF-β actions from the viewpoint of its intracellular signaling pathway and cross-talk with other cytokines (3).

We have been studying a number of growth factors and cytokines that antagonize TGF-β/Smad signaling as well as their application in the treatment of hepatic fibrosis. For example, hepatocyte growth factor, which was originally identified as a potent mitogen for adult rat hepatocytes, has subsequently been revealed to suppress experimental hepatic fibrosis (4, 5). We have recently shown that hepatocyte growth factor suppresses profibrogenic signal transduction via nuclear export of Smad3 with galectin-7 (6). Interferon-γ (IFN-γ), a pleiotropic cytokine produced by T lymphocytes and natural killer cells, is also antagonistic to TGF-β in the transcriptional regulation of extracellular matrix genes, including collagen type I (7–9). Antifibrotic effects of IFN-γ have been shown in several hepatic fibrosis models (10–12). Regarding the molecular mechanisms of inhibitory action, we have previously shown that nuclear translocation of YB-1 by IFN-γ antagonizes TGF-β/Smad signaling in the regulation of collagen gene expression in vitro (9). In addition, Dooley et al. (13) observed that YB-1 is a potent inducer of Smad7 expression in activated hepatic stellate cells and that it could be used to antagonize TGF-β in liver, kidney, and other tissues during chronic stages of fibroproliferative diseases. Adenovirus-mediated overexpression of YB-1 driven by a collagen enhancer suppressed the progression of hepatic fibrosis and enhanced the antifibrotic effects of IFN-γ (14). Furthermore, we have demonstrated that a novel small compound HSc025...
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stimulates nuclear translocation of YB-1 and improves skin and pulmonary fibrosis (15).

This study was conducted to elucidate the mechanism by which HSc025 promotes nuclear translocation of YB-1 resulting in the blockage of TGF-β signaling. A combination of proteomics studies and immunoprecipitation followed by glutathione S-transferase (GST)-pulldown assays suggested that HSc025 interrupts the interaction between YB-1 and poly(A)-binding protein (PABP)2 through its direct binding to YB-1. Moreover, we demonstrated that HSc025 improves liver injury and fibrosis induced by carbon tetrachloride (CCL4) injections in mice. These findings provide novel insights into possible treatment strategies for hepatic fibrosis using YB-1 modulators.

EXPERIMENTAL PROCEDURES

Cell Cultures, Plasmids, and Reagents—Normal human dermal fibroblasts were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (FCS). Rat hepatic stellate cells were isolated and purified from the livers of normal rats as described previously (16). Briefly, livers were excised, digested with Pronase and collagenase, filtered through a nylon mesh, and then centrifuged. The pellet was resuspended in Nycodenz solution, and hepatic stellate cells were suspended in DMEM supplemented with 10% FCS. Normal human dermal fibroblasts were dialyzed against extraction buffer containing 20 mM Hepes-KOH (pH 7.9), 100 mM NaCl, 1 mM MgCl₂, 0.2 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 0.1% Nonidet P-40 at 4 °C. For purification of HSc025-binding proteins, 0.5 mg of cytoplasmic extracts was first incubated with 1 mg of nonimmobilized beads for 2 h at 4 °C. Unbound extracts were incubated with nonimmobilized beads again and then reacted with HSc025-immobilized beads three times. Protein-bound beads were washed with extraction buffer five times in each cycle, and bound proteins were eluted with Laemmli dye, separated by SDS-PAGE, and then visualized by silver staining.

Immunoprecipitation and Microsequencing—Cytoplasmic extracts were prepared from human fibroblasts as described previously (9). Immunoprecipitation was performed using rabbit polyclonal anti-YB-1 antibodies and protein G-Sepharose beads (Amersham Biosciences). Proteins bound to beads were resolved by 10% SDS-PAGE, and then visualized by silver staining. Desired bands were excised, in-gel digested with trypsin, and solvent extracted, and the resulting MS/MS spectra were searched using the MASCOT search engine as described previously (6).

Bacterially Expressed Recombinant Proteins—The His₆-tagged full length of YB-1 as well as its deletion mutants, SET and APRIL recombinant proteins, were produced using the pET system (Novagen) as described previously (9). The full-length PABP fused to glutathione S-transferase (GST) was expressed in *Escherichia coli* and purified as described previously (9).

GST-Pulldown Assay and Western Blot Analysis—GST-fused proteins bound to glutathione-Sepharose beads and His₆-tagged YB-1 protein were incubated in the absence or presence of HSc025 for 2 h at 4 °C, and the bound proteins were detected by Western blotting using an anti-His₆ monclonal antibody (Qiagen, Hilden, Germany) (9).

