

World Journal of *Gastroenterology*

World J Gastroenterol 2017 August 28; 23(32): 5829-6008



EDITORIAL

- 5829 Role of tissue microenvironment resident adipocytes in colon cancer
Tabuso M, Homer-Vanniasinkam S, Adya R, Arasaradnam RP

REVIEW

- 5836 Ophthalmic manifestations in patients with inflammatory bowel disease: A review
Troncoso LL, Biancardi AL, de Moraes Jr HV, Zaltman C
- 5849 Laparoscopic appendectomy for acute appendicitis: How to discourage surgeons using inadequate therapy
Hori T, Machimoto T, Kadokawa Y, Hata T, Ito T, Kato S, Yasukawa D, Aisu Y, Kimura Y, Sasaki M, Takamatsu Y, Kitano T, Hisamori S, Yoshimura T
- 5860 Long non-coding RNAs in hepatocellular carcinoma: Potential roles and clinical implications
Niu ZS, Niu XJ, Wang WH

MINIREVIEWS

- 5875 Nano albumin bound-paclitaxel in pancreatic cancer: Current evidences and future directions
Giordano G, Pancione M, Olivieri N, Parcesepe P, Velocci M, Di Raimo T, Coppola L, Toffoli G, D'Andrea MR

ORIGINAL ARTICLE

Basic Study

- 5887 Comparison between tocotrienol and omeprazole on gastric growth factors in stress-exposed rats
Nur Azlina MF, Qodriyah HMS, Chua KH, Kamisah Y
- 5895 (-)-Epigallocatechin-3-gallate enhances poly I:C-induced interferon- λ 1 production and inhibits hepatitis C virus replication in hepatocytes
Wang YZ, Li JL, Wang X, Zhang T, Ho WZ
- 5904 Effects and mechanism of adenovirus-mediated phosphatase and tension homologue deleted on chromosome ten gene on collagen deposition in rat liver fibrosis
Xie SR, An JY, Zheng LB, Huo XX, Guo J, Shih D, Zhang XL

Retrospective Study

- 5913 Integrating *TYMS*, *KRAS* and *BRAF* testing in patients with metastatic colorectal cancer
Ntavatzikos A, Spathis A, Patapis P, Machairas N, Peros G, Konstantoudakis S, Leventakou D, Panayiotides IG, Karakitsos P, Koumariou A

Clinical Trials Study

- 5925 Characterizing gastrointestinal stromal tumors and evaluating neoadjuvant imatinib by sequencing of endoscopic ultrasound-biopsies

Hedenström P, Nilsson B, Demir A, Andersson C, Enlund F, Nilsson O, Sadik R

Observational Study

- 5936 Novel predictors for lymph node metastasis in submucosal invasive colorectal carcinoma

Yim K, Won DD, Lee IK, Oh ST, Jung ES, Lee SH

- 5945 Changes with aging in gastric biomarkers levels and in biochemical factors associated with *Helicobacter pylori* infection in asymptomatic Chinese population

Shan JH, Bai XJ, Han LL, Yuan Y, Sun XF

Prospective Study

- 5954 Modified *Helicobacter* test using a new test meal and a ¹³C-urea breath test in *Helicobacter pylori* positive and negative dyspepsia patients on proton pump inhibitors

Tepeš B, Malfertheiner P, Labenz J, Aygen S

- 5962 Real time endoscopic ultrasound elastography and strain ratio in the diagnosis of solid pancreatic lesions

Okasha H, Elkholy S, El-Sayed R, Wifi MN, El-Nady M, El-Nabawi W, El-Dayem WA, Radwan MI, Farag A, El-sherif Y, Al-Gemeie E, Salman A, El-Sherbiny M, El-Mazny A, Mahdy RE

- 5969 Efficacy and safety of sofosbuvir and daclatasvir in treatment of kidney transplantation recipients with hepatitis C virus infection

Xue Y, Zhang LX, Wang L, Li T, Qu YD, Liu F

Randomized Controlled Trial

- 5977 New botanical drug, HL tablet, reduces hepatic fat as measured by magnetic resonance spectroscopy in patients with nonalcoholic fatty liver disease: A placebo-controlled, randomized, phase II trial

Jeong JY, Sohn JH, Baek YH, Cho YK, Kim Y, Kim H

Randomized Clinical Trial

- 5986 Randomized clinical trial comparing fixed-time split dosing and split dosing of oral Picosulfate regimen for bowel preparation

Jun JH, Han KH, Park JK, Seo HL, Kim YD, Lee SJ, Jun BK, Hwang MS, Park YK, Kim MJ, Cheon GJ

META-ANALYSIS

- 5994 Systematic review and meta-analysis of colon cleansing preparations in patients with inflammatory bowel disease

