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Role of histone deacetylases(HDACs) in progression and reversal of liver fibrosis

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ABSTRACT

Liver fibrosis refers to a reversible wound healing process response to chronic liver injuries. Activation of hepatic stellate cells (HSCs) is closely correlated with the development of liver fibrosis. Histone deacetylases(HDACs) determine the acetylation levels of core histones to modulate expression of genes. To demonstrate the link between HDACs and liver fibrosis, CCl4-induced mouse liver fibrosis model and its spontaneous reversal model were established. Results of the current study demonstrated that deregulation of liver HDACs may involved in the development of liver fibrosis. Among 11 HDACs tested in our study (Class I, II, and IV HDACs), expression of HDAC2 was maximally increased in CCl4-induced fibrotic livers but decreased after spontaneous recovery. Moreover, expression of HDAC2 was elevated in human liver fibrotic tissues. In this regard, the potential role of HDAC2 in liver fibrosis was further evaluated. Our results showed that administration of HSC-T6 cells with transforming growth factor-beta1 (TGF-β1) resulted in an increase of HDAC2 protein expression in dose- and time-dependent manners. Moreover, HDAC2 deficiency inhibited HSC-T6 cell proliferation and activation induced by TGF-β1. More importantly, the present study showed HDAC2 may regulate HSCs activation by suppressing expression of Smad7, which is a negative modulator in HSCs activation and liver fibrosis. Collectively, these observations revealed that HDAC2 may play a pivotal role in HSCs activation and liver fibrosis while deregulation of HDACs may serve as a novel mechanism underlying liver fibrosis.

1. Introduction

Liver fibrosis, characterized by excessive accumulation of extracellular matrix (ECM) components, is a reversible wound healing process response to chronic liver injuries induced by various etiological factors such as drug abuse, metabolic syndrome, autoimmune disease (Bataller and Brenner, 2005; Hernandez-Gea and Friedman, 2011). With the development of fibrosis, liver parenchyma and vascular structures might be damaged and liver function would be distorted, eventually result in liver cirrhosis or hepatocellular carcinoma (HCC) (Friedman, 2008b; Popov and Schuppan, 2009). Hepatic stellate cells (HSCs), the resident vitamin A-storing cell type, have been recognized to be the primary ECM producers during liver fibrosis (Friedman, 2008b; Puche et al., 2013). Numerous studies have concluded that HSCs activation is the critical event in the development of liver fibrosis (Moreira, 2007). Upon liver insults, the resting state HSCs lose vitamin A-containing lipid droplets and trans-differentiate into myofibroblasts-like cells with significant up-regulation of COL1α1 and α-SMA, which is considered as HSCs activation (Moreira, 2007; Friedman, 2010; Hernandez-Gea and Friedman, 2011; Mallat and Lotersztajn, 2013). Lots of growth factors and inflammatory cytokines, such as platelet-derived growth factor (PDGF) and transforming growth factor-beta1 (TGF-β1),can drive the activation of HSCs, thereby promoting liver fibrogenesis (Friedman, 1999). Therefore, blocking the activation and function of HSCs is regarded as a crucial goal to establish therapeutic strategies for liver fibrosis. Nevertheless, some *in vivo* and *in vitro* studies have raised new insights into the concept of liver fibrosis reversal, indicating that excessive ECM could be degraded and almost-normal liver architecture could be remodeled based on the withdrawal of injury (Kisseleva et al., 2012; Troeger et al., 2012; Wu et al., 2015). These studies are significant as they show that activated HSCs can also revert their phenotype towards a quiescent-like phenotype, eventually lead to remission even reversal of liver fibrosis. Moreover, She H et al. have confirmed that activated HSCs can be polarized into the lipid-storing phenotype by MDI (adipogenic differentiation mixture, 0.5 mM isobutylmethylxanthine, 1 μM dexamethasone, and 1 μM

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insulin) treatment (She et al., 2005). However, we are still at the beginning understanding of the mechanisms of HSCs activation and reversal because of its complexity.

