

# World Journal of *Gastroenterology*

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## Basic Study

# Effects and mechanism of adenovirus-mediated phosphatase and tension homologue deleted on chromosome ten gene on collagen deposition in rat liver fibrosis

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## Abstract

### AIM

To evaluate the effects of phosphatase and tension homologue deleted on chromosome ten (*PTEN*) gene on collagen metabolism in hepatic fibrosis and the underlying mechanisms.

### METHODS

Rat primary hepatic stellate cells (HSCs) and human

LX-2 cells were transfected with adenovirus containing cDNA constructs encoding wild-type *PTEN* (Ad-PTEN), *PTEN* mutant *G129E* gene (Ad-G129E), and RNA interference constructs targeting the *PTEN* sequence. PTEN short hairpin RNA to up-regulate and down-regulate the expression of *PTEN*. HSCs were assayed using fluorescent microscopy, real-time polymerase chain reaction, and western blotting. Moreover, a CCl<sub>4</sub>-induced rat hepatic fibrosis model was established to investigate the *in vivo* effects. Hematoxylin and eosin, and Masson's trichrome were used to assess the histological changes. The expression of collagen I and III was assessed using immunohistochemistry and western blot analysis.

### RESULTS

Elevated expression of *PTEN* gene reduced serum levels of alanine transaminase and aspartate transaminase, decreased collagen deposition in the liver, and reduced hepatocyte necrosis. In contrast, knockdown of *PTEN* expression had an opposite effect, such as increased collagen deposition in the liver, and was molecularly characterized by the increased expression of matrix metalloproteinase (MMP)-13 ( $P < 0.01$ ) and MMP-2 ( $P < 0.01$ ), as well as decreased expression of the tissue inhibitor of metalloproteinase (TIMP)-1 ( $P < 0.01$ ) and TIMP-2 ( $P < 0.01$ ).

### CONCLUSION

These data indicated that gene therapy using recombinant adenovirus encoding PTEN might be a novel way of treating hepatic fibrosis.

**Key words:** Collagen metabolism; Hepatic stellate cells; Phosphatase and tension homologue deleted on chromosome ten; PTEN; Gene therapy; Hepatic fibrosis

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**Core tip:** Phosphatase and tension homologue deleted on chromosome ten (PTEN) has a negative relation with the activation and proliferation of hepatic stellate cells (HSCs), which is the central event in liver fibrogenesis as HSCs are the major source of collagens and matrix metalloproteinases in fibrotic liver. In this study, adenoviruses containing cDNA constructs encoding wild-type PTEN (Ad-PTEN) and *PTEN* mutant *G129E* gene (Ad-G129E) were constructed to over-express the *PTEN* gene in both rat primary HSCs and human LX-2 cells as well as in the CCl<sub>4</sub>-induced rat liver fibrosis model. The adenovirus-mediated over-expression of the *PTEN* gene attenuated extracellular matrix (ECM) synthesis (collagens I and III) and promoted ECM degradation, representing a possible novel anti-fibrosis therapy.

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## INTRODUCTION

Cirrhosis, with its manifestation of liver fibrosis, represents a major medical problem worldwide<sup>[1,2]</sup>. Hepatic stellate cells (HSCs) are one of the cell types that play a critical role in the development and maintenance of liver fibrosis. Under fibrogenic conditions, HSCs undergo a complex activation process with morphological and phenotypic changes from quiescent vitamin A-storing cells to activated myofibroblast-like cells under fibrogenic conditions, resulting in increased synthesis and deposition of extracellular matrix (ECM) components, such as collagen I<sup>[3,4]</sup>.

Phosphatase and tension homologue deleted on chromosome ten (*PTEN*) is the first tumor-suppressing gene found to inhibit the proliferation and promote the apoptosis of tumor cells<sup>[5,6]</sup>. PTEN has pleiotropic effects including pulmonary fibrosis, renal fibrosis, and cardiac interstitial fibrosis<sup>[7-10]</sup>. The absence of PTEN in specific hepatic cells leads to not only hepatocellular carcinoma but also nonalcoholic steatohepatitis, which has been found to be associated with hepatic fibrosis<sup>[11]</sup>.

A previous study showed that the expression of PTEN was decreased in rat fibrotic liver tissues and HSCs induced by bile duct ligation *in vivo*<sup>[12]</sup>. During the reversal of liver fibrosis, pretreated *PTEN* mRNA and protein expression normalized, showing the relationship between PTEN and the severity of rat hepatic fibrosis<sup>[13]</sup>. The study presented herein investigated the *in vitro* and *in vivo* effects of PTEN on liver fibrosis using adenoviral transduction of wild-type *PTEN* (Ad-PTEN), mutant *PTEN* (Ad-G129E), and *PTEN* short hairpin RNA (PTEN shRNA) to better characterize the molecular mechanisms of PTEN in liver fibrosis.

## MATERIALS AND METHODS

### Animals

Adult male Wistar rats weighing 350-400 g were obtained from the Experimental Animal Center of Hebei Medical University, Hebei Province, China. The study was performed in compliance with the national ethical guidelines for the care and use of laboratory animals, following the internationally accepted principles for laboratory animal use and care as found in the United States' guidelines (National Institutes of Health publication #85-23, revised in 1985).

### Isolation of rat primary HSCs and cell culture

Rat primary HSCs were isolated from normal healthy

male Wistar rats using *in situ* recirculating perfusion technology, as described in a previous study<sup>[14]</sup>. Then, Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum was used for cell culture; passages 2-3 were used in this study. Human LX-2 cells were obtained from Mount Sinai School of Medicine, authorized by Dr. Friedman.

### Recombinant adenovirus and transfection

Adenovirus containing cDNA constructs encoding wild-type *PTEN* (Ad-PTEN) with green fluorescent protein (GFP), *PTEN* mutant G129E gene (Ad-G129E) with GFP, and the empty virus control (Ad-GFP) were kindly provided by Prof. Junshan Zhu from the Third Military Medical University in China. RNA interference targeting *PTEN* sequence shRNA with enhanced GFP was established by Wuhan Genesil Biotechnology Co., Ltd (Wuhan, China). The transfection was performed as described in a previous study<sup>[15]</sup>.

The rat primary HSCs and human LX-2 cells were divided into five groups: (1) control group, with serum-free antibiotic-free DMEM; (2) Ad-GFP group, with Ad-GFP transfection; (3) Ad-PTEN group, with Ad-PTEN transfection; (4) Ad-G129E group, with Ad-G129E transfection; and (5) *PTEN* shRNA group, with *PTEN* shRNA transfection.

