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Original Article

Peroxiredoxin-1 attenuates hepatic fibrosis by inhibiting TGF-β1/Smad3 and ROS signal pathways

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Abstract: By activation of the hepatic stellate cells (HSC), transforming growth factor β1 (TGF-β1) makes a major contribution to the development of hepatic fibrosis in response to increases in intracellular reactive oxygen species (ROS). However, our understanding of factors regulating the TGF-β1 action in the above process remains limited. The purpose of this study was to identify the potential role of Peroxiredoxin-1 (Prx1) in TGF-β1 induced activation of HSCs and hepatic fibrosis. Protein and mRNA expression of Prx1 in the rats of dimethylnitrosamine (DMN)-induced hepatic fibrosis model were determined by Western blot and RT-PCR. siRNA and overexpression plasmid were transfected specifically in HSC cell lines, CFSC or LX-2 cells, to assess the role of Prx1 in hepatic fibrosis. The intracellular ROS was tested by dichlorofluorescein fluorescence analysis. Prx1 protein and mRNA expression was significantly reduced in DMN rats with a concurrent increase in TGF-β1 and phosphorylation of Smad3 (p-Smad3). In both CFSC and LX-2 cells, knocking down Prx1 led to increased ROS production and activation of HSCs, while overexpressing Prx1 ameliorated these events. Prx1-derived suppression of TGF-β1/Smad3 signaling, thereby leading to modulation of the pro-oxidant/anti-oxidant redox balance. As a result, Prx1 plays a protective role against activation of HSCs, suggesting Prx1 is potential therapeutic target for hepatic fibrosis.

Keywords: Peroxiredoxin-1, ROS, hepatic fibrosis, TGF-β signaling

Introduction

Hepatic fibrosis is the common pathologic change like a wound healing response to a variety of acute or chronic insults [1]. Cholestasis, ethanol, viral infections and hepatotoxic drugs could induce progressive hepatic fibrosis, which may advance to cirrhosis over decades. It represents a major worldwide medical problem associated with severe complication and significant morbidity. However, how to prevent hepatic fibrosis remains elusive. Understanding the mechanisms of hepatic fibrosis is essential in establishing common interventional strategies out of different etiological treatment.

The activation of hepatic stellate cells (HSCs) into matric-producing myofibroblast-like phenotype are known as a central event in hepatic fibrogenesis [2]. HSCs activation is characterized by enhanced contractility, increased migratory capacity, deposition of the extra cellular matrix (ECM) proteins such as fibronectin (FN) and collagen I or III, upregulation of α-smooth muscle alpha actin (α-SMA) [3]. Especially, the expression of α-SMA directly correlates with activation of myofibroblasts [4]. Binding to its cognate receptors, transforming growth factor β1 (TGF-β1) initiates phosphorylation on the Smad3, which in turn enhances the transcription of α-SMA and collagen. So that, TGF-β1 can efficiently induce the activation of HSCs and act as a major fibrogenic cytokine [5].

A lot of factors have been documented to affect the activation of HSCs including oxidative stress (OS), apoptosis, inflammation, paracrine stimulation [6, 7]. Among these factors, OS has always been suggested to play an important role in pathogenesis and proved in almost every kind of clinical and experimental liver disease, including virus hepatitis, non-alcoholic fatty liver disease and autoimmune Hepatitis [8-10]. Moreover, OS-related molecules may act as

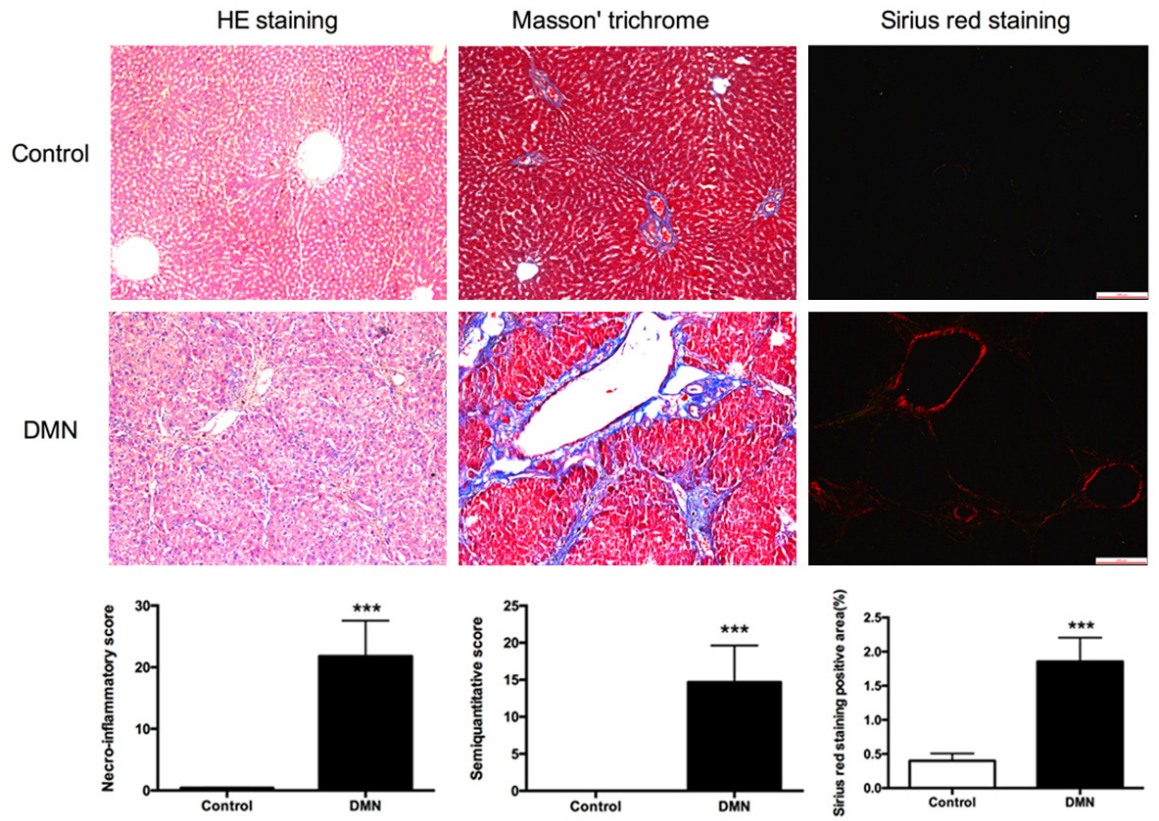

Figure 1. Pathology change of DMN-induced hepatic fibrosis. Control and DMN group liver tissue stained with HE, Masson's trichrome or Sirius red staining (×100). Typical images and quantifications (means ± SD) were shown in each parts. **P*<0.05; ***P*<0.01; ****P*<0.001 in comparison to the control group.

mediators that modulate different pathways responsible for the progression of liver fibrosis. However, the mechanisms underlying this process remains unclear.