Animal Model—Animals used in this study received human care in compliance with the National Institutes of Health guidelines. Hepatic fibrosis was induced in C57BL/6 mice by intraperitoneal injections of 0.1 ml/kg body weight of CCl₄ twice a week for 8 weeks as described previously (19). The mice were treated with either 3, 15, and 75 mg/kg/day of HSc025 or vehicle for 4 weeks after injections of carbon tetrachloride for 4 weeks. At the end of the experiment, liver weights and serum ALT levels were determined. In addition, sections prepared from excised liver specimens were subjected to hematoxylin and eosin (H&E) or Azan-Mallory staining.

Histological Analysis—H&E sections were randomly selected and blindly analyzed for the degree of necrosis or hepatocellular vacuolization as markers of hepatic injury. To evaluate the degree of necrosis, a scoring system based on the severity in the centrilobular zone was employed (0; none, 1; less than one hepatocyte layer, 2; one to two layers, 3; three layers and more). Hepatocellular vacuolization was graded based on the proportion of affected hepatocytes (0; none, 1; <25%, 2; 25–50%, 3; 50–75%, 4; >75%). The degree of hepatic fibrosis was semi-quantified using Azan-Mallory-stained sections.

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2 The abbreviations used are: PABP, poly(A)-binding protein; APRIL, acidic protein rich in leucine; ALT, alanine aminotransferase; CSD, cold shock domain.
Hydroxyproline Assays—Acid-insoluble collagen contents were determined as described previously (20). Briefly, excised livers were freeze-dried, weighed, and homogenized in phosphate buffer (pH 7.4). After centrifugation, sodium citrate buffer (pH 3.5) was added to pellets, and the acid-insoluble pellets were hydrolyzed with HCl for 20 h at 105 °C, and chloramine T solution was added to desiccated hydrolysates.

After adding Ehrlich’s solution, each sample was incubated for 15 min at 65 °C and read at 550 nm. Various amounts of hydroxyproline were used as standards.

Statistical Analysis—Values are expressed as mean ± S.D. The Student’s t test was used to evaluate statistical differences between groups, and the Mann-Whitney test was used for comparison of histological findings. A p value of less than 0.05 was considered significant.

RESULTS

HSc025 Suppresses Collagen Gene Expression in Activated Hepatic Stellate Cells—We previously showed that acceleration of nuclear translocation of YB-1 represses collagen gene expression (9). Moreover, adenovirus-mediated YB-1 expression under the control of the collagen enhancer/promoter significantly suppressed the progression of hepatic fibrosis (14). Random screening of a chemical library and modification of hit compounds using the YB-1-dependent collagen promoter assay system revealed that cinnamoyl derivatives activate the nuclear translocation of YB-1. Based on these findings, we recently assessed the actions of a novel form, HSc025 (Fig. 1A), and we showed it to repress TGF-β-induced collagen gene expression in human dermal fibroblasts (15). In this study, we first examined the inhibitory effects of HSc025 on extracellular matrix gene expression in rat hepatic stellate cells using real time RT-PCR assays. Purified hepatic stellate cells were treated with HSc025 for 6 days, and the expression levels of extracellular matrix genes were determined. As shown in Fig. 1B, exposure of cells to HSc025 significantly ameliorated the spontaneously enhanced gene expression for collagen types I and IV. In addition, consistent with the results of a previous study (15), treatment of cells with HSc025 markedly reduced fibronectin gene expression (Fig. 1B). However, no inhibitory effects were evident on cell proliferation as measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (data not shown).

HSc025 Binds to the C-tail Region of YB-1—The results described above led us to investigate the molecular mechanism responsible for nuclear translocation of YB-1 by HSc025. We first searched for interacting proteins using HSc025-immobilized affinity beads (18). After removal of nonspecifically bound proteins by two treatments with nonimmobilized resins, the interacting proteins were purified using HSc025-immobilized resins and identified by mass spectrometry. Interestingly, YB-1 itself was identified as one of the HSc025-binding proteins. Others included several acidic proteins such as SET and APRIL (acidic protein rich in leucine) (data not shown).

To define the interacting regions of YB-1 with HSc025, pulldown assays using recombinant proteins were performed (Fig. 2A). As shown in Fig. 2B, YB-1 specifically bound to HSc025 resin, and this binding was completely eliminated in the presence of free HSc025. Although SET proteins were associated with HSc025 resin, these interactions were not significantly inhibited in the presence of HSc025. Similarly, APRIL showed a very weak interaction with HSc025 (Fig. 2B).

