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*Original Article*

# BMP-7 attenuates liver fibrosis via regulation of epidermal growth factor receptor

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**Abstract:** The aim of this study was to elucidate the effect of bone morphogenetic protein-7 (BMP-7) on liver fibrosis induced by carbon tetrachloride (CCl4) in vivo and on the hepatic stellate cells (HSC) activation in vitro. In vivo, thirty male ICR mice were randomly allocated to three groups, the control group (n = 6), the CCl4 group (n = 18) and the BMP-7+CCl4 group (n = 6). The model of liver fibrosis was induced by intraperitoneal injection with CCl4 three times per week lasting for 12 weeks in CCl4 group and the BMP-7+CCl4 group. After 8 weeks injection with CCl4, mice were intraperitoneal injected with human recombinant BMP-7 in BMP-7+CCl4 group. Meanwhile, mice in the CCl4 group were only intraperitoneal injection with equal amount of saline. The degree of liver fibrosis was assessed by HE and Masson's staining. PCR and western blot were used to detect mRNA and protein levels. In BMP-7+CCl4 group, serum levels of alanine aminotransferase (ALT) and aminotransferase (AST) were decreased and serum albumin (Alb) was increased. Meanwhile, the expressions of transforming growth factor-β1 (TGF-β1) and α-smooth muscle actin (α-SMA) were down-regulated by BMP-7 intervention as compared to the CCl4 group ($P < 0.05$). Furthermore, BMP-7 also suppressed the expression of epidermal growth factor receptor (EGFR) and phosphorylated-epidermal growth factor receptor (pEGFR). HE and Masson stain showed that liver damage was alleviated in BMP-7+CCl4 group. In vitro study, expression of EGFR, TGF-β1 and α-SMA were down regulated by BMP-7 dose-dependently, indicating it might effect on suppression of HSC activation. Therefore, our data indicate BMP-7 was capable of inhibiting liver fibrosis and suppressing HSCs activation, and these effects might rely on its crosstalk with EGFR and TGF-β1. We suggest that BMP-7 may be a potential reagentfor the prevention and treatment of liver fibrosis.

**Keywords:** Carbon tetrachloride, liver fibrosis, bone morphogenetic protein-7, epidermal growth factor receptor, hepatic stellate cell

## Introduction

Liver fibrosis is a common pathological process resulted by various chronic hepatic injuries, which is characterized by remodeling of extracellular matrix (ECM) and excessive deposition of collagen. The activation of quiescent hepatic stellate cells (HSCs) to a myofibroblast-like phenotype is considered as the pivotal events of the pathogenesis of liver fibrosis. ECM synthesis and deposition are regulated by many factors. Transforming growth factor-β (TGF-β) is a major profibrogenic cytokine for fibroblasts recruitment, myofibroblast differentiation, epithelial-mesenchymal transition (EMT) [1, 2] and ECM deposition. Increasing TGF-β1 could inhibit the synthesis of ECM degradation enzymes, as plasminogen activator inhibitor type 1 (PAI-1) [3] and tissue inhibitor of metalloproteinases (TIMPs).

Bone morphogenetic protein-7 (BMP-7), a 30-35 kDa protein, was originally identified as signal that can induce ectopic bone and cartilage upon nonsystemic injection in animals [4] N.Y. Its role in the embryonic development has been confirmed by several studies [5, 6] and mice with BMP-7 KO exhibit embryonic lethality [7, 8]. Meanwhile, BMP-7 can repair the tubular injury in the model of fibrotic kidney by inhibiting EMT [9]. Recent studies indicated BMP-7 could negative regulate the actions of TGF-β1 [9, 10]. Epidermal growth factor receptor (EGFR), a membrane-bound receptor tyrosine kinase,

**Table 1.** PCR primers

| Gene | Primer sequence (5'→3') | Product size (bp) | Annealing temperature |
|---|---|---|---|
| β-actin (mus) | Forward: AACAGTCCGCCTAGAAGCAC | 281 | 57°C |
| | Reverse: CGTTGACATCCGTAAAGACC | | |
| TGF-β1 (mus) | Forward: GCCCTCGGGAGCCACAAACC | 277 | 60°C |
| | Reverse: GCAGCAGGAGTCGCGGTGAG | | |
| α-SMA (mus) | Forward: CCCTGCTCTGCCTCTAGCACACA | 231 | 60°C |
| | Reverse: TCCTGACCACTAGAGGGGGCCA | | |
| β-actin (rat) | Forward: CACCCGCGAGTACAACCTTC | 207 | 61°C |
| | Reverse: CCCATACCCACCATCACACC | | |
| EGFR (rat) | Forward: CATCCAGTGCCATCCAGAAT | 163 | 61°C |
| | Reverse: CTTCCAGACCAGGGTGTTGT | | |
| TGFβ1 (rat) | Forward: CACCATCCATGACATGAACC | 404 | 54°C |
| | Reverse: TCATGTTGGACAACTGCTCC | | |
| α-SMA (rat) | Forward: GCTCTGTAAGGCGGGCTTTG | 558 | 56°C |
| | Reverse: ACGAAGGAATAGCCACGCTCA | | |

was involved in organ fibrosis, such as lung [11, 12], cardiovascular and kidney [13]. Studies have revealed that expression of EGFR could be altered directly by BMPs [14]. However, the regulation of EGFR by BMP-7 in liver fibrosis has not been fully investigated.

We hypothesized that BMP-7 may be helpful to prevent the development of liver fibrosis. In this study, we evaluated the anti-fibrotic properties of BMP-7 on liver fibrosis induced by carbon tetrachloride (CCl4) in vivo and on the HSCs activation in vitro. Meanwhile, the crosstalk between BMP-7, TGF-β1 and EGFR was also explored.

