

Regulatory, diagnostic, and therapeutic roles of microRNAs in chronic liver diseases

Razieh Kazemzadeh¹, Maryam Kheirollahi², Seyed Ali Mard³, Akram Ahangarpour⁴, Feryal Savari⁵

(1) Department of Physiology, Ahvaz University of Medical Sciences, Ahvaz, Iran; (2) Department of Physiology, Kerman University of Medical Sciences, Kerman, Iran; (3) Clinical Sciences Research Institute, Alimentary Tract Research Center, Department of Physiology, The school of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; (4) Medical Basic Sciences Research Institute, Physiology Research Center, Department of Physiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; (5) Department of Medical Basic Sciences, Shoushtar Faculty of Medical Sciences, Shoushtar, Iran.

Abstract

Fibrogenesis is initially performed during tissue damage to protect the remaining tissues from the progressive death of epithelial cells, infiltration of immune and inflammatory cells, and local degrading enzymes. Inflammation can lead to excessive extracellular matrix deposition by fibroblasts and the induction of fibrosis in many organs, such as the liver. MiRNAs are small non-coding RNAs that mediate mRNA repression or destabilization, leading to translational repression. Owing to the wide range of roles of miRNAs in the development of fibrosis, especially liver fibrosis, many studies have focused on their diagnostic, regulatory, and therapeutic roles. In this study, we used medical science and general databases, including PubMed, Elsevier, Scopus, Nature, and Google Scholar, to find valid studies on the different roles of miRNAs in liver fibrosis. Because a large number of miRNAs with regulatory, diagnostic, and therapeutic roles in diseases associated with liver fibrosis have been identified and reported in this study, special attention to these elements is needed in the future of healthcare systems. (*Acta gastroenterol. belg.*, 2024, 87, 403-412).

Keywords: Liver fibrosis, MiRNAs, liver disease, diagnose.

Introduction

When a tissue is damaged or inflamed, it initiates a series of repair processes that, if out of control, can lead to tissue fibrosis. Fibrogenesis is initially performed to protect the remaining tissues from the progressive death of epithelial cells, infiltration of immune and inflammatory cells, and local degrading enzymes. Inflammation can lead to excessive extracellular matrix deposition by fibroblasts and the induction of fibrosis, which is generally considered a wound healing response. Various factors such as infectious agents, heat, mechanical damage, and autoimmune diseases can cause fibrogenesis and mediate tissue fibrosis through cytokines, chemokines, and immune and inflammatory factors. Interleukin one, six and thirteen, plus transforming growth factor-beta (TGF- β) are important factors in this process. Fibrogenesis plays a major role in many diseases, and multiorgan fibrogenesis is fatal. Liver fibrosis, idiopathic pulmonary fibrosis, and renal or skin fibrosis (1) are among the cases that we can name (2). Unfortunately, despite the wide pathological role of fibrogenesis in the aforementioned diseases, especially liver diseases, there are no comprehensive and integrated studies on reliable and valid diagnostic or therapeutic factors in this field.

Although miRNAs were first discovered in the nematode *Caenorhabditis elegans*, they are highly con-

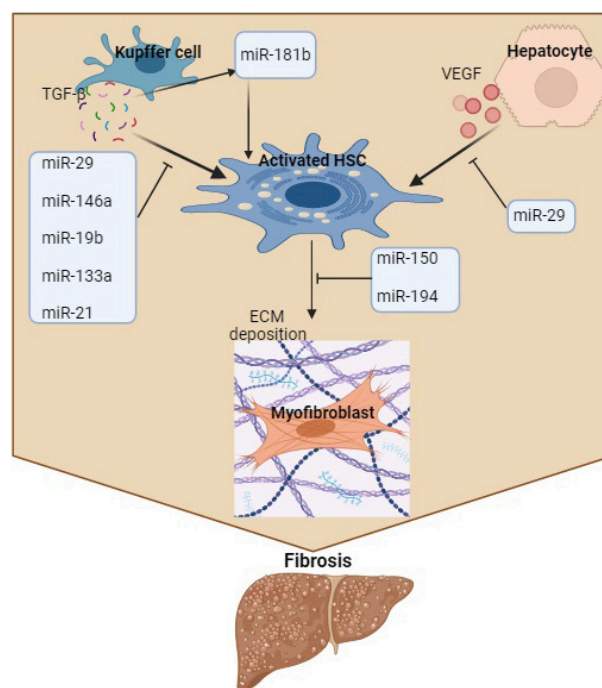


Figure 1. — The role of miRNAs in fibrosis. miR: microRNA; VEGF: Vascular endothelial growth factor; TGF- β : transforming growth factor-beta; HSC: Hepatic stellate cells; ECM: extracellular matrix.

served among species. MiRNAs are small non-coding RNAs with a length between 21 and 25 nucleotides that mediate mRNA repression or destabilization and lead to translation repression. Therefore, they are important regulators of gene expression and affect more than 60% of mRNAs (2,3). MicroRNAs are secreted from cells bound to proteins or enclosed in vesicles (4) and pair incomplete bases to the 3' end of mRNA and cause translation suppression. It has been known for decades that the complexity of gene expression is controlled by epigenetic mechanisms (such as what was said about the function of miRNAs) and the human genome itself

