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Brg1 promotes liver fibrosis via activation of hepatic stellate cells

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ABSTRACT

Liver fibrosis, an important health concern associated to chronic liver injury that provides a permissive environment for cancer development, is characterized by the persistent deposition of extracellular matrix components mainly derived from activated hepatic stellate cells (HSCs). Brg1, the core subunit of the SWI/SNF chromatin remodeling complex, has been proved to associated with nonalcoholic steatohepatitis which may progress to cirrhosis. Herein, we determined whether Brg1 regulates liver fibrosis and examined its mechanism by focusing on HSCs activation. In this study, we demonstrate that Brg1 is elevated in human and mouse fibrotic liver tissues and Brg1 mediate the profibrotic response in activated HSCs. Our data indicate that Brg1 regulates the activation of HSCs through TGFβ/Smad signal pathway. Moreover, Brg1 deficiency mice displayed decreased HSCs activation in vitro and liver fibrogenesis after chronic damage by CCl_4 administration. In addition, Brg1 expression is positively correlated with liver fibrosis in cirrhotic patients and may be a prognostic factor in HCC. Collectively, we demonstrate that Brg1 promotes liver fibrosis by activating HSCs and may represent a potential target for anti-fibrotic therapies.

1. Introduction

Liver fibrosis, the critical pre-stage in the development of liver cirrhosis, is defined as the accumulation of excessive amounts of extracellular matrix(ECM) in the liver parenchyma [1]. Liver fibrosis develops on the basis of chronic liver injury including hepatitis B and C, alcoholic liver disease or non-alcoholic steatohepatitis [2]. The fibrotic process may lead to hepatic transplantation eventually or promote a favorable microenvironment for cancer development [3]. Recent evidence indicates that liver fibrosis can be reversed even in advanced stage [4]. Thus, a greater understanding of the molecular mechanisms governing liver fibrosis regression is needed to facilitate the development of antifibrotic therapeutic approaches.

Activation of quiescent hepatic stellate cells(HSCs) to myofibroblasts is central to the fibrogenic process in chronic liver disease [5]. In the healthy liver, HSCs are retinoid and lipid-containing stromal cells located in the space of Disse. Liver injuries result in the activation of quiescent HSCs, which undergo dramatic phenotypic changes and transdifferentiate into myofibroblasts with upregulated alpha-smooth muscle actin(α-SMA) expression and ECM production(e.g. collagen, hyaluronic acid and fibronectin) [6]. TGF-β/Smad signal pathway plays a prominent role in the regulation of ECM formation [7]. It has been shown that local induction of TGF-β1 from the autocrine or paracrine pathway is crucial for the activation of HSCs and the production of ECM proteins leading to liver fibrosis [8].

Chromatin remodeling is one of the most important epigenetic mechanisms regulating gene expression. The Swltch/Sucrose Nonfementable (SWI/SNF) nucleosome repositioning complex regulates gene expression using the energy derived from ATP hydrolysis to disrupt histone-DNA interactions, resulting in transcriptional activation or repression [9]. Brg1, the core subunit of the SWI/SNF chromatin remodeling complex, is essential for DNA repair, differentiation and organ development [10,11]. Previous studies have showed that depletion of Brg1 significantly ameliorated hepatic pathology in nonalcoholic steatohepatitis(NASH) mice [12] and NASH is considered to be a potentially health-threatening disease that may progress to cirrhosis in 10–15% of patients [13]. However, the functional role of Brg1 in liver fibrosis and its molecular mechanism remain to be elucidated.

In the present study, we aimed to investigate the contribution of Brg1 for liver fibrosis in vitro and in vivo. Our results revealed that Brg1 is an interesting target to block HSC transformation by inhibiting TGFβ/Smad-α-SMA/Col1a1 signal pathway in vitro and the suppression of Brg1 diminishes experimental liver fibrosis after chronic administration of CCl_4. Thus, Brg1 may represent a potential target for anti-fibrotic strategies.

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2. Materials and methods

2.1. Cell lines and cell culture

Human hepatic stellate cells LX-2 were cultured at 5% CO_2 and 37 °C in Dulbecco's Modified Eagle Medium (DMEM) that is supplemented with 10% fetal bovine serum (HyClone, Logan, Utah, USA).

2.2. Clinical tissue samples

Normal liver tissues and cirrhotic liver tissues were collected from liver angioma and liver cirrhosis patients, respectively. The informed consent was obtained from all participant before sample collection. This study was approved by the Huazhong University of Science and Technology Research Ethics Committee.

2.3. Western blot analysis and real-time PCR assay

Western bolt analysis and real-time PCR assay was conducted as previously described [14]. The antibodies to Brg1 and GAPDH were purchased from Santa Cruz Company(Santa Cruz, CA, USA) and the antibodies to α-SMA, Col1a1 and SMAD3 were purchased from Cell Signaling Technology(Danvers, Massachusetts, USA). Brg1 inhibitors PFI-3 came from Selleck (Shanghai, China). The primer sequences for RT-PCR were listed in Supplementary Table 1.

