Parasitol Res (2015) 114:4251–4257
DOI 10.1007/s00436-015-4663-0

ORIGINAL PAPER

Schistosoma japonicum protein SjP40 inhibits TGF-β1-induced activation of hepatic stellate cells

Xiaolei Sun[1] · Lingbo Zhang[1] · Jianxin Wang[1,2] · Jinling Chen[1] · Dandan Zhu[1] · Pei Shen[1,2,3] · Xue He[1,4] · Jing Pan[1] · Wenxia Peng[1] · Yinong Duan[1]

Received: 27 April 2015 / Accepted: 4 August 2015 / Published online: 14 August 2015

Abstract SjP40 is a major egg antigen of *Schistosoma japonicum*. In the present study, the authors investigated the effect of SjP40 in vitro on transforming growth factor-β1 (TGF-β1)- stimulated hepatic stellate cells (HSCs). LX-2, an immortalized human HSC line, was treated with purified recombinant SjP40 (rSjP40) in the presence or absence of TGF-β1. Quantitative real-time polymerase chain reaction and western blot analysis were performed to determine messenger ribonucleic acid and protein of fibrogenic genes and TGF-β signaling pathway. The results showed that expression of fibrogenic genes was significantly reduced by rSjP40. Furthermore, rSjP40 also suppressed the TGF-β1-induced upregulation of Smads and ERK proteins. We also found that the effect of rSjP40 on HSCs was similar to SB431542, an inhibitor of type I TGF-β receptor. In conclusion, the data suggest that SjP40 attenuates HSC activation, which might be, at least in part, mediated by inhibiting the TGF-β and ERK signaling pathways.

Electronic supplementary material The online version of this article (doi:10.1007/s00436-015-4663-0) contains supplementary material, which is available to authorized users.

✉ Yinong Duan
yinongduan@aliyun.com

[1] Department of Pathogen Biology, School of Medicine, Nantong University, 19 Qixiu Road, Nantong 226001, Jiangsu, People's Republic of China

[2] Laboratory Medicine Center, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, People's Republic of China

[3] School of Medical Science and Laboratory Medicine, Jiangsu University, Zhenjiang 212013, Jiangsu, People's Republic of China

[4] Department of Pathology, the Second Affiliated Hospital of Nanjing Medical University, 121 Jiangjiayuan, Nanjing 210011, Jiangsu, People's Republic of China

Keywords *Schistosoma japonicum* · P40 · Hepatic stellate cells · Hepatic fibrosis · TGF-β1 · ERK

Introduction

Hepatic fibrosis represents a wound-healing process which occurs in most types of chronic liver injury (Guyot et al. 2006). The most characteristic feature of hepatic fibrosis is an excessive deposition of extracellular matrix (ECM) components, due to an imbalance between the synthesis and degradation of ECM (Tsukada et al. 2006). The activation of hepatic stellate cells (HSCs) is a key event in the development of hepatic fibrosis. In the course of hepatic fibrosis, many factors have been identified as mediators of HSC activation. Increasing evidence shows that transforming growth factor-β1 (TGF-β1) is the most potent fibrogenic cytokine to initiate HSC activation (Breitkopf et al. 2006; Inagaki and Okazaki 2007). It has been reported that TGF-β1 stimulates the synthesis of collagen type I alpha I (COL1A1), the major component of ECM. Thus, inhibition of the TGF-β signal pathway has become an effective strategy for the prevention and treatment of hepatic fibrosis (Derynck and Zhang 2003).

Previous studies have demonstrated that schistosome eggs or soluble egg antigens (SEA) exhibited an anti-fibrogenic activity on TGF-β1-activated HSCs (Anthony et al. 2010, 2013; Duan et al. 2014). It has been reported by our lab that SEA from *Schistosoma japonicum* (*S. japonicum*) inhibited the proliferation of HSCs and suppressed the activation of HSCs through the peroxisome proliferator-activated receptor γ (PPARγ) and TGF-β signal pathway (Duan et al. 2014). Since SEA has been identified as a complex of immunogenic glycoproteins in which the P40 peptide is a major component (Asahi and Stadecker 2003; Cass et al. 2007; Stadecker et al. 2001), we aimed to investigate the effect of P40 of

S. japonicum (SjP40) on the human HSC line (LX-2). We also explored the potential mechanism by which rSjP40 could inhibit the activation of LX-2 cells.

Materials and methods

Reagents

Recombinant human TGF-β1, which was obtained from CHO cells, was purchased from Sigma-Aldrich (T7039, USA). SB431542 (Selleck Chemicals, USA), which is a potent and selective inhibitor of the TGF-β receptor I (TβR I), was demonstrated to suppress TGF-β1-induced extracellular matrix in different types of cells (Islam et al. 2014; Laping et al. 2002; Zhao et al. 2006). Polyclonal antibodies against alpha-smooth muscle actin (α-SMA), pro-collagen type I alpha 1 (pro-COL1A1), TβR I, TGF-β receptor II (TβR II), p-Smad2/3, Smad4, and ERK were obtained from Santa Cruz Biotechnology, USA. Primary antibody for p-ERK was purchased from Cell Signaling Technology (USA).