Correspondence to: Feryal Savari, Department of Medical Basic Sciences, Shoushtar Faculty of Medical Sciences, Shoushtar, Iran. Phone: +989166101504; Fax: +986113362411. Email: feryal.savari@gmail.com

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consists of very few coding genes. Nowadays, owing to the wide role of miRNAs in the development of fibrosis, especially liver fibrosis, many studies have focused on the diagnostic, regulatory, and therapeutic roles of miRNAs (2). For example, non-invasive diagnosis of fibrosis and liver diseases, like non-alcoholic steatohepatitis (NASH), is still a challenge, and common diagnostic methods such as biopsy, are expensive and painful (4), which reminds us of the necessity of using new and potential methods.

Among the known miRNAs that are effective against liver fibrosis, the following can be mentioned: 17-5p, 33a, 122, 132, 133a, 214, miR-29a, miR-138, miR-143, miR-34a, miR-22.

These miRNAs can affect molecules such as Mothers against decapentaplegic homolog 7 (SMAD7), Peroxisome proliferator-activated receptor α (PPAR α), prolyl 4-hydroxylase subunit alpha-1 (P4HA1), methyl-CpG-binding protein MECP2, collagen, and connective tissue growth factor (CTGF) (2,3). the overall role of miRNAs in the cell and the fibrosis-inducing signaling pathways they can affect is shown in Figure 1.

Owing to the lack of integrated and comprehensive studies on the diagnostic, regulatory, and therapeutic effects of miRNAs in chronic liver diseases, we have dedicated this research to this issue and the potential roles of miRNAs.

Methods

We used medical science databases and general databases including PubMed, Elsevier, Scopus, Nature, and Google Scholar to find valid studies. The population of the experiment, the year of conducting the research, the absence of contradictory results, and easy access to the article were among the criteria for entering the study in our research. It is worth noting that we also paid attention to the references that were in the selected articles of our research and used them.

1. Cirrhosis

The final stage of chronic liver diseases is cirrhosis, which results in the death of over one million individuals worldwide each year (1,5) such as obesity, non-alcoholic fatty liver disease, high alcohol consumption, hepatitis B or C infection, autoimmune diseases, cholestatic diseases, and iron or copper overload. Cirrhosis develops after a long period of inflammation that results in replacement of the healthy liver parenchyma with fibrotic tissue and regenerative nodules, leading to portal hypertension. The disease evolves from an asymptomatic phase (compensated cirrhosis). Liver cells, particularly hepatic stellate cells (HSCs), undergo apoptosis and regeneration under the influence of various factors, such as infection, cytokines, and miRNAs in liver cirrhosis. HSCs, also known as Ito cells, are located in the diss space and can present lipid antigens to T lymphocytes. They are also responsible for storing vitamin A (6,7) liver perisinusoidal

stellate cells play an important role as a main store of body retinol (vitamin A) (8). HSCs and fibroblasts are the main cells that produce extracellular matrix (ECM) (9). HSCs and Fibroblasts are the main cells that produce extracellular matrix (ECM), which is deposited in response to inflammation. Although excessive ECM deposition can lead to fibrosis, it serves as a protective mechanism under physiological conditions and typically ends at the proper time. However, chronic inflammation can also lead to excessive ECM deposition and fibrosis (6). Some of the most common complications of cirrhosis include hepatocellular carcinoma, gastrointestinal bleeding due to portal hypertension, ascites, portal vein thrombosis, and hepatic encephalopathy (10).

MiR-29 acts as an anti-fibrotic mediator by inhibiting angiogenic factors such as vascular endothelial growth factor (VEGF) (11) probably via PI3K/AKT and JAK/STAT pathways (12). Conversely, the miR-126 family directly inhibits the negative regulators of the VEGF pathway and promotes angiogenesis. Sprouty-related, equine herpesvirus-1 domain-containing protein 1 (SPRED1) and phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2) are negative regulators of the VEGF pathway (13). In HSCs isolated from laboratory mice with liver fibrosis, miRNA-150 and miRNA-194 levels decreased. It has been previously reported that these two miRNAs inhibit HSC activation and ECM production through inhibition of c-myc and *rac1* expression (14). In contrast, in HSCs isolated from laboratory animals with liver fibrosis, the levels of several miRNAs are decreased, such as miR-29 (by affecting TGF- β , inflammatory signals such as lipopolysaccharide (LPS) and nuclear factor kappa B (NF- κ B)), miR 19b (through regulation of TGF- β signaling components, reduction of TGF- β receptor II (TGF- β RII) and SMAD3 expression), miR-146a (by inhibiting TGF- β 1 mediated epithelial-mesenchymal transition in hepatocytes), and miR-133a (by mediating TGF- β -dependent depression of collagen synthesis), and restoration of these miRNAs reduces liver fibrogenesis (15,16,17,18). Therapeutic agents that target miRNAs that affect TGF- β and VEGF may be key to treating portal hypertension in patients with liver cirrhosis. Specifically, miR-19a and miR-34a have a significant relationship with portal vein thrombosis, while miR-21 can be considered a biomarker of portal vein thrombosis (19). In addition, miRNA-122 decreases in cirrhosis, and elevated miR-181b expression has been suggested as a potential diagnostic biomarker for cirrhosis (4).

