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MicroRNA in hepatic fibrosis and cirrhosis

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1. ABSTRACT

Hepatic fibrosis is caused by an imbalance between production and dissolution of extracellular matrix after chronic and inflmmatory injury, when hepatic stellate cells are stimulated to proliferate and secret extracellular matrix. The most common causes of liver fibrosis are chronic viral hepatitis B and C. Cirrhosis is the most advanced stage of fibrosis, which usually develop into hepatocellular carcinoma (HCC). microRNAs participate the pathogenesis of hepatic fibrosis and cirrhosis or even the onset of HCC. In this review, we will summarize the role of miRNA in the pathogenesis of viral hepatitis fibrosis, non-alcoholic steatohepatitis fibrosis, primary biliary cirrhosis and HCC onset, especially in the regulation of stellate cells.

2. INTRODUCTION

Hepatic fibrosis is a histological stage triggered by chronic and inflmmatory injury, during which liver damage causes hepatic stellate cells (HSCs) to be over active and then the extracellular matrix (ECM) is overproduced, degraded deficiently, or both (1). The excessive collagen fiber deposits in the extra-cellular spaces of the liver cells, resulting in the liver cells ischemia and hardened. The hepatic fibrosis includes congenital and acquired types. In this artical, we focus on the acquired hepatic fibrosis. The most common causes of liver fibrosis are chronic viral hepatitis B and C. Cirrhosis is the most advanced stage of fibrosis, in which fibro-connective tissue is hyperplasia and pseudo-lobule is formed in liver parenchyma and the liver lobular structures are demaged,

causing blood flow changes and the potential development of liver failure. In worldwide, 30% of cirrhosis is attributed to hepatitis B and 27% is attributed to hepatitis C (2).

The microRNA (miRNA) is a small non-coding RNA molecule with 22 nucleotides, which functions in transcriptional and post-transcriptional regulation of gene expression (3). In alcoholic liver disease, a common cause of hepatic fibrosis and cirrhosis, the miR-155 and miR-132 in hepatocytes have been found to increased expresse both *in vitro* and *in vivo* with mice (4). *In vitro* study with mice later found miR-666 and miR-708 targeted aquaporin-1 were decreased in cirrhosis (5). In the rat model of dimethylnitrosamine-induced hepatic fibrosis, the rno-miR-34 family is upregulated and participates liver fibrosis via targeting acyl-CoA synthetase long-chain family member 1, an enzyme invovled into lipid biosynthesis and fatty acid degradation (6). Study based on human liver tissues further showed the expression of miR-155, miR-454, miR-582-5p, let-7f-1*, miR-181d, and miR-500 were increased in cirrhosis, which were negative correlation with activity of hepatic cytochrome P4503A, a member of the cytochrome P450 family of oxidizing enzymes, involving in drug metabolism and synthesis of cholesterol, steroids, and other lipids components (7). By inhibiting miR-21 expression, the experimental hepatic fibrosis can be alleviate (8). These foundings suggest miRNAs participate the pathogenesis of hepatic fibrosis and cirrhosis, which may be invovled into the dyfuntion of injuried liver.

Through the hepatic fibrosis can be classified as viral hepatitis fibrosis, parasitic infection fibrosis, alcoholic fibrosis, biliary fibrosis, metabolic fibrosis, intoxication fibrosis, mal-nutritional fibrosis and cardiogenic fibrosis, the miRNA-related pathogenesis mostly are focused on viral hepatitis fibrosis, non-alcoholic steatohepatitis fibrosis and primary biliary cirrhosis. Cirrhosis usually progresses into hepatocellular carcinoma (HCC), which causes 745,000 deaths worldwide per year (9) about half of them in China. About 80% to 90% patients with HCC is present cirrhosis (10). Thus, we will discuss the microRNA in the progress of HCC from cirrhosis. Therefore, in this review, we will summarize the role of miRNA in the pathogenesis of viral hepatitis fibrosis, non-alcoholic steatohepatitis fibrosis, primary biliary cirrhosis and HCC onset.