## Materials and methods

### *Animal grouping and treatment*

Thirty male ICR mice initially weighing 18-22 g were purchased from the Shanghai Laboratory Animal Center (Shanghai, China). All animals were allowed free access to standard water and food. Animals were randomly allocated to three groups, the control group (n = 6), the CCl4 group (n = 18) and the BMP-7+CCl4 group (n = 6). The model of liver fibrosis was induced by intraperitoneal injection with CCl4 (1 ug/g body weight dissolved 1:4 in corn oil) three times a week for 12 weeks. In order to study the dynamic liver change during fibrogenesis, the CCl4 group was further divided into four subgroups according to the different time points: 4 weeks (n = 6), 8 weeks (n = 6), 12 weeks (n = 6). Mice in BMP-7+CCl4 group were intraperitoneally injection with BMP-7 at a dose of 300 pg/g body weight, three times a week, starting at 8 weeks after the first administration of CCl4 and last for 4 weeks. At the end of the experiment, mice of BMP-7+CCl4 group were all sacrificed. On week 4, 8 and 12 after CCl4 treatment, the animals of CCl4 group were killed. Liver and blood samples of each group were collected. All animal protocols were approved by institutional animal committee of Wenzhou Medical College and experiments were performed in accordance with the guidelines for the care and use of experiment animals by the National Institutes of Health.

### *Histologic evaluation*

The partial liver of each mouse was fixed in 10% neutral formalin and then embedded with paraffin. All specimens were sliced at 4-μm thickness. To further assess histological change, hematoxylin and eosin (HE) and Masson's staining were used. Images of different groups were examined blindly by a pathologist.

### *Serum biochemical analysis*

The mice were anesthetized with urethane (1.2 g/kg, intraperitoneal injection (i.p.)), the blood samples from abdominal aorta were drawn into heparinized injectors and centrifuged at 3000 rpm at 4°C for 10 min. Serum alanine asninotrasferase (ALT), aspartate aminotransferase (AST) and serum albumin (Alb) levels were measured by the first Affiliated Hospital, Wenzhou Medical College (Wenzhou, China). After finishing with the blood collection, the experimental animals were sacrificed, liver samples were dissected and washed with ice-cold saline, then they were immediately stored at -80°C for further analysis. The largest right lobe of each liver was excised and fixed in a 10% formalin solution for histopathologic analyses.

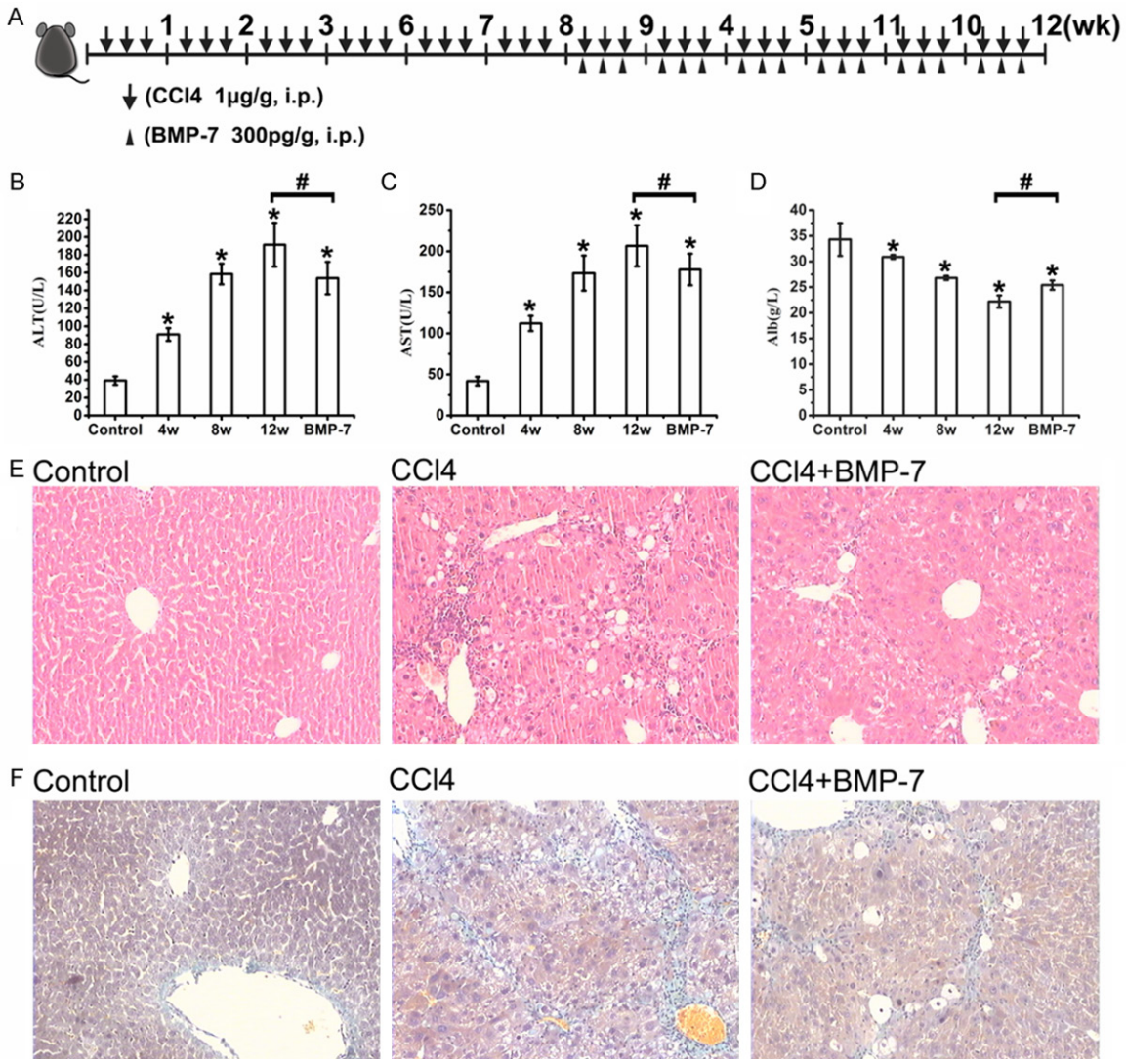


**Figure 1.** Effects of BMP-7 on liver function and histological changes by CCl4 in mice. (A) Mice in the carbon tetrachloride (CCl4) group were intraperitoneal injection with CCl4 (1 ug/g body weight dissolved 1:4 in corn oil) three times a week for 12 weeks. Mice in the bone morphogenetic protein-7 (BMP-7)+CCl4 group were intraperitoneal injection with BMP-7 (300 pg/g) three times a week, starting at 8 weeks after the first administration of CCl4 and last for 4 weeks. The activities of alanine aminotransferase (ALT) (B) aspartate aminotransferase (AST) (C) and albumin (Alb) (D) were assayed by using an automated blood chemistry analyzer. (*significant compared to vehicle-treated group, $P < 0.05$; #significant compared to 12-week subgroup, $P < 0.05$). (E) Liver sections were stained with hematoxylin and eosin (HE) in mice treated with vehicle, CCl4, CCl4+BMP-7 at 12 weeks. (F) Masson's trichrome staining was used to detect the accumulated collagen in liver sections from vehicle, CCl4, CCl4+BMP-7 at 12 weeks.

*RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)*

Liver tissues of every mouse was snap frozen and stored at -80°C until processing. Total RNA was extracted from liver samples by RNAiso Plus reagent (Aidlab Biotechnologies Co., China) according to the manufacturer's protocol. The isolated RNA was then reverse transcribed to cDNA. The cDNA was then amplified by polymerase chain reaction (PCR) in a final volume of 25 μl which contained 12.5 μl of 2X Master Mix, 0.5 μl of forward primers, 0.5 μl of reverse primers, 2.5 μl of cDNA sample and 1 μl of RNase-free $dH_2O$. The assay conditions performed in this study were as follows: initial denaturation at 94°C for 5 min, denaturation at 94°C for 30 s, annealing for 30 s, and extension at 72°C for 45 s, for a total of 30 cycles, then a final extension at 72°C for 10 min was

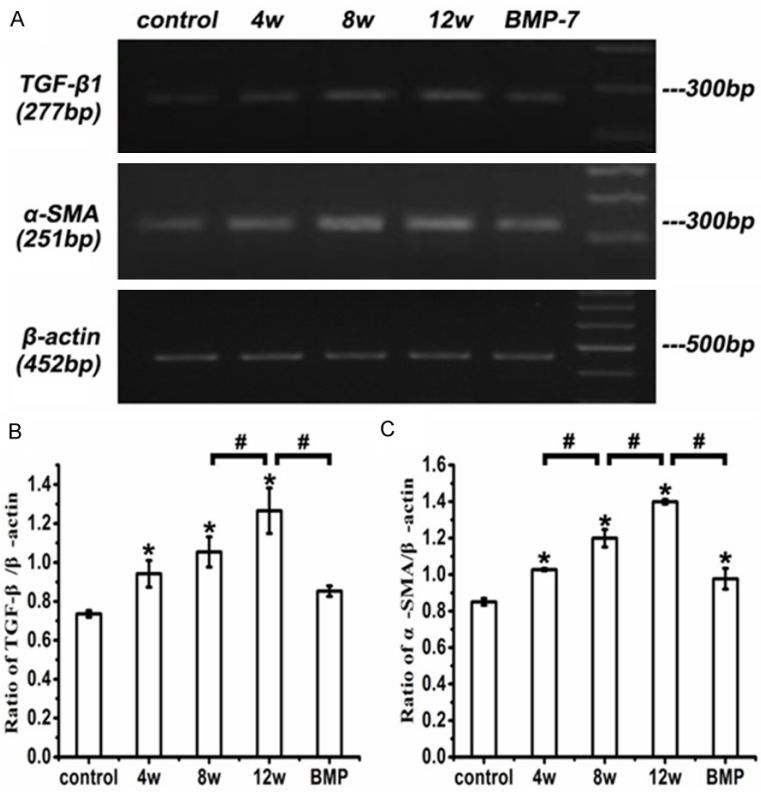


**Figure 2.** Effects of BMP-7 on fibrogenic gene expressions in CCL4-treated mice. (A) Reverse transcription polymerase chain reaction was employed to investigate mRNA level of transforming growth factor β1 (TGFβ1) (B) and alpha smooth muscle actin (α-SMA) (C) in the liver. Data represent the mean ± SD of 6 mice (*significant compared to control group, $p < 0.05$; #significant compared between the subgroups of CCl4 group and BMP-7+CCl4 group, #$p < 0.05$).

performed. The primer sequences and annealing temperatures used in this study are listed in **Table 1**.

*Protein isolation and western blotting*

For analysis protein contents of EGFR, TGF-β1 and α-SMA, total protein of mice livers was isolated using lysis buffer and protein concentration was estimated by BCA protein assay kit according to its manufacturer's protocol. Following heat denaturation, the samples from whole liver lysates (20 μg/lane) were subjected to SDS-polyacrylamide electrophoresis gel (SDS-PAGE) and subsequently electro-transferred onto a nitrocellulose membrane. The membrane was then blocked with 5% bovine serum albumin (BSA) for 90 min at room temperature. The respective primary antibodies against EGFR (1:1000; Cell Signaling, 3265), pEGFR (1:1000; Cell Signaling, 3777), TGF-β1 (1:200; Santa Cruz, sc-52893), α-SMA (1:200; Boster, BM0002) and β-actin (1:2000; Anbo, E0012) in blocking solution were used for incubation with the membrane overnight at 4°C. After being washed with Tris-Buffered-Saline with Tween (TBST) four times for 7 min each, the membrane was incubated with the secondary antibody at room temperature for 90 min. Then target proteins were visualized using a chemiluminescent horseradish peroxidase substrate (Millipore Corporation, Billerica, USA).

*Cell culture and treatment*

The immortalized rat HSCs cell line, HSC-T6 cells, was purchased from XiangYa Central Experiment Laboratory (Changsha, Hunan province, China). The cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere with 5% $CO_2$. The culture medium was changed to serum-free DMEM containing 0.25% bovine serum albumin for 24 hours and then further incubated with different concentration (2, 5, 10 ng/ml) of recombinant TGF-β1 (Peprotech, 100-21).