MiR-21 reduces TGF- β expression and consequently suppresses HSC activation (20). TGF- β 1 induces the expression of miR-181a and miR-181b, and miR-181b can enhance HSC proliferation by regulating p27 and cell cycle (6). MiR-214-5p increases the expression of genes related to fibrosis, such as MMP-2, MMP-9, α -SMA, and TGF- β 1, in cells and can cause the activation of HSC and the progression of liver fibrosis (21). MiR-221 and miR-222 are increased in the human liver in parallel

Table 1. — Regulatory, diagnostic and therapeutic miRNAs in cirrhosis

Liver disease	Etiology	Complications	Regulatory miRNAs	Diagnostic miRNAs	Therapeutic miRNAs
Cirrhosis	Hepatic stellate cells (HSCs), undergo apoptosis and regeneration under the influence of various factors, such as infection, cytokines, and miRNAs in liver cirrhosis (6,7,8)	Hepatocellular carcinoma, gastrointestinal bleeding, ascites, portal vein thrombosis, hepatic encephalopathy (10)	MiR-29 (11), MiR-126 (13), MiR-221 (22), MiR-222 (22), MiR-21 (20), MiR-571 (23), MiR-214-5-p (21)	MiR-194 (14), MiR-150 (14), MiR-19 (15), MiR-146-a (18), MiR-133-a (17), MiR-34-a (19), MiR-19-a (19), MiR-181-b (4), MiR-122 (4), MiR-155 (25), MiR-223 (27)	MiR-21 (20), MiR-181-b (6), MiR-214-5-p (21), MiR-222 (22)
MiR: microRNA					

with the progression of fibrosis and are upregulated in mouse models of liver fibrosis. Inducing the expression of miR-222, TGF- α , or TNF- α causes miR-222 to bind to the CDKN1B (p27) 3'-translated region (UTR), and leading to the regulation of protein expression (22) miR-571 expression is altered in response to TGF- β in human hepatocytes and HSCs (23).

Patients with cirrhosis who have ascites and hepatorenal syndrome have significantly lower levels of MiR-122 compared to those with cirrhosis who do not exhibit these complications. Serum miR-122 levels are also associated with survival in patients with cirrhosis, independent of patient age (24). MiR-155 is a useful diagnostic marker for spontaneous bacterial peritonitis in patients with ascites, a complication of cirrhosis (25) calprotectin, and microRNA-155 (miR-155). Additionally, MiR-155 and MiR-223 levels are increased in patients with cirrhosis and spontaneous bacterial peritonitis who develop ascites (26,27). These miRNAs are likely to play a role in the immune response in ascites. The levels of miRNAs in animal models of induced hepatic encephalopathy show changes in the blood and cerebral cortex (28,29). Other miRNAs related to fibrosis, whose values are affected by cirrhosis, include miRNAs 21, 221, 222, 214-5-p, and miRNA-571. The mechanism of action of these agents is discussed in the discussion section (6). Because cirrhosis is the final stage of chronic liver diseases, doctors have little time to treat fibrosis. Therefore, it is important to use the capacity of miRNAs to diagnose and treat disease complications, and pay attention to their role in disease progression. Table 1 briefly mentions the etiology, complications, and regulatory, diagnostic and therapeutic microRNAs of liver cirrhosis.

2. Non-alcoholic fatty liver disease (NAFLD)/ Non-alcoholic steatohepatitis (NASH)

Non-alcoholic fatty liver (NAFLD) refers to the accumulation of fat in the liver cells, which is related to insulin resistance. In other words, when the volume and mass of fat stored in the liver is greater than 5%, the patient is diagnosed with fatty liver (30). One-third of the world's young adults are affected by NAFLD and their

number is increasing (31). The prevalence of the disease is higher in obese people and those with type 2 diabetes, reaching 70-90% (32). Complications of NAFLD include steatosis, steatohepatitis, fibrosis, and eventually the need for liver transplantation in the final stages of the disease. Fibrosis causes NAFLD progression (31). Currently, there is no definitive treatment for NAFLD (32,33) and Changes in lifestyle, diet and exercise are the only available options (34).

The progression of chronic liver steatosis towards hepatocyte death, along with the appearance of inflammatory processes in the liver, creates a condition called non-alcoholic steatohepatitis (NASH), which can lead to progressive liver fibrosis, cirrhosis, and hepatocellular carcinoma (34). NASH was first described by Ludwig et al. in 1980 (35). It is a stage in the spectrum of NAFLD (36) that affects approximately 75% of patients, most of whom are female and obese, with nearly all having diabetes. Apart from liver enlargement, which is detectable in many NASH patients, no other common clinical signs are observed until the typical symptoms and complications of cirrhosis develop (35).

