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REVIEW

miR-33a, an important marker and putative therapeutic target in chronic HBV-induced fibrosis

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To investigate the roles and mechanisms of miR-33a in liver fibrosis, miR-33a expression in whole liver and serum samples was measured from chronic hepatitis B (CHB) patients by quantitative real-time PCR (qRT-PCR). In addition, Human and murine primary liver fibrosis-associated cells were isolated and treated with transforming growth factor-β1 (TGF-β1). We found that miR-33a expression levels in liver tissue significantly increased with a fibrosis progression manner in the human liver. Furthermore, serum miR-33a levels associated positively with progressing process of hepatic fibrosis. miR-33a was in particular increased in hepatic stellate cells (HSC) than other liver fibrosis-associated cells. Stimulation of HSCs with TGF-β1 leads to a critical increase of miR- 33a. Increasing miR-33a levels increased (whereas inhibiting miR-33a weakened) the activation role of TGF-β1 in LX-2 cells, which might be a potential mechanism through moderating Smad7 expression. Altogether, data suggest that miR-33a may be a novel marker for HSC activation and hepatic fibrosis progress, suggesting a new therapeutic target in liver fibrosis.

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Liver fibrosis is the late stage of different chronic liver diseases, and in China, chronic hepatitis B virus (HBV) infection has been identified as the main cause of liver fibrosis. Fibrosis is characterized by excessive production and deposition of extracellular matrix (ECM) proteins, mainly the type I collagen that is synthesized by hepatic stellate cells (HSCs) after various fibrogenic stimulation [1, 2]. Growing evidence has demonstrated that transforming growth factor-β1 (TGF-β1) plays a central role in liver fibrosis [3, 4]. However, despite advanced knowledge in understanding the pathophysiology of hepatic fibrosis, the underlying mechanisms of this process remain unclear.

A microRNA (miRNA) is a small newly discovered endogenous non-coding RNA molecule that contains 18–25 nucleotides and perform as post-transcriptional regulators of target genes by binding to the 3'untranslated region (3'UTR) mRNA [5–7]. microRNA-33a (miR-33a) recently has become a hot research small miRNA and it has been demonstrated that it not only regulates lipid homeostasis [8–11], but also may be exerting as a potent tumor suppressor [12], cell proliferation and cycling [13]. Moreover, Li *et al*. [14] recently demonstrated that miR-33a-associated HSC activation and extracellular matrix production was, at least in part, modulated by the activation of the PI3K/Akt pathway and PPAR-α in vitro, and they found that the expression of miR-33a was associated with the progression of liver fibrosis. However, the role and mechanism of miR-33a on

hepatic fibrosis is largely unknown.

In this work we investigated the profibrogenic effects and associated mechanisms of miR-33a in hepatic fibrosis. Our results showed that serum and hepatic miR-33a levels increased with the progression of liver fibrosis in CHB patients and expression of miR-33a was significantly upregulated in primary hepatocytes and hepatic stellate cells from C57BL/6 murine hepatic fibrosis models as previously described [15-17]. Moreover, miR-33a expression in HSCs is higher than another fibrosis-associated cell hepatocytes (e.g., LX-2, HepG2 and Huh7 cells), both of which were isolated from human and mice liver. Interestingly, the expression pattern of miR-33a in the human or mice hepatocyte was not altered after TGF-β1 treatment. These results confirm that miR-33a expression was specifically expressed in the activation process of hepatic stellate cells, and suggest that miR-33a is regulated in whole liver extracts in mice and human hepatic fibrosis, which was not dependent on the type of experimental model of liver fibrosis.

A growing amount of evidence has demonstrated that TGF-β1 and its activation role of HSCs plays a central role in the progression of hepatic fibrosis [18]. Our results shown that TGF-β1 modulates miR-33a expression in a dose- and time-dependent manner in HSCs, increasing miR-33a levels increased (whereas inhibition miR-33a decreased) TGF-β1-induced collagen I (Col1A1) and alpha-smooth muscle actin (a-SMA) protein levels in HSCs, mRNA expression of Col1A1 and a-SMA was exerted in a manner similar to miR-33a expression. These results indicate that the profibrotic effects of miR-33a might be modulated by modulation of TGF-β1-associated signaling pathway. TGF-β1 predominantly activates hepatic stellate cells (HSC) via the TGF-β1/Smad signaling pathway, thus resulting in liver fibrosis [19, 20]. Our results first showed that TGF-β1-induceded miR-33a expression and miR-33a alternately stimulated TGF-β1- induced HSC fibrogenic activation by targeting the inhibitory Smad, Smad7. However, Smad7 inhibition might not be the only mechanisms about miR-33a profibrogenic effects. Thus, it needs further investigation in the future.

According to our results, we assume that miR-33a is, at least in part, a novel signaling pathway that modulates TGF-β1-dependent induction of ECM genes (e.g., ColA1, a-SMA) in hepatic stellate cells during liver fibrosis. To our knowledge, this study is the first clinical investigation to identify miR-33a is a hepatic fibrogenesis associated microRNA molecules. Overall, our findings imply that miR-33a plays a central role in the pathogenesis of hepatic fibrosis and is a new marker for clinical diagnosis, which may be developed as a potential therapeutic target in treating liver fibrosis. However, in the current study, the scale of this study was too small, a large sample size is needed for future investigations.

In summary, we found that miR-33a might be a new marker for hepatic stellate cell activation and hepatic fibrogenesis processes in both humans and mice. MiR-33a could be used to exploit future diagnostic and therapeutic tools to treat liver fibrosis.

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