To analyze whether BMP-7 has an effect on HSC activation, and cells were pretreated with or without different concentrations (500, 1000, 2000 ng/ml) BMP-7 (Peprotech, 120-03) 30 min before the treatment of 5 ng/ml recombinant TGF-β1 and lasted for 48 hours, during which the medium was not changed. Stock solutions of recombinant TGF-β1 and BMP-7 were Phosphate Buffer Saline (PBS) containing 10% bovine serum albumin and kept at -80°C. The equivalent vehicle (PBS with 10% bovine serum albumin) was added to control samples. After indicated time interval, the cellular total RNA and protein was extracted and the expression of EGFR, TGF-β1 and α-SMA were analyzed by RT-PCR and Western blot as described

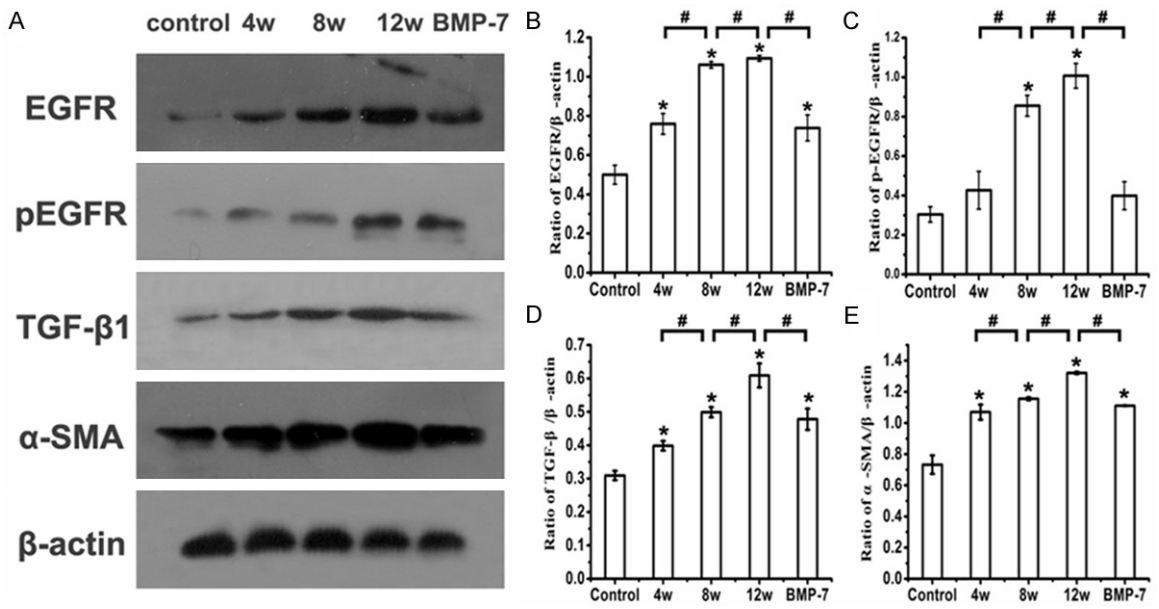


**Figure 3.** Effect of BMP-7 on the expression of α-SMA, TGF-β1, EGFR and pEGFR in CCL4-treated mice. (A) Western blotting was assessed to investigate protein level of epidermal growth factor receptor (EGFR) (B), phosphoepidermal growth factor receptor (pEGFR) (C), transforming growth factor β1 (TGFβ1) (D) and alpha smooth muscle actin (α-SMA) (E) in the liver. Data represent the mean ± SD of 6 mice (*significant compared to control group, $p < 0.05$; #significant compared between the subgroups of CCl4 group and BMP-7+CCl4 group, #$p < 0.05$).

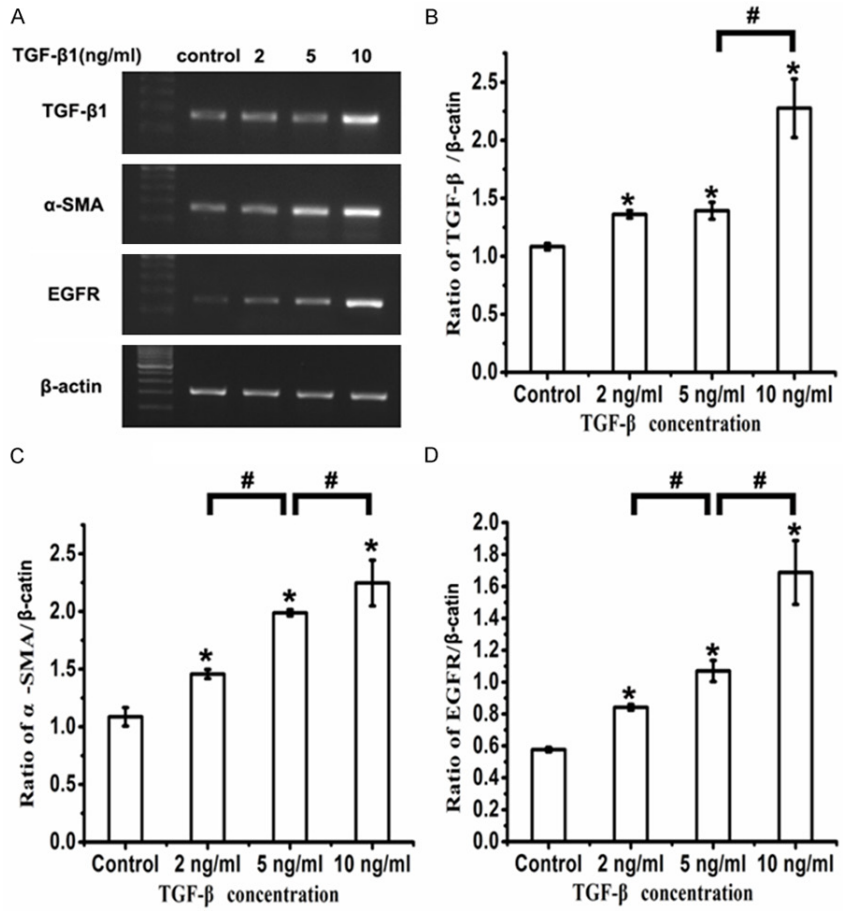


**Figure 4.** Effects of different concentration of TGFβ1 on gene lever of TGFβ1, α-SMA and EGFR in HSC-T6. (A) HSC-T6 cells were incubated with different concentration (2, 5, 10 ng/ml) of recombinant TGF-β1 for 48 h, then cells were subjected to reverse transcription polymerase chain reaction (PCR) analysis. The mRNA levels of transforming growth factor β1 (TGFβ1), (B) alpha smooth muscle actin (α-SMA) (C) and epidermal growth factor receptor (EGFR) (D) in HSC-T6 cells. Data represent the mean ± SD and the experiments were repeated three times. (*significant compared to control group, $p < 0.05$; #significant compared between TGF-β1 treated group, #$p < 0.05$).

above, respectively. The primer sequences used in this study are listed in **Table 1**.

