The American Journal of Pathology, Vol. ■, No. ■, ■ 2015

ELSEVIER

The American Journal of PATHOLOGY

ajp.amjpathol.org

Matricellular Protein Periostin Contributes to Hepatic Inflammation and Fibrosis

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Accepted for publication November 5, 2014.

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Periostin actively contributes to tissue injury, fibrosis, atherosclerosis, and inflammatory diseases; however, its role in hepatic fibrosis is unclear. Herein, we revealed that periostin expression was significantly up-regulated in carbon tetrachloride— and bile duct ligation—induced mice with acute and Q4 chronic liver fibrosis. Deficiency in periostin abrogated the development of liver fibrosis in mice. Carbon tetrachloride treatment significantly increased α-smooth muscle actin, fibronectin, and collagen I levels in wild-type mice, which were unaffected in periostin-knockout mice. Periostin-deficient mice showed a significantly reduced area of collagen deposition and decreased levels of serum alanine aminotransferase and aspartate aminotransferase compared with wild-type mice after 2 weeks of carbon tetrachloride administration. Chemokine ligand 2, IL-6, IL-1β, tumor necrosis factor-α, and tissue inhibitor of met- Q5 alloproteinases 1 mRNA levels were significantly lower in periostin-deficient mice than in wild-type mice after carbon tetrachloride treatment. Periostin colocalized with hepatic stellate cell—derived collagen I and α-smooth muscle actin in mouse acute and chronic fibrotic liver tissues. Transforming growth factor (TGF)-β1 markedly induced periostin expression in primary mouse hepatic stellate cells. Periostin-deficient mice showed significantly lower levels of TGF-β1 and TGF-β2 compared with wild-type mice after carbon tetrachloride treatment. High levels of periostin in patients with acute or chronic hepatitis correlated with TGF-β1 and TGF-β2 expression in serum from patients with hepatitis. Data indicate that periostin is a novel mediator of hepatic fibrosis development. *(Am J Pathol 2015, ■: 1—12; http://dx.doi.org/10.1016/j.ajpath.2014.11.002)*

Q6 Liver fibrosis is a common consequence of chronic liver damage, such as damage due to hepatotropic viruses (mainly hepatitis B and C viruses), toxins, excessive alcohol intake, and autoimmunity. Liver fibrosis is characterized by alteration of tissue architecture and deposition of collagen-rich extracellular matrix (ECM). Chronic inflammatory stimuli can cause hepatocyte death, and the apoptotic hepatocytes, in turn, stimulate the hepatic resident macrophages (ie, Kupffer cells) and the recruited inflammatory leukocytes to secrete proinflammatory cytokines and profibrogenic factors, which activate collagen-producing hepatic stellate cells (HSCs). These immunological and tissue repair responses result in excessive collagen deposition and compromised liver function.[1—3] HSCs are a major source of ECM proteins in the damaged liver. Under physiological conditions, HSCs maintain a quiescent phenotype. After liver injury, the quiescent HSCs can be activated by several fibrogenic stimuli, including transforming growth factor β (TGF-β), platelet-derived growth factor, and inflammatory cytokines that are mainly secreted by Kupffer cells. The activated HSCs transdifferentiate into

Supported by National Nature Science Foundation of China grants Q3 81372841, 31171339, 31071302, and J1310027/J0106, the Programme of Introducing Talents of Discipline to Universities grant B12001, the 111 Project of Education of China grant B06016, the Fundamental Research Funds for the Central Universities, the Program for New Century Excellent Talents in University grant NCET-11-0296, and the Scientific Fund for Distinguished Young Investigator of Fujian Province grant 2010J06013.

Y.H., W.L., and H.X. contributed equally to this work.

Disclosures: None declared.

http://dx.doi.org/10.1016/j.ajpath.2014.11.002

myofibroblasts and produce excessive ECM proteins, which result in excessive collagen deposition and subsequent liver fibrosis.[1–3]