Peroxiredoxin-1 (Prx1) is a member of the peroxiredoxin family that detoxifies H_2O_2, mediates signaling and regulates metabolism [11]. Evidence is emerging for a critical role of Prx1 in protection from lung, kidney, and potentially liver fibrosis which are associated with OS [12-14]. However, whether Prx1 plays a role in the development of hepatic fibrosis is currently unknown, and the underlying mechanisms remains poorly understood. In this study, we would demonstrate the role of Prx1 in hepatic fibrosis and explore the molecular mechanisms under this.

Materials and methods

Antibodies and reagents

The antibodies respectively against Prx1, α-SMA, phospho-Smad3, Smad2/3, and β-Actin were purchased from Abcam (Cambrige, UK). Anti-Flag antibodies and 2', 7'-Dichlorofluorescin diacetate (DCFH-DA) were obtained from Sigma (Saint Louis, MO). The antibody against fibronectin was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The Horseradish Peroxidase (HRP)-conjugated secondary antibody in Western blotting was manufactured by the Jackson Laboratory (Bar Harbor, Maine). Trizol reagent, RevertAid First Strand cDNA Synthesis Kit, Lipofectamine 2000, Penicillin, Streptomycin were all purchased from Life Technologies (Carlsbad, CA). TGF-β1 (Peprotech, Rocky Hill, CT), SYBR green real-time PCR kit (Takara, Dalian, Liaoning, China), siRNA (Genepharma, Shanghai, China), the overexpression plasmid (Yingrun, Changsha, Hunan, China), and TBARS Assay kit (R&D Systems, Minneapolis, MN) were respectively purchased from the indicted sources. Dulbecco's modified eagle medium (DMEM), Fetal Bovine Serum (FBS) and Opti-MEM were purchased from Gibco (Waltham, MA).

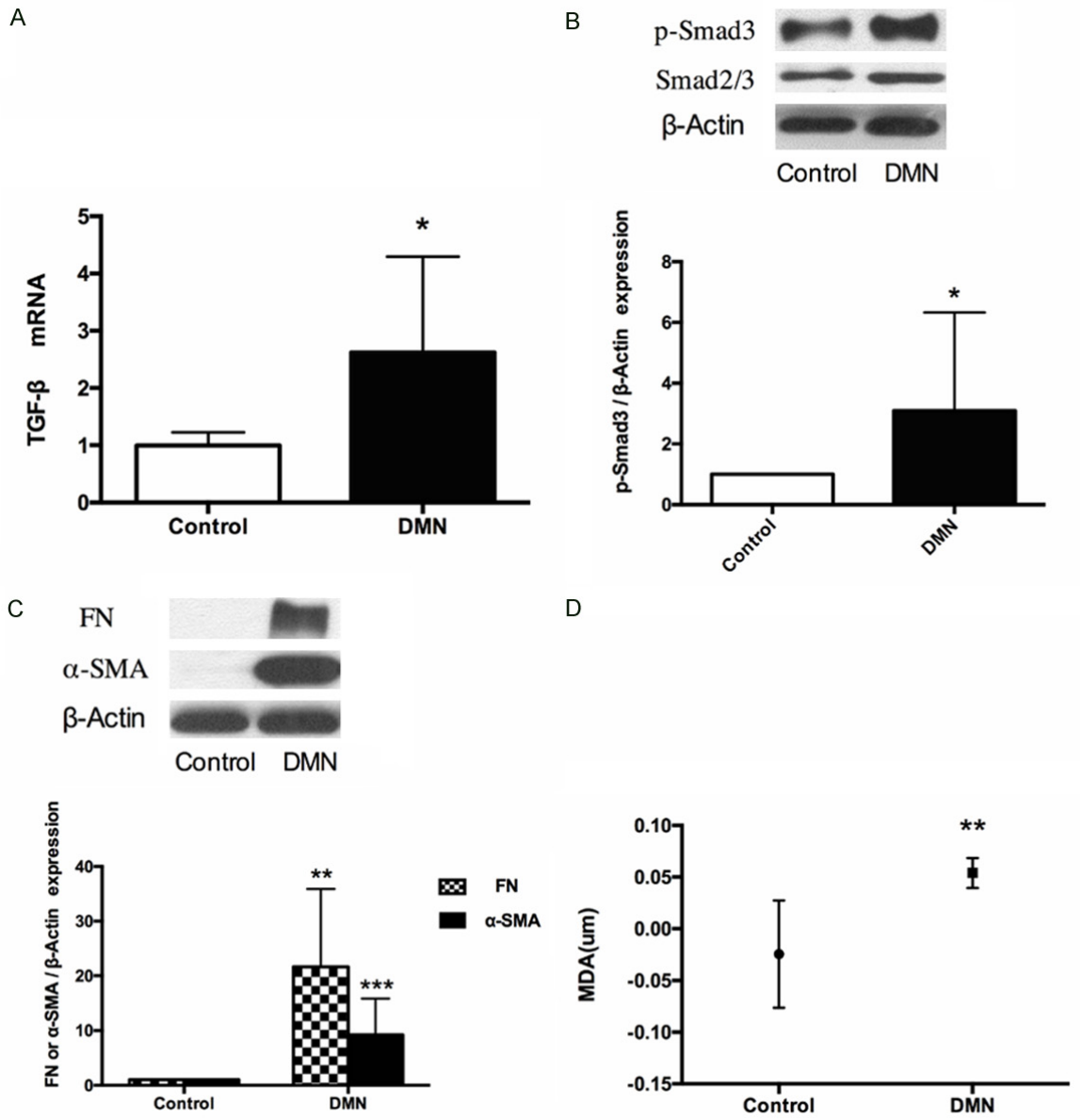

Figure 2. Feature of DMN-induced hepatic fibrosis. A: TGF-β1 mRNA in liver from the control and DMN group tested by Real-time PCR. B: Western blot analysis of p-Smad3 in the liver of the indicated rats. C: Western blot analysis of α-SMA and FN in the liver of the indicated rats. D: The MDA level were tested in control and DMN groups. Typical images and quantifications (means ± SD) were shown in each parts. **P*<0.05; ***P*<0.01; ****P*<0.001 in comparison to the control group.