### Real-time polymerase chain reaction assay

A real-time polymerase chain reaction assay was performed using a previously established protocol<sup>[13,16]</sup>. Primer Express 5.0 was used to design the following primers: *PTEN* (rat), forward 5'-GGAAAGGACGGACTGGTGTA-3' and reverse 5'-GGAAAGGACGGACTGGTGTA-3'; *PTEN* (human), forward 5'-ACCGCCAAATTTAATTGTCAG-3' and reverse 5'-GGGTCCTGAATTGGAGGAA T-3'; glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) (rat), forward 5'-GGCAAGTTCACGGCAG-3' and reverse 5'-CGCCAGTAGACTCCACGACAT-3'; and *GAPDH* (human), forward 5'-ACTTTGGTATCGTGAAGGACT-3' and reverse 5'-GTAGAGGCAGGGATGATGTTCT-3'. The primers were synthesized by SBS Genetech Co., Ltd (Beijing, China). The mRNA expression of genes was normalized to *GAPDH*.

### Western blot assay

Western blotting was performed as described in a previous study<sup>[12]</sup>. Anti-PTEN, anti- $\alpha$ -smooth muscle actin, anti-collagen I, anti-collagen III, anti-matrix metalloproteinase (anti-MMP)-13, anti-MMP-2, anti-tissue inhibitor of metalloproteinase (anti-TIMP)-1 and anti-TIMP-2 antibodies (1:200), and anti-GAPDH antibody (1:500) were used as primary antibodies.

### Animal model

The CCl<sub>4</sub>-induced rat hepatic fibrosis model was established as described in a previous study<sup>[13]</sup>. Rats were randomly divided into pretreatment and treatment groups. Pretreatment with recombinant

adenovirus ( $2 \times 10^9$  pfu/100  $\mu$ L/rat) through tail vein injection was conducted on rats once a week by administering CCl<sub>4</sub> for 7 wk. Treatment with adenovirus ( $2 \times 10^9$  pfu/100  $\mu$ L/rat) through tail vein injection was performed on rats once a week starting in the fourth week postadministration of CCl<sub>4</sub> for 4 wk. Recombinant adenoviruses used were Ad-GFP, Ad-PTEN, Ad-G129E, and *PTEN* shRNA.

### Pathology and immunohistochemical and immunofluorescent staining on liver tissue

Hematoxylin and eosin (H&E) staining and Masson's trichrome (MT) staining were performed to assess the histological changes and fibrosis in liver tissues. Immunohistochemical staining was used to further check the deposition of collagens I and III in the fibrotic liver; the procedure was performed as described in a previous study<sup>[12]</sup>. Immunofluorescent staining was also performed on frozen liver sections as described in a previous study to check the changes in the expression of *PTEN* in liver tissue<sup>[13]</sup>.

## RESULTS

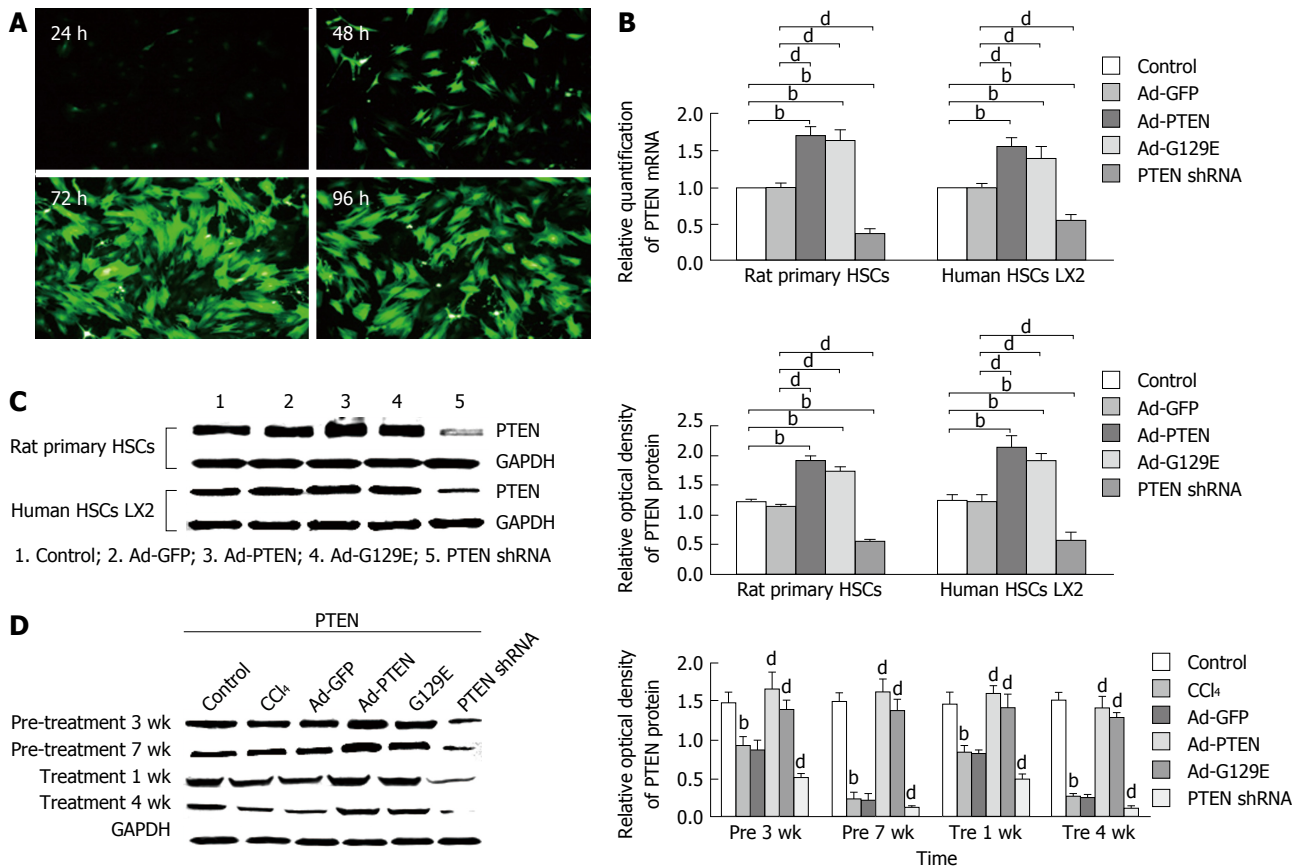
### Establishment of adenoviral transfection to modulate the expression of *PTEN*