Production and purification of rSjP40

The open reading frame of the SjP40 gene from *S. japonicum* eggs was cloned into a pET-28a (+) vector and transformed into *Escherichia coli* BL21 (DE3). Then the recombinant rSjP40 protein was expressed and purified by the Ni-NTA His•Bind Resin (Novagen, USA) according to the instructions. After identified by western blot, the endotoxin of rSjP40 recombinant protein was removed using polymyxin B-agarose beads (Sigma, USA) following the suggested protocol.

Cell culture and treatment

The immortalized human HSC line LX-2 cells were preserved in our lab (Wang et al. 2014) and were routinely cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) supplemented with 10 % heat-inactivated fetal bovine serum (FBS, Hyclone, USA). Cultures were incubated at 37 °C in a humidified atmosphere of 5 % CO_2, and the medium was changed daily. For quantitative real-time PCR (qRT-PCR) and western blot assay, LX-2 cells were seeded at a density of 1×10^5 cells/ml into 6-well plates and incubated for 24 h, then rSjP40 was added to the cells and incubated for 48 h in the presence or absence of other reagents (10 ng/ml of TGF-β1 or 10 μg/ml of SB431542; Zhang et al. 2014; Zhu et al. 2014).

RNA extraction and qRT-PCR

Total RNA was extracted from cultured cells using the Trizol RNA isolation reagent (Invitrogen, USA) according to the manufacturer's instructions. Extracted total RNA was then reverse-transcribed to cDNA using the RevertAid First-Strand cDNA Synthesis Kit with Oligo (dT) 18 primers (Thermo Fisher Scientific, USA). An Eco Real-Time PCR Sequence Detection System (Illumina, USA) was used for qRT-PCR. The PCR products were amplified using SYBR Premix Ex Taq Kit (Takara, Japan) with specific primers for target genes. The expression levels of all the transcripts were normalized to that of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the same samples.

Western blot analysis

LX-2 cells exposed to rSjP40, TGF-β1, or SB431542 were cultured for up to 48 h in DMEM with 10 % FBS. The group of cells treated with PBS or DMSO was used as the control. Cells were harvested and lysed on ice by RIPA lysis buffer with 1 % phenylmethylsulfonyl fluoride (PMSF), and the supernatant was collected after centrifugation. Then the equal amounts of protein were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Merck, Germany). The membranes were blocked with 5 % non-fat milk in TBST, and incubated with primary antibodies at 4 °C overnight and subsequently with horseradish peroxidase (HRP)-conjugated secondary antibodies and visualized using a chemiluminescence kit (Merck, Germany). Band quantification analysis was performed by GeneTools software from Syngene (UK). GAPDH was used as the internal control.

Statistical analysis

All data were expressed as mean±SEM of three independent experiments. The statistical analyses were performed with one-way analysis of variance (ANOVA). Differences were considered significant at $p<0.05$.

Results

rSjP40 inhibits α-SMA and pro-COL1A1 expression in LX-2 cells

LX-2 cells were used to observe the effects of rSjP40 in the present study, and the expression of α-SMA and pro-COL1A1 in LX-2 cells was detected first. As noted in Fig. 1a, we found that treatment of LX-2 cells with rSjP40 at concentrations ranging from 5 to 20 μg/ml for 48 h resulted in the inhibition of protein expression levels of α-SMA and pro-COL1A1 in a dose-dependent manner. The messenger ribonucleic acid (mRNA) expression levels of α-SMA and pro-COL1A1 were also decreased in a dose-dependent manner