Cell culture and transfection

Rat HSCs of CFSC-2G and human HSCs of LX-2 cells were provided from the Department of Cell Biology, Central South University, China. Cells were cultured in DMEM supplemented with 10% FBS, 100 μg/mL streptomycin and 100 U/mL penicillin (Life Technologies) at 37°C in a humidified atmosphere with 5% CO_2 and 95% air. The cells were seeded on 6-well culture plates in complete medium for 24 h. Transfections were performed with Prx1 siRNA or overexpression plasmid using Lipofectamine 2000 according to the manufacturer's instructions. The sequences for the rat Prx1 siRNA oligos were as follows: sense 5'-CACCAUUGCUCAGGAUUAUTT-3' and antisense 5'-AUAAUCCUGAGCAAUGGUGTT-3'. The sequences for the human Prx1 siRNA oligos were: sense 5'-GCCGAAUUGUGGUGUCUUAUU-3' and antisense

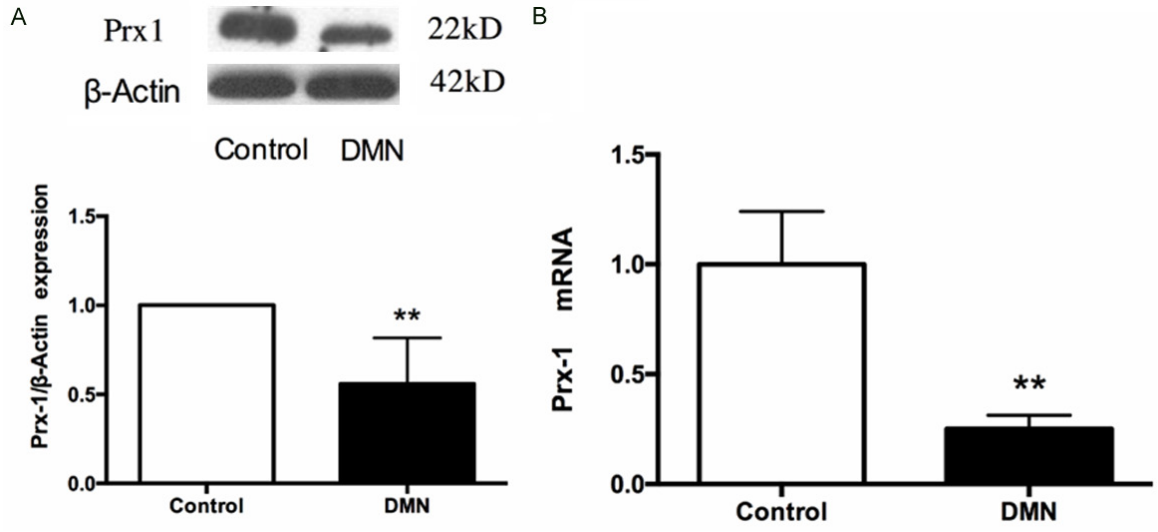

Figure 3. Prx1 reduced in DMN-induced hepatic fibrosis. A: Western blot analysis of Prx1 in the liver of the indicated rats. B: Real-time PCR analysis of Prx1 mRNA in liver from the control and DMN groups. Typical images and quantifications (means ± SD) were shown in each parts. **P*<0.05; ***P*<0.01; ****P*<0.001 in comparison to the control group.

5'-UAAGACACCACAAUUCGGCUU-3'. pCDNA3.1-Prx1-Flag (rat), pCDNA3.1-Prx1-Flag (human) and the empty vector pCDNA3.1 were used for overexpression and negative control vectors, respectively. The HSCs were incubated with TGF-β1 (10 ng/mL) for 24 h to induce transdifferentiation, 15 min to activate Smad3, or 30 min to examine intracellular ROS levels, respectively.

Animals

Male albino rats of the Wistar strain weighing between 220 and 250 g were obtained from Silaike Laboratory Animal Co. (Shanghai, China). Animals were housed in a pathogen-free environment with a 12-hour light-dark cycle and unrestricted access to standard rat chow diet and water. Animal care was provided in compliance with the university's guidelines. All experimental protocols were approved by the Ethics Review Committees for Animal Experimentation of Central South University. For the purpose of this study, animals were randomly divided into two groups: control (n = 8), and DMN treatment (n = 8). Hepatic fibrosis was induced via intraperitoneal injections of DMN at a dose of 1 μL (diluted 1:100 with 0.15 M NaCl) per 100 g body weight. The injections were given on the first three consecutive days of each week for a period of 4 weeks. The control animals received the same injection of 0.15 M sterile NaCl without DMN. Rats were euthanized at the end of 4 weeks. Their livers were rapidly removed, and a portion of liver was fixed with 4% paraformaldehyde before embedding in paraffin. Paraffin sections (4 μm) were used for haematoxylin and eosin (H&E) and Masson's trichrome procedures. The remaining tissue was snap-frozen in liquid nitrogen for Western blot and Real-time PCR analyses.

Histological analysis

The paraffin sections (4 μm) were stained with H&E, Masson's trichrome or Sirius red staining. To determine the degree of necroinflammatory liver injury, histological grading were blindly performed by an independent pathologist as previously described [15]. To quantify interstitial collagen deposition, Masson's trichrome-stained sections and Sirius red staining sections were graded according to previously published procedures [16].