Direct estimation of the prevalence of NASH at the national or global population level is almost impossible because a definitive diagnosis requires liver biopsy, which is rarely performed in patients. The prevalence of NASH has been estimated to be between 1.4% and 15%, using a biopsy case series from clinic outpatients or living donors for liver transplantation (37).

Recently, the discovery of the role of miRNAs in the differentiation of fat cells, insulin resistance, lipid metabolism in liver cells, and inflammation indicated their importance in the pathogenesis of fatty liver (38). Specifically, miR-16 has been shown to increase in parallel with the progression of NAFLD and has been introduced as a regulator of apoptosis, suggesting its potential to regulate hepatocyte apoptosis during NAFLD pathogenesis and have therapeutic potential (39). MiR-29c and miR-122 have also been reported to regulate insulin resistance and lipid metabolism, and play a role in the pathogenesis of fatty liver (40). This result has also confirmed in mice (41). MiR-216 and miR-302a have been found to play an important role in

the development of fatty liver and may also play a role in the elongation of palmitate to stearate. miR-34a has been shown to be increased in the serum of patients with NAFLD and reduce the biological function of sirtuin-1, which aggravates the accumulation of hepatic lipids (42,43) cancer, and possibly aging. Despite extensive studies on SIRT1 function, how SIRT1 levels are regulated remains relatively unknown. Here, we report that the nuclear bile acid receptor farnesoid X receptor (FXR(44)hyperlipidemia, and atherosclerosis in type 1 diabetic mice, we hypothesize that polyphenol-activated SIRT1 acts upstream of AMPK signaling and hepatocellular lipid metabolism. Here we show that polyphenols, including resveratrol and the synthetic polyphenol S17834, increase SIRT1 deacetylase activity, LKB1 phosphorylation at Ser428, and AMPK activity. Polyphenols substantially prevent the impairment in phosphorylation of AMPK and its downstream target, ACC (acetyl-CoA carboxylase). Suppression of miR-34a significantly reduces fat accumulation caused by free fatty acids (FFA) in liver cells and can be considered as a therapeutic option. Accordingly, it has been shown that intravenous injection of an miR-34a inhibitor reduces the progression of hepatic steatosis caused by a high-fat diet. In addition, suppression of endogenous miR-24 increases Insig1 and inhibits fat accumulation in the liver. MiR-21 also directly targets HMG-CoA reductase (HMGCR) and consequently regulates triglyceride and cholesterol metabolism in the liver (45). In conclusion, the potential role of miRNAs in the diagnosis and treatment of fatty liver disease has been demonstrated by considering all these factors together.

Circulating microRNAs are consistently associated with NAFLD, including miR-122, miR-21, miR-34a, miR-192, miR-193, and the miR-17-92 miRNA cluster (4). It has been found that miR-200, identified as a biomarker in different types of tumors, also plays a role in the pathogenesis of NAFLD through the MAPK pathway (31). MiRNA-122, miR-34a, and miR-21 are increased in NAFLD, and miR-34a is a diagnostic biomarker. Different members of the miRNA family, miR-17-92, are associated with NAFLD and NASH (4). Levels of miR-122, miR-34a and miR-16 are enhanced in patients with NAFLD (42).

On the other hand, MiR-192 appears to be significantly upregulated in NASH compared to NAFLD, whereas miR-193 differentiates NASH from fibrosis (4). MiR-372-3p and miR-373-3p expression is downregulated during NASH, whereas miR-34a is upregulated (2). MiR-372-3p regulates the expression of the fibrotic factor AEBP1 in NASH (46), while miR-34a activates miR-34a/Sirtuin 1(SIRT1)/p53 pro-apoptotic pathway (47). The increase in the levels of microRNAs (miRNA-122 and miRNA-192) in the experiment of Lee et al. caused the positive regulation of the expression of genes related to fibrosis, such as α -smooth muscle actin (α -SMA), collagen type 1 alpha 1 (coll1 α 1), and transforming growth factor β (TGF- β), leading to steatohepatitis and

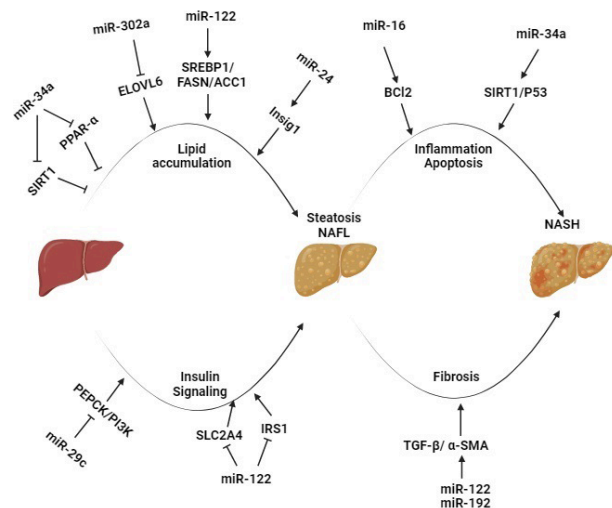


Figure 2. — The role of miRNAs in NAFLD and NASH. miR: microRNA; SREBP1: Sterol regulatory element-binding transcription factor; PPAR- α : Peroxisome proliferator-activated receptor alpha; FASN: Fatty acid synthase; ACC1: Acetyl-CoA carboxylase; NASH: Non-alcoholic steatohepatitis; NAFL: Non-alcoholic fatty liver; PEPCK: Phosphoenolpyruvate carboxykinase, PI3K: Phosphoinositide 3-kinases; TGF- β : transforming growth factor-beta; α -SMA: α -smooth muscle actin.