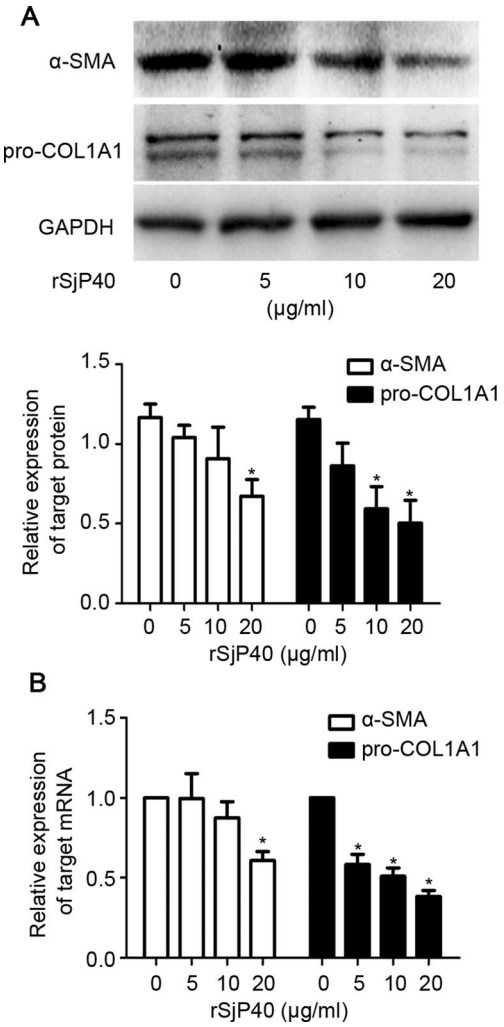

Fig. 1 Inhibitory effect of rSjP40 on α-SMA and pro-COL1A1 expression in LX-2 cells. **a** Western blot analysis for protein expression levels of α-SMA and pro-COL1A1. GADPH was used as the internal standard. **b** qRT-PCR analysis for transcript expression levels of α-SMA and pro-COL1A1. GADPH was used as the internal standard for normalization. *$p<0.05$, compared with control (0 μg/ml)

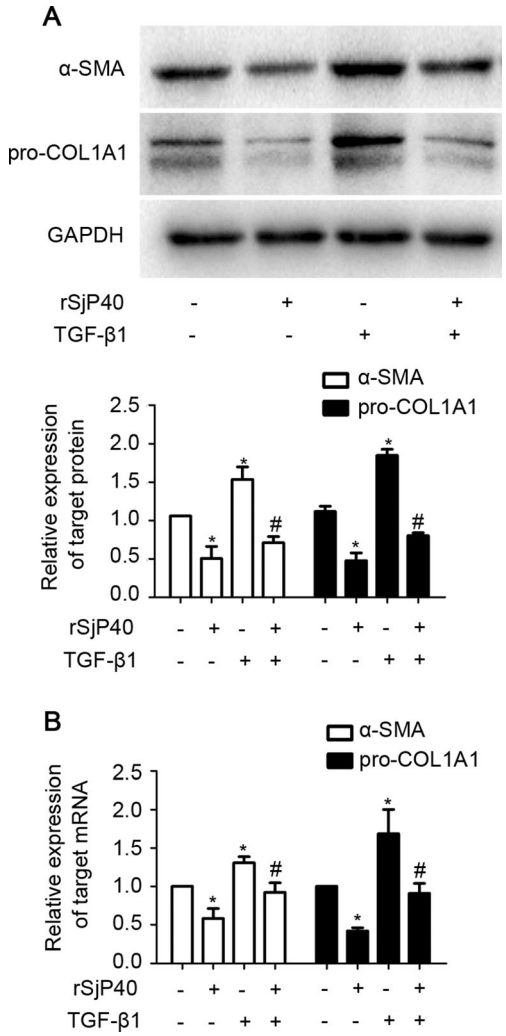

Fig. 2 rSjP40 reduces the expression of fibrotic genes in TGF-β1-activated HSCs. LX-2 cells were subjected to the treatment with rSjP40(20 μg/ml) and/or TGF-β1 (10 ng/ml) for 48 h. **a** Western blot was performed to determine protein expression levels of α-SMA and pro-COL1A1. GADPH was used as an invariant control. **b** qRT-PCR was assessed to investigate mRNA expression levels of α-SMA and COL1A1. GAPDH was used as the invariant control for calculating fold changes in mRNA levels. *$p<0.05$ vs control (rSjP40-TGF-β1−); #$p<0.05$ vs group of TGF-β1 treatment (rSjP40-TGF-β1+)

after rSjP40 treatment (Fig. 1b). In addition, we also observed that the cells treated with rSjP40 exhibited a more elongated cell phenotype, while activated LX-2 cells with no treatment displayed a flat phenotype (Fig. S1).

rSjP40 attenuates the expression of fibrogenic genes in TGF-β1-activated LX-2 cells

Given that the expression changes of α-SMA and collagen was observed after rSjP40 treatment, we explored whether rSjP40 was able to inhibit the activation of HSCs provoked by TGF-β1. LX-2 cells were treated with TGF-β1 (10 ng/ml) and/or rSjP40 (20 μg/ml) for 48 h and the expression levels of mRNA and protein were examined using the methods of qRT-PCR and western blot. As shown in Fig. 2a, TGF-β1 increased the expression of α-SMA protein in LX-2 cells, compared with that of the control group. Co-treatment with TGF-β1 and rSjP40 resulted in the decrease of α-SMA protein expression. Similar to α-SMA, rSjP40 suppressed pro-COL1A1 expression in TGF-β1-activated LX-2 cells. The results of qRT-PCR of α-SMA and pro-COL1A1 expression were consistent with the protein results (Fig. 2b).

rSjP40 inhibits the activation of LX-2 cells through TGF-β/Smad signaling pathway