Western blot

Liver tissue, CFSC-2G and LX-2 cell lysates were prepared using a lysate buffer (20 mM Tris-HCl (pH 7.4), 4% SDS, and 10% glycerol). Twenty micrograms of lysate protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions, and transferred onto polyvinylidene difluoride membranes (Millipore,

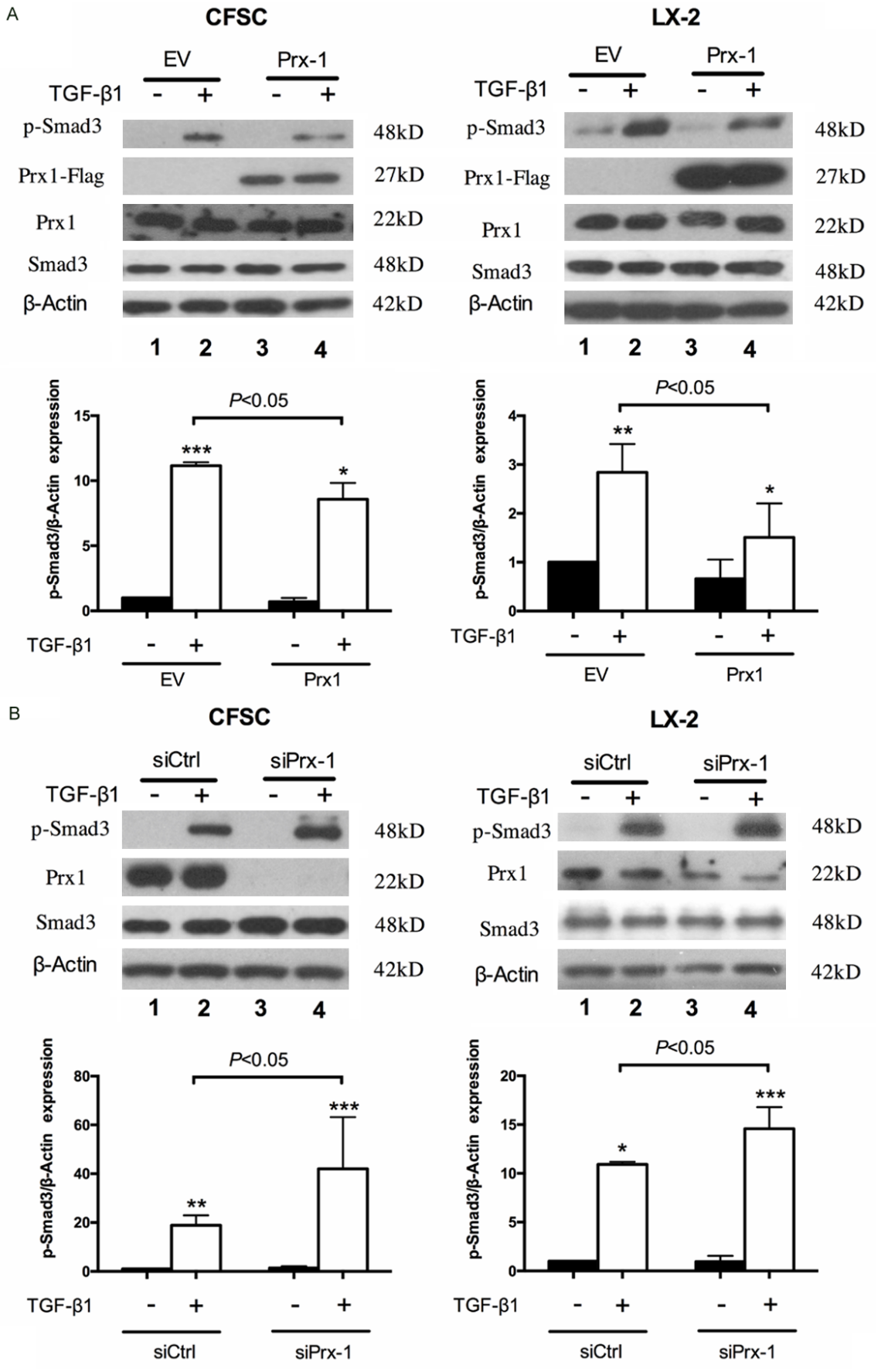
A
CFSC
LX-2
EV
Prx-1
TGF-β1
p-Smad3
Prx1-Flag
Prx1
Smad3
β-Actin
48kD
27kD
22kD
42kD
1 2 3 4
p-Smad3/β-Actin expression
P<0.05
EV
Prx1
B
siCtrl
siPrx-1

Figure 4. Prx1 suppresses TGF-β1-derived Smad3 phosphorylation of HSCs. A: EV and Prx1 overexpression CFSC-2G or LX-2 cells were treated with TGF-β1 (10 ng/ml) for 10 min, followed by Western blot examination for p-Smad3. B: siCtrl and siPrx1 CFSC-2G or LX-2 cells were treated with TGF-β1 (10 ng/ml) for 10 min, followed by Western blot examination for p-Smad3. Experiments were repeated three times; typical images and quantifications (mean ± standard deviation (SD)) are shown. **P*<0.05; ***P*<0.01; ****P*<0.001 in comparison to the respective untreated cells.

Billerica, MA). The membranes were immersed in a blocking solution composed of 5% skim milk and TBS-T (0.05% Tween 20, 20 mM Tris-HCl, and 150 mM NaCl (pH 7.6)) for 1 h at room temperature before incubation with the primary antibodies at 4°C overnight. The HRP secondary antibodies were added for 1 h at room temperature, followed by removing the primary antibodies and rinsing the membranes. Upon completion of three washes with TBS-T, signals were developed using the ECL Plus Western Blotting Detection Reagents (Advansta, Menlo Park, CA) and X-ray film (Kodak, Rochester, NY). Bands were quantified using Image J. Primary antibodies used were anti-Prx1 (1:2000), anti-α-SMA (1:8000), anti-phospho-Smad3 (1:2000), and anti-Smad2/3 (1:2000), anti-Fibronectin (1:800), anti-β-Actin (1:5000), anti-Flag (1:1000) respectively.

Real-time PCR

Total RNA was isolated from liver tissue using Trizol reagent according to the manufacturer's instruction. The first strand cDNAs were synthesized from 1 μg of total RNA in a 20 μL reaction using RevertAid First Strand cDNA Synthesis Kit. The specific primers used for Prx1, TGF-β1 and β-actin were designed based on the gene sequences and synthesized by Generay Biotech (Shanghai, China). The Prx1 primer pair consisted of forward primer 5'-GGCTCACGGTTGGTTCTGTT-3' and reverse primer 5'-GGGAGCAGGATGCCCAATTT-3'; TGF-β1 primer pair consisted of forward primer 5'-CAACAATTCCTGGCGTTACCTT-3' and reverse primer 5'-AAGCCCTGTATTCCGTCTCCTT-3'; and β-actin primers were forward primer 5'-CACCCGCGAGTACAACCTTC-3' and reverse primer 5'-CCCATACCCACCATCACACC-3'. Real-time reverse transcription polymerase chain reaction (RT)-PCR quantification for individual target mRNA expression was performed using the CFX96 Real-time Detection System (Bio-Rad, Hercules, CA) involving a TaKaRa SYBR green real-time PCR kit. The amount of specific mRNA in each sample was calculated from the standard curve and normalized to the β-actin mRNA. The comparative $2^{-\Delta\Delta CT}$ method was used for quantification and statistical analysis.