Adenoviral transfection using Ad-GFP was performed initially to establish the feasibility to modulate the expression of *PTEN* in rat primary HSCs and human LX-2 cells. This study showed that an adenovirus multiplicity of infection of 50 for 72 h gave the best transfection in rat primary HSCs (94.46% efficiency; Figure 1A) and human LX-2 cells (89.89% efficiency). At 72 h posttransfection, the mRNA and protein expression of *PTEN* in both rat primary HSCs and human LX-2 cells significantly increased in the Ad-PTEN group (mRNA: 1.698, 1.547 and protein:  $1.91 \pm 0.09$ ,  $2.13 \pm 0.01$ , respectively) and Ad-G129E group (mRNA: 1.624, 1.479 and protein:  $1.74 \pm 0.08$ ,  $1.98 \pm 0.12$ , respectively) compared with the Ad-GFP group (mRNA: 0.994, 0.998 and protein:  $1.15 \pm 0.04$ ,  $1.21 \pm 0.14$ , respectively) ( $P < 0.01$ ), and significantly decreased in the *PTEN* shRNA group (mRNA: 0.357, 0.548 and protein:  $0.56 \pm 0.04$ ,  $0.58 \pm 0.13$ , respectively) (Figure 1B and C).

### *PTEN* negatively regulated collagen metabolism

Collagen deposition in fibrotic liver tissues mainly comprised collagens I and III. The protein expression of collagens I and III in rat primary HSCs was found to be significantly decreased in the Ad-PTEN ( $0.32 \pm 0.05$  and  $0.18 \pm 0.02$ , respectively) and Ad-G129E groups compared with the CCl<sub>4</sub> control and Ad-GFP groups (Figure 2A and B). In contrast, the expression of collagens I and III significantly increased in the *PTEN* shRNA group compared with the CCl<sub>4</sub> control and Ad-GFP groups. A similar tendency was also found in experiments using human LX-2 cells (Figure 2A and C).





**Figure 1** Effective modulation of the expression of phosphatase and tension homologue deleted on chromosome ten *in vitro* and *in vivo*. **A**: Representative immunofluorescent image showing adenoviral transfection efficiency using Ad-GFP in rat primary HSCs; **B**: The mRNA expression of PTEN was quantitated for rat primary HSCs (left panel) and human LX-2 cells (right panel). Data are expressed as a relative value, and the error bars represent SE; **C**: Representative western blot showing the protein expression of PTEN (left panel), quantitated and expressed as relative optical density. The error bars represent SE. <sup>b</sup> $P < 0.01$  vs the control group; <sup>d</sup> $P < 0.01$  vs the Ad-GFP group; **D**: Representative western blot showing that the protein expression of PTEN in rat livers treated with Ad-PTEN was significantly enhanced in pre 1 wk, pre 3 wk, pre 5 wk, and pre 7 wk, compared with the Ad-GFP and control CCl<sub>4</sub> groups.  $n = 3$  for all experiments. GFP: Green fluorescent protein; HSCs: Hepatic stellate cells; PTEN: Phosphatase and tension homologue deleted on chromosome ten.

MMP-13 and MMP-2 play a critical role in the metabolism of collagens I and III. In rat primary HSCs, a significantly higher expression of MMP-13 and MMP-2 was found in the Ad-PTEN and Ad-G129E groups compared with the PTEN shRNA and CCl<sub>4</sub> control groups. As expected, the expression of MMP-13 and MMP-2 was significantly reduced in the PTEN shRNA group compared with the control and Ad-GFP groups (Figure 2A and B). Similar regulation of MMP-13 and MMP-2 was also found using human LX-2 cells (Figure 2A and C).

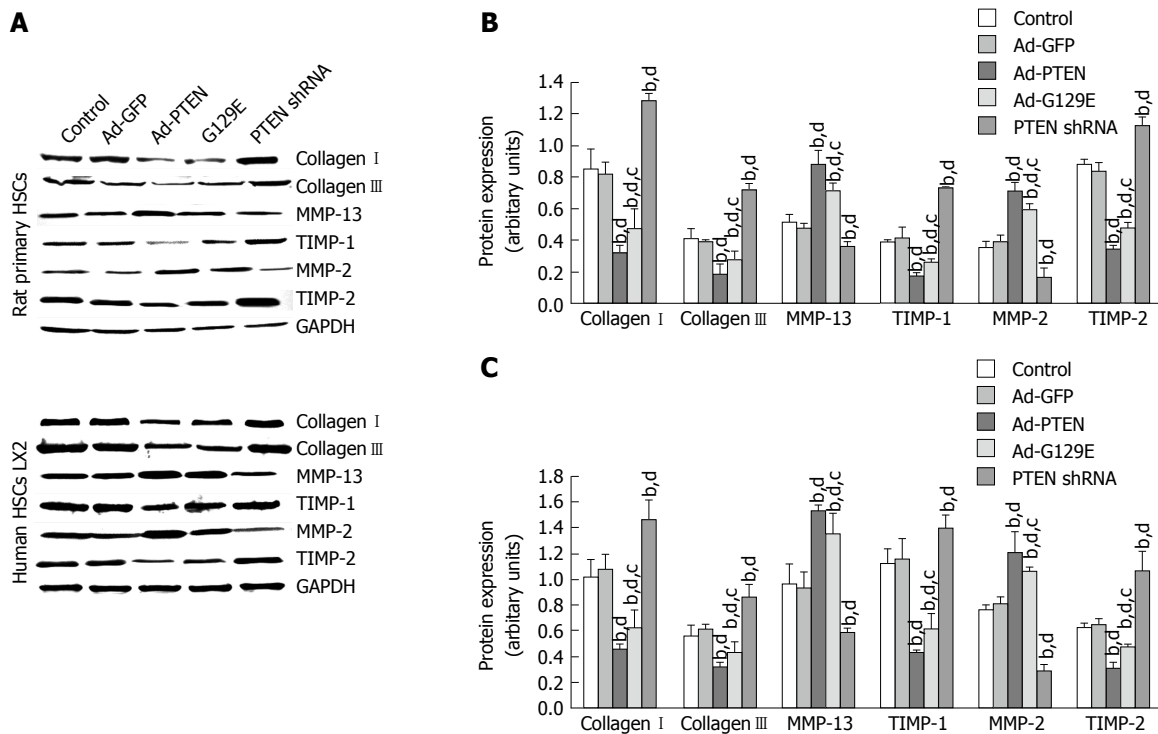
The degree of collagen deposition was due to the balance between collagenases and their inhibitors. The inhibitors of MMP-13 and MMP-2 were additionally measured by western blotting at 72 h posttransfection in both rat primary HSCs and human LX2 HSCs. The expression of TIMP-1 and TIMP-2 was significantly down-regulated by Ad-PTEN and Ad-G129E compared with the CCl<sub>4</sub> control and Ad-GFP groups (Figure 2A and B). Transfection of PTEN shRNA up-regulated these inhibitors of collagenases compared with the control and Ad-GFP groups (Figure 2).