To elucidate the underlying molecular mechanisms, we further examined the expression changes of the TGF-β signaling cascades. As shown in Fig. 3a, rSjP40 inhibited expression

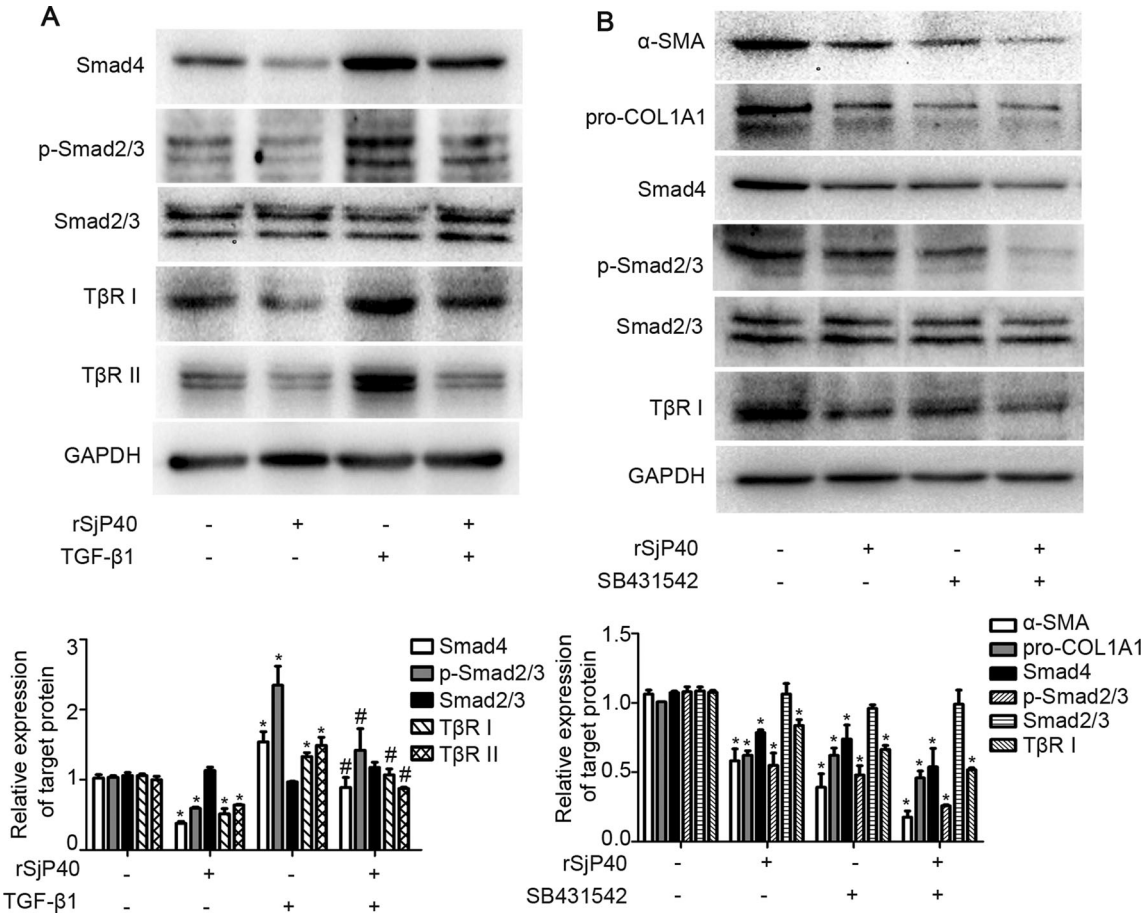

Fig. 3 Effect of rSjP40 on TGF-β1/Smad signaling pathway. **a** LX-2 cells were treated with rSjP40 in the presence or absence of TGF-β1. The results showed that rSjP40 modulated the expressions of TβR I, TβR II, p-Smad2/3, and Smad4 in TGF-β1 activated LX-2 cells. *$p<0.05$ vs control (rSjP40-TGF-β1−); #$p<0.05$ vs group of TGF-β1 treatment (rSjP40-TGF-β1+). **b** LX-2 cells were treated with rSjP40 in the presence or absence of SB431542. The results showed that rSjP40, similar to SB431542, inhibited pro-COL1A1 and α-SMA expression by blocking of TGF-β receptors. *$p<0.05$, compared with control (rSjP40-SB431542−)