liver fibrosis (48). It has been observed that miR-122 and miR-192 levels were increased in mice fed with choline-deficient l-amino acid (CDAA) was used as a steatohepatitis-inducing diet (49). MiR-34a, miR-122, and miR-192 levels are independently associated with liver steatosis and fibrosis. Jampoka et al. showed that the decrease in miR-29a serum level was significantly negatively correlated with NASH (50). These studies show the important role of miRNAs in the pathogenesis of NASH and the possibility of their potential use as clinical biomarkers.

According to HOE et al. study, an increase in liver fibrosis in mice is likely to decrease the level of anti-fibrotic miR-223 and upregulate the target gene (TAZ). TAZ is involved in the progression of fibrosis and NASH (51). These data emphasize the antifibrotic therapeutic potential of miR-223. In addition, miR-690 directly inhibits fibrogenesis in HSC and inflammation in hepatocytes, as the injection of miR-690 into NASH mice inhibits all features of the NASH phenotype. These studies emphasize the therapeutic potential of miRNAs, including miR-690 (52).

Increased levels of serum miRNA-122, -192, and -21 were significantly associated with NASH in Becker et al.'s study and were suggested as a biomarker in NASH patients (53). An overview of the roles of miRNAs in NAFLD and NASH is shown in Figure 2. Table 2 and table 3 briefly mentions the etiology, complications, and regulatory, diagnostic and therapeutic microRNAs of NAFLD and NASH, respectively.

3. Alcoholic fatty liver disease (AFLD)

Alcohol is a psychoactive substance that is responsible for a significant burden of disease and death worldwide (54) regional and country consumption of alcohol, patterns of drinking, health consequences and policy responses in Mewomber States. It represents a continuing effort by the World Health Organization (WHO) (55). According to the World Health Organization, alcohol consumption was estimated to cause 3.3 million deaths in 2013, making it one of the top 20 leading causes of death globally. Alcohol consumption is also a major risk factor for a range of diseases, including nervous system disorders, digestive diseases, cancer, injuries, cardiovascular disease, fetal alcohol syndrome, diabetes, infectious diseases, and liver disease. Alcoholic fatty liver (AFLD), a complication of alcohol consumption, is often associated with steatosis and fibrosis and can lead to severe alcoholic hepatitis or alcoholic cirrhosis. Apart from hepatomegaly, AFLD is often asymptomatic, but can be treated and reversed with lifestyle changes. Similar to NAFLD, AFLD involves the accumulation of fat in liver cells and increased production of cytoplasmic triglycerides. The induction of tumor necrosis factor- α (TNF- α), reduction of fatty acid oxidation, and increase in lipogenesis in liver cells due to alcohol consumption cause steatosis and the pathogenesis of AFLD. AFLD-induced fibrosis also significantly increases the risk of hepatocellular carcinoma (HCC). Liver biopsy is sometimes to diagnose AFLD or differentiate between AFLD and alcoholic steatohepatitis (ASH) (56,57).

MiRNAs play a crucial role in regulating the complex interactions between inflammatory signals by controlling the expression of cytokines and other pathway components. They also have significant modulatory effects on macrophage activation and differentiation. Recent studies have shown even more significant effects of miRNAs in ALD development, including their involvement in intercellular communication, secretion in exosomes,

expression of enzymes linked to alcohol metabolism, and modulation of pro-inflammatory pathways such as the B-cell translocation gene signaling pathway by miR-497 (58).