Restellini S, Kherad O, Bessissow T, Ménard C, Martel M, Taheri Tanjani M, Lakatos PL, Barkun AN

CASE REPORT

- 6003** Postoperative inflammation as a possible cause of portal vein thrombosis after irreversible electroporation for locally advanced pancreatic cancer

Su JJ, Su M, Xu K, Wang PF, Yan L, Lu SC, Gu WQ, Chen YL

LETTERS TO THE EDITOR

- 6007** Comment on "Efficacy and adverse events of cold *vs* hot polypectomy: A meta-analysis"

Sun HH, Huang SL, Bai Y

ABOUT COVER

Editorial board member of *World Journal of Gastroenterology*, Mitsushige Sugimoto, MD, PhD, Associate Professor, Division of Digestive Endoscopy, Shiga University of Medical Science Hospital, Otsu 520-2192, Japan

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1375 experts in gastroenterology and hepatology from 68 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

World Journal of Gastroenterology (*WJG*) is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central and Directory of Open Access Journals. The 2017 edition of Journal Citation Reports® cites the 2016 impact factor for *WJG* as 3.365 (5-year impact factor: 3.176), ranking *WJG* as 29th among 79 journals in gastroenterology and hepatology (quartile in category Q2).

FLYLEAF

I-IX Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Fen-Fen Zhang*
Proofing Editor-in-Chief: *Lian-Sheng Ma*
Responsible Science Editor: *Yuan Qi*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
 ISSN 1007-9327 (print)
 ISSN 2219-2840 (online)

LAUNCH DATE
 October 1, 1995

FREQUENCY
 Weekly

EDITORS-IN-CHIEF
Damian Garcia-Olmo, MD, PhD, Doctor, Professor, Surgeon, Department of Surgery, Universidad Autonoma de Madrid; Department of General Surgery, Fundacion Jimenez Diaz University Hospital, Madrid 28040, Spain

Stephen C Strom, PhD, Professor, Department of Laboratory Medicine, Division of Pathology, Karolinska Institutet, Stockholm 141-86, Sweden

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach,

CA 90822, United States

EDITORIAL BOARD MEMBERS
 All editorial board members resources online at <http://www.wjgnet.com/1007-9327/editorialboard.htm>

EDITORIAL OFFICE
 Jin-Lei Wang, Director
 Yuan Qi, Vice Director
 Ze-Mao Gong, Vice Director
World Journal of Gastroenterology
 Baishideng Publishing Group Inc
 7901 Stoneridge Drive, Suite 501,
 Pleasanton, CA 94588, USA
 Telephone: +1-925-2238242
 Fax: +1-925-2238243
 E-mail: editorialoffice@wjgnet.com
 Help Desk: <http://www.fpublishing.com/helpdesk>
<http://www.wjgnet.com>

PUBLISHER
 Baishideng Publishing Group Inc
 7901 Stoneridge Drive, Suite 501,
 Pleasanton, CA 94588, USA
 Telephone: +1-925-2238242
 Fax: +1-925-2238243
 E-mail: bpoffice@wjgnet.com
 Help Desk: <http://www.fpublishing.com/helpdesk>

<http://www.wjgnet.com>

PUBLICATION DATE
 August 28, 2017

COPYRIGHT
 © 2017 Baishideng Publishing Group Inc. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
 All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS
 Full instructions are available online at <http://www.wjgnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION
<http://www.fpublishing.com>

Basic Study

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

Shu-Rui Xie, Jun-Yan An, Li-Bo Zheng, Xiao-Xia Huo, Jian Guo, David Shih, Xiao-Lan Zhang

Shu-Rui Xie, Jun-Yan An, Li-Bo Zheng, Xiao-Xia Huo, Jian Guo, Xiao-Lan Zhang, Department of Gastroenterology, The Second Hospital of Hebei Medical University, Hebei Key Laboratory of Gastroenterology, Hebei Institute of Gastroenterology, Shijiazhuang 050000, Hebei Province, China

Shu-Rui Xie, Department of Gastroenterology, Xingtai People's Hospital, Xingtai 054031, Hebei Province, China

David Shih, Inflammatory Bowel and Immunobiology Research Institute, F. Widjaja Foundation, Cedars-Sinai Medical Center, Los Angeles, CA 90048, United States

Author contributions: Xie SR and An JY contributed equally to this work; Xie SR and An JY performed the majority of the experiments; Zheng LB and Guo J assisted with various experiments and helped to analyze the data; Huo XX and Zhang XL drafted and edited the manuscript; Shih D performed critical revision of the manuscript.

Supported by the National Natural Science Foundation of China, No. 30872513.

Institutional review board statement: This study was reviewed and approved by the Second Hospital of Hebei Medical University Institutional Review Board.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Experimental Animal Center of Hebei Medical University (No. 911102).