Flow cytometry detection of intracellular ROS

Intracellular ROS were measured using the DCFH-DA assay. CFSC-2G and LX-2 cells were seeded at a density of 10^5 cells/well in a 6-well plate in complete medium for 24 h, followed by transfection for 48 h. After incubation with DCFH-DA (10 μM) and stimulation with TGF-β1 (10 ng/ml) in 5% CO_2 at 37°C for 30 min, the cells were washed three times with PBS and analyzed within 30 min using FACScan (Becton Dickinson, San Jose, CA) with excitation set at 488 nm and emission at 525 nm.

MDA assay

The serum Malondialdehyde (MDA) levels were determined by TBARS assay kit according to the manufacturer's instruction.

Statistical analysis

All data are presented as mean ± SD (standard derivation). Statistical analysis was performed using SPSS 20.0 software (IBM Corp, Armonk, NY). Difference between two groups was performed using Student's t-test. Differences among groups were tested by one-way ANOVA. Multiple comparison tests were applied only when a significant difference was determined by the ANOVA. P<0.05 was considered statistically significant.

Results

Reduction of Prx1 expression in DMN-induced hepatic fibrosis

To examine the role of Prx1 in liver fibrosis in vivo, we established the hepatic fibrosis model by intraperitoneal injection of DMN. This is a well-established rat model for hepatic fibrosis, which is characterized by differentiation and proliferation of HSCs, and increases in ECM deposition. Consistent with this concept, we observed damages in the portal area architecture, derangement of liver cells, infiltration of inflammatory cells, and deposition of collagen

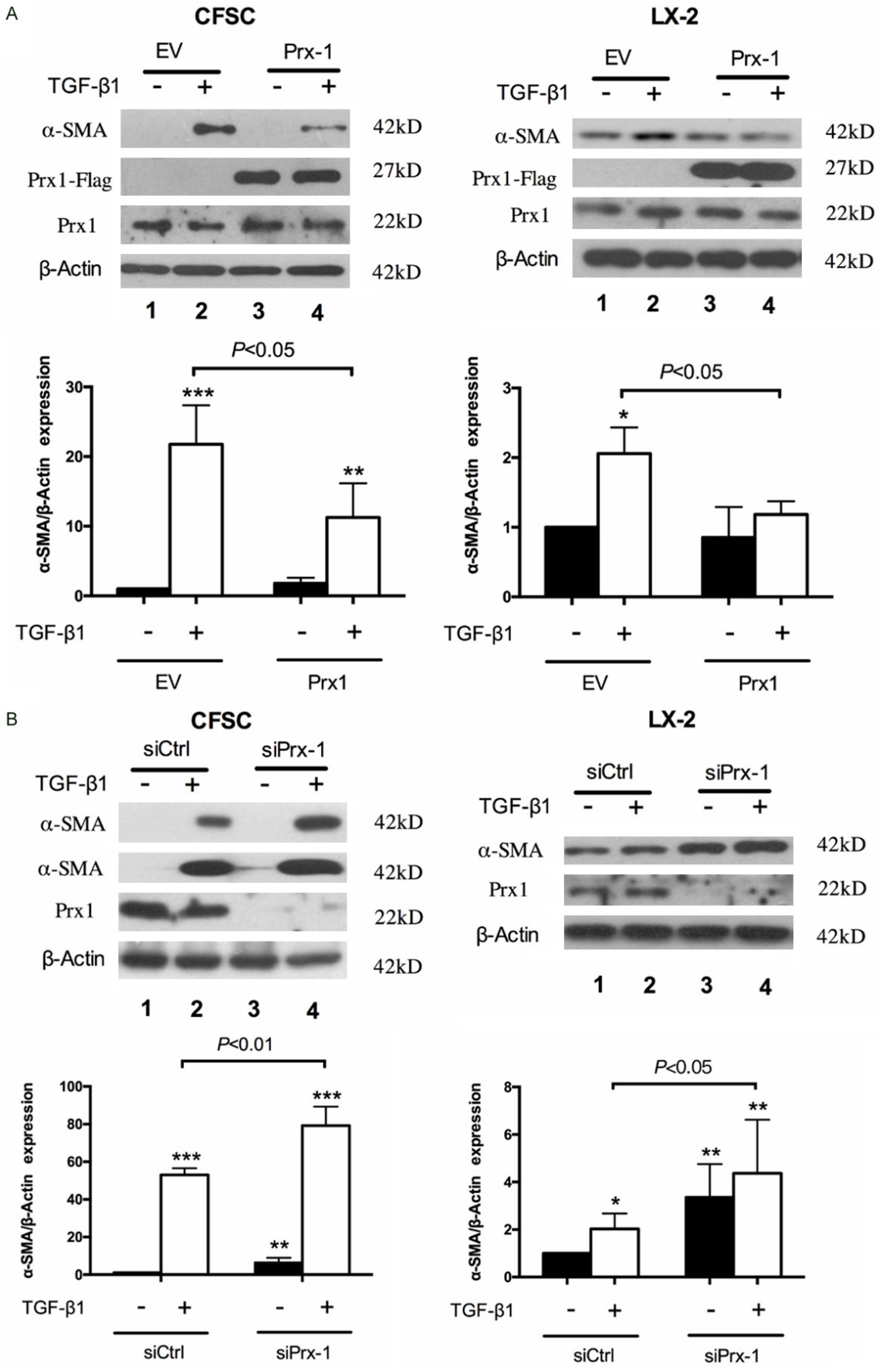
A
CFSC
EV
Prx-1
TGF-β1
α-SMA
Prx1-Flag
Prx1
β-Actin
42kD
27kD
22kD
42kD
1 2 3 4
LX-2
P<0.05
α-SMA/β-Actin expression
EV
Prx1
B
siCtrl
siPrx-1
P<0.01