### PTEN had a protective effect on CCl<sub>4</sub>-induced rat liver fibrosis

The increased expression of PTEN gene in the Ad-PTEN and Ad-G129E groups was significantly reduced, whereas the reduced PTEN gene expression in the PTEN shRNA group was increased, along with the serum levels of alanine transaminase and aspartate transaminase compared with the CCl<sub>4</sub> control and Ad-GFP groups, indicating improved liver function (Tables 1 and 2).

H&E and MT staining confirmed that the increased expression of PTEN in the Ad-PTEN and Ad-G129E groups led to reduced hepatocyte necrosis and collagen deposition in liver tissue compared with that in the control groups (Figure 3). Immunofluorescent staining for PTEN was performed to see whether the improved pathology was associated with changes in the expression of the PTEN gene. It was found that the expression of the PTEN gene significantly increased with Ad-PTEN and Ad-G129E recombinant adenovirus in both the prevention and treatment groups (Figure 3).

Moreover, the total protein was isolated from liver

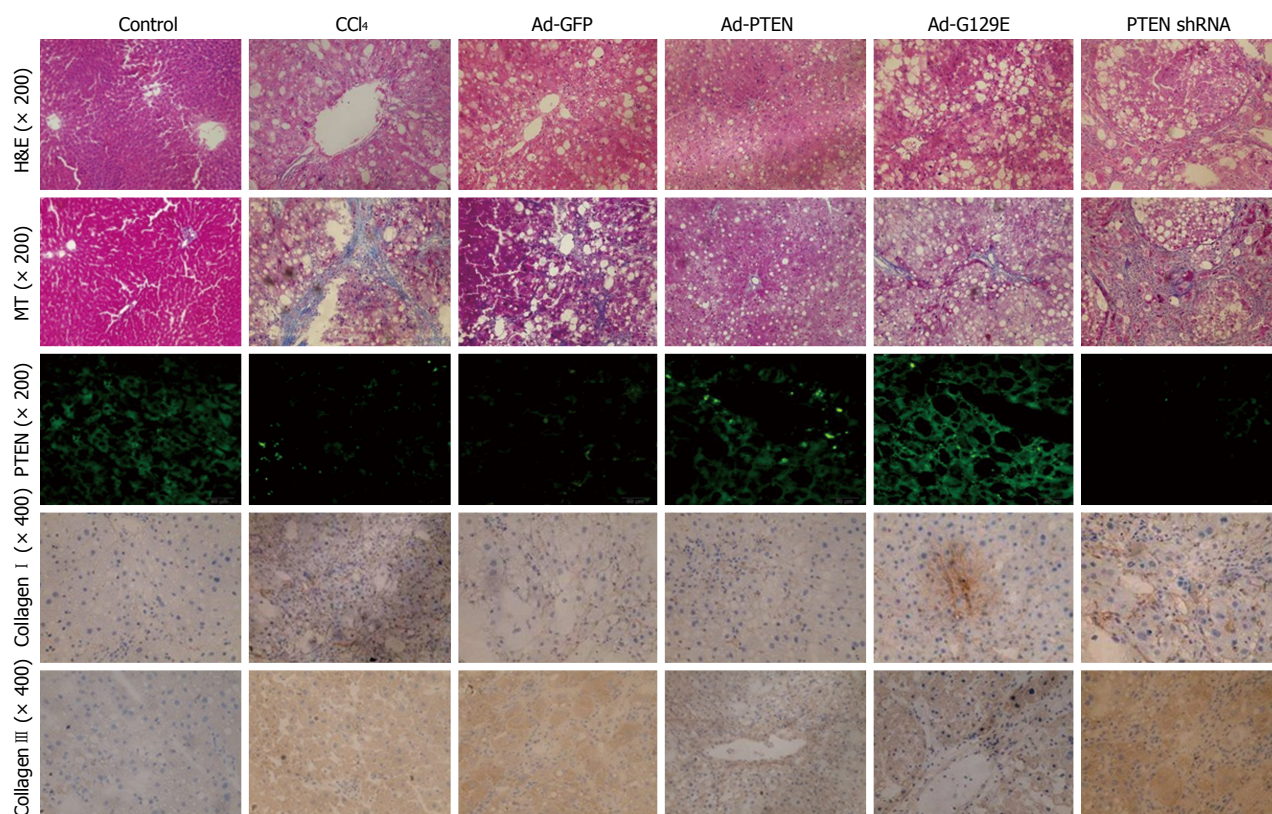


**Figure 2** Phosphatase and tension homologue deleted on chromosome ten has a negative effect on collagen deposition *in vitro*. A: Representative western blots of factors involved in the metabolism of fibrosis at 72 h posttransfection are shown in (A) and quantitated for rat primary HSCs in (B) and for human HSC LX2 cells in (C). Data in (B) and (C) are represented as mean  $\pm$  SE.  $n = 3$  for all groups. <sup>b</sup> $P < 0.01$  vs the control group; <sup>c</sup> $P < 0.05$  vs Ad-PTEN group; <sup>d</sup> $P < 0.01$  vs the Ad-GFP group. HSC: Hepatic stellate cell; PTEN: Phosphatase and tension homologue deleted on chromosome ten.