Conflict-of-interest statement: We declare that there are no conflicts of interest to disclose.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at xiaolanzh@126.com. Participants gave informed consent for data sharing.

Open-Access: This article is an open-access article which was

selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Xiao-Lan Zhang, MD, PhD, Professor, Department of Gastroenterology, The Second Hospital of Hebei Medical University, Hebei Key Laboratory of Gastroenterology, Hebei Institute of Gastroenterology, 215 West Heping Road, Shijiazhuang 050000, Hebei Province, China. xiaolanzh@hb2h.com
Telephone: +86-311-66007370
Fax: +86-311-66007370

Received: January 26, 2017

Peer-review started: February 4, 2017

First decision: April 21, 2017

Revised: June 19, 2017

Accepted: July 22, 2017

Article in press: July 24, 2017

Published online: August 28, 2017

Abstract**AIM**

To evaluate the effects of phosphatase and tension homologue deleted on chromosome ten (*PTEH*) 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 CCl₄-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

© **The Author(s) 2017.** Published by Baishideng Publishing Group Inc. All rights reserved.

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 CCl₄-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.

Xie SR, An JY, Zheng LB, Huo XX, Guo J, Shih D, Zhang XL. Effects and mechanism of adenovirus-mediated phosphatase and tension homologue deleted on chromosome ten gene on collagen

deposition in rat liver fibrosis. *World J Gastroenterol* 2017; 23(32): 5904-5912 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v23/i32/5904.htm> DOI: <http://dx.doi.org/10.3748/wjg.v23.i32.5904>

INTRODUCTION

Cirrhosis, with its manifestation of liver fibrosis, represents a major medical problem worldwide^[1,2]. 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^[3,4].

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^[5,6]. PTEN has pleiotropic effects including pulmonary fibrosis, renal fibrosis, and cardiac interstitial fibrosis^[7-10]. 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^[11].

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*^[12]. 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^[13]. 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^[14]. 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^[15].

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^[13,16]. Primer Express 5.0 was used to design the following primers: *PTEN* (rat), forward 5'-GGAAAGGACGGACTGGTGTA-3' and reverse 5'-GGAAAGGACGGACTGGTGTGA-3'; *PTEN* (human), forward 5'-ACCGCCAAATTTAATGTCAG-3' and reverse 5'-GGGTCCTGAATTGGAGGAAAT-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^[12]. Anti-*PTEN*, anti- α -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 CCl₄-induced rat hepatic fibrosis model was established as described in a previous study^[13]. Rats were randomly divided into pretreatment and treatment groups. Pretreatment with recombinant

adenovirus (2×10^9 pfu/100 μ L/rat) through tail vein injection was conducted on rats once a week by administering CCl₄ for 7 wk. Treatment with adenovirus (2×10^9 pfu/100 μ L/rat) through tail vein injection was performed on rats once a week starting in the fourth week postadministration of CCl₄ 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^[12]. 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^[13].

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 ± 0.09 , 2.13 ± 0.01 , respectively) and Ad-G129E group (mRNA: 1.624, 1.479 and protein: 1.74 ± 0.08 , 1.98 ± 0.12 , respectively) compared with the Ad-GFP group (mRNA: 0.994, 0.998 and protein: 1.15 ± 0.04 , 1.21 ± 0.14 , respectively) ($P < 0.01$), and significantly decreased in the *PTEN* shRNA group (mRNA: 0.357, 0.548 and protein: 0.56 ± 0.04 , 0.58 ± 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 ± 0.05 and 0.18 ± 0.02 , respectively) and Ad-G129E groups compared with the CCl₄ 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 CCl₄ control and Ad-GFP groups. A similar tendency was also found in experiments using human LX-2 cells (Figure 2A and C).

