Research Article

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Cartilage oligomeric matrix protein participates in the pathogenesis of liver fibrosis

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Background & Aims: Liver fibrosis is characterized by significant accumulation of extracellular matrix (ECM) proteins, mainly fibrillar collagen-I, as a result of persistent liver injury. Cartilage oligomeric matrix protein (COMP) is largely found in the ECM of skeletal tissue. Increased COMP expression has been associated with fibrogenesis in systemic sclerosis, lung fibrosis, chronic pancreatitis, cirrhosis and hepatocellular carcinoma. We hypothesized that COMP could induce fibrillar collagen-I deposition and participate in matrix remodeling thus contributing to the pathophysiology of liver fibrosis.

Methods: Thioacetamide (TAA) and carbon tetrachloride (CCl_4) were used to induce liver fibrosis in wild-type (WT) and *Comp*$^{-/-}$ mice. *In vitro* experiments were performed with primary hepatic stellate cells (HSCs).

Results: COMP expression was detected in livers from control WT mice and was upregulated in response to either TAA or CCl_4-induced liver fibrosis. TAA-treated or CCl_4-injected *Comp*$^{-/-}$ mice showed less liver injury, inflammation and fibrosis compared to their corresponding control WT mice. Challenge of HSCs with recombinant COMP (rCOMP) induced intra- plus extracellular collagen-I deposition and increased matrix metalloproteinases (MMPs) 2, 9 and 13, albeit similar expression of transforming growth factor beta (TGFβ) protein, in addition to *Tgfβ*, tumour necrosis factor alpha (*Tnfα*) and tissue inhibitor of metalloproteinases-1 (*Timp1*) mRNAs. We demonstrated that COMP binds collagen-I; yet, it does not prevent collagen-I cleavage by MMP1. Last, rCOMP induced collagen-I expression in HSCs via CD36 receptor signaling and activation of the MEK1/2-pERK1/2 pathway.

Conclusion: These results suggest that COMP contributes to liver fibrosis by regulating collagen-I deposition.

Lay summary: Cartilage oligomeric matrix protein (COMP) induces fibrillar collagen-I deposition via the CD36 receptor signaling and activation of the MEK1/2-pERK1/2 pathway, and participates in extracellular matrix remodeling contributing to the pathophysiology of liver fibrosis.

Introduction

A key event participating in the pathogenesis of liver fibrosis is the significant increase in extracellular matrix (ECM) deposition in response to persistent liver damage of various etiologies. Indeed, the presence of fibrosis is considered a prognostic marker for progression of chronic liver injury eventually resulting in cirrhosis and hepatocellular carcinoma in many patients [1,2]. During fibrogenesis the liver undergoes significant changes in the quality, quantity, composition and arrangement of the ECM components with a major increase in fibrillar collagen, primarily collagen-I [3], disrupting the hepatic architecture and compromising the normal liver function [4]. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are at the forefront of ECM remodeling and changes in their physiological balance also lead to disruption of the ECM dynamics hence contributing to scarring [5].

Although several cell types participate in the development of liver fibrosis, hepatic stellate cells (HSCs), located in the space of Disse between the sinusoidal endothelial cells and the hepatocytes, are the primary cells responsible for fibrillar collagen-I deposition. Following the onset of liver injury, they differentiate into myofibroblasts and acquire contractile, migratory, pro-inflammatory and pro-fibrogenic features [6]. HSC activation is driven by multiple mediators such as reactive oxygen species, chemokines, growth factors, matrix stiffness, matricellular proteins and damage-associated molecular patters, which are also

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCl_4, carbon tetrachloride; COMP, cartilage oligomeric matrix protein; *Comp*$^{-/-}$, cartilage oligomeric matrix protein global knockout mice; CV, central vein; ECM, extracellular matrix; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H&E, hematoxylin and eosin; HSC(s), hepatic stellate cell(s); IHC, immunohistochemistry; IOD, integrated optical density; MMP(s), matrix metalloproteinase(s); MO, mineral oil; rCOMP, recombinant COMP; TAA, thioacetamide; TGFβ, transforming growth factor-β; TIMP1, tissue inhibitor of metalloproteinase-1; WT, wild-type.

secreted by neighboring cells and signal to drive scarring by HSCs in an autocrine and/or paracrine fashion [7,8].

Cartilage oligomeric matrix protein (COMP), also referred to as thrombospondin-5, is a glycoprotein mainly found in the ECM of cartilage, synovium, ligaments and tendons [9]. It has five identical subunits linked by disulfide bonds forming a large protein of 524 kDa [10]. COMP can bind collagen-I, II and IX [11–13] with high affinity via its C-terminal domain, as well as other ECM components such as fibronectin [14], matrilins [15], proteoglycans [16] and heparin [17].

Increased COMP has been associated with fibrogenesis in systemic sclerosis [18], skin keloids [19,20], vascular atherosclerosis [21], lung fibrosis [22] and chronic pancreatitis [23] as well as with other conditions such as rheumatoid arthritis [24,25], osteoarthritis [26,27], pseudoachondroplasia [28], acute trauma [29] and systemic lupus erythematosus [30,31]. Recently, COMP has been proposed as a novel non-invasive marker for assessing cirrhosis and the risk of hepatocellular carcinoma [32] and as a biomarker of liver fibrosis in chronic hepatitis C [33]. However, to date, the role of COMP and its mechanism of action in the context of liver fibrosis have not been fully defined. Since COMP could promote scarring, our aim was to study the potential involvement of COMP in the development of liver fibrosis and to dissect its ability to modulate pathological collagen-I deposition, thereby contributing to the onset and/or progression of liver fibrosis.