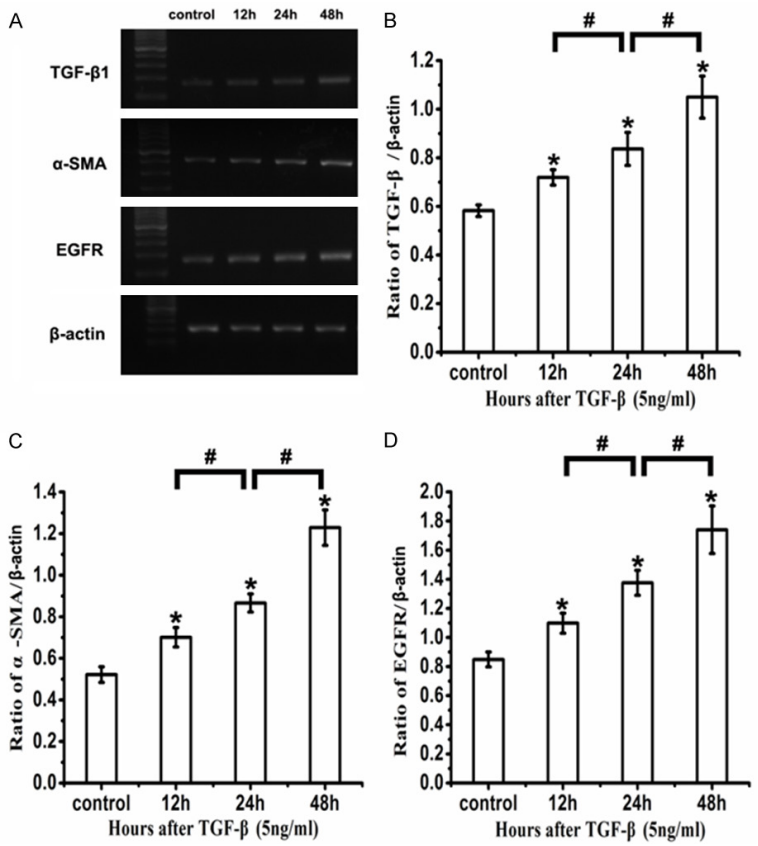


**Figure 5.** Gene lever of TGFβ1, α-SMA and EGFR in HSC-T6 after different stimulation time of TGFβ1. (A) HSC-T6 cells were incubated with of recombinant TGF-β1 (5 ng/ml) for different time (12, 24, 48 h). Then cells were subjected to reverse transcription polymerase chain reaction (PCR) analysis. The mRNA level of transforming growth factor β1 (TGFβ1) (B) alpha smooth muscle actin (α-SMA) (C) and epidermal growth factor receptor (EGFR) (D) in HSC-T6 were evaluated. Data represent the mean ± SD and the experiments were repeated three times. (*significant compared to control group, $p < 0.05$; #significant compared between TGF-β1 treated group, #$p < 0.05$).

## Results

*Effects of BMP-7 on serum concentrations of ALT, AST and Alb*

As shown in **Figure 1**, after administration of CCl4, ALT and AST levers in the CCl4 group were gradually increased, meanwhile, Alb levers were gradually decreased, as compared to the control group ($P < 0.05$). However, BMP-7 was found to significantly reverse those changing tendency of serum markers induced by CCl4 with distinct decreased aminotransferases levers and increased Alb lever.

*Effects of BMP-7 on histopathologic characteristics*

Histological examination using HE and Masson's staining were employed to show the extent of liver damage (shown in **Figure 1**). HE staining for the control group showed normal architecture, whereas the CCl4 group exhibited fatty degeneration, necrosis and inflammation of hepatocytes. However, treatment with BMP-7 markedly improved the hepatic morphology and architecture with less pseudolobules and inflammatory cell infiltration compared with CCl4 group (**Figure 1**).

Masson's staining for the control group showed normal architecture, while the CCl4 group exhibited extensive liver bridging fibrosis and substantial collagen deposition. However, in the BMP-7+CCl4 group had less bridging fibrosis and collagen.

*Effect of BMP-7 on fibrosis-related genes in CCL4-treated mice*

In order to further evaluate the antifibrotic efficacy of BMP-7, we examined genes expression of the key fibrotic markers such as TGF-β1 and α-SMA by RT-PCR. We observed that both the TGF-β1 and α-SMA mRNA are increased gradually in CCl4 group compared to the control group ($P < 0.05$). However, gene lever of TGF-β1 and α-SMA in BMP-7+CCl4 group were significantly less compared with the CCl4 group ($P < 0.05$) (**Figure 2**).

*Effect of BMP-7 on the expression of α-SMA, TGF-β1, EGFR and pEGFR in CCL4-treated mice*

As showed in **Figure 3**, α-SMA and TGF-β1 were significantly increased in CCl4 group compared to control group. However, treatment with BMP-7 significantly inhibited those elevations. In order to further evaluate the mechanism of BMP-7 treatment, the dynamic liver expression of EGFR and phosphorylated EGFR were also examined using western blotting to evaluate the effect of BMP-7 on EGFR in mice. The levels of EGFR and pEGFR in CCl4 group were significantly higher than those in the control group (*P*

