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Role of MicroRNAs in Hepatic Fibrosis Progression

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ABSTRACT

Acute liver injury induced deviation in liver architecture are transient and often reversible. While chronic liver diseases lead to progressive net accumulation of extracellular matrix (ECM) in the liver leading to hepatic fibrosis. If hepatic fibrosis is untreated, hepatic fibrosis can lead to cirrhosis and consequence of organ failure and death. Hepatic stellate cells (HSCs) activation plays a significant role in the pathogenesis of hepatic fibrosis. MicroRNAs are short, non-coding, single-stranded RNA and their modulation upon chronic liver injury are said to involve in the activation and proliferation of HSCs that causes synthesis and accumulation of the enormous amount of ECM proteins by HSCs in liver hepatic fibrosis consequently. This review summarizes the modulation of key miRNAs pertaining to hepatic fibrosis.

INTRODUCTION

Epidemiological studies revealed that chronic liver disease (CLD) is one of the important sources of health and economic burden worldwide. According to the Global Burden of Disease Study in 2010, about 1.75 million deaths were attributable to CLD regardless of any etiology (Udompap *et al.,* 2015). The chronic liver disease is responsible for 2 million deaths annually. The main etiological factors associated with this mortality were found to be alcoholic liver disease and hepatitis B and C viral infections (Udompap *et al.,* 2015). Hepatic fibrosis is one of the common pathological sequel of all CLD and responsible for significant morbidity and mortality worldwide (Ezhilarasan *et al.,* 2017).

Hepatic fibrosis

Hepatic fibrosis is commonly preceded by CLD and result from the progressive accumulation and decreased degradation or remodeling of the extracellular matrix (ECM), any etiology, including viral infection, alcoholic liver disease and non-alcoholic steatohepatitis (Ezhilarasan *et al.,* 2015). In response to chronic liver injury, hepatic stellate cells (HSCs) undergo transdifferentiation of quiescent into contractile, proliferative and highly activated myofibroblast (MFB) like phenotype and this phenotypic activation is demonstrated in experimental and clinical studies and is considered as central event of hepatic fibrosis (Tsuchida and Friedman, 2017; Ezhilarasan *et al.,* 2016; Ezhilarasan *et al.,* 2012). If hepatic fibrosis is untreated, hepatic fibrosis causes distortion of liver architecture leading to organ contraction, nodular formation, liver cirrhosis, and hepatocellular carcinoma (HCC) and ultimately death (Jung and Yim, 2017).

MicroRNAs and chronic liver diseases

MicroRNAs (miRNA or miR) are short, 18 to 25 nucleotide long, non-coding, single-stranded RNA that function as regulatory molecules and said to involve in a series of vital processes including cell growth and differentiation, apoptosis, metabolism and the pathogenesis of various human diseases (Kitano and Bloomston, 2016). A single miRNA is shown to regulate several genes and vice versa. Previous studies have demonstrated the significant role of miRNA in liver diseases (Hayes and Chayama, 2016). It has been mentioned that even a

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small alteration in miRNA expression may significantly affect the battery of gene expressions and thus alter the transcriptome (Kerr *et al.,* 2011). In liver, the interference of miRNA expressions has been implicated in varieties of CLD.

miRNA expression is considered tissue or a specific organ. For instance, in liver, miR-122 is one of the most abundant in hepatocytes (~70%). miRNAs present outside the hepatocytes such as in neutrophils, HSCs are also implicated for their role in CLD. For instance, miR-22 is an important regulator to block neutrophil infiltration in alcoholic liver diseases (ALD) (Li *et al.,* 2016). Studies have also documented that miR-122, 155, 34a, 21, 146a and 125b are increased in the circulation of CLD patients (Shigehara *et al.,* 2011; Weber *et al.,* 2010).

