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SENP2 alleviates CCl_4-induced liver fibrosis by promoting activated hepatic stellate cell apoptosis and reversion

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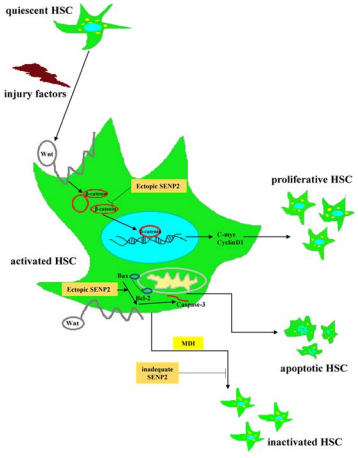
GRAPHICAL ABSTRACT



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ABSTRACT

SUMOylation and deSUMOylation, a dynamic process, is proved to be involved in various fibrotic diseases. Here, we found SENP2, one of deSUMOylation protease family member, was decreased in CCl_4-induced mice fibrotic liver tissues, primary HSCs and restored after spontaneously recovery. In addition, HSC-T6 cells with TGF-β1 treatment resulted in a significant reduction of SENP2. Ectopic expression of SENP2 hindered cells activation and proliferation induced by TGF-β1 while knockdown of SENP2 showed an opposite effect. Importantly, SENP2 promoted apoptosis of HSC-T6 cells activated by TGF-β1. Furthermore, restoration of SENP2 was observed in inactivated HSCs after adipogenic differentiation mixture (MDI) treatment. Inadequate SENP2 inhibited the reversion of HSC-T6 cells, featured as aberrant expressions of α-SMA and col1a1, two markers of liver fibrosis. It has been reported SENP2 was a suppressant regulator of Wnt/β-catenin signal pathway. Similarly, we found

Abbreviations: SENP2, SUMO-specific protease 2; ECMs, extracellular matrixs; HSCs, hepatic stellate cells; α-SMA, α-smooth muscle actin; col1a1, type I collagen; H&E, hematoxylin and eosinstaining; Sirius, sirius red staining; MDI, adipocyte differentiation cocktail; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IHC, Immunohistochemistry; ALD, alcoholic liver disease; NAFLD, nonalcoholic fatty liver disease; TGF-β1, transforming growth factor-β1

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SENP2 has a negative effect on β-catenin as well as its downstream genes C-myc and CyclinD1 in liver fibrosis. Collectively, our data indicated SENP2 may be involved in HSCs apoptosis and reversion in liver fibrosis.

1. Introduction

Liver fibrosis, a worldwide severe problem, mostly originates from chronic liver injury which caused by various factors such as hepatitis viral infection, schistosomiasis, nonalcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) (Zhang et al., 2016). Excessive deposition of extracellular matrixs (ECMs), a prominent characteristic of liver fibrosis, ultimately distorts normal liver architecture if not be treated appropriately (Zhang et al., 2016). Noteworthily, a growing body of evidence suggested that liver fibrosis is a reversible process with phenotype change of HSCs (Chen et al., 2016; Liu et al., 2016; Liu et al., 2013). HSCs, the main cell type which produce ECMs, play an essential role in initiation, progression, and reversion of liver fibrosis. Under physiological status, HSCs keep a quiescent type in the perisinusoidal space of disse (Puche et al., 2013). In respond to pathological damage, quiescent HSCs lose lipid droplets, subsequently proliferate and transdifferentiate to myofibroblasts, which characterized by substantial up-regulation α-SMA and col1a1 (Higashi et al., 2017). If accumulation of collagens persist excessively, the parenchyma and vascular architecture of liver will be destroyed. Up to now, removal of pro-fibrotic factors such as sustaining drug injury (CCl_4 or alcohol) could reverse liver fibrosis in experimental rodent fibrosis models (Kisseleva and Brenner, 2013; Liu et al., 2016; Wu et al., 2015). It is accepted that promoting HSCs go apoptosis or invert to a quiescent phenotype is a critical step in fibrosis reversion. Some researchers reported that activated HSCs, treatment with an MDI *in vitro* could contributes to morphologic and biochemical reversal of activated HSCs to quiescent cells (Kisseleva and Brenner, 2011; Tsukamoto, 2005). All this researches indicated that the plasticity of HSC was indispensable in liver fibrosis reversion.