3. HEPATITIS B VIRUS-RELATED HEPATITIS FIBROSIS AND CIRRHOSIS

As we mentioned before, hepatitis B viral hepatitis is a significant cause of hepatic fibrosis and cirrhosis, the miRNAs in the hepatitis B virus (HBV)-related hepatitis fibrosis and cirrhosis have been concerned. The expression of miRNAs is found a different pattern in tissues between chronic hepatitis and liver cirrhosis, though no differences were found between HBV-positive and hepatitis C virus (HCV)-positive tissues (11). Moreover, the miR-602 expression was higher in chronic hepatitis B, liver cirrhosis or HCC than in normal ones, and this miRNA is cancerigenic in HBV-related hepatocarcinogenesis (12). The similar trande of expression was also found in miR-885-5p that its expression was increased in sera from patients with chronic hepatitis B, liver cirrhosis or HCC, and furthermore, miR-885-5p, miR-574-3p, miR-224, miR-215 and miR-146a were all up-regulated in the patients with liver cirrhosis or HCC, compared with from healthy controls (13), suggesting miRNAs may participate the development of liver cirrhosis or even HCC from chronic hepatitis B.

In addition to the expression of miRNAs invovling into the HBV-related liver cirrhosis and hepatocarcinogenesis, the mutations of miRNAs are associated with hepatic cirrhosis and HCC based on study in Korean population. Bae and collegues (14) observed that miR-101-1 rs7536540 single nucleotide polymorphism was associated with development of liver cirrhosis and HCC in patients with HBV. Later, the miR-196a-2 rs12304647 CC genotype was found to protect patients with chronic hepatitis and cirrhosis from HCC, compared with AA or AC genotypes (15). Furthermore, the miR-323b polymorphism is associated with the persistent infection of HBV in patients with chronic hepatitis or HCC (16). Since all these foundings on miRNAs polymorphism are based on Korean, future study with different ethnic groups may provide further information on the miRNAs polymorphism in HBV-related cirrhosis.

4. HEPATITIS C VIRUS-RELATED HEPATITIS FIBROSIS AND CIRRHOSIS

In the HCV-related hepatitis fibrosis, miRNAs change has been found to be associated with aggressive fibrosis progression in HCV patients after liver transplantation (17). A cohort study with HCV patients observed a 18-miRNA signature can distinguish hepatitis cirrhosis, dysplastic nodules and HCC lesions (18). Study based on chronic HCV patients observed that HCV can induced the expression of miR-200c, and then lead to a decreased expression of FAS associated phosphatase 1, resulting in an increased expression of collagen and fibroblast growth factor (19). Thus, miRNAs can participate the formation of hepatic fibrosis by targeting certain functional protein, triggered by HCV (Figure1).

miR-122 is the miRNA that is specifically expressed in liver and is also the most abundantly expressed miRNA in the liver (20,21). In HCV infection, miR-122 down-regulate RNA replication by inhibiting miR-122 (22). miR-122 enhances the replication of hepatitis C virus (23). The seed domain of miR-122 can also interact the complementary sequences in 5'untranslated region (UTR) of HCV RNA, resulting in up-regulating the translation and replication of the HCV genome (24-27). In liver transplant recipients for HCV cirrhosis, high HCV titer at recurrence was associated with higher level of miR-122 (28). Even the interaction between miR-122 with mutation in a seed domain and HCV RNA is essential for the enhancement of viral replication (29). Moreover, the level of serum miR-122 can predict the survival of patients with hepatic fibrosis (30). Thus, miR-122 play a crucial role in the HCV injury of liver.