levels of TβR I, TβR II, p-Smad2/3, and Smad4 in LX-2 cells. The increased expression levels of TβR I, TβR II, p-Smad2/3, and Smad4 induced by TGF-β1 treatment were strongly down-regulated by rSjP40 (Fig. 3a). rSjP40 showed no effect on the expression of total Smad2/3 protein (Fig. 3a). Based on the observation that rSjP40 inhibited the expression of TGF-β receptors and its signal pathway, we hypothesized that the function of rSjP40 was similar to SB431542, a specific inhibitor of TβR I. As shown in Fig. 3b, TβR I expression in LX-2 cells was antagonized after treatment with rSjP40 or SB431542 (10 μg/ml) for 48 h. Likewise, both rSjP40 and SB431542 caused the reduction of p-Smad2/3 and Smad4. Consequently, rSjP40 suppressed the production of pro-COL1A1 and α-SMA mimicking SB431542 due to the blockade of TGF-β cascades. The results also showed that rSjP40 also amplified the inhibitory effect of SB431542 on TGF-β signaling cascades, to a certain extent. Taken together, these data further revealed that rSjP40 was capable of inhibiting TGF-β1-induced HSC activation by blocking the TGF-β1 receptor, thereby partly disruptting the TGF-β/Smad signaling pathway.

rSjP40 inhibits TGF-β1-induced activation of LX-2 cells through ERK signaling pathway

Previous study has shown that TGF-β1 enhanced the expression of ERK and then increased the phosphorylation and activation of Smads (Derynck and Zhang 2003). The results (Fig. 4a) in the present study also showed that TGF-β1 enhanced the expression of ERK in LX-2 cells. rSjP40 decreased the expression of p-ERK in LX-2 cells and rSjP40 also notably reduced the increased expression of p-ERK induced by TGF-β1 (Fig. 4a). However, rSjP40 showed no effect on the expression of total ERK protein (Fig. 4a). Both rSjP40 and SB431542 reduced the expression of p-ERK in LX-2 cells (Fig. 4b). rSjP40 suppressed TGF-β1-induced HSC activation by disrupting the TGF-β/ERK signaling pathway partly.

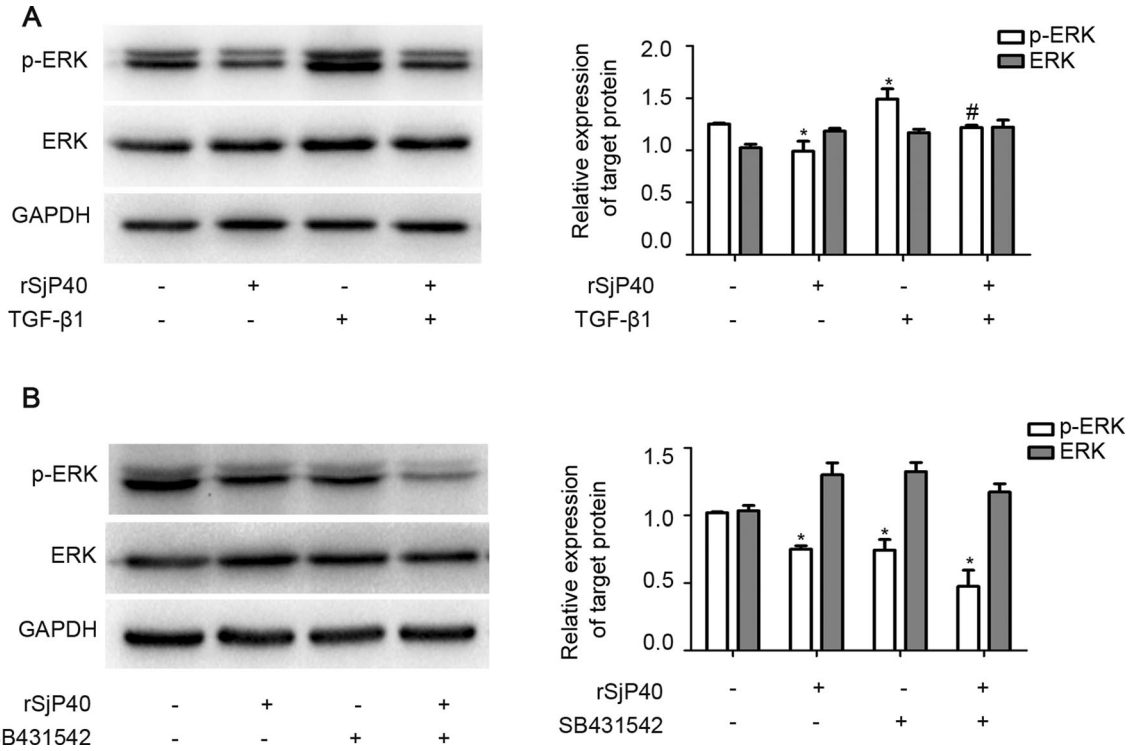

Fig. 4 Effect of rSjP40 on TGF-β1/ERK signaling pathway. **a** LX-2 cells were treated with rSjP40 in the presence or absence of TGF-β1. The results showed that rSjP40 modulated the expression of p-ERK in TGF-β1 activated LX-2 cells. *$p<0.05$ vs control (rSjP40-TGF-β1-); #$p<0.05$ vs group of TGF-β1 treatment (rSjP40-TGF-β1+). **b** LX-2 cells were treated with rSjP40 in the presence or absence of SB431542. The results showed that rSjP40, similar to SB431542, inhibited p-ERK expression in LX-2 cells. *$p<0.05$, compared with control (rSjP40-SB431542−)