Matricellular proteins are a family of secreted ECM proteins that includes osteopontin (OPN), tenascins, cysteine-rich acidic secreted protein (SPARC), CCNs, thrombospondins, and periostin. These non-structural ECM proteins are important during embryonic development but are typically restricted to tissue remodeling and wound repair in the normal adults.[4–6] Periostin (encoded by *Postn*) was originally identified in MC3T3-E1 osteoblast cells as a critical regulator of bone and tooth development and maintenance.[7,8] Periostin is mainly expressed in collagen-rich fibrous connective tissues, including heart valves, tendons, corneas, the perichondrium, and the periodontal ligament, which are subjected to constant mechanical stresses during embryonic development or pathogenesis.[9–11] Current evidence in animal models and patients demonstrates that periostin is also involved in the pathobiology of various inflammatory diseases, including tissue injury, fibrosis, arthritis, atherosclerosis, and other inflammatory diseases.[12–16] Periostin is a critical mediator of the healing process of myocardial infarction,[17,18] and plays a critical role in fibrosis of bronchial asthma and chronic allergic skin inflammation.[13,19] Periostin-deficient mice show a reduction in pulmonary fibrosis.[14] Interestingly, as a critical developmental factor and a secreted ECM protein, periostin is also overexpressed in various types of human cancers and contributes to tumorigenesis and metastasis.[5,20–23] Collectively, these findings suggest that periostin actively contributes to tissue injury, inflammation, fibrosis, and tumor initiation and progression.[15,24] Interestingly, a recent report reveals that periostin actively contributes to obesity-induced hepatosteatosis.[25] However, whether periostin is involved in liver fibrosis progression has not yet been studied.[26]

Our aim was to explore the role of periostin in liver fibrosis. Herein, by using murine experimental models of liver fibrosis, we demonstrate that periostin is up-regulated in carbon tetrachloride– and bile duct ligation (BDL)–induced acute and chronic liver fibrosis and that liver fibrosis is dramatically decreased in periostin-deficient mice. The level of periostin is significantly elevated in the serum of patients with acute or chronic hepatitis. We also demonstrate that TGF-β signaling is involved in periostin-related liver fibrosis. Our observations identify periostin as a potential diagnostic or therapeutic target for hepatic fibrosis.

Materials and Methods

Experimental Models of Liver Fibrosis

All experimental procedures involving animals were performed in accordance with animal protocols approved by the Institutional Animal Care and Use Committee of Xiamen University (Xiamen, China). Heterozygous B6;129-*Postn*^{tm1Jmol}/J (*Postn*$^{+/-}$) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Wild-type and periostin-deficient mice were generated from crossing *Postn*$^{+/-}$ mice with *Postn*$^{+/-}$ mice. Littermate controls were used in all experiments. All mice were housed in a specific pathogen-free environment. In the carbon tetrachloride–induced acute fibrosis model, male mice were injected i.p. with carbon tetrachloride (diluted 1:5 in sterile mineral oil) or vehicle (mineral oil) at a dose of 2.5 mL/kg body weight twice per week for 2 weeks. In the carbon tetrachloride–induced chronic fibrosis model, male mice were injected i.p. with the same dose of carbon tetrachloride twice per week for 8 weeks. Mice were euthanized 72 hours after the final i.p. injection. To perform BDL, the common bile duct was ligated distally. We performed the sham operation similarly, except that the bile duct was not ligated. Mice were euthanized 1 or 3 weeks after BDL.

Cell Culture

The immortalized mouse macrophage cell line RAW264.7 was routinely cultured in Dulbecco's modified Eagle's medium (Gibco) with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C and saturated with 5% CO_2 in a humidified atmosphere.

IHC and Immunofluorescence

Immunohistochemistry (IHC) and immunofluorescence staining were performed as described.[27] The primary antibodies used included antibodies to periostin (AdipoGen), fibronectin (BD Biosciences), α-smooth muscle actin (α-SMA; Santa Cruz Biotechnology), collagen I, β-actin, and glyceraldehyde-3-phosphate dehydrogenase (Millipore).

Immunoblotting Analysis

Total proteins were extracted from liver tissues. Immunoblotting analysis was performed as described.[27]

Real-Time PCR

Total RNA was extracted from liver tissues or cells using TRIzol reagent, according to the manufacturer's instructions (Invitrogen). Reverse transcription was performed as previously described.[27] The expression levels of the mRNAs were determined by real-time PCR using SYBR Premix Ex Taq (Takara) and the predesigned primers listed in Table 1. [T1]

Hematoxylin and Eosin and Sirius Red Staining

Before staining, paraffin-embedded sections (5 μm thick) were dewaxed and hydrated. For hematoxylin and eosin staining, nuclei were stained with hematoxylin, followed by staining with eosin. For Sirius red staining, the dewaxed sections were dyed with Picrosirius Red solution (0.1%

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