It has been demonstrated that miR-122, miR-123 and miR181b-3p have been shown to inhibit AFLD and alcoholic liver disease (ALD), whereas miR-155 and miR-217 promote disease progression (3). Downregulation of miR-122 induces the release of tumor necrosis factor- α from Kupffer cells and inflammation, which is mediated by lipopolysaccharide attachment to Toll-like receptor 4 (TLR4). In patients with ALD and mice, the beneficial role of miR-122 is modulated by granule-like transcription factor 2 (GRHL2) to regulate hypoxia-inducible factor 1 alpha (HIF1a) (59). The MALAT1/miR-181a-5p axis also involve in regulating of collagen production and inflammation via activating TLR4/NF- κ B signaling (60). Mice lacking miR-155 are immune to alcohol-induced steatosis and inflammation. Inhibition of miR-155 increases peroxisome proliferator-activated receptor response element (PPRE), peroxisome proliferator-activated receptors (PPAR- α), as miR-155 target, and decreases MCP1 production, increases PPAR γ expression in liver macrophages, decreases lipid metabolism-related genes expression such as Fatty Acid-Binding Protein 4 (FABP4), Liver X receptor alpha (LXR α), Acetyl-CoA carboxylase (ACC1) and LDL receptor (LDLR), decreases the number of CD163⁺ and CD206⁺ macrophages and neutrophils, increases C/EBP β , decreases the expression of pro-fibrotic genes, decreases hydroxyproline and α -SMA caused by carbon tetrachloride (CCl₄), inhibits TLR4 activity and increases the expression of FoxO3 (61,62). Other miRNAs, such as let-7, miR-19b, miR-26a, miR-27a, miR-34a, miR-103, miR-27a104, miR-214, miR-212, miR-200, miR-199, miR-182, miR-223; and miR-217 are involved in the pathogenesis of alcoholic liver diseases and act on molecules such as TGF- β RII, Col1 α 2, MeCP2, FASLG, DR5, Crebl2, SIRT1, CASP2, Caveolin-1. (46) Some of

Table 2. — Regulatory, diagnostic and therapeutic miRNAs in NAFLD

Liver disease	Etiology	Complications	Regulatory miRNAs	Diagnostic miRNAs	Therapeutic miRNAs
NAFLD	accumulation of fat in the liver cells, related to insulin resistance (30)	steatosis, steatohepatitis, fibrosis, need for liver transplantation (31)	MiR-16 (39), MiR-29-c (40), MiR-122 (40), MiR-302-a (42), MiR-216 (42), MiR-21 (45)	MiR-122 & MiR-193, MiR-192 & MiR-17-92, MiR-34-a (4), MiR-200 (31)	MiR-16(42), MiR-34-a(45), MiR-24(45)

MiR: microRNA; NAFLD: non-alcoholic fatty liver disease.

Table 3. — Regulatory, diagnostic and therapeutic miRNAs in NASH

Liver disease	Etiology	Complications	Regulatory miRNAs	Diagnostic miRNAs	Therapeutic miRNAs
NASH	progression of chronic liver steatosis towards hepatocyte death, along with the appearance of inflammatory processes in the liver (34)	fibrosis, cirrhosis, and hepatocellular carcinoma (34)	MiR-192 & MiR-193(4), MiR-372-3p(46), MiR-373-3p(2), MiR-34-a(47)	MiR-29-a(50), MiR-122 & MiR-192 & MiR-21 (53)	MiR-223 (51), MiR-690(52)

MiR: microRNA; NASH: non-alcoholic steatohepatitis.

these miRNAs, including miR-27a, miR-192, miR-30a, and miR-182, have a diagnostic value (63,64).

To date, no comprehensive studies have supported the therapeutic role of miRNAs in ALD. However, some miRNAs may serve as potential therapeutic targets. For example, in rats treated with ethanol, the levels of importin α 5 and miR-181b-3p were found to be alleviated, indicating that miRNAs may play a therapeutic role (65). Miravirsin, an inhibitor of miR-122, has shown promising results for the treatment of chronic hepatitis C and ALD. Miravirsin works by hybridizing to mature miR-122 and blocking its interaction with HCV RNA (66,67). There are indirect data suggesting that inhibition of miR-155 can reduce in KC sensitivity to LPS-mediated activation (68). MiR-21, which promotes hepatocyte regeneration (69); miR26a, which protects hepatocytes from the development of fibrosis (70); miR-223, which inhibits neutrophil activation and liver infiltration (71) and how miR-223, one of the most abundant microRNAs (miRNAs; and miR-378, which exerts a stall signal in HSC (72), are all potential therapeutic target for ALD. However, more studies are needed before their clinical application. Table 4 briefly mentions the etiology, complications, and regulatory, diagnostic and therapeutic microRNAs of AFLD.

4. Viral Hepatitis

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are non-cytopathic and hepatotropic members of the hepadnavirus and flavivirus families, respectively. These viruses spread through contact with infected blood and body fluids, and cause acute and chronic necroinflammatory diseases of the liver. Hepatitis A virus (HAV) is also a member of picornavirus family, an RNA virus that infects 1.4 million people in the world every year and killed more than seven thousand people in 2016. Almost half of these cases were reported in Asia. The enterohepatic cycle of HAV continues until the body responds with a strong immune response to antibodies (73,74). In addition, persistent HBV infection is associated with various degrees of chronic liver disease and, in most cases, leads to cirrhosis and hepatocellular carcinoma (HCC). More than 350 million people worldwide are infected with HBV, and every

year, almost one million people die of this disease and its complications. Unlike HBV infection, 70%-90% of acute HCV infections remain persistent. Approximately 170 million people worldwide are chronically infected with HCV and experience complications similar to end-stage liver cirrhosis and HCC. Although a highly effective vaccine against HBV infection has been developed and used over the past 20 years, there has been no vaccine for HCV to date (73,74).