To define the interacting regions of YB-1 with HSc025, YB-1 deletion mutants were prepared, as described previously (9), and incubated with HSc025 resin. YB-1 protein has three independent domains as follows: an N-terminal region, a DNA binding region called a cold shock domain (CSD), and a C-tail region (Fig. 2C). The CSD is a highly conserved nucleic acid recognition domain, whereas the C-tail region is thought to...
interact with other cellular proteins (9). The full-length YB-1 protein showed interaction with HSc025, but a deletion mutant deprived of the C-terminal region (N + CSD) did not (Fig. 2C). Although both C and ΔC regions of YB-1 showed interaction with HSc025, further deletion of the amino acid sequence between 204 and 271 led to a complete diminution of binding to HSc025. These results indicated that HSc025 directly binds to the C-tail region of YB-1.

**HSc025 Interferes with the Interaction between YB-1 and PABP**—A previous study suggested the existence of an anchoring protein responsible for retention of YB-1 in the cytoplasm (21). Because HSc025 directly binds to the C-tail region of YB-1, we anticipated that HSc025 might have effects on associations between YB-1 and any anchoring proteins. To identify the proteins that retain YB-1 in the cytoplasm, we performed immunoprecipitation of HSc025-treated cytoplasmic extracts with anti-YB-1 antibodies. Consistent with the results of a previous study (22), a number of proteins that interact with YB-1 were detected by silver staining (Fig. 3A). However, only one protein with molecular mass of 70 kDa disappeared dramatically on treatment with HSc025 for 1 or 6 h (Fig. 3A). The relevant band was excised, analyzed by mass spectrometry, and identified as PABP. In addition, immunoprecipitation was performed after incubation of nontreated cytoplasmic extracts with HSc025. Interestingly, HSc025 stimulated the dissociation of PABP from YB-1 in a dose-dependent manner (Fig. 3B). Both YB-1 and PABP are known to be major components of messenger ribonucleoprotein particles (23). We then investigated whether HSc025 interrupts the binding between YB-1 and PABP in the absence of mRNA. To this end, we performed GST pulldown assays using GST-fused PABP protein (Fig. 3C). As shown in Fig. 3D, binding between YB-1 and PABP was observed, whereas YB-1 did not associate with GST itself. When HSc025 was added to a solution containing YB-1 and PABP, the interaction between YB-1 and PABP totally disappeared (Fig. 3D). Although the N + CSD region of YB-1 had no interaction with PABP, association between the C-tail region of YB-1 and PABP was observed. As expected, this interaction was completely blocked in the presence of HSc025 (Fig. 3D). Taken together, these results suggested that HSc025 interrupts the binding between YB-1 and PABP through its direct binding to the C-tail region of YB-1.

**Displacement of PABP Antagonizes Fibrotic Signaling**—The above results suggested that the displacement of PABP by HSc025 accelerates nuclear translocation of YB-1 and subsequently represses fibrotic signaling. To examine this possibility, we employed the RNA interference technique using siRNA. As shown in Fig. 4A, transfection of human fibroblasts with PABP-specific double-stranded RNA. As shown in Fig. 4A, transfection of human fibroblasts with PABP siRNA decreased the amounts of cytoplasmic YB-1, whereas it increased the nuclear YB-1 content (Fig. 4B). As expected, down-regulation of PABP expression significantly inhibited basal and TGF-β-stimulated collagen gene expression (Fig. 4C) but had no effect on the expression level of YB-1 (data not shown). Collectively, these results strongly suggest that the HSc025-induced displacement of PABP promoted nuclear translocation of YB-1, resulting in transcriptional repression of collagen gene.

**HSc025 Treatment Prevents the Progression of Hepatic Fibrosis**—We have recently demonstrated that oral administration of HSc025 prevents tissue fibrosis in murine models of systemic sclerosis (15). In the last set of experiments, we examined whether HSc025 suppressed the progression of hepatic fibrosis. Mice were treated with HSc025 for 4 weeks after initial CCl4 injections for 4 weeks. Although oral administration of HSc025 had no effect on body weight or food intake throughout the experiments (data not shown), treatment of mice with HSc025 reduced the relative liver weight in a dose-dependent manner (Fig. 5A). The serum ALT levels were also significantly decreased in HSc025-treated mice as compared with control animals (Fig. 5B). Ne-
A previous study indicated that the progression of fibrosis is induced by repeated CCl₄ injections (Fig. 5). In a similar study (19), progressive hepatic fibrosis was induced by repeated CCl₄ injections. Consistent with these findings, another study showed that treatment with HSc025 at a dose of 75 mg/kg/day significantly decreased the hydroxyproline content. These results suggested that HSc025 treatment prevents liver injury and hepatic fibrosis.