Discussion

Hepatic fibrosis is the major pathological change in schistosomiasis, and previous studies have shown that schistosome eggs trapped in hepatic sinusoids are widely thought to trigger fibrotic processes (Anthony et al. 2012; Bartley et al. 2006; Burke et al. 2009; Chang et al. 2006). Nevertheless, it has been found recently that schistosome eggs prevented HSC fibrogenic response, with the down-regulation of α-SMA and COL1A1 by co-culturing HSCs and eggs (Anthony et al. 2010, 2013). Our previous studies also demonstrated that *S. japonicum* SEA engaged in anti-fibrotic activity by inhibiting HSC activation and inducing HSC apoptosis (Duan et al. 2014; Wang et al. 2014). Since SEA is a complex mixture, the main component(s) of SEA eliciting an anti-fibrotic role within HSCs must be investigated. P40, a 40-kDa peptide, is a major component of schistosome eggs, named as SmP40 in *Schistosoma mansoni* (*S.mansoni*) or SjP40 in *S. japonicum* (Liu et al. 2006; Zhou et al. 2010). SmP40 likely revealed the anti-pathology action in schistosomiasis, since stimulation with SmP40 in PBMC resulted in decreased IL-5 expression and increased IL-10 expression, both of these effects were associated with reduced fibrosis (Abouel-Nour et al. 2006). However, these findings have not established whether P40 could inhibit the activation of HSCs directly. LX-2 cells are the most attractive HSC cell line for fibrosis research, since they are spontaneously immortalized during culturing, and they retain all the features of activated HSCs, including the expression of α-SMA and the excessive secretion of ECM (Castilho-Fernandes et al. 2011; Xu et al. 2005). Hence, in the present study, we explored the effects of rSjP40 on LX-2 cells, and demonstrated that rSjP40 suppressed HSC activation and the expression levels of α-SMA, and pro-COL1A1 in LX-2 cells were all reduced.

Since TGF-β signaling plays an important role in SEA and eggs of schistosome-induced inhibition of HSC activation, subsequent experiments were also carried out to examine the effect of rSjP40 on TGF-β1-induced myofibroblast transition. We found that co-treatment with rSjP40 and TGF-β1 down-regulated synthesis of α-SMA and pro-COL1A1, compared with the group with TGF-β1 treatment alone. In the Smad-dependent pathway, TGF-β1 causes a significant increase in the number of TβR II and TβR I on the cell surface (Huang et al. 2011). TGF-β1 binds to the receptors and phosphorylates Smad2 and Smad3, which form a complex with Smad4 and translocate into the nucleus, and then regulate specific gene transcription (Schiller et al. 2004). Hence, TβR I and TβR II play a pivotal role in initiation of TGF-β signal transduction. Previous study has reported that blocking of TβR I reduced collagen gene expression in both acute and chronic models of liver fibrosis (de Gouville et al. 2005). In the present

study, we also found that rSjP40 inhibited the expression of TβR I, TβR II, p-Smad2/3, and Smad4 in TGF-β1-activated LX-2 cells. Then the activity of rSjP40 was compared with that of SB431542, a kind of TβR I inhibitor which has been used in previous studies to interrupt the TGF-β signal pathway (Islam et al. 2014; Zhang et al. 2014). We also demonstrated that the inhibitory effects of rSjP40 and SB431542 on TGF-β signal associated proteins and target genes were parallel. rSjP40 even amplified the inhibitory effect of SB431542 on TGF-β signaling cascades.

It is to be noted that multiple complex signaling pathways are involved in TGF-β1-induced HSC activation. In addition to Smad-mediated transcription signaling, TGF-β1 and the TGF-β receptors also modulate the activation of ERK, p38, and JNK MAP kinases (Derynck and Zhang 2003; Mu et al. 2012). It has been demonstrated that TGF-β1 induced HSC activation through ERK-MAPK signaling pathway (Liu et al. 2011). Abundant data have also demonstrated that the blockade of HSC activation and liver fibrosis is due to inhibiting the activation of ERK. For example, salvianolic-acid B performed an anti-fibrosis effect by inhibiting phosphorylation of ERK in HSCs stimulated with TGF-β1 (Lv et al. 2010). AKD-FD reduced the expression of ERK, p38, and JNK in fibrotic liver in rats, thereby decreasing the deposition of ECM (Peng et al. 2013). In the present research, the results revealed that treatment with TGF-β1 upregulated the expression of p-ERK protein in LX-2 cells. Blocking of TβR I with both rSjP40 and SB431542 diminished the expression of p-ERK protein in LX-2 cells. All the results indicated that the function of rSjP40 on TGF-β1-activated HSCs is associated with the ERK pathway.