Histone deacetylases(HDACs) promote the deacetylation of lysine residues on histones and play important roles in various diseases (Livyatan and Meshorer, 2013; Tang et al., 2013). It has been identified that the balance between the acetylated and deacetylated status of core histones is modulated by histone acetyltransferases (HATs) and HDACs (Kirpich et al., 2012). Until now at least 18 mammalian HDAC proteins, categorized into four sub-families depending on similarity to yeast orthologs, have been identified (Martin et al., 2012). The Zn^{2+}-dependent HDAC family consists of 11 members currently, which are divided into three classes (class I, II, and IV) based on the structure, sequence homology and domain organization (Wang et al., 2015). Class I HDACs contain HDAC1, 2, 3 and 8, and share high sequence homology with yeast HDAC retinoblastoma protein (Rpd3) in their catalytic sites (Yang and Seto, 2003). In general, Class I HDACs are ubiquitously tissue distributed and mainly localized in the nucleus of the cell (Yang and Seto, 2003). Class II HDACs include HDAC 4, 5, 6, 7,9 and 10, and closely relate to yeast HDA1 band. According to the number of catalytic domains, this class is further sub-divided into class IIa (HDAC 4, 5, 7and 9) with one catalytic domain and class IIb (HDAC 6 and 10) with two catalytic domains (Koppel and Timmusk, 2013). Different from Class I HDACs, the whole Class II members display a tissue-specific distribution in mammals and are predominantly localized in the cytoplasm but can 'shuttle' between the cytoplasm and nucleus in response to cellular signals (Kouzarides, 2000). HDAC 11 is currently the exclusive HDAC in class IV localizing in the nucleus and its catalytic domain resembles the actives sites of both class I and class II enzymes (Bagui et al., 2013). In comparison, Class III HDAC enzymes (also known as sirtuins, silent information regulator, SIRT1–7) are NAD^{+}-dependent and share sequence homology with the yeast *Sir2* gene (Haigis and Sinclair, 2010).

Of note, emerging evidence reveals a potential character of HDACs in liver fibrosis (Mannaerts et al., 2013; Pannem et al., 2014). As demonstrated, several HDACs are reported to participate in modulating the activation of HSCs and the progression of liver fibrosis (Mannaerts et al., 2010; Qin and Han, 2010). However, we are still at the beginning cognitive of the varieties features of HDACs in liver fibrosis. For the first time, we probed the mRNA levels of Class I, II, IV HDACs in CCl4-induced murine liver fibrosis model and its spontaneous reversal model. Interestingly, we found that treatment and withdrawal of CCl_4 led to liver fibrosis and its reversal were correlated with the deregulation of liver HDACs. To further confirm the contribution of disturbed HDACs in the progression of liver fibrosis, the potential role of HDAC2 in TGF-β1 stimulated HSC-T6 cells (rat hepatic stellate cells) was studied, because among all HDACs tested in our study, HDAC2 was maximally increased in the progression, but decreased in the reversal stage, of liver fibrosis. And protein expression of HDAC2 in human liver fibrotic tissues was also obviously elevated. It is worth mentioning that the role of HDAC2 in liver fibrosis is still beyond our understanding while HDAC2 plays a crucial role in HCC (Zhang et al., 2014b; Ler et al., 2015). Accumulating evidence also indicates the potential role of HDAC2 in the development of renal fibrosis and pulmonary fibrosis (Noh et al., 2009; Huang et al., 2013; Lee et al., 2014). In this regard, we investigated the link between HDAC2 and liver fibrosis, further verified the functional role of HDAC2 in liver fibrosis. Taken together, our findings may cast light on HDACs in progression and reversal of liver fibrosis, thus may be of great significance in developing novel therapeutic strategies for liver fibrosis.