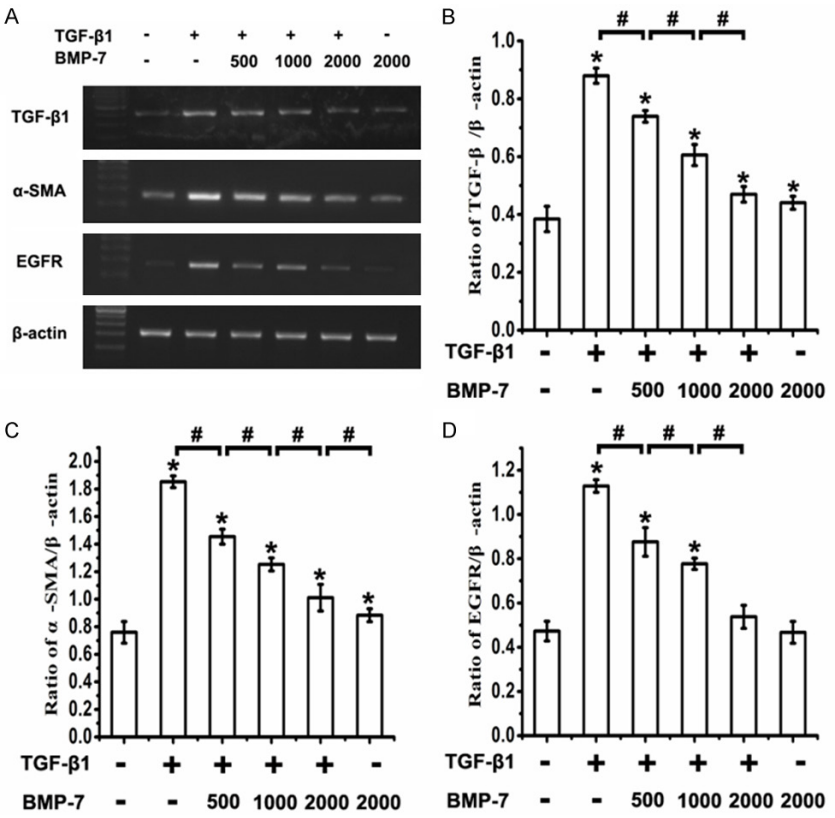


**Figure 6.** Effects of BMP-7 on gene lever of TGF-β1, α-SMA and EGFR in HSC-T6. (A) HSC-T6 cells were incubated with recombinant TGF-β1 (5 ng/ml) and different concentrations (500, 1000, 2000 ng/ml) of BMP-7 for 48 h. Then cells were subjected to reverse transcription polymerase chain reaction analysis. The mRNA levels of transforming growth factor β1 (TGFβ1) (B) alpha smooth muscle actin (α-SMA) (C) and epidermal growth factor receptor (EGFR) (D) in HSC-T6 were evaluated. Data represent the mean ± SD, and the experiments were repeated three times. (*significant compared to vehicle-treated group, p < 0.05; #significant compared between BMP-7-treated group, #p < 0.05).

< 0.05). However, treatment with BMP-7 significantly attenuated the increased expression of EGFR and phosphorylated EGFR (pEGFR). Moreover, linear correlation analysis showed a positive correlation between the expression of EGFR/pEGFR and TGF-β1 (rs = 0.895, 0.859, *P* < 0.05).

*Effect of BMP-7 on fibrosis-related genes in HSC-T6*

In order to assess the effect of BMP-7 on the activation of HSC-T6 in vitro, we analyzed gene expression of the key fibrosis mediator and marker such as TGF-β1 and α-SMA mRNA at different time point (12 h, 24 h, 48 h) after stimulation of HSC-T6 cells with 5 ng/ml of TGF-β1 as well as 48 hours after stimulation with different concentration of TGF-β1 (2, 5, 10 ng/ml). Our results showed that TGF-β1 stimulation result in an enhanced gene and protein expression of EGFR, TGF-β1 and α-SMA in a dose- and time- dependent manner as to the control group (*P* < 0.05) (**Figures 4** and **5**). However, pre-application of hepatic stellate cells with BMP-7 significantly reversed these changes (**Figure 6**).

*Effect of BMP-7 on the expression of α-SMA, TGF-β1, EGFR and pEGFR in HSC-T6*

As show in **Figures 7** and **8**, the proteins expression of α-SMA, TGF-β1, EGFR all elevated after stimulation with different concentration of TGF-β1 in HSC-T6 in a dose- and time- dependent manner as compared to the control (*P* < 0.05). However, treatment with BMP-7 significantly attenuated these changes (**Figure 9**). In addition, this effect had a correction with the concentration of BMP-7. Moverover, EGFR was more highly phosphorylated in the TGF-β1 alone stimulation cells as to the control, but the tyrosine phosphorylation pattern was decreased in the BMP-7/TGF-β1 co-treated cells.

## Discussion

Liver fibrosis is one of the processes that occur when the liver is damaged. Effective antifibrotic treatments are not yet available for human use, and numerous efforts are directed at the development of liver-specific antifibrotic therapies. The proliferation and differentiation of HSC to myofibroblast-like cells and deposition of extracellular matrix (ECM) and collagen are its key characteristic in liver fibrosis [15-19]. The progression of liver fibrosis was found to be mediated by various cytokines especially TGF-β1