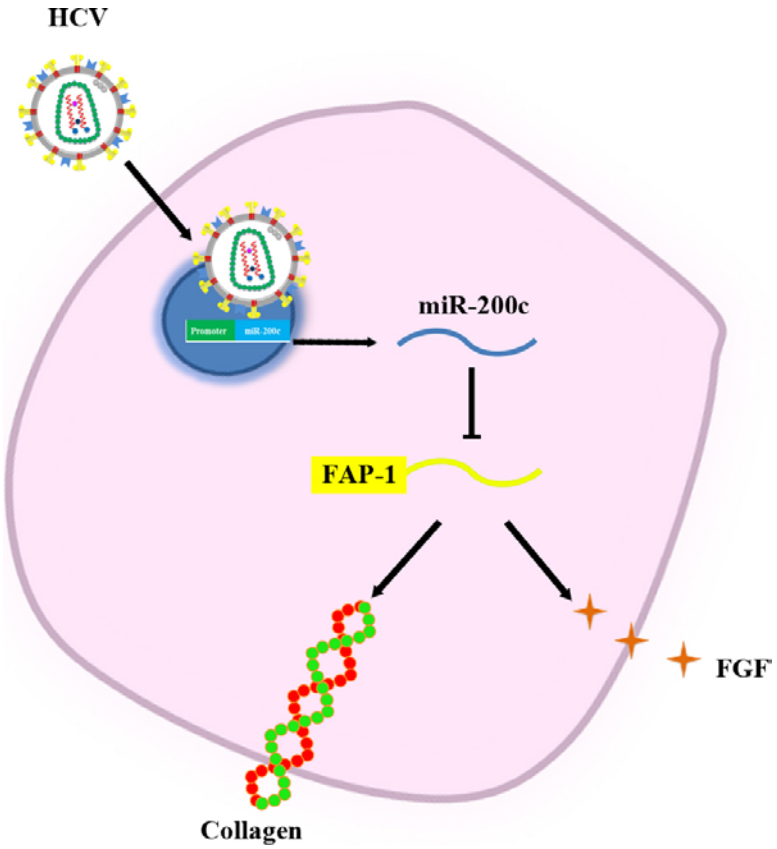

Figure 1. Hepatitis C virus (HCV) induces hepatitis fibrosis by regulating microRNA (miR)-200c. HCV can induced the expression of miR-200c, and then lead to a decreased expression of FAS associated phosphatase 1 (FAP-1), resulting in an increased expression of collagen and fibroblast growth factor.

In HCC patients with HCV infection and liver cirrhosis, miR-122 as well as miR-100 and miR-10a were increased expressed while miR-198 and miR-145 was decreased (31). But the serum level of miR-122 was found no difference between HCC patients and patients with liver cirrhosis (32). These suggest miR-122 expression may be abnormal in the stage of liver cirrhosis from HCV infection. Thus miR-122 may be a biomarker for HCV patients to become hepatic fibrosis.

5. NON-ALCOHOLIC STEATOHEPATITIS FIBROSIS AND CIRRHOSIS

In the non-alcoholic steatohepatitis fibrosis and cirrhosis, only miR-122 was found to associated with the hepatic injuries. Evern before the aminotransferases becoming abnormal after damaged by viral, alcohol, and chemical, there is an increased level of plasma miR-122 (33). In mice model of non-alcoholic steatohepatitis, the serum level of miR-122 is sensitive to early detect hepatotoxicity and liver injury (34). The possible mechanism of hepatic fibrosis occurance may be partially due to the miR-122a targeting the Klf6 transcript (35). More knowledge on the mechanism of miR-122 in non-alcoholic steatohepatitis fibrosis and cirrhosis is still need to further investigate.

6. PRIMARY BILIARY CIRRHOSIS

miR-122 is such a significant in liver that it invovled into the pathogenesis of hepatic fibrosis from not only non-alcoholic steatohepatitis fibrosis but also primary biliary cirrhosis, or even the occurance of HCC, which will be discussed later. In primary biliary cirrhosis, the miR-122a expression is decreased, as well as miR-26a, while miR-328 and miR-299-5p are increased expressed (36). All these altered expressed miRNAs in liver target genes participating cell proliferation, apoptosis, inflammation, oxidative stress, and metabolism (36). In the biliary epithelium of patients with primary biliary cirrhosis, miR-506 is increased expressed, binds the 3'UTR region of Cl-/HCO3- anion exchanger 2 mRNA, inhibiting this protein translation, resulting in the inactivation of this protein and dyfunction of biliary secretion (37) (Figure2).