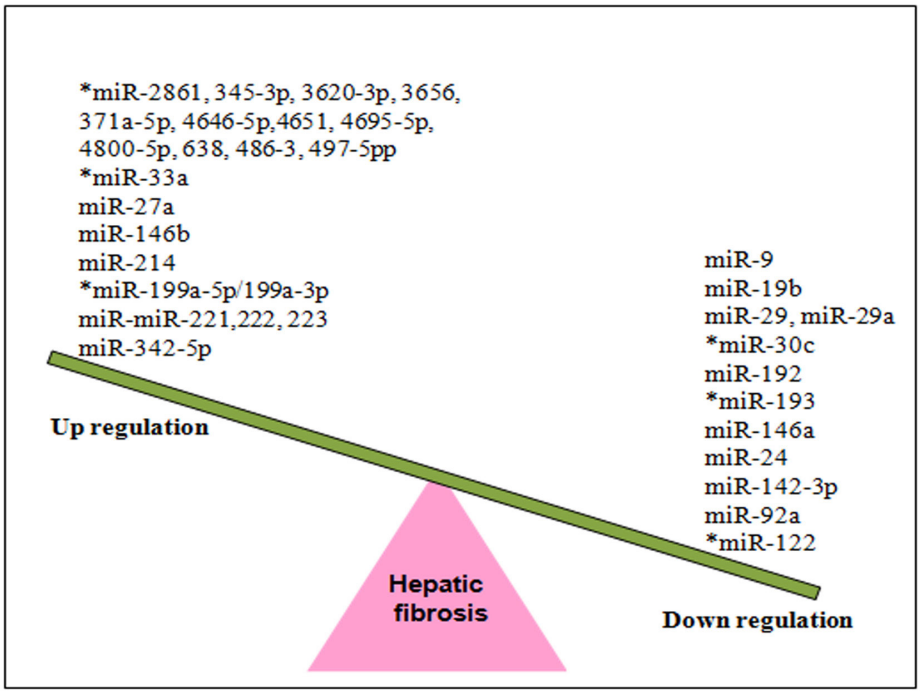

Fig. 1: Modulation of some of the key miRNAs in liver during the progression of hepatic fibrosis. *clinical studies.

Role of microRNAs in hepatic fibrosis

As mentioned before, HSCs play a significant role in the progression and onset of hepatic fibrosis. Therefore, studies have identified HSCs specific miRNAs that are responsible for early events of hepatic fibrosis (Figure 1). The first miRNA expression profile in human HSCs was reported by Coll *et al.* (2015). They have identified key miRNAs pertain to the maintenance of the quiescent phenotype of HSCs. It is shown that miR-192 downregulation during the activation of qHSCs in experimental studies and that over expression of miR-192 found to have a key role in the HSCs activation by reducing the perpetuating potentials of HSCs (Coll *et al.,* 2015).

Role of miRNAs in TGF-β signaling and ECM synthesis

Transforming growth factor beta (TGF-β) is a profibrogenic cytokine that drives hepatic fibrosis by inducing HSC proliferation and ECM production (Xu *et al.,* 2016). In CCl_4-induced hepatic fibrosis mice model, miR-29 mediates the regulation of liver fibrosis. miR-29 is said to regulate TGF-β- and nuclear factor-κB (NF-κB)-dependent decrease of miR-29 family members in HSC with consequent increase of ECM related gene expressions. The above findings were well correlated with clinical studies in which patients with advanced liver cirrhosis showed significant decrease in miR-29a levels in their serum than that of normal subjects or patients with early fibrosis (Roderburg *et al.,* 2011) Further, it has been identified that miR-30c and miR-193 are also responsible for TGF-β-dependent regulation of controlling ECM genes expressions in CCl_4-induced fibrosis model. The miR-30c and miR-193 have been shown to downregulate during the hepatic fibrosis in experimental and human liver (Roy *et al.,* 2015). These studies clearly indicate the fact that miR-29a, miR-30c, and miR-193 have the predominant role in the TGF-β mediated ECM related gene expressions and activation of HSCs and hepatic fibrosis.

It is a well-established fact that TGF-β is one of the important fibrogenic cytokine responsible for the activation of quiescent HSCs. Inhibitory effect of miR-19b *in vitro* analyses of HSCs has been reported. TGF-β signaling is inhibited by the miR-19b. It was observed that the downregulation of miR-19b leading to activation of HSCs while it's upregulation shows concomitant fibrosis regression in rat liver. Similarly, miR-19b expression was markedly downregulated in the onset of hepatic fibrosis in human (Lakner *et al.,* 2012). The miR-146a is shown to downregulate in liver fibrotic tissues and it was suggested as a novel regulator to modulate HSC activation during TGF-β1 induction by targeting SMAD4 (He *et al.,* 2012). Regulation of miR-33a expression in liver tissue was also implicated in the hepatic fibrosis. Particularly, miR-33a expression increased only in HSCs and not in other fibrosis associated cells such as Kupffer cells, hepatocytes etc. The upregulation of miR-33a expression activates the TGF-β1

signalling in HSCs (Huang *et al.,* 2015). Ge *et al.* (2014) reported that modulation of 48 miRNAs was found in porcine serum-induced hepatic fibrotic rats. Among 48 miRNAs the expressions levels of miR-27a and miR-146b have been significantly increased with concomitant increase in fibrotic marker gene expressions such as TGF-β and collagen 1. The functional role of miR-9 was investigated in a CCl_4-induced mouse model of liver fibrosis. Interestingly, miR-9 level was found to be downregulated in fibrotic liver tissue and in activated HSCs. miR-9 limits liver fibrosis by controlling the perpetuating nature of activated HSCs by directly targeting multidrug resistance-associated protein 1 (MRP1/ABCC1) (Sun *et al.,* 2017). Inhibition of miR-24 has directly increased the TGF-β1 expression consequently increasing hepatic fibrosis (Hall *et al.,* 2017).