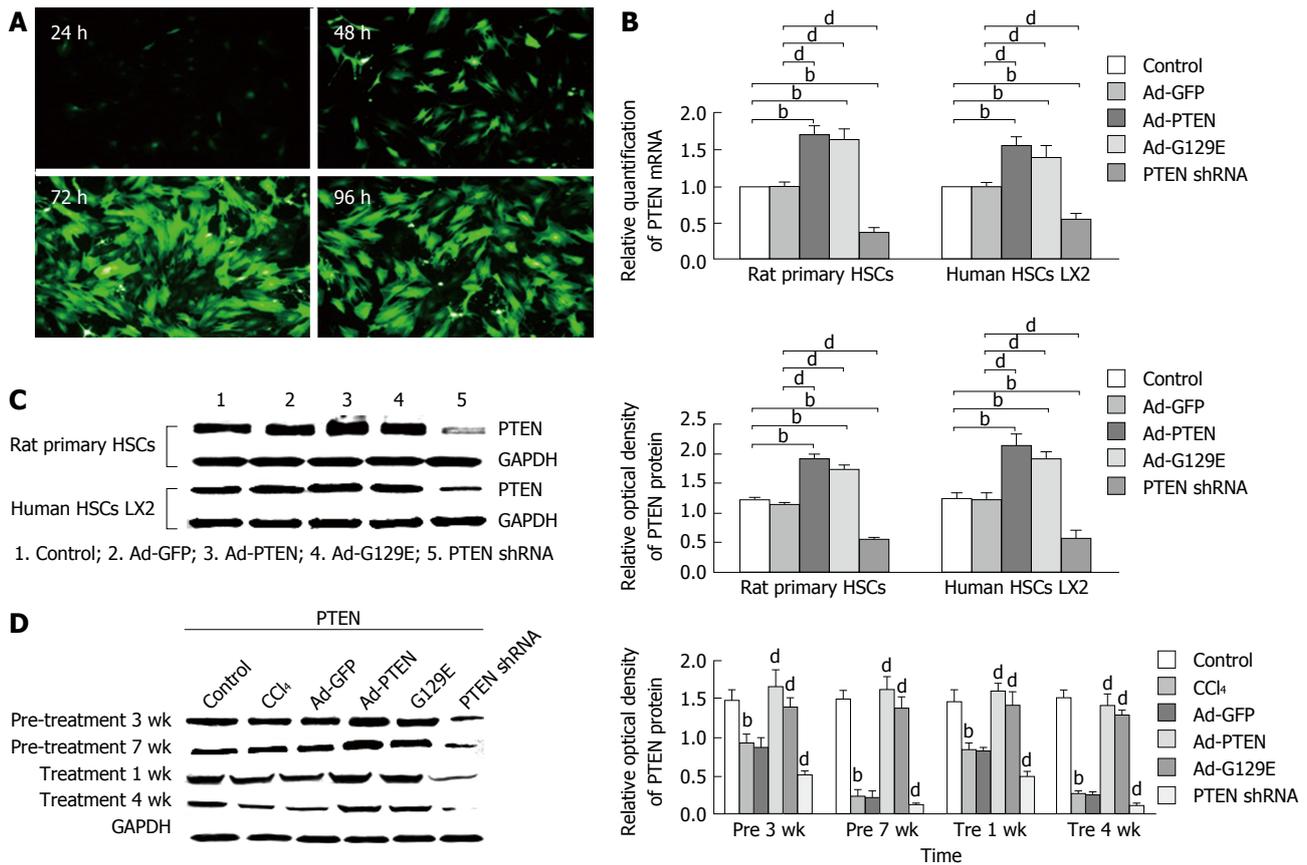


Figure 1 Effective modulation of the expression of phosphatase and tension homologue deleted on chromosome ten *in vitro* and *in vivo*. A: Representative immunofluorescent image showing adenoviral transfection efficiency using Ad-GFP in rat primary HSCs; B: The mRNA expression of PTEN was quantitated 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. ^b $P < 0.01$ vs the control group; ^d $P < 0.01$ 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 CCl₄ 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 CCl₄ 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 CCl₄ 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 CCl₄-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 CCl₄ 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