Materials and methods

General methodology

Details on general methodology such as Western blot analysis, H&E staining, collagen-I immunohistochemistry (IHC), Sirius red/fast green staining and measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities have been described in our previous publications [34,35]. The source of commercially available antibodies can be found in Supplementary Table 1. The collagen-I antibody was generated and provided by Dr. Schuppan (University of Mainz, Mainz, Germany). The qPCR primers used are shown in Supplementary Table 2.

Cell culture and treatments

Due to the almost complete homology in all the proteins from our study in rat and mice, all the *in vitro* experiments were carried out with rat HSCs due to their higher yield. Primary rat HSCs were isolated as previously [34,36]. HSCs were seeded on 6-well plates (300,000 cells/well) in Dulbecco's modified Eagle's medium (DMEM)/F12 supplemented with 10% FBS, fungizone, penicillin and streptomycin. The medium was replaced with serum-free medium 12 h before treatment with 0–25 nM of human recombinant COMP (rCOMP) (R&D Systems, Minneapolis, MN). In some experiments, 1 μM of PD98059 (a mitogen-activated protein kinase MEK1/2 inhibitor) (Calbiochem, San Diego, CA) and 33 nM of mouse CD36 neutralizing antibody clone FA6-152 or mouse IgG1 kappa isotype control antibody clone MOPC-21 (StemCell Technologies Inc, Vancouver, BC, Canada), were added to the cells 1 h prior to incubation with rCOMP. The optimal concentration for collagen-I induction was determined experimentally.

Mice

Comp$^{-/-}$ mice (C57BL/6J) and their wild-type (WT) littermates were donated by Dr. Åke Oldberg (Lund University, Lund, Sweden) [37]. The targeting vector constructed to generate these mice disrupted exon 8 in the *Comp* gene. Colonies were established by intercrossing *Comp*$^{+/-}$ mice and control littermates (referred to as WT) were used in all experiments. *Comp*$^{-/-}$ mice do not show any anatomical, histological or ultrastructural abnormalities [37].

Induction of liver fibrosis

Two models of liver fibrosis were used in 10 wks old male *Comp*$^{-/-}$ and WT mice. In the first model, mice were given thioacetamide (TAA, Sigma, St. Louis, MO) at a dose of 300 mg/L in the drinking water or equal volume of water in the control group for 4 months. In the second model, mice were injected carbon tetrachloride (CCl_4, Acros Organics, New Jersey, NJ) dissolved in mineral oil (MO) at a dose of 0.5 ml/kg i.p. or equal volume of MO in the control group twice a week for 1 month. Thus, 4 groups (n = 6 mice/group) were included in each model: WT + Water or MO; WT + TAA or CCl_4; *Comp*$^{-/-}$ + water or MO and *Comp*$^{-/-}$ + TAA or CCl_4. Mice were sacrificed under ketamine/xylazine anesthesia 48 h after TAA withdrawal or after the last injection of CCl_4 to avoid an acute response over chronic liver injury. All mice received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health. Protocols were approved by the Icahn School of Medicine at Mount Sinai Institutional Animal Care and Use Committee.

Please see the Supplementary material for additional information.

Statistical analysis

Data are expressed as mean ± standard deviation. Statistical comparisons among groups and treatments were performed using the paired Student's *t* test and the two-factor analysis of variance. All the *in vitro* experiments were performed in triplicate at least four times. A representative image or blot is shown in all Figs.

Results

COMP expression is upregulated in the TAA and in the CCl_4 models of liver fibrosis in WT but is absent in Comp$^{-/-}$ *mice*

To determine whether COMP expression increased in liver fibrosis, WT and *Comp*$^{-/-}$ mice were either administered TAA or water for 4 months or injected CCl_4 or MO for 1 month; both are well-established models of drug-induced liver fibrosis [34,35]. Cytochrome P450 activity, the key enzyme involved in the metabolism of these two drugs was similar in WT and *Comp*$^{-/-}$ mice (Supplementary Fig. 1A and B). IHC analysis revealed positive staining for COMP in WT mice, which was increased in zones 1 and 3 by either TAA treatment or CCl_4 injection, yet it was totally absent in *Comp*$^{-/-}$ mice (Fig. 1A and B). To identify the source of hepatic COMP, frozen liver sections were immunostained with COMP or arginase-I (hepatocyte marker), desmin (HSC marker) or F4/80 (macrophage marker). Co-localization studies demonstrate that COMP is mostly expressed in hepatocytes but also to a minor extent in Kupffer cells and HSCs (Fig. 1C) and it is induced by TAA (Fig. 1D) or CCl_4 in WT but not in *Comp*$^{-/-}$ mice (not shown). This was also confirmed by qPCR (not shown).

TAA-treated and CCl_4-injected Comp$^{-/-}$ *are protected from liver injury compared to control WT mice*

Since COMP was induced following TAA treatment or CCl_4 injection, to further investigate the role of COMP in liver fibrosis, liver sections were processed for H&E staining. TAA-treated or CCl_4-injected *Comp*$^{-/-}$ showed less periportal and pericentral necrosis and inflammation compared to their corresponding control WT mice (Fig. 2A and B). Moreover, TAA-treated and CCl_4-injected *Comp*$^{-/-}$ had less hepatic macrophage infiltration (Supplementary Fig. 2A and B) yet, there was no difference in the number of neutrophils (not shown). Importantly, rCOMP did not induce migration of macrophages *in vitro* (Supplementary Fig. 3A).

The expression of inflammation markers was significantly reduced in TAA-treated and CCl_4-injected *Comp*$^{-/-}$ compared to