Moreover, the profibrogenic role of miR-214 has been previously studied using transgenic mouse model (PDGF-C TG) in which platelet-derived growth factor C (PDGF-C) is over expressed resulting in hepatic fibrosis. The upregulation of miR-214 has been well correlated with the progression of hepatic fibrosis in PDGF-C TG mice. The miR-214 was found to involve in the progression of liver fibrosis by modulating the TGF-β related signaling pathways. Therefore, locked nucleic acid (LNA)-anti-miR-214 was injected into PDGF-C TG mice to evaluate the fibrosis regression. Interestingly, LNA-anti-miR-214 treatment significantly reduced the profibrotic gene expressions such as collagen 1a2 and 4a1, TGF-β1 and α-smooth muscle actin (α-SMA) with a concomitant decrease in the levels of p-extracellular signal-regulated kinases 1/2 and p-AKT (Okada *et al.,* 2015). It was also found that upregulation of miR-199a-5p/199a-3p and miR-221/222 in the human liver in a fibrosis progression-dependent manner. In particular, miR-221/222 was upregulated in LX-2 cells (human hepatic stellate cell line), also increased during the course of culture-dependent activation of mouse primary HSCs and also increased in the experimental mouse models of liver fibrosis (Ogawa *et al.,* 2012). This study demonstrated the upregulation of miR-221/222 in primary HSCs and immortalized HSCs cell line and even in experimental models.