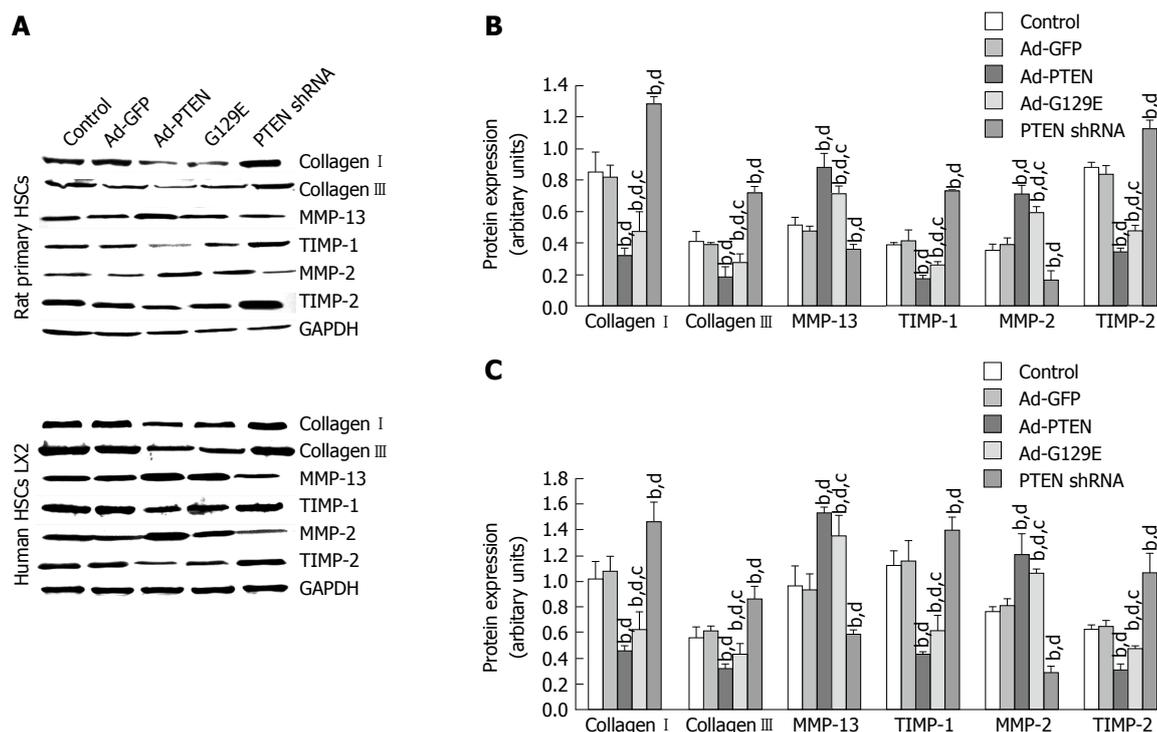


Figure 2 Phosphatase and tension homologue deleted on chromosome ten has a negative effect on collagen deposition *in vitro*. A: Representative western blots of factors involved in the metabolism of fibrosis at 72 h posttransfection are shown in (A) and quantitated for rat primary HSCs in (B) and for human HSC LX2 cells in (C). Data in (B) and (C) are represented as mean ± SE. *n* = 3 for all groups. ^b*P* < 0.01 vs the control group; ^c*P* < 0.05 vs Ad-PTEN group; ^d*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 CCl₄ in prevention groups at different time points

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

Data are presented as mean ± SD for *n* = 3. ^a*P* < 0.01 vs CCl₄, Ad-GFP; ^b*P* < 0.05 vs CCl₄, 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.