Specific antibodies against hepatitis A virus (anti-HAV) and anti-hepatitis A virus immunoglobulin (Ig) M can be detected in the serum and are common tools for hepatitis A diagnosis. Serological diagnosis of HBV can be misleading because some hepatitis B patients, despite HBsAg clearance in the body, do not show any hepatitis B virus surface antibody (HBsAb) (74). Third-generation enzyme immunoassays (EIAs) detect antibodies against different hepatitis C virus antigens after 2-6 months of the disease period (75).

No specific treatment for HAV has been reported, and treatment is mainly symptomatic. Clinicians have suggested viral suppression therapy (for example, nucleoside/nucleotide analogs and PEG-interferon) in patients with HBV (76,77). Antiviral therapy is considered for almost all patients with HCV, such as interferon alpha, ribavirin, and direct-acting antivirals (DAAs). Eradication of the virus and reduction of cirrhosis and related complications, such as portal hypertension, HCC, the need for liver transplantation and death, are among the treatment goals considered for HCV (74).

MiRNAs are important factors in HCV pathogenesis and act on critical host cellular factors required for HCV replication and enhanced cell growth. In the altered expression of miRNAs, the control of signaling pathways such as immune response, proliferation, and apoptosis can be observed. miRNAs play a role in the communication between different liver cells. Many studies have acknowledged that circulating miRNAs can be used as biomarkers of disease progression and can be targeted due to their therapeutic potential. (78) liver cirrhosis, hepatocellular carcinoma, and a major reason for liver transplantation. Discovery of direct acting antiviral (DAA

MiRNAs affect the cell cycle and pathogenesis of hepatitis by directly and indirectly interacting with

Table 4. — Regulatory, diagnostic and therapeutic miRNAs in AFLD

Liver disease	Etiology	Complications	Regulatory miRNAs	Diagnostic miRNAs	Therapeutic miRNAs
AFLD	accumulation of fat in liver cells and increased production of cytoplasmic triglycerides (56,57)	steatosis, fibrosis, hepatomegaly, hepatocellular carcinoma (HCC) (56,57)	miR-122 & miR-123 & miR-181-b-3p & miR-34-a (3), miR-181-a-5p (60), miR-26-a & miR-19-b & miR-let7 & miR-199 & miR-200 & miR-212 & miR-214 & miR-103 & miR-223 & miR-217 & miR-497 (58)	miR-30-a & miR-192 & miR-27-a & miR-182 (63,64)	miR-155 (68), miR-181-b-3p (65), miR-122 (59), miR-21 (69), miR-26-a (70), miR-223 (71), miR-378 (72)
MiR: microRNA; AFLD: alcoholic fatty liver disease					

the viral genome, related proteins, and cell signaling molecules.

It has been found that miRNAs can be used as biomarkers and therapeutic tools for liver diseases and hepatitis (79). Eight miRNAs, including miRNA206, miRNA141-3, miRNA433-3, miRNA1228-5, miRNA199a, miRNA122-5, miRNA192-5, and miRNA26a5 were been introduced by Tan et al. as miRNAs related to hepatitis B that have diagnostic potential (79). Some studies have reported that miRNA222 and miRNA2224 are increased in hepatitis B and C (80,81,82,83), but contradictory results have been reported in other studies (84) miRNA-222 and tumor suppressor miRNA-145 in malignant hepatocytes. Methods: Expressions of miRNA-21, miRNA-222 and miRNA-145 were measured in HepG2 cells transfected with HBx-plasmid (genotype D. It has been proven that miRNA155 increases hepatitis C and has diagnostic value.

miR-122 is involved in the regulation of hepatic metabolic pathways (e.g., cholesterol biosynthesis) and exacerbates hepatitis C virus replication (85). During chronic hepatitis C, miR-34a, miR-16, miR-143, and miR-223 are also upregulated, whereas miR-122 expression is downregulated during fibrosis progression (86,42). In patients with chronic HBV infection, the expression of miR-185 increases, whereas the expression of miR-29, -143, -21, and miR-223 decreases during the progression of fibrosis. In general, it has been shown that miR-29, miR-143, miR-185, miR-21, miR-221, miR-222 and miR-223 have fibrotic or anti-fibrotic roles in viral hepatitis (86). A deeper understanding of the relationship between miRNA regulation and the immune response in liver diseases and hepatitis may lead to the use of miRNAs as diagnostic and therapeutic tools.

It has been proven that miRNA155 increases hepatitis C and has diagnostic value (87). miRNA122 is a promising therapeutic tool for chronic hepatitis C virus infection (79). However, further studies are required to the clinical and widespread use of miRNAs in hepatitis. Table 5 briefly mentions the etiology, complications, and regulatory, diagnostic and therapeutic microRNAs of viral hepatitis.