**Table 1** Impact of adenovirus-mediated phosphatase and tension homologue deleted on chromosome ten on liver function in rat liver fibrosis induced by CCl<sub>4</sub> in prevention groups at different time points

| Group            | ALT (U/L)                         | AST (U/L)                         | ALB (g/L)                     | TBIL ( $\mu$ mol/L)           | DBIL ( $\mu$ mol/L)           |
|------------------|-----------------------------------|-----------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Pre 1 wk         |                                   |                                   |                               |                               |                               |
| Control          | 58.09 $\pm$ 6.54                  | 147.23 $\pm$ 10.25                | 28.95 $\pm$ 2.14              | 2.15 $\pm$ 0.54               | 1.12 $\pm$ 0.32               |
| CCl <sub>4</sub> | 414.45 $\pm$ 29.37                | 339.12 $\pm$ 45.37                | 27.20 $\pm$ 2.35              | 2.12 $\pm$ 0.38               | 1.70 $\pm$ 0.21               |
| Ad-GFP           | 426.24 $\pm$ 30.76                | 289.37 $\pm$ 20.76                | 27.92 $\pm$ 3.12              | 2.13 $\pm$ 0.31               | 1.56 $\pm$ 0.12               |
| Ad-PTEN          | 404.23 $\pm$ 26.78                | 283.76 $\pm$ 23.45 <sup>a</sup>   | 29.12 $\pm$ 4.25              | 1.98 $\pm$ 0.34               | 1.42 $\pm$ 0.31               |
| Ad-G129E         | 409.98 $\pm$ 43.24                | 289.76 $\pm$ 35.43 <sup>a</sup>   | 28.82 $\pm$ 3.23              | 2.00 $\pm$ 0.34               | 1.50 $\pm$ 0.21               |
| PTEN shRNA       | 2970.11 $\pm$ 267.34 <sup>b</sup> | 1690.25 $\pm$ 200.34 <sup>b</sup> | 27.10 $\pm$ 3.23              | 4.03 $\pm$ 0.78 <sup>b</sup>  | 2.67 $\pm$ 0.56 <sup>b</sup>  |
| Pre 3 wk         |                                   |                                   |                               |                               |                               |
| Control          | 60.71 $\pm$ 5.34                  | 150.54 $\pm$ 12.34                | 27.65 $\pm$ 3.28              | 2.34 $\pm$ 0.23               | 1.28 $\pm$ 0.77               |
| CCl <sub>4</sub> | 532.21 $\pm$ 34.02                | 804.12 $\pm$ 67.34                | 25.31 $\pm$ 4.32              | 17.31 $\pm$ 1.21              | 7.78 $\pm$ 2.01               |
| Ad-GFP           | 589.34 $\pm$ 26.45                | 787.45 $\pm$ 43.21                | 26.70 $\pm$ 3.15              | 15.04 $\pm$ 2.12              | 7.34 $\pm$ 1.29               |
| Ad-PTEN          | 422.37 $\pm$ 34.23 <sup>a</sup>   | 478.43 $\pm$ 50.34 <sup>a</sup>   | 27.31 $\pm$ 2.43              | 5.78 $\pm$ 1.01 <sup>a</sup>  | 3.24 $\pm$ 0.78 <sup>a</sup>  |
| Ad-G129E         | 506.45 $\pm$ 57.32                | 526.43 $\pm$ 32.98 <sup>a</sup>   | 26.59 $\pm$ 1.37              | 10.54 $\pm$ 2.76 <sup>a</sup> | 6.21 $\pm$ 2.37               |
| PTEN shRNA       | 3440.32 $\pm$ 342.32 <sup>b</sup> | 3090.39 $\pm$ 228.23 <sup>b</sup> | 24.41 $\pm$ 2.34 <sup>b</sup> | 28.37 $\pm$ 4.78 <sup>b</sup> | 14.56 $\pm$ 2.42 <sup>b</sup> |
| Pre 5 wk         |                                   |                                   |                               |                               |                               |
| Control          | 59.22 $\pm$ 7.34                  | 145.38 $\pm$ 11.32                | 28.37 $\pm$ 2.56              | 2.34 $\pm$ 0.78               | 1.43 $\pm$ 0.22               |
| CCl <sub>4</sub> | 672.34 $\pm$ 17.37                | 817.56 $\pm$ 46.32                | 26.70 $\pm$ 3.02              | 15.23 $\pm$ 2.78              | 7.65 $\pm$ 1.22               |
| Ad-GFP           | 597.45 $\pm$ 33.21                | 753.24 $\pm$ 32.12                | 26.30 $\pm$ 1.76              | 16.30 $\pm$ 3.56              | 8.72 $\pm$ 2.11               |
| Ad-PTEN          | 456.43 $\pm$ 32.12 <sup>a</sup>   | 566.76 $\pm$ 53.21 <sup>a</sup>   | 28.54 $\pm$ 2.12 <sup>a</sup> | 9.31 $\pm$ 2.12 <sup>a</sup>  | 4.50 $\pm$ 0.89 <sup>a</sup>  |
| Ad-G129E         | 492.37 $\pm$ 40.65 <sup>a</sup>   | 705.43 $\pm$ 63.21 <sup>a</sup>   | 27.21 $\pm$ 1.35              | 10.56 $\pm$ 1.23 <sup>a</sup> | 5.61 $\pm$ 1.23 <sup>a</sup>  |
| PTEN shRNA       | 2060.21 $\pm$ 325.34 <sup>b</sup> | 2490.34 $\pm$ 532.12 <sup>b</sup> | 24.23 $\pm$ 1.23 <sup>b</sup> | 17.31 $\pm$ 2.67 <sup>b</sup> | 9.45 $\pm$ 2.12 <sup>b</sup>  |
| Pre 7 wk         |                                   |                                   |                               |                               |                               |
| Control          | 60.21 $\pm$ 3.19                  | 152.12 $\pm$ 15.34                | 28.34 $\pm$ 1.15              | 2.38 $\pm$ 0.59               | 1.48 $\pm$ 0.26               |
| CCl <sub>4</sub> | 605.23 $\pm$ 85.37                | 1110.32 $\pm$ 215.32              | 22.76 $\pm$ 3.21              | 15.26 $\pm$ 1.23              | 8.65 $\pm$ 0.32               |
| Ad-GFP           | 623.45 $\pm$ 56.34                | 1134.23 $\pm$ 121.24              | 23.43 $\pm$ 1.21              | 14.87 $\pm$ 1.02              | 9.34 $\pm$ 0.98               |
| Ad-PTEN          | 456.45 $\pm$ 32.12 <sup>a</sup>   | 183.23 $\pm$ 34.23 <sup>a</sup>   | 26.73 $\pm$ 2.23 <sup>a</sup> | 4.32 $\pm$ 0.97               | 2.62 $\pm$ 0.42               |
| Ad-G129E         | 545.87 $\pm$ 43.21 <sup>a</sup>   | 694.32 $\pm$ 25.34 <sup>a</sup>   | 25.63 $\pm$ 1.23 <sup>a</sup> | 4.56 $\pm$ 0.32               | 2.54 $\pm$ 0.23               |
| PTEN shRNA       | 1825.23 $\pm$ 28.23 <sup>b</sup>  | 1878.45 $\pm$ 56.87 <sup>b</sup>  | 21.90 $\pm$ 3.23 <sup>b</sup> | 17.23 $\pm$ 3.56 <sup>b</sup> | 9.08 $\pm$ 1.32 <sup>b</sup>  |