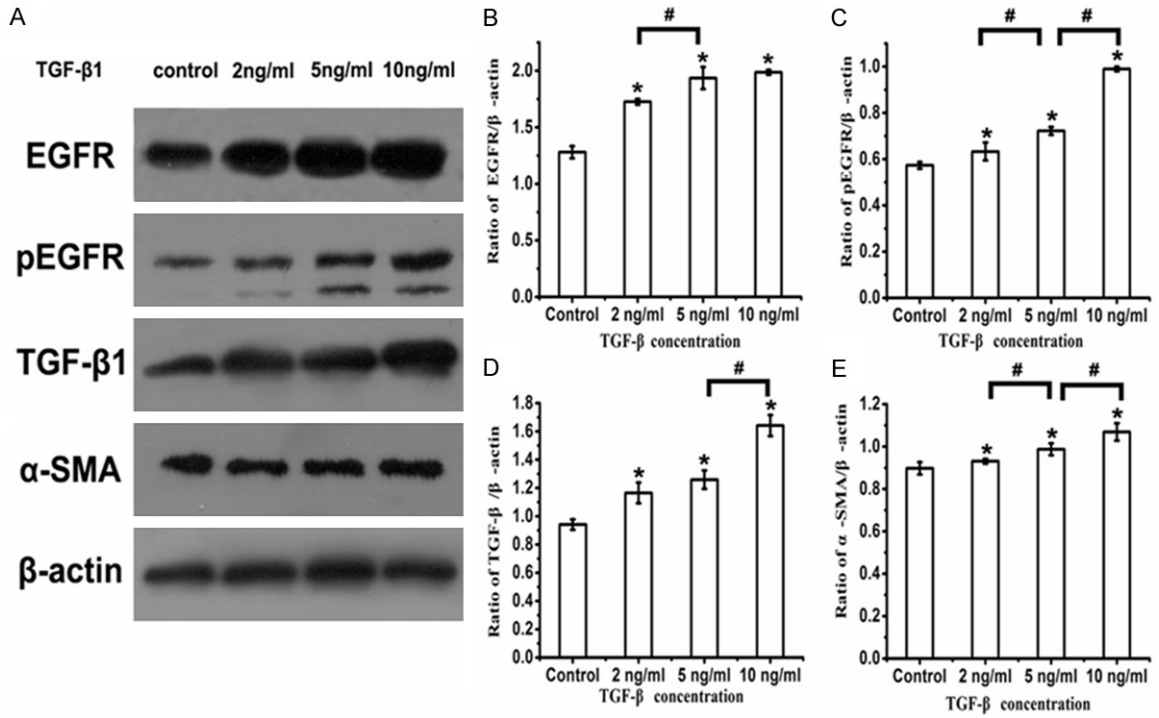


**Figure 7.** Effect of different concentration of TGFβ1 on protein expressions of EGFR, pEGFR, TGFβ1, α-SMA in HSC-T6. (A) HSC-T6 cells were incubated with different concentration (2, 5, 10 ng/ml) of recombinant transforming growth factor β1 (TGF-β1) for 48 h. Then cells were subjected to western blot analysis. The protein level of epidermal growth factor receptor (EGFR) (B) phosphoepidermal growth factor receptor (pEGFR) (C) transforming growth factor β1 (TGFβ1) (D) and alpha smooth muscle actin (α-SMA) (E) in HSC-T6 were evaluated. Data represent the mean ± SD, and the experiments were repeated three times. (*significant compared to control group, $p < 0.05$; #significant compared between TGF-β1 treated group, #$p < 0.05$).

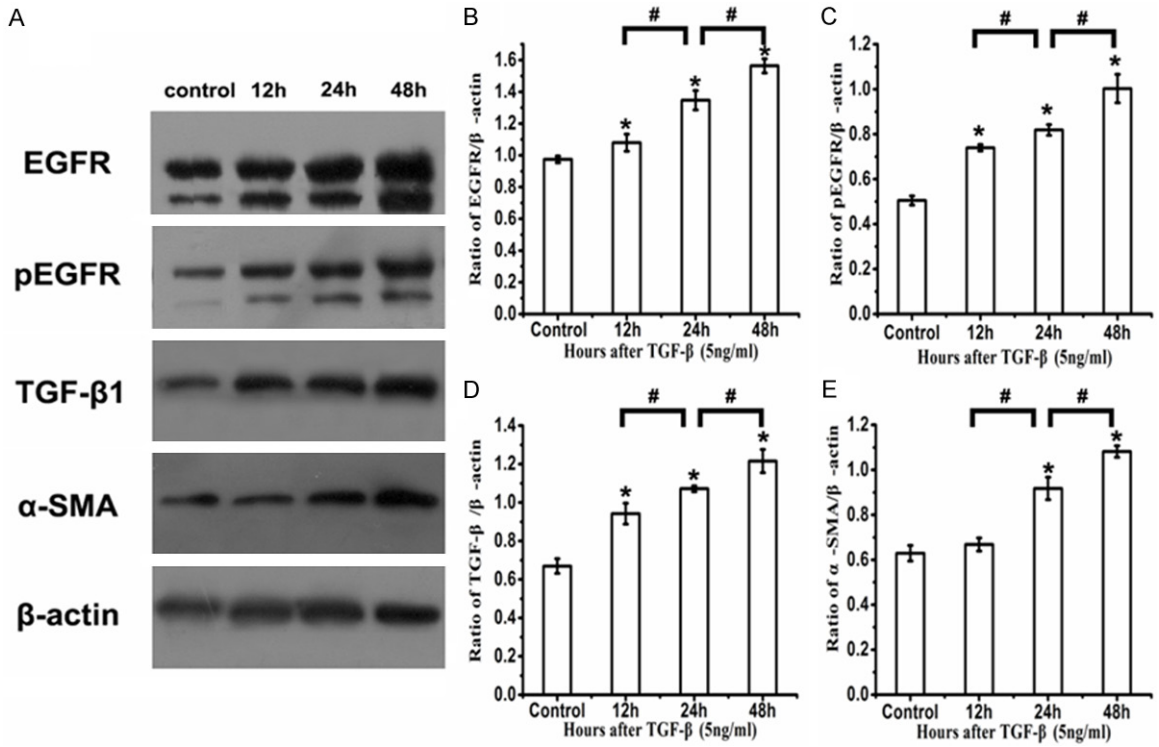

**Figure 8.** Protein expression of EGFR, pEGFR, TGFβ1 and α-SMA in HSC-T6 after different stimulation time of TGFβ1. (A) HSC-T6 were incubated with of recombinant transforming growth factor β1 (TGF-β1, 5 ng/ml) for different time (12, 24, 48 h). Then cells were subjected to western blot analysis. The protein level of epidermal growth factor receptor (EGFR) (B) phosphoepidermal growth factor receptor (pEGFR) (C) TGFβ1 (D) and alpha smooth muscle actin (α-SMA) (E) in HSC-T6 were evaluated. Data represent the mean ± SD, and the experiments were repeated three times. (*significant compared to control group, $p < 0.05$; #significant compared between TGF-β1 treated group, #$p < 0.05$).

