MMP-13 deletion attenuates N-nitrosodimethylamine-induced hepatic fibrosis in mice

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Abstract

The pathogenesis of hepatic fibrosis is a dynamic process involving several cell types and molecular events. Connective tissue growth factor (CTGF) is a major profibrogenic molecule and plays a significant role in the pathogenesis of hepatic fibrosis. Since matrix metalloproteinase-13 (MMP-13) is absent in mice, MMP-13 is responsible for cleavage and activation of CTGF. Here, we elucidated the role of MMP-13 and CTGF in the pathogenesis of hepatic fibrosis using MMP-13 knockout mice. Hepatic fibrosis was induced in wild-type and MMP-13 knockout mice through intraperitoneal injections of N-nitrosodimethylamine (NDMA) in doses of 1 mg/100 g body weight on 3 consecutive days of every week over a period of 4 weeks. NDMA administrations resulted in marked elevation of serum ALT, AST, hyaluronic acid (HA), TGF-β1 and procollagen-Ill peptide in wild-type mice. There was marked activation of hepatic stellate cells, deposition of collagen and HA in the liver. However, all these changes were attenuated in NDMA administered MMP-13 knockout mice. Immunohistochemical staining demonstrated marked upregulation of CTGF in the necrotic and fibrotic areas of NDMA-treated wild-type mice but not in the knockout. Semiquantitative and qRT-PCR for collagen I, α-smooth muscle actin (α-SMA), CTGF and TGF-β1 mRNA demonstrated marked upregulation in NDMA treated wild-type mice, but not in similarly treated MMP-13 knockout mice. Western blotting showed increased levels of collagen I, α-SMA, CTGF and TGF-β1 mRNA in wildtype fibrotic mice, but not in knockout. Our results demonstrated that MMP-13 plays a significant role in the pathogenesis of hepatic fibrosis through cleavage and activation of CTGF. Furthermore, our study indicates that blocking of CTGF using effective molecules has potential therapeutic application to prevent hepatic fibrosis.

Introduction

Hepatic fibrosis initiates with a chronic liver injury, either alcohol, drugs, virus, metabolic disorders or unknown reasons. The pathogenesis of hepatic fibrosis is always associated with oxidative stress and release of reactive oxygen species (ROS), which triggers a chain of molecular events that culminates in hepatic fibrosis. If the causative agent is not controlled or prevented hepatic fibrosis could lead to cirrhosis and ultimate death. The molecular pathogenesis of hepatic fibrosis is a very complex and dynamic process that involves several cell types and recruit of hepatic progenitor and inflammatory cells. The activation and transformation of resting hepatic stellate cells into myofibroblasts with the expression of several growth factors and connective tissue proteins could lead to the production of several growth factors and connective tissue proteins play the key role in the progression of hepatic fibrosis.

Connective tissue growth factor (CTGF), also known as CCN3, is a multi-domain, cysteine-rich protein, and is expressed in interstitial and perivascular cells. CTGF acts upregulated by transforming growth factor beta (TGF-β) and is involved in the repair of damaged tissues. Connective tissue growth factor stimulates the synthesis of collagen and other connective tissue proteins that accumulate in the extracellular matrix of the liver. This process leads to scarring, loss of normal hepatic architecture, and cirrhosis. Appropriate strategy to inhibit the cleavage and maturation of CTGF would be promising to prevent the progression of hepatic fibrosis into cirrhosis.

Materials and Methods

MMP-13 knockout and wild type male littermates in a C57BL/6J and 129Sv hybrid background were generated from the intercross between MMP-13−/− mice. Hepatic fibrosis was induced by serial intraperitoneal injections of N-nitrosodimethylamine (NDMA) in doses of 1 mg/100 g body weight in sterile NaCl0.150 g body weight. The injections were given on three consecutive days of each week over a period of 4 weeks. Control animals also received an equal volume of 15 M NaCl without NDMA. All the injected animals were sacrificed on day 28 of the experiment. Hepatic injury was assessed through hematoxylin and eosin staining as Masson’s trichrome staining. The activation of hepatic stellate cells, evaluated through immunohistochemical staining for the expression of α-smooth muscle actin (α-SMA), was also considered as a marker for the extent of fibrosis. Alcian blue masonry (ALT), aspartate transaminases (AST), hyaluronic acid (HA), and transforming growth factor (TGF-β1) were measured in serum. The expression of CTGF, TGF-β1, α-SMA and collagen type I were also determined both at mRNA and protein levels in the hepatic tissue.

Figure 1

NDMA induced model of chronic liver fibrosis in wild-type and MMP-13 knockout mice. Hepatic fibrosis was induced in wild-type and MMP-13 knockout mice through intraperitoneal injections of N-nitrosodimethylamine (NDMA) thrice a week over a period of 4 weeks. NDMA administrations resulted in marked elevation of serum ALT, AST, hyaluronic acid (HA), TGF-β1 and procollagen-Ill peptide in wild-type mice. There was marked activation of hepatic stellate cells, deposition of collagen and HA in the liver. However, all these changes were attenuated in NDMA administered MMP-13 knockout mice. Immunohistochemical staining demonstrated marked upregulation of CTGF in the necrotic and fibrotic areas of NDMA-treated wild-type mice, but not in similarly treated MMP-13 knockout mice. Western blotting showed increased levels of collagen I, α-SMA, CTGF and TGF-β1 mRNA in wild-type fibrotic mice, but not in knockout. Our results demonstrated that MMP-13 plays a significant role in the pathogenesis of hepatic fibrosis through cleavage and activation of CTGF. Furthermore, our study indicates that blocking of CTGF using effective molecules has potential therapeutic application to prevent hepatic fibrosis.

Conclusions

• Serial administrations of NDMA produced centrilobular necrosis and well developed fibrosis in mouse liver within 28 days.

• Treatment with NDMA resulted in increased levels of serum ALT, AST, HA, and TGF-β1, activation of hepatic stellate cells and upregulation of CTGF, TGF-β1, α-SMA and collagen type I in wild-type mice.

• Treatment with NDMA and MMP-13 knockout mice showed inhibition of fibrosis, marked reduction in the activation of hepatic stellate cells and upregulation of CTGF, TGF-β1, α-SMA and collagen type I compared to similarly treated wild type mice.

• MMP-13 plays a crucial role in the pathogenesis of hepatic fibrosis through activation of CTGF

• Effective blocking of CTGF has potential therapeutic implication to prevent hepatic fibrosis.