7. HEPATOCELLULAR CARCINOMA

In cirrhotic and hepatitis-positive livers, more than 200 precursor and mature miRNAs were analyzed, and the results showed a global increase in the transcription of these miRNA genes, which may prognosticate the occurance of HCC (38). Study based on Chinese patients with cirrhosis found that miR-196a2 polymorphism may

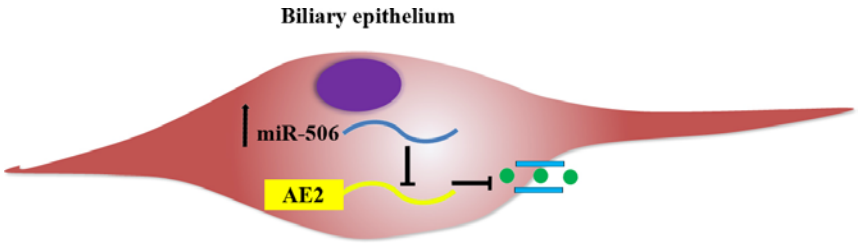

Figure 2. The microRNA (miR)-506 in the primary biliary cirrhosis. In the biliary epithelium of patients with primary biliary cirrhosis, miR-506 is increased expressed, binds the 3'UTR region of Cl-/HCO3- anion exchanger 2 (AE2) mRNA, inhibiting this protein translation, resulting in the inactivation of this protein and dyfunction of biliary secretion.

promote HCC occurance from cirrhosis via regulating mature miR-196a expression (39). In clinical, an increased expression of miR-183 is associated with HCC onset from cirrhosis and with TNM stage (40). Thus, miRNAs are invovled into the mechanism of HCC onset from hepatic fibrosis and may indicate canceration of cirrhosis.

Study with human liver tissues showed that a set of 12 miRNAs, including miR-21, miR-221/222, miR-34a, miR-519a, miR-93, miR-96, and let-7c, was associated with the development of HCC from normal liver through cirrhosis (41). Lendvai and collegues (23) found that miR-21, miR-221, miR-222 and miR-199a, differently expressed in HCC, had a decreased expression in patients at the stages of chronic HCV and fibrosis and HCC patients. The similar miRNAs, namely miR-21, miR-122, miR-192, miR-223, miR-26a, miR-27a and miR-801, also found differently expressed between HCC patients from healthy, chronic HBV and cirrhosis (42). From thses foundings, it can find that certain miRNAs, especially miR-21, may play a key role in the HCC occurance from chronic hepatitis, no matter HBV or HCV.

Since the significant role of miR-21 in the tumorigenesis as mentioned above, the possible mechanism is concerned. The transforming growth factor (TGF)-beta was found to be a key signalling in the miR-21 regulation that up-regulates miR-21 by producing a microprocessor complex containing Smad proteins (43). In turn, the miR-21 up-regulates TGF-beta signalling by targeting Smad7, a negative regulator of TGF-beta, resulting in an increased fibrogenesis (44). When artificial miRNA reduces the mRNA expression of TGF-beta 1, the hepatic fibrosis is observed to decreased (45). The miR-181a was over-expressed in cirrhosis and HCC, which directly mediates the TGF-beta induced hepatocyte epithelial-mesenchymal-transition (46), suggesting miRNA may be the brigde between TGF-beta and cirrhosis or even HCC.

Moreover, a decreased expression of miR-29 was found to associated with severe hepatic fibrosis (43). The miR-29 promoter contains several binding sites of the Smad proteins and the Ap1 complex (43). The miR-29 reduces hepatic fibrosis after bile duct-ligation by regulating extrinsic pathway of apoptosis (47). However, during process of fibrogenesis, the Hedgehog signaling regulates the proliferation of MF-HSCs irrespective of miRNA-29 (48).