Figure 5. Prx1 restrains α-SMA expression of HSCs. B: EV and Prx1 overexpression CFSC-2G or LX-2 cells were treated with TGF-β1 (10 ng/ml) for 24 h, followed by Western blot examination for α-SMA. A: siCtrl and siPrx1 CFSC-2G or LX-2 cells were treated with TGF-β1 (10 ng/ml) for 24 h, followed by Western blot examination for α-SMA. Experiments were repeated three times; typical images and quantifications (mean ± standard deviation (SD)) are shown. **P*<0.05; ***P*<0.01; ****P*<0.001 in comparison to the respective untreated cells.

fibers in DMN group compared to control rats using H&E, Masson's trichrome staining and Sirius red staining (**Figure 1**). As expected, an elevation of the TGF-β1 mRNA was also demonstrated in the liver of DMN-treated mice (**Figure 2A**). Furthermore, the phosphorylated form of Smad3 (p-Smad3) was concomitantly increased in DMN livers (**Figure 2B**), which was associated with robust upregulations of fibronectin (FN) and α-SMA (**Figure 2C**). Furthermore, in comparison to control rates, DMN treatment let to an increase in melonaldehyde (MDA) (**Figure 2D**), indicating an elevation of ROS levels in the liver. Collectively, we have established a liver fibrosis model that is associated with activation of the TGF-β1 pathway and an increase in ROS.

By using this model, we demonstrated a downregulation of Prx1 expression at both mRNA and protein levels in DMN rats in comparison to control rats (**Figure 3A**, **3B**). As Prx1 is a well-known anti-oxidant enzyme, and because oxidative stress is a major fibrogenic factor, our observed Prx1 reduction (**Figure 3A**, **3B**) and the concomitant elevation of the TGF-β1 pathway (**Figure 2A-C**) in DMA rat liver reveal a reverse correlation between Prx1 expression and activation of the TGF-β1 pathway during the development of hepatic fibrosis.

Prx1 represses TGF-β1 signaling

Prx1 has been demonstrated to protect fibrosis in the lung and kidney [12, 13]. To investigate potential contributions of Prx1 to liver fibrosis, we first examined an impact of Prx1 on TGF-β1 signaling in two HSC cell lines (CFSC and LX-2 cells), the cell type of origin of liver fibrosis [17, 18]. While Prx1 overexpression in either cell line did not affect the basal level of Smad3 phosphorylation (p-Smad3) (**Figure 4A**, comparing the respective lane 1 to lane 3), TGF-β1-stimulated p-Smad3 was significantly attenuated in Prx1 overexpressing cells in comparison to the respective empty vector (EV) lines (**Figure 4A**, comparing lane 2 to the respective lane 4). To further demonstrate a role of Prx1 in reducing TGF-β1-initiated p-Smad3, Prx1 was knocked-down in CFSC and LX-2 cells (**Figure 4B**, top panel, comparing lane 1 to the respective lane 3); Prx1 knockdown significantly sensitized p-Smad3 upon stimulation with TGF-β1 (**Figure 4B**, comparing lane 2 to the respective lane 4). Consistent with Prx1 overexpression, Prx1 downregulation was without a clear effect on the basal levels of p-Smad3 in both cell lines (**Figure 4B**, up panel, see basal levels of p-Smad3 lane 1 vs lane 3). This would be expected as the basal levels of p-Smad3 in both cell lines are low. Since p-Smad3 is a major TGF-β1 target [5], the above observations support a role of Prx1 in inhibiting TGF-β1 signaling.

In addition of the production of p-Smad3, TGF-β1 signaling transactivates multiple targets, including α-SMA. The expression of α-SMA in HSCs reflects of their transition towards a myofibroblast-like phenotype [3]. To further demonstrate Prx1-derived inhibition of TGF-β1 signaling, we were able to show a reduction of α-SMA in Prx1-overexpressing CFSC-2G and LX-2 cells comparing to the respective EV lines, when the cells were stimulated with TGF-β1 (**Figure 5A**, comparing lane 1 to the respective lane 3). Conversely, knockdown of Prx1 significantly elevated α-SMA expression compared to the control cells upon stimulated with TGF-β1 (**Figure 5B**, comparing lane 2 to the respective lane 4). Collectively, we provide comprehensive evidence demonstrating an important role of Prx1 in downregulating TGF-β1-mediated signaling.

Prx1 suppresses TGF-β1-mediated ROS production

ROS induces liver fibrosis, a process in which TGF-β1 signaling plays a critical role. This knowledge suggests a positive feedback loop between ROS and TGF-β1 signaling in the pathogenesis of liver fibrosis [6]. The observed role of Prx1 in inhibiting the TGF-β1 signaling implies a possibility that Prx1 reduces TGF-β1-induced ROS production. In supporting this scenario, we were able to show a reduction of ROS in Prx1 overexpression CFSC and LX-2 cells compared to the respective EV cells with TGF-β1 stimulating (**Figure 6A**) and an elevation of ROS production in Prx1 knockdown CFSC and