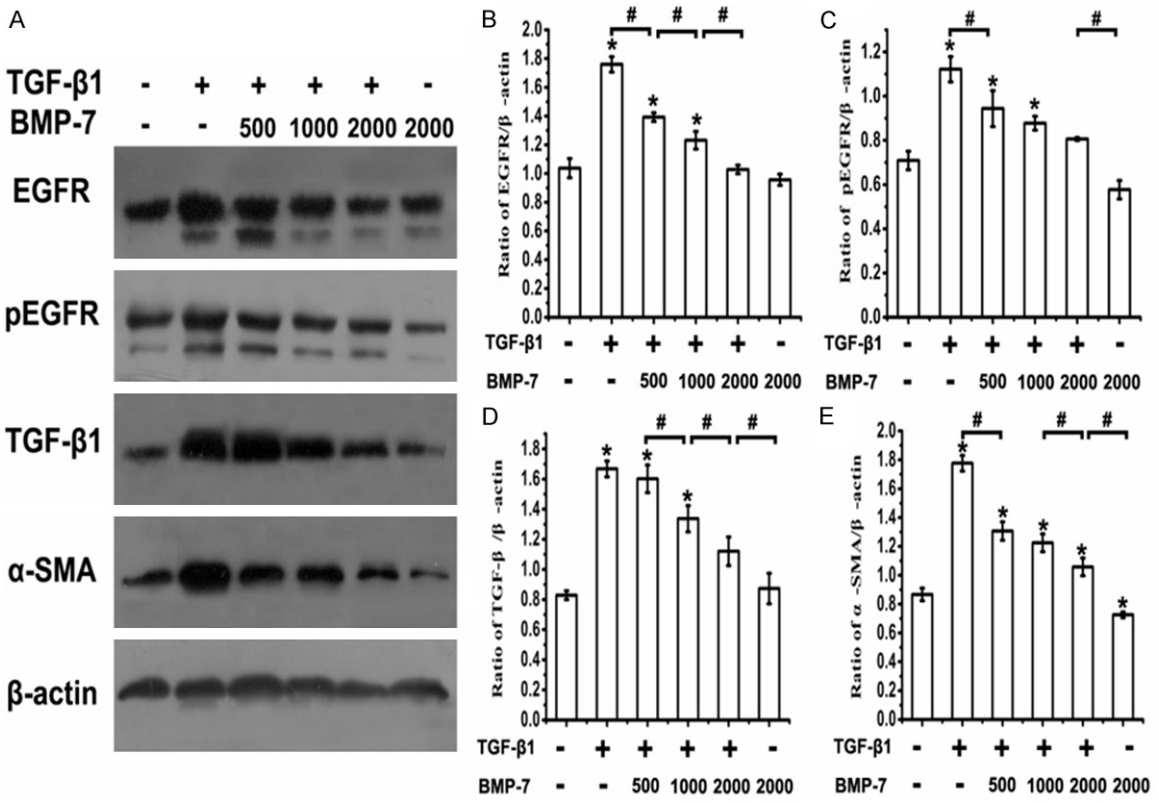


**Figure 9.** Effects of BMP-7 on protein expression of EGFR, pEGFR, TGF-β1 and α-SMA in HSC-T6. (A) HSCs were incubated with recombinant transforming growth factor β1 (TGF-β1, 5 ng/ml) and different concentrations (100, 1000, 2000 ng/ml) of bone morphogenetic protein-7 (BMP-7) for 48 h. Then cells were subjected to western blot analysis. The protein level of epidermal growth factor receptor (EGFR) (B) phosphoepidermal growth factor receptor (pEGFR) (C) TGFβ1 (D) and alpha smooth muscle actin (α-SMA) (E) in HSC-T6 were evaluated. Data represent the mean ± SD and the experiments were repeated three times. (*significant compared to control group, $p < 0.05$; #significant compared between BMP-7 treated group, #$p < 0.05$).

which was accordance with our research. In our study, a liver fibrosis model was successfully established through intraperitoneal injection with CCl4 in mice, as evidenced by histological evaluation and distinct increases of the plasma aminotransferases levels in parallel with the upregulated expression of TGF-β1 and α-SMA.

Bone morphogenic protein 7 (BMP-7), a member of TGF-β superfamily, was described originally for its ability to accelerate bone formation [20]. In the adults, it is mainly produced by the bone and kidney. Studies found that in several animal models BMP-7 was renoprotective and could promote regeneration and ameliorated organ fibrosis in kidney [21]. However, whether BMP-7 has similar effect on liver fibrogenesis and its potential mechanism is still unknown. It has showed that BMP-7 was capable of binding to its receptor, which could lead to phosphorylation of Smad1, Smad5 and as well as Smad8 and then form heterodimerisation with Smad4 and translocated into the nucleus. As a result, Smad3 which was stimulated by TGF-β1 was found to be suppressed, so as to suppressed the TGF-β1 signaling pathway [22]. In our study, we found that BMP-7 showed an antifibrotic efficacy to the pathogenesis of hepatic fibrosis.

The increased aminotransferases levels and expression of TGF-β1 and α-SMA induced by CCl4 and histological change improved in the BMP-7+CCl4 groups, suggesting that BMP-7 protected the mice against liver fibrosis induced by CCl4 to some extent. Furthermore, the study by Shou et al. revealed BMPs could alter expression of EGFR directly, through transcriptional and post-translational mechanisms [14]. Our date showed expression of EGFR was upregulated in CCl4 fibrosis. Moreover, the phosphorylation type of EGFR also showed the similar elevated tendency, suggesting the involvement of EGFR in the regulation of liver fibrogenesis. Thus, EGFR might be a potential novel marker for assessing the progress of fibrosis. However, the levers of both EGFR and phosphorylated EGFR showed a dramatic decrease after treatment with BMP-7, suggesting that there was a cross talk between BMP-7 and EGFR signaling, a potential mechanism of the protective role of BMP-7 in liver fibrosis.

In addition, we examined the effects of TGF-β1 and BMP-7 on HSC-T6. The expression of α-SMA, a marker of HSC, was found to be upregulated in the treatment of TGF-β1 and decreased when added into the BMP-7, suggesting that BMP-7 has an effect on HSC activation. Furthermore, mRNA and protein levers of EGFR and phosphorylated EGFR, decreased when administration of rhBMP-7, which was accordance with our study in vivo.

In conclusion, we show that administration of BMP-7 suppressed the gene and protein expression of α-SMA and TGF-β1 in cultured HSC-T6 and in CCl4 induced liver fibrosis model of mice. Furthermore, the expression of EGFR was increased both in the progress of liver fibrosis and in vitro HSC-T6 cells, which could be reversed by treatment of human recombinant BMP-7. Therefore, EGFR, a potential biomarker for fibrogenesis, could be regulated by BMP-7 in the progression of liver fibrosis.

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## Disclosure of conflict of interest

None.

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