**DISCUSSION**

Hepatic fibrosis represents the generic wound healing response to a wide range of underlying injurious processes, including excessive alcohol consumption, chronic viral hepatitis, nonalcoholic steatohepatitis, hemochromatosis and immune-mediated liver injury. Although there are currently no approved antifibrotic agents for hepatic fibrosis in humans, several potential therapeutic approaches have been tested, largely classifiable into the following: 1) inhibition of activation, proliferation, and fibrogenesis of hepatic stellate cells; 2) enhancement of extracellular matrix degradation; and 3) attenuation of hepatic inflammation. Ideal therapies will be those that are orally available, well tolerated during chronic usage, and not simply preventing the progression of fibrosis but rather regressing scarring, leading to stabilization or improvement in liver function (25). In this study, we have demonstrated that a novel small compound HSc025 inhibited collagen gene expression in hepatic stellate cells, and oral administration of HSc025 to mice with CCl₄-induced hepatic fibrosis exhibited repression of extracellular matrix deposition as well as attenuation of hepatic inflammation.

TGF-β regulates many aspects of cellular functions and consequently has diverse effects on a variety of cell types and tissues. In the liver, TGF-β inhibits proliferation of hepatocytes, while stimulating proliferation and activation of hepatic stellate cells (26). We have previously shown that YB-1 abrogates TGF-β signaling through its direct interaction with Smad3 followed by repression of collagen gene expression (9). More recently, we showed that a novel small compound HSc025 stimulates nuclear translocation of YB-1 and improves skin and pulmonary fibrosis (15). HSc025 rapidly induced the nuclear translocation of YB-1, but the translocation returned to the basal levels within 24 h. HSc025 had no effects on YB-1 gene expression levels (15). To understand the mechanism by which HSc025 activates nuclear translocation of YB-1, we first tried to identify proteins with which it associates using chemical compound-immobilized resins as described previously (18). All the proteins identified by mass spectrometry analyses were acidic except for YB-1. We found that HSc025 directly binds to YB-1, although direct association of HSc025 with SET or APRIL was not observed. Consistent with our findings, another acidic protein YBAP1 is reported to associate with YB-1 in the chicken DT40 cells (21). Previous studies showed that SET and APRIL are nucleo-cytoplasmic shuttling proteins interacting with the nuclear export factor CRM1 (27, 28). Thus, it is possible that both SET and APRIL contribute to the nuclear import and export of YB-1. The effects of SET or APRIL on the HSc025-induced translocation of YB-1 are now under investigation.

A previous study suggested the existence of an anchoring protein responsible for retention of YB-1 in the cytoplasm (21). Thus, we tried to identify the anchoring protein for YB-1 that is dissociated in the presence of HSc025. The results of immunoprecipitation assays demonstrated that the treatment...
FIGURE 5. Oral administration of HSc025 attenuates liver injury and hepatic fibrosis. Mice were chronically treated with 0.1 ml/kg body weight of CCl4 once a week for 8 weeks. They were then orally administered 3, 15, or 75 mg/kg/day HSc025 or vehicle for 4 weeks after injection of carbon tetrachloride for 4 weeks. They were killed 72 h after the last CCl4 injection and subjected to the following: A, determination of relative liver weight that was normalized against body weight; B, measurement of activity of serum ALT; C, H&E staining (original magnification, ×100; bar, 20 μm). D, histological examination. H&E sections were randomly selected and blindly analyzed for the degree of necrosis or hepatocellular vacuolization as measures of hepatic injury. E, Azan-Mallory staining (original magnification, ×100; bar, 20 μm). F, measurement of fibrotic areas. After Azan-Mallory staining, the degree of hepatic fibrosis was semiquantified by measuring the relative areas of fibrosis with the aid of computer software. G, quantification of hydroxyproline in acid-insoluble liver pellets. Hydroxyproline content was determined as described under “Experimental Procedures.” The asterisk signifies that the values are significantly different between the groups. **, p < 0.01; *, p < 0.05. Cont, control.
of human fibroblasts with HSc025 stimulated the dissociation of YB-1 from PABP in a time-dependent manner (Fig. 3A). In addition, incubation of cytoplasmic extracts with HSc025 also enhanced this dissociation (Fig. 3B). Furthermore, the direct interaction between YB-1 and PABP was abolished in the presence of HSc025 (Fig. 3D). These results suggested that HSc025 physically disrupts the interaction between YB-1 and PABP. Several lines of evidence indicate that YB-1 is a major general translation regulator through mRNA structure arrangement and packaging. PABP is an evolutionarily conserved protein, which binds to the poly(A) tract with a periodicity of ~27 nucleotides. Recently, Svitkin et al. (29) have demonstrated that the major mRNA ribonucleoprotein YB-1 has a pivotal function in the regulation of eukaryotic translational initiation factor 4G activity by PABP, suggesting functional relations between YB-1 and PABP. Moreover, our experiments using PABP siRNA clearly indicated that the displacement of PABP promoted nuclear translocation of YB-1 and subsequently inhibited basal and TGF-β-stimulated collagen gene expression (Fig. 4). Collectively, it can be postulated that HSc025 interferes with the association between YB-1 and PABP, and the complex of YB-1/shuttling proteins is thereby dissociated from PABP and translocates from the cytoplasm to the nucleus, where YB-1 represses collagen gene transcription.