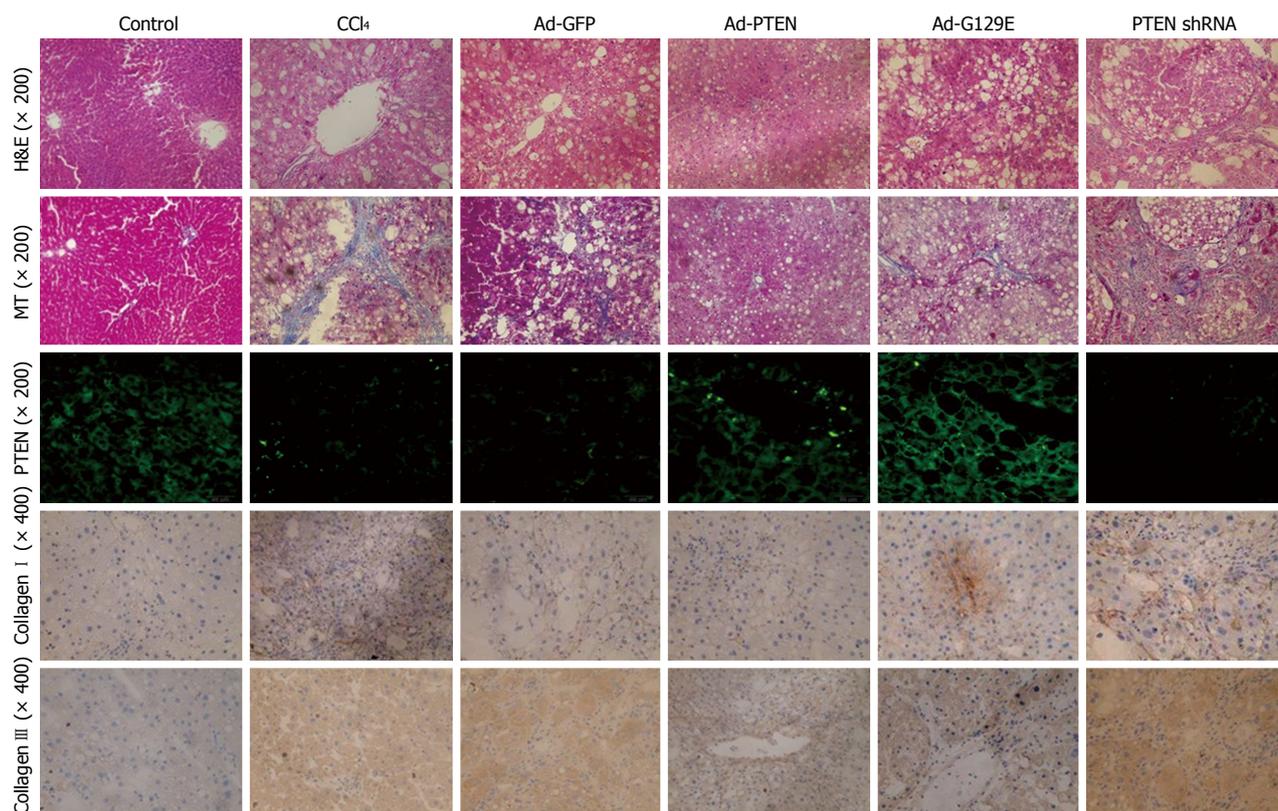


Figure 3 Phosphatase and tension homologue deleted on chromosome ten has protective effects on CCl₄-induced rat hepatic fibrosis *in vivo*. H&E stain (× 200) and MT stain (× 200) showed reduced hepatic cell necrosis and collagen deposition in liver tissue by over-expressed PTEN gene in Ad-PTEN and Ad-G129E 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^[13], the protein expression of PTEN was significantly decreased in rats treated with CCl₄. 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^[1,2]. 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^[1,17-19]. PTEN has been found to be involved in myocardial fibrosis, renal fibrosis, and lung fibrosis^[8-10]. A previous study found that higher expression of PTEN reduced the number of activated HSCs to negatively regulate fibrogenesis *in vivo*^[12,13]. This suggested that PTEN may also regulate the accumulation of ECM components in liver fibrosis because ECM is mainly produced from activated HSCs^[1].

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 CCl₄-induced liver fibrosis.

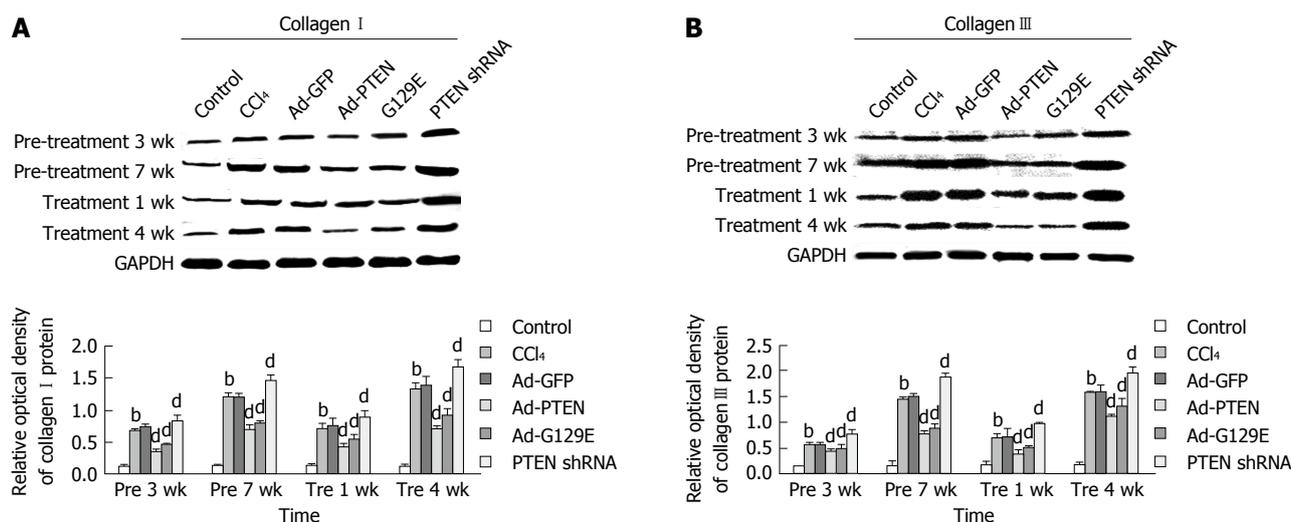


Figure 4 Phosphatase and tension homologue deleted on chromosome ten reduced collagen deposition in CCl₄-induced fibrotic hepatic tissues. Representative western blot of collagen I (A) and collagen III (B) expressions. Collagen I and collagen III expressions were quantitated in the bottom panels and represented as mean ± SE. ^b*P* < 0.01 vs the control group; ^d*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 CCl₄ in treatment groups at different time points

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

Data are presented as mean ± SD for *n* = 3. ^a*P* < 0.05 vs CCl₄, Ad-GFP, Ad-PTEN; ^b*P* < 0.01 vs CCl₄, 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^[20]. 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^[21]. MMP-2 mainly serves to degrade the collagen in

the basement membrane^[22]. 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^[20,23]. In this study, MMP-13 and MMP-2, the major collagenases

involved in collagen degradation^[24,25], 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^[26-28]. In this study, treatment with recombinant adenovirus carrying a highly expressed *PTEN* gene in rats with CCl₄-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 CCl₄-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.