2.4. Chromatin immunoprecipitation (ChIP) assay

ChIP assay were performed in accordance with the manufacturer's protocols (EpiQuik Chromatin Immunoprecipitation Kit, Epigentek Group Inc.). To examine changes in SMAD3-binding activity at the α-SMA and Col1a1 promoter, ChIP assays were conducted with the anti-SMAD3 antibody(9523, Cell Signaling Technology). The primer sequences for ChIP are listed in Supplementary Table 1.

2.5. Luciferase assays

Luciferase activity assays were performed using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA), in accordance with the manufacturer's protocol. LX-2 cells were transfected with α-SMA and Col1a1 promoter luciferase reporter constructs and siBrg1 or siControl. Luciferase assays were performed 72 h after transfection using the Dual-Luciferase Assay System. Firefly luciferase activity was normalized to the corresponding Renilla luciferase activity.

2.6. IHC analysis

IHC analysis was simultaneously conducted by two pathologists using a multiple viewing microscope to evaluate the staining of Brg1, α-SMA, Col1a1. Staging of fibrosis was assessed according to Ishak [15] on the sections with Sirius red. The quantification of IHC staining was evaluated by an IRS system as previously described [16]. Brg1, α-SMA and Col1a1 were determined as low (IRS:0–4) and high (IRS:6–12).

2.7. RNA interference and adenovirus system

siRNA duplexes targeting the human *Brg1* gene(siBrg1) and nonsense control siRNA were synthesized and purified by RiboBio (Ribobio, Guangzhou, China). RNA oligonucleotides were transfected using Lipofectamine RNAiMAX Regent (Invitrogen, Carlsbad, CA, USA) and the expression level of Brg1 was quantified 72 h after transfection. Adenovirus-expression Brg1-targeted short hairpin RNA (shBrg1) and control adenovirus (shCon) was previously constructed in our laboratory.

2.8. Administration of AAV vectors in mice

AAV8 vectors encoding murine Brg1-targeted shRNA(shBrg1) or GFP were purchased from Vigene Biosciences (Jinan, Shandong, China). AAV8-shBrg1 or AAV8-GFP vectors were administered by tail vein injection at a dose of 10×10^{11} vector genomes(vg)/mice in a total volume of 200ul.

2.9. Chronic carbon tetrachloride(CCl_4) liver injury model

C57BL/6 mice were treated intraperitoneal injected twice weekly for 4 weeks with CCl_4 at 2 μl/g body weight or olive oil vehicle. The mice were divide into four groups and every group had 5 mice. Mice were sacrificed and the tissues were harvested 48 h after the final CCl_4 injection.

2.10. Determination of ALT and HA levels

Serum mouse ALT levels and HA levels were determined using ALT Test Kit (Huili Biotech, Changchun, Jilin, China) and Hyaluronan Quantikine ELISA Kit (R&D Systems Inc., Minneapolis, MN, USA) following the manufacturer's instructions.

2.11. Statistical analysis

Collagen distribution was analyzed by Image Pro Plus software (Media Cybernetics, Bethesda, MD) to quantify the area of tissue occupied by positive staining. Data were presented as mean ± S.D. and two groups of data were statistically analyzed by two-tailed *t*-tests using Graphpad Prism 5 software. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Brg1 is overexpressed in liver fibrosis both in human and mice model

To investigate the expression of Brg1 in liver fibrosis, we performed the NCBI Gene Expression Omnibus (GEO) dataset parameters in normal liver tissues and cirrhosis tissues. The result showed that Brg1 was significantly higher in cirrhosis than in normal tissues (Fig. 1A). Interestingly, we found that in hepatocellular carcinoma (HCC), the most common progression of cirrhosis, the expression level of Brg1 was much higher than cirrhosis. Furthermore, the expression of Brg1 increased with the stage of HCC increased (Supplementary Figure 1A and B). It reveals that Brg1 overexpression seems to be early features of cirrhosis and continuing increase of Brg1 contributes to HCC.

To further investigate the relationship between the expression of Brg1 and liver fibrosis, We used the chronic administration of CCl_4 as a model to generate liver damage and fibrogenesis in mice. C57BL/6 mice were administered twice weekly i.p. CCl_4 for 4 weeks followed by tissue harvests 48 h after the final CCl_4 injection (Fig. 1B). Hepatic fibrosis was assessed by morphometric analysis of Sirius Red and α-SMA (Fig. 1C and D). The results showed that Brg1 was overexpressed both in protein and mRNA levels in liver fibrosis mice model. Taken together, Brg1 was overexpressed during progression of liver fibrosis.

3.2. Brg1 mediates the profibrotic response in activated HSCs

Considering the role of Brg1 in NASH in previous study [12] and the expression of Brg1 in our liver fibrosis mice, we have reasons to hypothesize that Brg1 may play an important role in the regulation of liver fibrosis progression. To validate this hypothesis, depletion or overexpression of Brg1 was performed to investigate the profibrotic response in LX-2 cells, a human-activated HSC cell line. siRNA-mediated depletion of Brg1 resulted in a significantly reduction of α-SMA (a marker for activated HSCs) and collagen type1(Col1a1, a prominent