Overall, these observations lead to the suggestion that SjP40 attenuates TGF-β1-induced HSC activation in vitro and the anti-fibrotic effect of SjP40 may be associated with the suppression of the TGF-β signal pathway. More studies are necessary to clarify other biological actions of P40 from schistosomes.

Acknowledgments This work was supported by National Natural Science Foundation of China (Grant Numbers 81471975, 81171589, and 81401683), the Jiangsu provincial Natural Science Foundation (Grant Number BK20140435), the Natural Science Research Project of the Colleges and Universities of Jiangsu Province (Grant Number 14KJD180002), the Natural Science Foundation of Nantong City (Grant Number BK2014033), and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Conflicts of interest The authors declared that no conflicts of interest existed in this study.

References

Abouel-Nour MF, Lotfy M, Attallah AM, Doughty BL (2006) *Schistosoma mansoni* major egg antigen Smp40: molecular modeling and potential immunoreactivity for anti-pathology vaccine development. Mem Inst Oswaldo Cruz 101(4):365–372

Anthony B, Mathieson W, de Castro-Borges W, Allen J (2010) *Schistosoma mansoni*: egg-induced downregulation of hepatic stellate cell activation and fibrogenesis. Exp Parasitol 124(4):409–420

Anthony BJ, Ramm GA, McManus DP (2012) Role of resident liver cells in the pathogenesis of schistosomiasis. Trends Parasitol 28(12):572–579

Anthony BJ, James KR, Gobert GN, Ramm GA, McManus DP (2013) Schistosoma eggs induce a proinflammatory, anti-fibrogenic phenotype in hepatic stellate cells. Plos One 8(6):e68479

Asahi H, Stadecker MJ (2003) Analysis of egg antigens inducing hepatic lesions in schistosome infection. Parasitol Int 52(4):361–367

Bartley PB, Ramm GA, Jones MK, Ruddell RG, Li Y, McManus DP (2006) A contributory role for activated hepatic stellate cells in the dynamics of *Schistosoma japonicum* egg-induced fibrosis. Int J Parasitol 36(9):993–1001

Breitkopf K, Godoy P, Ciuclan L, Singer MV, Dooley S (2006) TGF-beta/Smad signaling in the injured liver. Z Gastroenterol 44(1):57–66

Burke ML, Jones MK, Gobert GN, Li YS, Ellis MK, McManus DP (2009) Immunopathogenesis of human schistosomiasis. Parasite Immunol 31(4):163–176

Cass CL, Johnson JR, Califf LL, Xu T, Hernandez HJ, Stadecker MJ, Yates JR 3rd, Williams DL (2007) Proteomic analysis of *Schistosoma mansoni* egg secretions. Mol Biochem Parasitol 155(2):84–93

Castilho-Fernandes A, de Almeida DC, Fontes AM, Melo FU, Picanco-Castro V, Freitas MC, Orellana MD, Palma PV, Hackett PB, Friedman SL, Covas DT (2011) Human hepatic stellate cell line (LX-2) exhibits characteristics of bone marrow-derived mesenchymal stem cells. Exp Mol Pathol 91(3):664–672

Chang D, Ramalho LN, Ramalho FS, Martinelli AL, Zucoloto S (2006) Hepatic stellate cells in human schistosomiasis mansoni: a comparative immunohistochemical study with liver cirrhosis. Acta Trop 97(3):318–323

de Gouville AC, Boullay V, Krysa G, Pilot J, Brusq JM, Loriolle F, Gauthier JM, Papworth SA, Laroze A, Gellibert F, Huet S (2005) Inhibition of TGF-beta signaling by an ALK5 inhibitor protects rats from dimethylnitrosamine-induced liver fibrosis. Br J Pharmacol 145(2):166–177

Derynck R, Zhang YE (2003) Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature 425(6958):577–584

Duan Y, Gu X, Zhu D, Sun W, Chen J, Feng J, Song K, Xu F, He X, He X (2014) *Schistosoma japonicum* soluble egg antigens induce apoptosis and inhibit activation of hepatic stellate cells: a possible molecular mechanism. Int J Parasitol 44(3–4):217–224

Guyot C, Lepreux S, Combe C, Doudnikoff E, Bioulac-Sage P, Balabaud C, Desmouliere A (2006) Hepatic fibrosis and cirrhosis: the (myo)fibroblastic cell subpopulations involved. Int J Biochem Cell Biol 38(2):135–151

Huang T, David L, Mendoza V, Yang Y, Villarreal M, De K, Sun L, Fang X, Lopez-Casillas F, Wrana JL, Hinck AP (2011) TGF-beta signalling is mediated by two autonomously functioning TbetaRI: TbetaRII pairs. EMBO J 30(7):1263–1276