REFERENCES

- Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. *Annu Rev Pathol* 2011; **6**: 425-456 [PMID: 21073339 DOI: 10.1146/annurev-pathol-011110-130246]
- Friedman SL. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008; **134**: 1655-1669 [PMID: 18471545 DOI: 10.1053/j.gastro.2008.03.003]

- Wasmuth HE, Weiskirchen R. [Pathogenesis of liver fibrosis: modulation of stellate cells by chemokines]. *Z Gastroenterol* 2010; **48**: 38-45 [PMID: 20072995 DOI: 10.1055/s-0028-1109933]
- Atzori L, Poli G, Perra A. Hepatic stellate cell: a star cell in the liver. *Int J Biochem Cell Biol* 2009; **41**: 1639-1642 [PMID: 19433304 DOI: 10.1016/j.biocel.2009.03.001]
- Kim HA, Kim KJ, Seo KH, Lee HK, Im SY. PTEN/MAPK pathways play a key role in platelet-activating factor-induced experimental pulmonary tumor metastasis. *FEBS Lett* 2012; **586**: 4296-4302 [PMID: 23137704 DOI: 10.1016/j.febslet.2012.10.034]
- Shi Y, Paluch BE, Wang X, Jiang X. PTEN at a glance. *J Cell Sci* 2012; **125**: 4687-4692 [PMID: 23223894 DOI: 10.1242/jcs.093765]
- Nho RS, Hergert P. IPF fibroblasts are desensitized to type I collagen matrix-induced cell death by suppressing low autophagy via aberrant Akt/mTOR kinases. *PLoS One* 2014; **9**: e94616 [PMID: 24728102 DOI: 10.1371/journal.pone.0094616]
- White ES, Atrasz RG, Hu B, Phan SH, Stambolic V, Mak TW, Hogaboam CM, Flaherty KR, Martinez FJ, Kontos CD, Toews GB. Negative regulation of myofibroblast differentiation by PTEN (Phosphatase and Tensin Homolog Deleted on chromosome 10). *Am J Respir Crit Care Med* 2006; **173**: 112-121 [PMID: 16179636 DOI: 10.1164/rccm.200507-1058OC]
- Lan R, Geng H, Polichnowski AJ, Singha PK, Saikumar P, McEwen DG, Griffin KA, Koesters R, Weinberg JM, Bidani AK, Kriz W, Venkatachalam MA. PTEN loss defines a TGF- β -induced tubule phenotype of failed differentiation and JNK signaling during renal fibrosis. *Am J Physiol Renal Physiol* 2012; **302**: F1210-F1223 [PMID: 22301622 DOI: 10.1152/ajprenal.00660.2011]
- Singla DK. Akt-mTOR Pathway Inhibits Apoptosis and Fibrosis in Doxorubicin-Induced Cardiotoxicity Following Embryonic Stem Cell Transplantation. *Cell Transplant* 2015; **24**: 1031-1042 [PMID: 24594448 DOI: 10.3727/096368914X679200]
- Vinciguerra M, Veyrat-Durebex C, Moukil MA, Rubbia-Brandt L, Rohner-Jeanrenaud F, Foti M. PTEN down-regulation by unsaturated fatty acids triggers hepatic steatosis via an NF- κ Bp65/mTOR-dependent mechanism. *Gastroenterology* 2008; **134**: 268-280 [PMID: 18166358 DOI: 10.1053/j.gastro.2007.10.010]
- Hao LS, Zhang XL, An JY, Karlin J, Tian XP, Dun ZN, Xie SR, Chen S. PTEN expression is down-regulated in liver tissues of rats with hepatic fibrosis induced by biliary stenosis. *APMIS* 2009; **117**: 681-691 [PMID: 19703128 DOI: 10.1111/j.1600-0463.2009.02515.x]
- Zheng L, Chen X, Guo J, Sun H, Liu L, Shih DQ, Zhang X. Differential expression of PTEN in hepatic tissue and hepatic stellate cells during rat liver fibrosis and its reversal. *Int J Mol Med* 2012; **30**: 1424-1430 [PMID: 23041795 DOI: 10.3892/ijmm.2012.1151]
- Ma J, Li F, Liu L, Cui D, Wu X, Jiang X, Jiang H. Raf kinase inhibitor protein inhibits cell proliferation but promotes cell migration in rat hepatic stellate cells. *Liver Int* 2009; **29**: 567-574 [PMID: 19323783 DOI: 10.1111/j.1478-3231.2009.01981.x]
- Hao LS, Zhang XL, An JY, Yao DM, Karlin J, Fang SM, Jiang HQ, Bai WY, Chen S. Adenoviral transduction of PTEN induces apoptosis of cultured hepatic stellate cells. *Chin Med J (Engl)* 2009; **122**: 2907-2911 [PMID: 20092800]
- An J, Zheng L, Xie S, Dun Z, Hao L, Yao D, Shih DQ, Zhang X. Down-regulation of focal adhesion kinase by short hairpin RNA increased apoptosis of rat hepatic stellate cells. *APMIS* 2011; **119**: 319-329 [PMID: 21569089 DOI: 10.1111/j.1600-0463.2011.02720.x]
- Takashima M, Parsons CJ, Ikejima K, Watanabe S, White ES, Rippe RA. The tumor suppressor protein PTEN inhibits rat hepatic stellate cell activation. *J Gastroenterol* 2009; **44**: 847-855 [PMID: 19436944 DOI: 10.1007/s00535-009-0073-3]
- Povero D, Busletta C, Novo E, di Bonzo LV, Cannito S, Paternostro C, Parola M. Liver fibrosis: a dynamic and potentially reversible process. *Histol Histopathol* 2010; **25**: 1075-1091 [PMID: 20552556 DOI: 10.14670/HH-25.1075]