Data are presented as mean  $\pm$  SD for  $n = 3$ . <sup>a</sup> $P < 0.01$  vs CCl<sub>4</sub>, Ad-GFP; <sup>b</sup> $P < 0.05$  vs CCl<sub>4</sub>, Ad-GFP, Ad-PTEN. ALB: Albumin; ALT: Alanine transaminase; AST: Aspartate transaminase; DBIL: Direct bilirubin; PTEN: Phosphatase and tension homologue deleted on chromosome ten; TBIL: Total bilirubin.



**Figure 3** Phosphatase and tension homologue deleted on chromosome ten has protective effects on CCl<sub>4</sub>-induced rat hepatic fibrosis *in vivo*. H&E stain (× 200) and MT stain (× 200) showed reduced hepatic cell necrosis and collagen deposition in liver tissue by over-expressed PTEN gene in Ad-PTEN and Ad-G129E groups. Immunofluorescent staining for PTEN (green) showed increased PTEN expression with Ad-PTEN and Ad-G129E recombinant adenovirus in both the prevention group or the treatment group. Immunohistochemical staining (bottom 2 rows) showed decreased collagen I and collagen III expression in hepatic tissues (× 400) with over-expression of PTEN induced by exogenous wild-type PTEN or G129E gene, whereas collagen I and collagen III expressions was reduced by PTEN shRNA. H&E: Hematoxylin and eosin; MT: Masson's trichrome; PTEN: Phosphatase and tension homologue deleted on chromosome ten.

tissues in each group, and western blot analysis of the expression of PTEN was performed. Consistent with the findings from previous studies<sup>[13]</sup>, the protein expression of PTEN was significantly decreased in rats treated with CCl<sub>4</sub>. The protein expression of PTEN was significantly increased in rats treated with Ad-PTEN in the pretreatment and treatment groups compared with the control group (Figure 1D).

Then, western blot analysis was performed to check the protein expression of collagens I and III in the rat liver tissue at each time point. Compared with the Ad-GFP group, the expression of collagens I and III significantly decreased with the enhanced expression of PTEN in the Ad-PTEN group at pre 3 wk, pre 7 wk, pre 1 wk, and pre 4 wk (Figure 4). In contrast, the reduced expression of PTEN with *PTEN* shRNA significantly increased the expression of collagens I and III (Figure 4).

## DISCUSSION

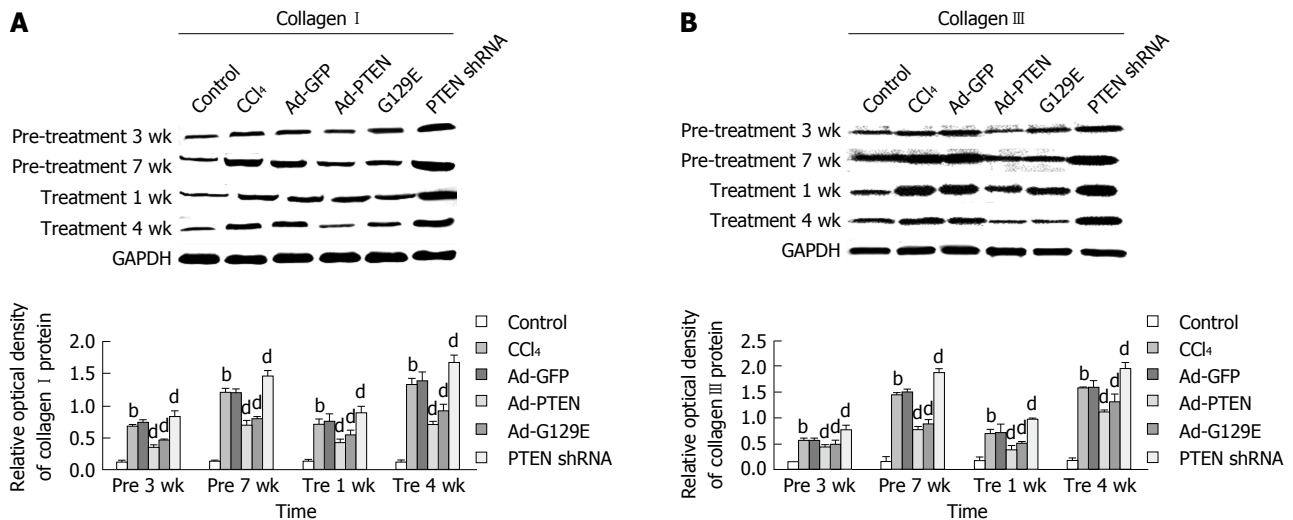
Hepatic fibrosis is the accumulation of ECM in response to chronic liver injury that ultimately leads to cirrhosis<sup>[1,2]</sup>. Cirrhosis is associated with increased morbidity and mortality and results in substantial

economic and social costs. At present, no effective therapy is available to treat or reverse hepatic fibrosis.

Under chronic injury, HSCs are activated to produce more ECM, mainly comprised of collagens I and III in the liver tissue. Moreover, HSCs also regulate the balance of MMPs and TIMPs, which determines the degree of collagen deposition in the liver<sup>[1,17-19]</sup>. PTEN has been found to be involved in myocardial fibrosis, renal fibrosis, and lung fibrosis<sup>[8-10]</sup>. A previous study found that higher expression of PTEN reduced the number of activated HSCs to negatively regulate fibrogenesis *in vivo*<sup>[12,13]</sup>. This suggested that PTEN may also regulate the accumulation of ECM components in liver fibrosis because ECM is mainly produced from activated HSCs<sup>[1]</sup>.