The dynamic balance between production and degradation of collagen is rigorously controlled by several growth factors and cytokines (1). We have previously shown that HSc025 inhibits TGF-β-induced collagen and fibronectin gene expression in human fibroblasts (15). In addition, orally administered HSc025 reduced hypodermal thickness and hydroxyproline content in the tight skin mouse and markedly decreased the histological fibrotic score and hydroxyproline content in the lung tissue with bleomycin-induced murine pulmonary fibrosis (15). Furthermore, oral administration of HSc025 at a dose of 15 mg/kg to Thy-1-induced kidney fibrosis rats significantly decreased the histological fibrotic score, collagen gene expression, and hydroxyproline content. Consistent with the results of these previous studies, we showed here that HSc025 inhibits collagen gene expression in cultured hepatic stellate cells, the major collagen producers in fibrotic liver (Fig. 1B). It also reduced the relative fibrotic areas and decreased hydroxyproline content in liver tissue in a murine model of hepatic fibrosis (Fig. 5, F and G). Overall, we conclude that oral administration of HSc025 to experimental fibrotic animals ameliorates organ fibrosis.

Treatment of mice with HSc025 significantly reduced the relative liver weight and decreased the serum ALT levels (Fig. 5, A and B). Our previous study showed that oral administration of HSc025 to normal rats at doses of up to 800 mg/kg for 14 days had no effect on the relative liver weight or serum ALT levels (supplemental Table). In addition, histological examination of several tissues, including liver in HSc025-treated rats, showed no significant alterations (data not shown). Furthermore, oral administration of HSc025 to normal dogs at doses of up to 1000 mg/kg for 14 days showed no adverse effects (data not shown). From these findings, administration of HSc025 itself does not seem to affect the liver weight, serum ALT levels, or liver morphology, and the decrease in the relative liver weight observed in HSc025-treated mice rather represents anti-inflammatory and anti-fibrotic effects of HSc025.

We have previously shown that oral administration of HSc025 exhibits direct anti-fibrotic effects on noninflammatory skin fibrosis in Tsk mice (15). On the other hand, this study revealed a significant decrease in the serum ALT levels and a reduction of hepatocellular necrosis/vacuolization in HSc025-treated mice (Fig. 5, B and D), suggesting that it exerts hepatotoxicity-protective effects as well. Kupffer cells, the liver’s tissue-specific macrophage population, are important effector cells in the inflammatory response (25). NF-κB activation in Kupffer cells drives the expression of a number of inflammatory genes. Raj et al. (30) showed that YB-1 regulates the association of p50/p65 to the NF-κB DNA-binding element. In addition, oxidative stress, through the generation of reactive oxygen species, plays an important role in producing liver damage and hepatic fibrosis (25). It is well known that reactive oxygen species participate in the p38 MAPK pathway, which is involved in inflammatory responses. A previous study suggested that Y-box proteins also regulate the redox signaling pathway (31). To determine the broad pharmacological profile of HSc025, we performed 100 distinct in vitro assays and found that HSc025 inhibits p38 MAPK activity. These findings raised the possibility that HSc025 represses hepatic injury independent of YB-1 action. The precise mechanisms by which HSc025 might exhibit cytoprotective effects against liver injury continue to be the topic for investigation.

In summary, we showed that HSc025 promotes nuclear translocation of YB-1 through direct binding to YB-1. This is followed by disruption of the association between YB-1 and PABP. Treatment of spontaneously activated stellate cells with HSc025 attenuated collagen gene expression, and oral administration of HSc025 exhibited antifibrotic efficacy and hepatocellular protection against liver injury in an experimental model of murine hepatic fibrosis. Because the results of these early preclinical studies demonstrated several advantages of HSc025, including bioavailability and a good safety profile, HSc025 or its analogs could be a promising candidate for clinical trials in treating organ fibrosis.

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