It has been reported sumoylation and desumoylation, a dynamic processes, was involved in various fibrotic diseases including cystic fibrosis, renal fibrosis, cardiac fibrosis and *etc* (Ahner et al., 2016; Khodzhigorova et al., 2012; Liu et al., 2017; Zhou et al., 2014). Sumoylation, a post-translational modification way by SUMOs, was proved to regulate amounts of proteins localization, stability, and interaction with other protein(Hay, 2005). Noteworthily, evidences showed sumoylation of Nrf2 and MafG contributes to a quiescent phenotype of HSCs in liver fibrosis. Besides, UBC9, the only known E2-conjugating enzyme involved in SUMOylation, was high expressed in activated LX-2 cells (human HSCs) and silencing UBC9 could promote activated HSCs apoptosis (Fang et al., 2017). Intriguingly, SUMO-protein cleavage could be carried out by SUMO proteases, which controls biological processes ranging from cell division and embryonic development to tumor progression (Cheng et al., 2006; Cubenas-Potts et al., 2013; Dong et al., 2016; Hickey et al., 2012; Kang et al., 2010). To date, there were seven members of SENPs family was reported in mammals including SENP1-3 and SENP5-8 (Huang et al., 2015). SENP2, as a vital member, was reported to participate in regulating key signal pathways during multiple diseases progression. Of note, a number of studies have shown SENP2 inhibits the activity of β-catenin in different cancers (Kadoya et al., 2002; Shen et al., 2012; Shitashige et al., 2008; Tan et al., 2015). Ectopic expression of SENP2 repressed HepG2 cells growth via reinforcing the expression of WW domain-containing oxidoreductase, a novel inhibitor of the Wnt/β-catenin (Jiang et al., 2014). Importantly, plenty of reports have confirmed that Wnt/β-catenin is a pivotal signal pathway regulating HSCs proliferation in liver fibrosis (Cai et al., 2016; Ge et al., 2014). Therefore, whether SENP2, a suppressant regulator of Wnt/β-catenin, is involved in liver fibrosis remain to be studied. SENP2 also controlled adipogenesis by regulating PPAR-γ expression during preadipocyte differentiation (Chung et al., 2010). Interestingly, ectopic expression of PPAR-γ made for a quiescent phenotype change of HSCs which ultimately attenuated liver fibrosis (Zhang et al., 2013). Given the intimate relationship of SENP2 and PPAR-γ, we hypothesize that SENP2 may regulate the transdifferentiation of HSCs in liver fibrosis.

In the present study, we detected the expression of SENP2 was decreased in mice fibrotic tissues as well as primary HSCs and restored after spontaneously 6 weeks' recovery *in vivo*. Moreover, inadequacy of SENP2 was also observed in rat HSC-T6 cells activated by TGF-β1 *in vitro*. Overexpression of SENP2 hindered activation and proliferation of HSC-T6 cells by repressing the activity of β-catenin while knockdown of SENP2 showed an opposite effect. Importantly, we found ectopic expression of SENP2 resulted in an evident apoptosis of activated HSC-T6 cells. Besides, a significant regression of SENP2 was appeared in inactivated HSC-T6 cells reverted by MDI. Silencing SENP2 lead to an aberrant upregulation of col1a1 and α-SMA in MDI-reverted cells. Taken together, our findings suggested the essential role of SENP2 in progression and reversal of liver fibrosis and SENP2 may serve as a therapeutic target in the future.

2. Materials and methods

2.1. Mouse models of liver fibrosis

6–8 weeks old male C57BL/6J mice were purchased from the Experimental Animal Center of Anhui Medical University and all animal procedures were permitted by the University Animal Care and Use Committee. Mice were randomly divided into three groups (n = 15 per group), including vehicle group, CCl_4 group and reversal group. Liver fibrosis was established by intraperitoneal injection of CCl_4 (10% in olive oil) at a dose of 0.001 ml/g per mouse biweekly for 4 weeks. In the meantime, the same volume of olive oil was injected in vehicle mice group intraperitoneally. The reversal model was kept regular feeding for 6 weeks after CCl_4 cessation.

2.2. Serum chemistry

Whole blood samples were collected from abdominal aorta, centrifuged at 3000 rpm for 15 min. Then blood serum was extracted and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were detected by alanine aminotransferase/aminotransferase Assay Kits (Nanjing Jiancheng Bioengineering Institute, China) following the manufacturer's instructions. All experiments were performed in triplicate and repeated at least three times.

2.3. Isolation of HSCs

Primary HSCs isolation methods with some modifications were followed as previously described by liu et al. (Liu et al., 2016). Briefly, mice (n = 6 per group) were anesthetized with chloral hydrate via intraperitoneal injection. Then, fixed mice in experimental board and flushed out the blood entirely by perfusion buffer. Next, digested the liver solid architecture with digestion liquid which was composed of collagenase IV (Sigma, St. Louis, MO, USA) and Pronase (Sigma, St. Louis, MO, USA). Until digested appropriately, the liver tissue were dissociated and disrupted in 1% BSA solution. At last, cell suspensions were achieved by Nycodenz (Sigma, St. Louis, MO, USA) density gradient centrifugation according to manufacture protocol. Finally, isolated HSCs were extracted from the gradient interface.