- 19 **Gieling RG**, Wallace K, Han YP. Interleukin-1 participates in the progression from liver injury to fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G1324-G1331 [PMID: 19342509 DOI: 10.1152/ajpgi.90564.2008]
- 20 **Hemmann S**, Graf J, Roderfeld M, Roeb E. Expression of MMPs and TIMPs in liver fibrosis - a systematic review with special emphasis on anti-fibrotic strategies. *J Hepatol* 2007; **46**: 955-975 [PMID: 17383048 DOI: 10.1016/j.jhep.2007.02.003]
- 21 **Okazaki I**, Noro T, Tsutsui N, Yamanouchi E, Kuroda H, Nakano M, Yokomori H, Inagaki Y. Fibrogenesis and Carcinogenesis in Nonalcoholic Steatohepatitis (NASH): Involvement of Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Metalloproteinase (TIMPs). *Cancers (Basel)* 2014; **6**: 1220-1255 [PMID: 24978432 DOI: 10.3390/cancers6031220]
- 22 **Trebicka J**, Hennenberg M, Odenthal M, Shir K, Klein S, Granzow M, Vogt A, Dienes HP, Lammert F, Reichen J, Heller J, Sauerbruch T. Atorvastatin attenuates hepatic fibrosis in rats after bile duct ligation via decreased turnover of hepatic stellate cells. *J Hepatol* 2010; **53**: 702-712 [PMID: 20633948 DOI: 10.1016/j.jhep.2010.04.025]
- 23 **Ngu JM**, Teng G, Meijndert HC, Mewhort HE, Turnbull JD, Stetler-Stevenson WG, Fedak PW. Human cardiac fibroblast extracellular matrix remodeling: dual effects of tissue inhibitor of metalloproteinase-2. *Cardiovasc Pathol* 2014; **23**: 335-343 [PMID: 25060386 DOI: 10.1016/j.carpath.2014.06.003]
- 24 **Radbill BD**, Gupta R, Ramirez MC, DiFeo A, Martignetti JA, Alvarez CE, Friedman SL, Narla G, Vrabie R, Bowles R, Saiman Y, Bansal MB. Loss of matrix metalloproteinase-2 amplifies murine toxin-induced liver fibrosis by upregulating collagen I expression. *Dig Dis Sci* 2011; **56**: 406-416 [PMID: 20563750 DOI: 10.1007/s10620-010-1296-0]
- 25 **Matono T**, Koda M, Tokunaga S, Sugihara T, Ueki M, Murawaki Y. The effects of the selective mineralocorticoid receptor antagonist eplerenone on hepatic fibrosis induced by bile duct ligation in rat. *Int J Mol Med* 2010; **25**: 875-882 [PMID: 20428791]
- 26 **Podolska K**, Stachurska A, Hajdukiewicz K, Malecki M. Gene therapy prospects--intranasal delivery of therapeutic genes. *Adv Clin Exp Med* 2012; **21**: 525-534 [PMID: 23240459]
- 27 **Vicente T**, Peixoto C, Carrondo MJ, Alves PM. Virus production for clinical gene therapy. *Methods Mol Biol* 2009; **542**: 447-470 [PMID: 19565917 DOI: 10.1007/978-1-59745-561-9_24]
- 28 **Cai XG**, Xia JR, Li WD, Lu FL, Liu J, Lu Q, Zhi H. Anti-fibrotic effects of specific-siRNA targeting of the receptor for advanced glycation end products in a rat model of experimental hepatic fibrosis. *Mol Med Rep* 2014; **10**: 306-314 [PMID: 24804792 DOI: 10.3892/mmr.2014.2207]

P- Reviewer: Yuan YF **S- Editor:** Gong ZM **L- Editor:** Filipodia
E- Editor: Zhang FF





Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgooffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>



ISSN 1007-9327