Inagaki Y, Okazaki I (2007) Emerging insights into transforming growth factor beta Smad signal in hepatic fibrogenesis. Gut 56(2):284–292

Islam SS, Mokhtari RB, El Hout Y, Azadi MA, Alauddin M, Yeger H, Farhat WA (2014) TGF-beta1 induces EMT reprogramming of porcine bladder urothelial cells into collagen producing fibroblasts-like cells in a Smad2/Smad3-dependent manner. J Cell Commun Sign 8(1):39–58

Laping NJ, Grygielko E, Mathur A, Butter S, Bomberger J, Tweed C, Martin W, Fornwald J, Lehr R, Harling J, Gaster L, Callahan JF, Olson BA (2002) Inhibition of transforming growth factor (TGF)-beta1-induced extracellular matrix with a novel inhibitor of the TGF-beta type I receptor kinase activity: SB-431542. Mol Pharmacol 62(1):58–64

Liu F, Lu J, Hu W, Wang SY, Cui SJ, Chi M, Yan Q, Wang XR, Song HD, Xu XN, Wang JJ, Zhang XL, Zhang X, Wang ZQ, Xue CL, Brindley PJ, McManus DP, Yang PY, Feng Z, Chen Z, Han ZG (2006) New perspectives on host-parasite interplay by comparative transcriptomic and proteomic analyses of *Schistosoma japonicum*. PLoS Pathog 2(4):e29

Liu Y, Wang Z, Kwong SQ, Lui EL, Friedman SL, Li FR, Lam RW, Zhang GC, Zhang H, Ye T (2011) Inhibition of PDGF, TGF-beta, and Abl signaling and reduction of liver fibrosis by the small molecule Bcr-Abl tyrosine kinase antagonist Nilotinib. J Hepatol 55(3): 612–625

Lv Z, Song Y, Xue D, Zhang W, Cheng Y, Xu L (2010) Effect of salvianolic-acid B on inhibiting MAPK signaling induced by transforming growth factor-beta1 in activated rat hepatic stellate cells. J Ethnopharmacol 132(2):384–392

Mu Y, Gudey SK, Landstrom M (2012) Non-Smad signaling pathways. Cell Tissue Res 347(1):11–20

Peng Y, Yang H, Zhu T, Zhao M, Deng Y, Liu B, Shen H, Hu G, Wang Z, Tao L (2013) The antihepatic fibrotic effects of fluorofenidone via MAPK signalling pathways. Europ J Clin Investig 43(4):358–368

Schiller M, Javelaud D, Mauviel A (2004) TGF-beta-induced SMAD signaling and gene regulation: consequences for extracellular matrix remodeling and wound healing. J Dermatol Sci 35(2):83–92

Stadecker MJ, Hernandez HJ, Asahi H (2001) The identification and characterization of new immunogenic egg components: implications for evaluation and control of the immunopathogenic T cell response in schistosomiasis. Mem Inst Oswaldo Cruz 96(Suppl):29–33

Tsukada S, Parsons CJ, Rippe RA (2006) Mechanisms of liver fibrosis. Clin Chim Acta 364(1–2):33–60

Wang J, Xu F, Zhu D, Duan Y, Chen J, Sun X, He X, Li P, Sun W, Feng J (2014) *Schistosoma japonicum* soluble egg antigens facilitate hepatic stellate cell apoptosis by downregulating Akt expression and upregulating p53 and DR5 expression. PLoS Negl Trop Dis 8(8):e3106

Xu L, Hui AY, Albanis E, Arthur MJ, O'Byrne SM, Blaner WS, Mukherjee P, Friedman SL, Eng FJ (2005) Human hepatic stellate cell lines, LX-1 and LX-2: new tools for analysis of hepatic fibrosis. Gut 54(1):142–151

Zhang Y, Li H, Hao J, Zhou Y, Liu W (2014) High glucose increases Cdk5 activity in podocytes via transforming growth factor-beta1 signaling pathway. Exp Cell Res 326(2):219–229

Zhao C, Chen W, Yang L, Chen L, Stimpson SA, Diehl AM (2006) PPARgamma agonists prevent TGFbeta1/Smad3-signaling in human hepatic stellate cells. Biochem Biophys Res Commun 350(2): 385–391

Zhou XH, Wu JY, Huang XQ, Kunnon SP, Zhu XQ, Chen XG (2010) Identification and characterization of *Schistosoma japonicum* Sjp40, a potential antigen candidate for the early diagnosis of schistosomiasis. Diag Microbiol Infect Dis 67(4):337–345

Zhu D, He X, Duan Y, Chen J, Wang J, Sun X, Qian H, Feng J, Sun W, Xu F, Zhang L (2014) Expression of microRNA-454 in TGF-beta1-stimulated hepatic stellate cells and in mouse livers infected with *Schistosoma japonicum*. Parasit Vectors 7:148