8. HEPATIC STELLATE CELLS IN HEPATIC FIBROSIS AND CIRRHOSIS

In the mechanism of miRNAs regulating hepatic fibrosis and cirrhosis, abnormality of cells in liver is regulated by miRNAs. For instance, endothelin-1 expression of rat liver sinusoidal endothelial cells was found to regulated by miR-199, while the human ones is regulated by not only miR-199 but also miR-155 (49). In the onset of HCC from cirrhosis, hepatoma-initiating cells may derive from hepatic progenitor cells stimulated by TGF-beta, which is regulated by microRNA-216a-modulated phosphatase (50). Among liver cells invovling hepatic fibrosis and cirrhosis, HSCs paly a crucial role. HSCs are resident vitamin A-storing cells in the perisinusoidal space of Disse between the sinusoidal endothelium and hepatocytes. In the process of hepatic fibrosis, hepatic injury stimulates HSCs to proliferate and secret ECM (1). The miR-132 can control the myofibroblast transdifferentiation of HSCs (51), suggesting the participatation of miRNAs during the hepatic fibrosis and cirrhosis invovling HSCs.

In the inhibition of hepatic fibrosis, The miR-19b inhibits HSC-mediated fibrogenesis (52). Further results showed possible mechanism of miRNAs inhibiting HSC-mediated fibrogenesis that the miR-16 inhibits HSC to proliferate and promotes it to apoptosis (53). And the miR-146a suppressed TGF-beta-induced HSC proliferation and increased HSC apoptosis by targeting Smad4 (54). Though miR-29b suppresses type I collagen, resulting in anti-fibrosis (55), miR-29a seems to inhibite hepatic fibrosis through increasing the activation of nuclear receptor farnesoid X receptor, which has potent antifibrotic activity in HSCs (56). The miR-335 inhibits HSC migration and reduced alfa-SMA and collagen type I, in turn, miR-335 is reduced during HSC activation and migration (57). Moreover, exosomal transfer of miR-214 from mouse or human HSCs suppresses alpha smooth muscle actin or collagen by targeting connective tissue growth factor (CCN2), resulting in down-regulating CCN2-dependent fibrogenesis (58).

On the other hand, miRNAs promote HSC-mediated fibrogenesis. The miR-214-5p is regulated by Twist-1 to stimulate the activation of HSCs and promote the progression of liver fibrosis (59). Furthermore, an feedback participates the development of hepatic fibrosis.

The microRNA-21, programmed cell death protein 4 and activation protein-1 constitute an autoregulatory feedback loop in HSCs to promote the hepatic fibrosis (60). Thus, miRNAs have a dual role of hepatic fibrosis in HSCs, further confirming the crucial role of HSCs in hepatic fibrosis.

9. CONCLUSIONS

In conclusion, miRNAs are invovled into not only the development of hepatic fibrosis and cirrhosis from viral hepatitis fibrosis, non-alcoholic steatohepatitis fibrosis and primary biliary cirrhosis, but also the onset of HCC secondary to cirrhosis. The HSCs play a significant role in the hepatic fibrosis and cirrhosis under a dual regulation by miRNAs. Further understanding of the miRNAs in hepatic fibrosis and cirrhosis may provide noval therapeutic targets for hepatic fibrosis and cirrhosis.

10. ACKNOWLEDGEMENTS

Xuan Xin and Yongxian Zhang are co-first authors.

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Abbreviations: HCC, hepatocellular carcinoma; ECM, extracellular matrix; HSCs, hepatic stellate cells; miRNA, microRNA; HBV, hepatitis B virus; UTR, untranslated region; CCN2, connective tissue growth factor

Key Words: Hepatocellular Carcinoma, Hepatic Fibrosis, Stellate Cells, Hepatocellular Carcinoma, Review

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