The expression of *PTEN* was modulated in this study using recombinant adenovirus that either increased or reduced the expression of PTEN. This study showed that reducing the expression of *PTEN* conferred worsened liver fibrosis through its modulation of collagens I and III, MMP-13 and MMP-2, and TIMP-1 and TIMP-2. These PTEN-dependent changes in collagen, collagenases, and collagenase inhibitors reduced collagen deposition that was associated with CCl<sub>4</sub>-induced liver fibrosis.





**Figure 4** Phosphatase and tension homologue deleted on chromosome ten reduced collagen deposition in CCl<sub>4</sub>-induced fibrotic hepatic tissues. Representative western blot of collagen I (A) and collagen III (B) expressions. Collagen I and collagen III expressions were quantitated in the bottom panels and represented as mean  $\pm$  SE. <sup>b</sup>*P* < 0.01 vs the control group; <sup>d</sup>*P* < 0.01 vs the Ad-GFP group. PTEN: Phosphatase and tension homologue deleted on chromosome ten.

**Table 2** Impact of adenovirus-mediated phosphatase and tension homologue deleted on chromosome ten on liver function in rat liver fibrosis induced by CCl<sub>4</sub> in treatment groups at different time points

| Group            | ALT (U/L)                         | AST (U/L)                         | ALB (g/L)                     | TBIL ( $\mu$ mol/L)           | DBIL ( $\mu$ mol/L)           |
|------------------|-----------------------------------|-----------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Tre 1 wk         |                                   |                                   |                               |                               |                               |
| Control          | 57.78 $\pm$ 8.23                  | 146.78 $\pm$ 12.23                | 27.99 $\pm$ 1.18              | 2.49 $\pm$ 0.55               | 1.65 $\pm$ 0.36               |
| CCl <sub>4</sub> | 684.32 $\pm$ 78.34                | 840.32 $\pm$ 32.25                | 25.26 $\pm$ 3.25              | 15.45 $\pm$ 3.23              | 6.56 $\pm$ 1.12               |
| Ad-GFP           | 645.65 $\pm$ 34.26                | 832.12 $\pm$ 40.32                | 24.54 $\pm$ 1.21              | 14.31 $\pm$ 1.21              | 7.21 $\pm$ 2.12               |
| Ad-PTEN          | 384.98 $\pm$ 36.23 <sup>b</sup>   | 398.43 $\pm$ 43.23 <sup>b</sup>   | 27.60 $\pm$ 2.34 <sup>b</sup> | 11.21 $\pm$ 1.21 <sup>b</sup> | 5.32 $\pm$ 1.46 <sup>b</sup>  |
| Ad-G129E         | 495.34 $\pm$ 45.12 <sup>b</sup>   | 546.32 $\pm$ 32.12 <sup>b</sup>   | 26.12 $\pm$ 2.43              | 13.23 $\pm$ 2.12              | 7.86 $\pm$ 1.23 <sup>b</sup>  |
| PTEN shRNA       | 2030.31 $\pm$ 112.34 <sup>a</sup> | 1730.54 $\pm$ 87.34 <sup>a</sup>  | 24.12 $\pm$ 2.34 <sup>a</sup> | 34.21 $\pm$ 5.34 <sup>a</sup> | 17.23 $\pm$ 2.12 <sup>a</sup> |
| Tre 2 wk         |                                   |                                   |                               |                               |                               |
| Control          | 57.46 $\pm$ 3.58                  | 142.34 $\pm$ 10.24                | 29.15 $\pm$ 3.21              | 2.68 $\pm$ 0.74               | 1.55 $\pm$ 0.46               |
| CCl <sub>4</sub> | 712.34 $\pm$ 34.54                | 843.23 $\pm$ 54.32                | 26.32 $\pm$ 1.34              | 14.32 $\pm$ 5.32              | 8.32 $\pm$ 2.12               |
| Ad-GFP           | 697.45 $\pm$ 45.76                | 876.34 $\pm$ 33.32                | 25.79 $\pm$ 2.32              | 13.34 $\pm$ 2.13              | 7.86 $\pm$ 1.23               |
| Ad-PTEN          | 338.12 $\pm$ 12.34 <sup>b</sup>   | 407.34 $\pm$ 32.12 <sup>b</sup>   | 27.56 $\pm$ 3.23 <sup>b</sup> | 6.56 $\pm$ 1.21 <sup>b</sup>  | 3.72 $\pm$ 0.78 <sup>b</sup>  |
| Ad-G129E         | 364.32 $\pm$ 34.56 <sup>b</sup>   | 408.67 $\pm$ 23.78 <sup>b</sup>   | 27.21 $\pm$ 1.22 <sup>b</sup> | 10.32 $\pm$ 1.21 <sup>b</sup> | 5.34 $\pm$ 1.09 <sup>b</sup>  |
| PTEN shRNA       | 2040.21 $\pm$ 126.32 <sup>a</sup> | 1860.32 $\pm$ 210.32 <sup>a</sup> | 25.60 $\pm$ 3.12 <sup>a</sup> | 27.12 $\pm$ 4.21 <sup>a</sup> | 14.78 $\pm$ 2.12 <sup>a</sup> |
| Tre 3 wk         |                                   |                                   |                               |                               |                               |
| Control          | 58.54 $\pm$ 6.58                  | 148.45 $\pm$ 13.27                | 29.12 $\pm$ 3.15              | 2.12 $\pm$ 0.21               | 1.48 $\pm$ 0.24               |
| CCl <sub>4</sub> | 1892.32 $\pm$ 113.12              | 2018.32 $\pm$ 123.45              | 22.14 $\pm$ 1.23              | 8.18 $\pm$ 1.10               | 5.21 $\pm$ 0.65               |
| Ad-GFP           | 1746.32 $\pm$ 98.23               | 1879.23 $\pm$ 119.34              | 23.10 $\pm$ 2.13              | 9.12 $\pm$ 1.23               | 5.72 $\pm$ 0.54               |
| Ad-PTEN          | 524.35 $\pm$ 34.12 <sup>b</sup>   | 690.35 $\pm$ 54.23 <sup>b</sup>   | 27.30 $\pm$ 3.21 <sup>b</sup> | 6.45 $\pm$ 0.32 <sup>b</sup>  | 4.12 $\pm$ 0.54 <sup>b</sup>  |
| Ad-G129E         | 576.23 $\pm$ 43.21 <sup>b</sup>   | 820.43 $\pm$ 67.32 <sup>b</sup>   | 25.20 $\pm$ 1.21 <sup>b</sup> | 8.12 $\pm$ 0.87 <sup>b</sup>  | 4.89 $\pm$ 0.81 <sup>b</sup>  |
| PTEN shRNA       | 1937.56 $\pm$ 32.21 <sup>a</sup>  | 2390.32 $\pm$ 112.23 <sup>a</sup> | 21.34 $\pm$ 2.34 <sup>a</sup> | 10.76 $\pm$ 1.23 <sup>a</sup> | 6.10 $\pm$ 0.54 <sup>a</sup>  |
| Tre 4 wk         |                                   |                                   |                               |                               |                               |
| Control          | 62.31 $\pm$ 8.12                  | 155.37 $\pm$ 13.27                | 29.91 $\pm$ 3.12              | 2.49 $\pm$ 0.32               | 1.65 $\pm$ 0.21               |
| CCl <sub>4</sub> | 594.39 $\pm$ 32.18                | 1110.32 $\pm$ 89.34               | 23.45 $\pm$ 2.12              | 17.32 $\pm$ 2.32              | 9.28 $\pm$ 1.23               |
| Ad-GFP           | 576.45 $\pm$ 23.43                | 886.43 $\pm$ 45.67                | 22.79 $\pm$ 1.21              | 13.45 $\pm$ 2.45              | 7.12 $\pm$ 1.34               |
| Ad-PTEN          | 490.32 $\pm$ 32.23 <sup>b</sup>   | 528.34 $\pm$ 32.12 <sup>b</sup>   | 25.31 $\pm$ 3.21 <sup>b</sup> | 7.34 $\pm$ 1.21 <sup>b</sup>  | 4.65 $\pm$ 0.98 <sup>b</sup>  |
| Ad-G129E         | 530.23 $\pm$ 22.56 <sup>b</sup>   | 628.43 $\pm$ 45.34 <sup>b</sup>   | 25.12 $\pm$ 1.23 <sup>b</sup> | 10.54 $\pm$ 2.32 <sup>b</sup> | 6.54 $\pm$ 1.21 <sup>b</sup>  |
| PTEN shRNA       | 1148.32 $\pm$ 54.32 <sup>a</sup>  | 1270.34 $\pm$ 121.28 <sup>a</sup> | 20.37 $\pm$ 3.12 <sup>a</sup> | 26.32 $\pm$ 3.56 <sup>a</sup> | 14.56 $\pm$ 2.13 <sup>a</sup> |