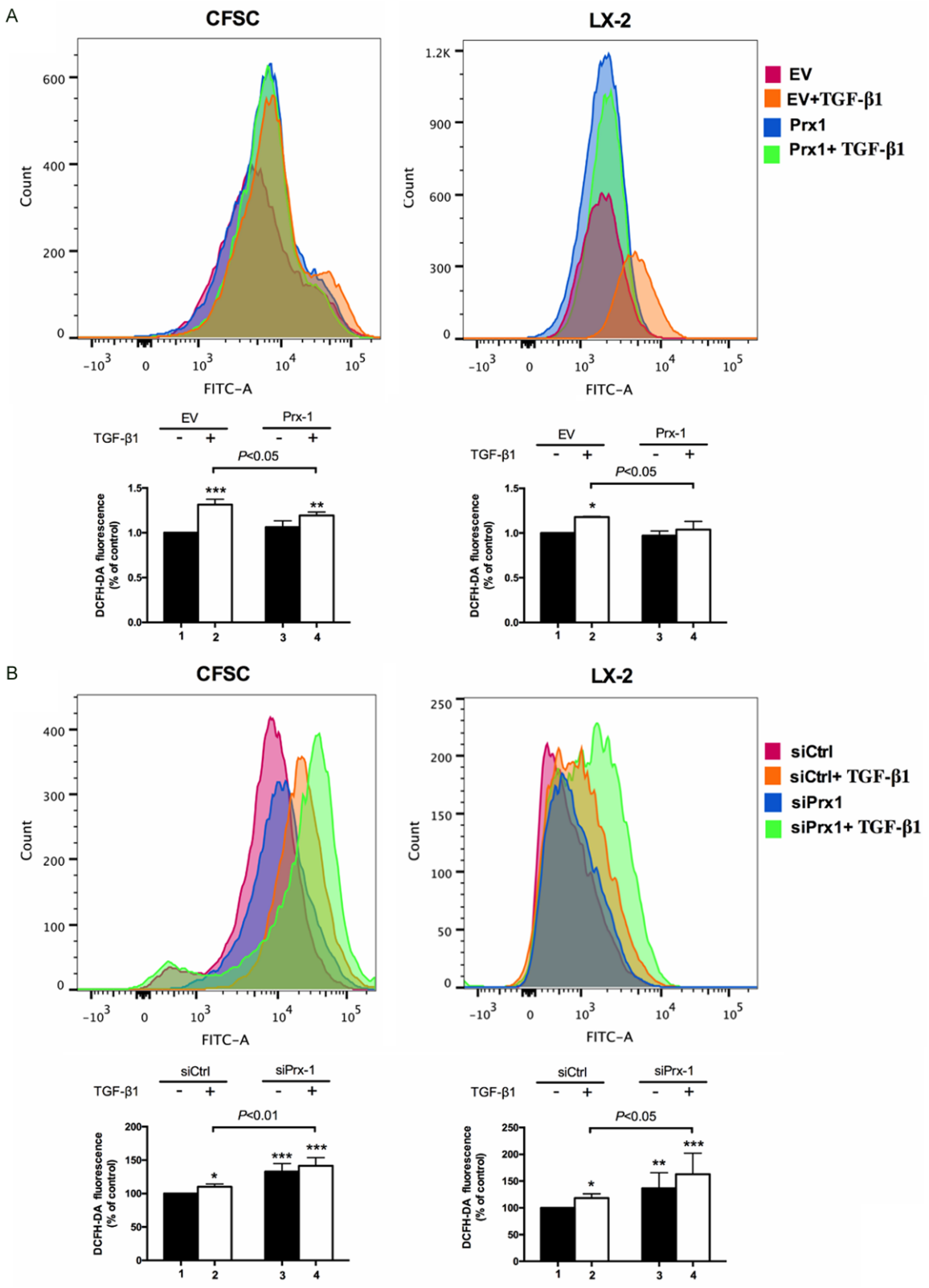

Figure 6. Prx1 suppresses TGF-β1-mediated ROS production of HSCs. B: EV and Prx1 overexpression CFSC-2G or LX-2 cells were treated with TGF-β1 (10 ng/ml) for 30 min, followed by followed by Flow cytometry detection of intracellular ROS. A: siCtrl and siPrx1 CFSC-2G or LX-2 cells were treated with TGF-β1 (10 ng/ml) for 30 min, followed by Flow cytometry detection of intracellular ROS. Experiments were repeated three times; typical images and quantifications (mean ± standard deviation (SD)) are shown. **P*<0.05; ***P*<0.01; ****P*<0.001 in comparison to the respective untreated cells.

LX-2 cells in comparison to the respective siCtrl lines (**Figure 6B**). Taken together, these observations support a role of Prx1 in attenuating the positive feedback loop between ROS and TGF-β1 signaling.

Discussion

Hepatic fibrosis is the common pathological process by all the kind of liver injury leading to hepatic cirrhosis. Oxidative stress plays a pivotal role in initiation and progression of hepatic fibrosis by activation of HSCs [19].

The molecular mechanisms about fibrogeniesis are not fully understood. In this study, we firstly demonstrated a significant downregulation of Prx1 in hepatic fibrosis. Prx1 is the most abundant Prx protein in mammalian cells [20]. Prxs reduce peroxide through the conserved cysteine (Cys) residue and function in multiple cellular processes by maintaining a proper cellular redox level. Indeed, accumulative evidence reveals Prx1 being involved in multiple processes, including differentiation, proliferation, inflammation, apoptosis, and tumorigenesis [21-24]. Recent developments also support an anti-fibrogenic role of Prx1. During the development of kidney fibrosis, a reduction of Prx1 was reported [13]. Mice deficient in Prx1 are sensitized to bleomycin-induced pulmonary inflammation and fibrosis [12]. However, the link between Prx1 and hepatic fibrosis remained to be determined.

In DMN-induced Rat hepatic fibrosis model, the portal area architecture was damaged with ECM deposition. We found that Prx1 protein and mRNA expression was significantly decreased in DMN rats. To identify the function of Prx1, we knocked down and overexpression Prx1 in CFSC and LX-2. Incubated with TGF-β1 which is the major fibrogenic factor, we found knocking down Prx1 exacerbated the TGF-β1 induced activation of HSCs and lifted the basal level of α-SMA, whereas Prx1 overexpression attenuated it. TGF-β1 subsequently initiates phosphorylation on the Smad proteins via binding to its cognate receptors, and regulates gene expression by p-Smad3 and p-Smad2 combined with Smad4 to from a heteromeric complex [5]. To further confirm that Prx1 plays a role in hepatic fibrosis through TGF-β1 pathway, we used the same in vitro model and found that Prx1 inhibiting phosphorylation of Smad3 induced by TGF-β1. These in vitro finding companied with decreased Prx1 expression in vivo was suggested that Prx1 prevent hepatic fibrosis by inhibition of HSCs activation.

Oxidative stress is one of the important pathogenesis of hepatic fibrosis which can both initiate and then perpetuate fibrosis. Oxidative disruption of lipids, proteins and DNA induces necrosis and apoptosis of hepatocytes, amplifies the inflammatory response, induces the cytokines released including TGF-β, activates the HSCs. ROS are also interact with several molecular pathways in fibrogenesis which is cover of TGF-β pathway, NADPH oxidase, MAPK pathway [25-27]. Curtailing oxidative stress as a therapeutic option in patients with hepatic fibrosis is under investigation. We found that ROS were higher in DMN rats. In vitro, ROS were enhanced in Prx1 knock down HSCs, and ROS were reduced in Prx1 overexpression HSCs. Taken together, these finding suggest significant interrelationships between Prx1 expression and oxidative stress in hepatic fibrosis.

Above all, we first provide the evidence of Prx1 inhibiting TGF-β1/Smad3 and ROS signal pathway in hepatic fibrosis. Our findings not only add another intriguing aspect of the multiple regulation mechanism of hepatic fibrosis, but also provide the potential therapeutics targeting Prx1 to prevent hepatic fibrosis in clinical.