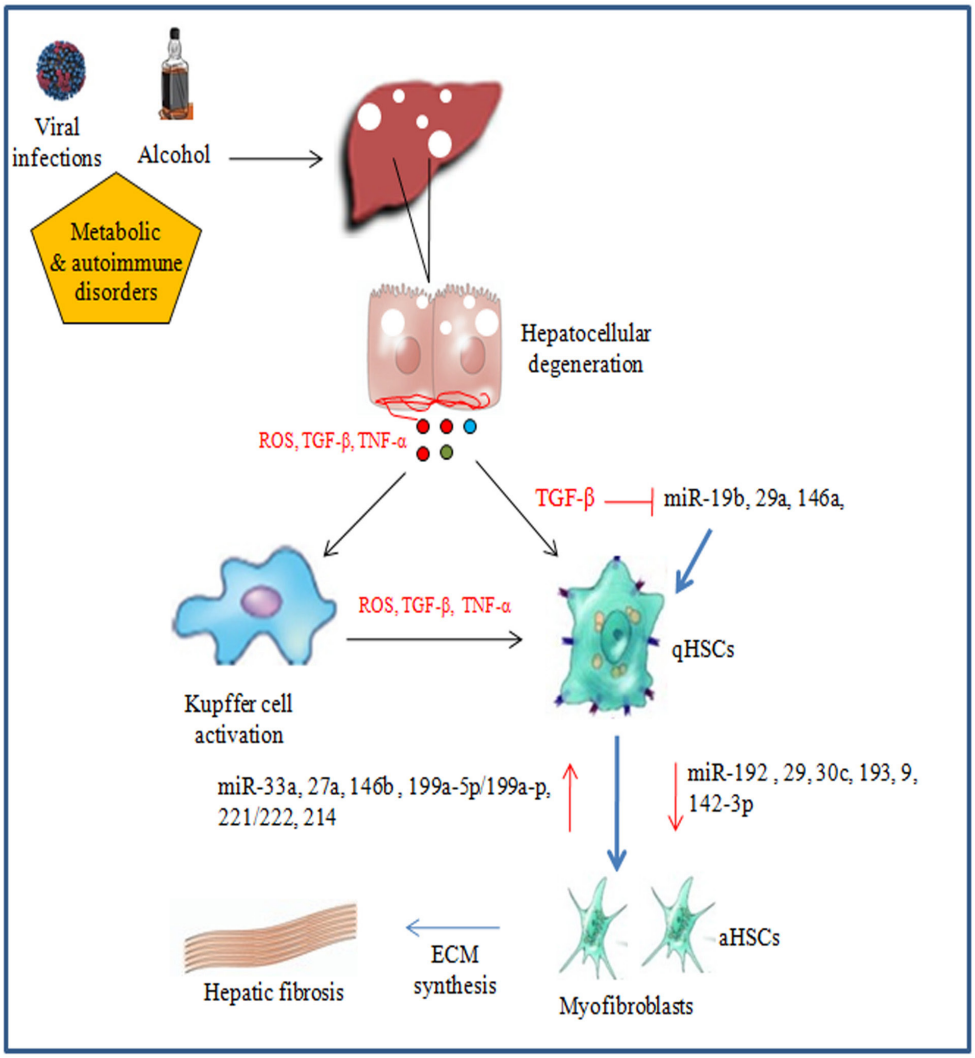

Fig. 2: Role of miRNAs in the progression of hepatic fibrosis due to various etiology. ROS-reactive oxygen species; TGF-β - Transforming growth factor-β; TNF-α - tumor necrosis factor-α; qHSCs - Quiescent hepatic stellate cells; aHSCs - Activ atedhepatic stellate cells, ECM-Extracellular matrix.

In a very recent study, leptin, an adipocyte-derived hormone has been reported to decrease the sterol regulatory element-binding protein-1c (SREBP-1c) and promotes the activation the HSCs. Further, miR-122 inhibited leptin-induced

liver fibrosis in a leptin-deficient mouse model (Zhai *et al.*, 2017). The miR-142-3p has recently identified as a novel regulator of activation of HSCs. The miR-142-3p level has been shown to reduce in the activated HSCs. Therefore, HSCs was transfected with mir-142-3p and studied for its effects against activated HSCs. This study reports that ectopic expression of miR-142-3p in activated HSCs caused a decrease in proliferation and viability and blocked HSCs activation (Yang *et al.*, 2017).

miRNAs and human hepatic fibrosis

In a recent study, miRNA expression pattern has been evaluated in liver tissue collected from the 40 chronic hepatitis B virus (HBV) associated hepatic fibrosis patients at stage S0-4. There were around 105 different miRNAs were found to modulated significantly in fibrotic tissues (Chen *et al.,* 2017a). In HBV associated hepatic fibrosis and cirrhotic patients, miR-30 has been directly regulated the expression of IL-6R, one of the potent pleiotropic cytokine that regulates cell growth and differentiation (Chen *et al.,* 2017b). In clinical studies, the paradigm of 43 hepatic miRNAs in NAFLD fibrosis model was reported previously. This study shows that differential expression of miR-17, miR-31, miR-150, miR-182, miR-183, miR-219a, miR-224, miR-378c, miR-378i, and miR-590 (Leti *et al.,* 2015). Schematic role of miRNAs in the activation of HSCs and onset of hepatic fibrosis is depicted in Figure 2.

Circulating miRNAs for early diagnosis of liver fibrosis

miRNAs released into the systemic circulation upon hepatocellular damage and have shown as a promising new class of tissue-specific biomarkers (Krauskopf *et al.,* 2017). Development of such a reliable non−invasive serum miRNA biomarkers may provide early detection of liver fibrosis and therefore studies have reported the modulation of miRNA expression in the circulation during the onset of hepatic fibrosis (Enache *et al.,* 2014). The miR-223 expression is shown to dysregulated in liver tissue of mice after induction of liver fibrosis. The levels were also found to concomitantly express highly in the serum during the fibrosis progression. The modulation of miR-223 has also been confirmed in clinical studies in which it was correlated with liver cirrhosis patients (Schueller *et al.,* 2017). The miR-122 levels found to decrease in circulation of patients with severe fibrosis due to hepatitis B and C virus infection and loss of liver cells were implicated in the decrease observed in the miR-122 in circulation during the fibrosis progression (Nakamura *et al.,* 2017; Trebicka *et al.,* 2013). This observation shows that analysis of miR-122 in serum could be one of the possible markers for early diagnosis of hepatic fibrosis due to hepatitis virus infections. In another study, plasma level of miR-142-3p significantly decreased in patients with hepatic cirrhosis (Yang *et al.,* 2017). Undoubtedly, these studies show that circulating microRNAs can be potential and emerging biomarkers for early diagnosis of CLD.

CONCLUSION

During the progression of hepatotoxicity, a variety of signaling pathways triggered by proinflammatory factors and profibrogenic cytokines involving in the process of hepatic fibrosis. Dysregulation of miRNAs has been implicated in the onset of hepatic fibrosis. Following chronic liver injury modulation of miRNAs triggered hepatic fibrosis is associated with a) direct activation, proliferation, and migration of HSCs, b) expression of several profibrogenic cytokines such as TGF-β and PGDF etc., c) high expressions of genes responsible for ECM synthesis such as different types of collagens. Since miRNA modulation has the myriad role in the activation of quiescent HSCs into myofibroblasts; miRNAs may represent novel therapeutic targets for the strategies of treatment of hepatic fibrosis.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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