Data are presented as mean  $\pm$  SD for *n* = 3. <sup>a</sup>*P* < 0.05 vs CCl<sub>4</sub>, Ad-GFP, Ad-PTEN; <sup>b</sup>*P* < 0.01 vs CCl<sub>4</sub>, Ad-GFP. ALB: Albumin; ALT: Alanine transaminase; AST: Aspartate transaminase; DBIL: Direct bilirubin; PTEN: Phosphatase and tension homologue deleted on chromosome ten; TBIL: Total bilirubin.

The human homologue of rat MMP-13 is MMP-1 and is expressed by HSCs, fibroblasts, Kupffer cells, and so forth<sup>[20]</sup>. MMP-13 remodels the surrounding tissue to clear room for deposition of newly synthesized ECM. The activity of MMP-13 can be inhibited by TIMP-1<sup>[21]</sup>. MMP-2 mainly serves to degrade the collagen in

the basement membrane<sup>[22]</sup>. TIMP-2 is essential for MMP-2 activation, as it can bind to pro-MMP-2 and then combine with MT1-MMP to activate pro-MMP-2. However, over-expressed TIMP-2 can inhibit MMP-2 activity, causing excessive collagen deposition<sup>[20,23]</sup>. In this study, MMP-13 and MMP-2, the major collagenases



involved in collagen degradation<sup>[24,25]</sup>, increased with a high expression level of the *PTEN* gene, which also down-regulated the expression of TIMP-1 and TIMP-2. The lower expression of PTEN could invert the ratio of MMP-13/TIMP-1 and MMP-2/TIMP-2 and cause severe collagen deposition. These data suggested that PTEN might regulate hepatic collagen metabolism through regulation of MMP-13, MMP-2, TIMP-1, and TIMP-2.

Gene therapy using adenovirus vector has been shown to be effective in modulating the expression of a gene of interest<sup>[26-28]</sup>. In this study, treatment with recombinant adenovirus carrying a highly expressed *PTEN* gene in rats with CCl<sub>4</sub>-induced hepatic fibrosis improved the liver function and reduced the collagen deposition in liver tissue.

In conclusion, these data demonstrated that PTEN might affect collagen deposition in the liver through MMP-13, MMP-2, TIMP-1, and TIMP-2. Gene therapy using recombinant adenovirus encoding wild-type *PTEN* may represent a novel way for treating hepatic fibrosis.

## COMMENTS

### Background

Phosphatase and tension homologue deleted on chromosome ten (PTEN) plays an essential role in the activation of hepatic stellate cells (HSCs), which are the major source of collagens and matrix metalloproteinases in the fibrotic liver. Liver fibrosis results from the excessive deposition of extracellular matrix (ECM) components, mainly comprising collagens I and III, which are produced by HSCs.

### Research frontiers

Previous studies have shown that PTEN had a negative relation with the activation and proliferation of HSCs, which is the central event in liver fibrogenesis. The collagen synthesis could be inhibited by over-expression of the *PTEN* gene. Adenoviruses containing cDNA constructs encoding wild-type *PTEN* (Ad-PTEN) and *PTEN* mutant G129E gene (Ad-G129E) were used in both rat primary HSCs and human LX-2 cells, as well as in the CCl<sub>4</sub>-induced rat liver fibrosis model.

### Innovations and breakthroughs

Recent reports highlighted the importance of collagen metabolism in HSCs. This novel study reported that the adenovirus-mediated over-expression of the *PTEN* gene could attenuate ECM synthesis and promote ECM degradation, which represents a potential tool for novel anti-fibrosis therapies.

### Applications

The results of this study indicated that the over-expressed *PTEN* gene might represent a novel tool for the treatment and reversal of hepatic fibrosis.

### Peer-review

It's an interesting topic about effects of the *PTEN* gene on collagen metabolism in hepatic fibrosis and the underlying mechanisms.

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