2. Materials and methods

2.1. Animals, mouse models of liver fibrosis, patients

Subjects of the study used for CCl_4 liver injury model were 8-week-old (half male and female) C57BL/6J mice, which were supplied by the Anhui Medical University Experimental Animal Center (Hefei, China). All animal experimental operating rules were reviewed and ratified by the University Animal Care and Use Committee. C57BL/6J mice were divided into three groups (eight mice per group) randomly, including control group, liver fibrosis group (model group) and reversal group. The reversal group was set up by subcutaneous injection of a 10% diluted concentration of CCl_4 in olive oil (0.02 ml/g/mouse) twice a week for 4 weeks. Control and model group mice were treated with same volume of olive oil in the same method and at the same time intervals. After 4 weeks, stop providing the olive oil and CCl_4 for all groups and control the mice for the establishment of spontaneous liver fibrosis reversal model for another 8 weeks, while the model group keeping the subcutaneous injection of a solution containing 10% CCl_4 in olive oil (similarly in 0.02 ml/g/mouse) twice a week to build liver fibrosis model for the last 4 weeks. All the experimental mice were sacrificed 24 h after the last injection. The liver tissues were harvested for further histopathological analysis by fixation in 10% formalin.

Human liver fibrosis tissue samples were gained from patients taking partial liver resection at the Department of Surgery, the First Affiliated Hospital of Anhui Medical University. Matched healthy volunteers with generally accepted normal aminotransferase activities, no history of liver related diseases, and tested no HBV, HCV or HIV infections were included as a normal control group in this study. The patient informed consents were provided written by patients or their guardians. The Health Human Research Ethics Committee of Anhui Medical University ratified all aspects of this study, which identify with the standards set by Helsinki Declaration. The fresh liver tissues were used for blot analysis.

2.2. Immunohistochemistry

Liver tissues were fixated in 10% formalin, embedded in paraffin and stained for routine histology. The sections were dewaxed in xylene and dehydrated in ethanol. Antigen retrieval was obtained in citrate by microwave processing for 15 min. The sections were further deparaffinized and treated with 0.3% hydrogen peroxide to block endogenous peroxidase activity for another 15 min. Then sections were further blocked by 2% bovine serum albumin followed closely by hatching with primary antibody against α-SMA (Bioss, China,1:200) and HDAC2 (cell Signaling, USA, 1:200) at 4 °C for 20 h. After the Phosphate Buffered Saline rinsing, sections were incubated at room temperature by biotinylated secondary antibody (Cowin Bioscience, China) at least for 60 min. The expression of α-SMA and HDAC2 was visualized by 3, 3′-diaminobenzidine tetrahydrochloride (DAB, Cowin Bioscience, China) staining. Soon afterwards the sections were dyeing with Mayer's hematoxylin for 30 s, dehydrated, and α-SMA or HDAC2 positive areas were observed within the region of fibrosis.

2.3. Cell culture

Rat hepatic stellate cell line HSC-T6 was provided by Shanghai Fumeng Gene Biological Corporation (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) was used to incubate HSC-T6 cells, supplemented with 10% fetal calf serum (FCS, Sijiqing, China), 100 U/ml penicillin, 2 mM L-glutamine and 100 mg/ml streptomycin. The HSC-T6 cells were cultured at 37 °C with 5% CO_2. When the cell density cultivate to approximately 80%, monolayers were incubated in serum-free DMEM in the presence of 10 ng/ml recombinant murine TGF-β1 (Peprotech, UK) or 100 nM trichostatin A (TSA, Sigma-Aldrich, USA) for 24 h. According to the experiment design, siRNA transfection was performed in HSC-T6 cells before TGF-β1 administration. MDI [0.5 m Misobutylmethylxanthine (Sigma-Aldrich, USA), 1 μM dexamethasone (Sigma-Aldrich, USA), and 167 nM insulin (Sigma-Aldrich, USA)] with 10% FCS was used to induce activated HSC-T6 cells revert to the quiescent state as described by She H et al.(She et al., 2005).