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Disclosure of conflict of interest

None.

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References

[1] Lee UE and Friedman SL. Mechanisms of hepatic fibrogenesis. Best Pract Res Clin Gastroenterol 2011; 25: 195-206.

[2] Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008; 134: 1655-1669.

[3] Greuter T and Shah VH. Hepatic sinusoids in liver injury, inflammation, and fibrosis: new pathophysiological insights. J Gastroenterol 2016; 51: 511-519.
[4] Nakatani T, Honda E, Hayakawa S, Sato M, Satoh K, Kudo M and Munakata H. Effects of decorin on the expression of alpha-smooth muscle actin in a human myofibroblast cell line. Mol Cell Biochem 2008; 308: 201-207.
[5] Massague J. TGFbeta signalling in context. Nat Rev Mol Cell Biol 2012; 13: 616-630.
[6] Torok NJ. Dysregulation of redox pathways in liver fibrosis. Am J Physiol Gastrointest Liver Physiol 2016; 311: G667-G674.
[7] Mazzolini G, Sowa JP and Canbay A. Cell death mechanisms in human chronic liver diseases: a far cry from clinical applicability. Clin Sci (Lond) 2016; 130: 2121-2138.
[8] Par A and Par G. Immune response and oxidative stress in hepatitis C virus infection. Orv Hetil 2015; 156: 1898-1903.
[9] Chen G, Ni Y, Nagata N, Xu L and Ota T. Micronutrient antioxidants and nonalcoholic fatty liver disease. Int J Mol Sci 2016; 17.
[10] Czaja AJ. Nature and implications of oxidative and nitrosative stresses in autoimmune hepatitis. Dig Dis Sci 2016; 61: 2784-2803.
[11] Jarvis RM, Hughes SM and Ledgerwood EC. Peroxiredoxin 1 functions as a signal peroxidase to receive, transduce, and transmit peroxide signals in mammalian cells. Free Radic Biol Med 2012; 53: 1522-1530.
[12] Kikuchi N, Ishii Y, Morishima Y, Yageta Y, Haraguchi N, Yamadori T, Masuko H, Sakamoto T, Yanagawa T, Warabi E, Ishii T and Hizawa N. Aggravation of bleomycin-induced pulmonary inflammation and fibrosis in mice lacking peroxiredoxin I. Am J Respir Cell Mol Biol 2011; 45: 600-609.
[13] Mei W, Peng Z, Lu M, Liu C, Deng Z, Xiao Y, Liu J, He Y, Yuan Q, Yuan X, Tang D, Yang H and Tao L. Peroxiredoxin 1 inhibits the oxidative stress induced apoptosis in renal tubulointerstitial fibrosis. Nephrology (Carlton) 2015; 20: 832-842.
[14] Bae SH, Sung SH, Cho EJ, Lee SK, Lee HE, Woo HA, Yu DY, Kil IS and Rhee SG. Concerted action of sulfiredoxin and peroxiredoxin I protects against alcohol-induced oxidative injury in mouse liver. Hepatology 2011; 53: 945-953.
[15] Di Sario A, Bendia E, Taffetani S, Marzioni M, Candelaresi C, Pigini P, Schindler U, Kleemann HW, Trozzi L, Macarri G and Benedetti A. Selective Na+/H+ exchange inhibition by cariporide reduces liver fibrosis in the rat. Hepatology 2003; 37: 256-266.
[16] Chevallier M, Guerret S, Chossegros P, Gerard F and Grimaud JA. A histological semiquantitative scoring system for evaluation of hepatic fibrosis in needle liver biopsy specimens: comparison with morphometric studies. Hepatology 1994; 20: 349-355.
[17] Gonzalez-Puertos VY, Hernandez-Perez E, Nuno-Lambarri N, Ventura-Gallegos JL, Lopez-Diazguerrero NE, Robles-Diaz G, Gutierrez-Ruiz MC and Konigsberg M. Bcl-2 overexpression in hepatic stellate cell line CFSC-2G, induces a pro-fibrotic state. J Gastroenterol Hepatol 2010; 25: 1306-1314.
[18] Kim HG, Han JM, Lee JS, Lee JS and Son CG. Ethyl acetate fraction of amomum xanthioides improves bile duct ligation-induced liver fibrosis of rat model via modulation of pro-fibrogenic cytokines. Sci Rep 2015; 5: 14531.
[19] Sanchez-Valle V, Chavez-Tapia NC, Uribe M and Mendez-Sanchez N. Role of oxidative stress and molecular changes in liver fibrosis: a review. Curr Med Chem 2012; 19: 4850-4860.
[20] Wood ZA, Schroder E, Robin Harris J and Poole LB. Structure, mechanism and regulation of peroxiredoxins. Trends Biochem Sci 2003; 28: 32-40.
[21] Ishii T, Warabi E and Yanagawa T. Novel roles of peroxiredoxins in inflammation, cancer and innate immunity. J Clin Biochem Nutr 2012; 50: 91-105.
[22] Lim JM, Lee KS, Woo HA, Kang D and Rhee SG. Control of the pericentrosomal H2O2 level by peroxiredoxin I is critical for mitotic progression. J Cell Biol 2015; 210: 23-33.
[23] Morinaka A, Funato Y, Uesugi K and Miki H. Oligomeric peroxiredoxin-I is an essential intermediate for p53 to activate MST1 kinase and apoptosis. Oncogene 2011; 30: 4208-4218.
[24] Neumann CA, Krause DS, Carman CV, Das S, Dubey DP, Abraham JL, Bronson RT, Fujiwara Y, Orkin SH and Van Etten RA. Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. Nature 2003; 424: 561-565.
[25] Liu RM and Gaston Pravia KA. Oxidative stress and glutathione in TGF-beta-mediated fibrogenesis. Free Radic Biol Med 2010; 48: 1-15.
[26] Barnes JL and Gorin Y. Myofibroblast differentiation during fibrosis: role of NAD(P)H oxidases. Kidney Int 2011; 79: 944-956.
[27] Kim YK, Bae GU, Kang JK, Park JW, Lee EK, Lee HY, Choi WS, Lee HW and Han JW. Cooperation of H2O2-mediated ERK activation with Smad pathway in TGF-beta1 induction of p21WAF1/Cip1. Cell Signal 2006; 18: 236-243.