5. Liver carcinoma

Rokitansky first described and introduced hepatocellular carcinoma in 1849 (88). Globally, more than six hundred thousand new cases of liver cancer have been

reported annually. Hepatocellular carcinoma (HCC) is one of the 10 most common cancers worldwide. Liver carcinoma is a common and fatal malignant and invasive tumor with metastasis; and for example, it is considered the second leading cause of death from cancer in China. In liver carcinoma caused by the hepatitis virus, the continuous process of liver cell necrosis and renewal with inflammation causes nodular regeneration, cirrhosis, progressive genomic errors in liver cells, and irregular growth and repair mechanisms, ultimately leading to hepatocyte dysplasia. Surgical removal of the liver, liver transplantation, chemotherapy, gene therapy, immunotherapy via weighing blood cells, and even the use of traditional medicine methods are common treatment options (89,90,91,92). Major risk factors for HCC are liver cirrhosis, chronic hepatitis B virus (HBV) infection, chronic hepatitis C virus infection, and chronic consumption of alcohol (93).

Since miR-4454 and miR-425 increase the proliferation, migration, and invasion ability of liver cancer cells (through pathways such as the Wnt/ β -catenin/EMT signaling pathway), inhibition of these miRNAs can be used as an effective therapeutic tool (94,95). It should be mentioned that it has been determined that mir 939 and 595 are specific biomarkers of hepatocellular carcinoma (96).

Histological changes of the liver of mice with hepatocellular carcinoma in Terindiak et al.'s experiment have been associated with various circulating levels of liver and plasma miRNAs, including miR-34a, miR-181a, and miR-200 (49).

In a study by Fornari et al. enhancement of circulating miRNAs was the most relevant event associated with HCC in experimental cells. Conversely, Zhou et al. (97) reported reduction of specific miRNAs in the same experimental cells (96) most studies have reported increased level of miRNA21 in HCC, however, Xu et al. (41) reported lower levels of miR-21 in patients with HCC (compared with patients with chronic hepatitis), and, Qi et al. (98) confirmed miR-21 didn't increase in HBV-related HCC patients.

Reduction in miR-122 expression has been observed in hepatocellular carcinoma (HCC), often in advanced tumors; however, upregulation of miR-122 has also been reported in HCV-derived HCC. These seemingly contradictory results indicate diverse regulatory mechanisms of miRNAs in the pathogenesis of hepatocellular carcinoma. Increased levels of miR-21,

Table 5. — Regulatory, diagnostic and therapeutic miRNAs in viral hepatitis

Liver disease	Etiology	Complications	Regulatory miRNAs	Diagnostic miRNAs	Therapeutic miRNAs
Viral hepatitis	Hepatitis virus infection (often A, B and C) (73,74)	cirrhosis, hepatocellular carcinoma (HCC), death (73,74)	MiR-185 & MiR-21 (86), MiR-222 & MiR-2224 (84), MiR-34-a & MiR-16 (42), MiR-143 & MiR-223 (86)	MiR-433-3 & MiR-141-3 & MiR-206 & MiR-192-5 & MiR-122-5 & MiR-199-a & MiR-1228-5 & MiR-26-a5 (79), MiR-155 (87)	MiR-122 (85)
MiR: microRNA.					

Table 6. — Regulatory, diagnostic and therapeutic miRNAs in liver carcinoma

Liver disease	Etiology	Complications	Regulatory miRNAs	Diagnostic miRNAs	Therapeutic miRNAs
Liver carcinoma	liver cell necrosis and renewal with inflammation causes nodular regeneration, cirrhosis, progressive genomic errors, and irregular growth and repair mechanisms(89,90, 91,92)	hepatocyte dysplasia (89,90,91,92)	MiR-181-a & MiR-34-a & MiR-200 (49), MiR-21 (41), MiR-122 (42)	MiR-939 & MiR-595(96), MiR-34-a & MiR-16 & MiR-21(42)	MiR-4454(94), MiR-425(95), MiR-132(99)

MiR: microRNA.

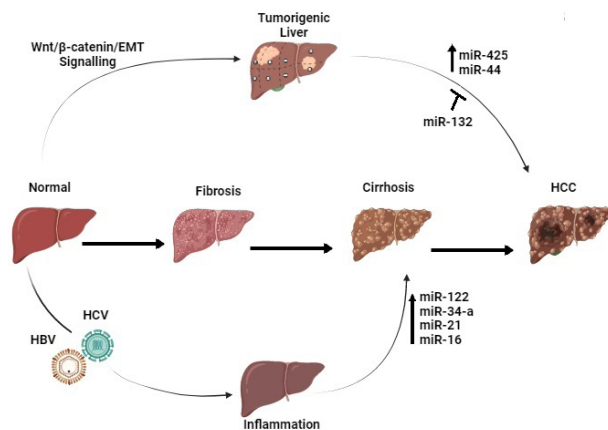


Figure 3. — Roles of miRNAs in HCC. miR: microRNA; EMT: Epithelial-mesenchymal transition; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HBV: hepatitis B virus.

miR-34a, and miR-16 have also been observed in the supernatants of HCV-infected cells (42). Based on these reports, we believe that these markers can be used as diagnostic biomarkers for hepatocellular carcinoma.

Although miRNAs are not yet widely used in the clinic for the treatment of hepatocellular carcinoma, there is abundant and reliable evidence from trials that have reported the targeting of miRNAs for the treatment of hepatocellular carcinoma. For example, miR-132 inhibits the proliferation of liver cancer cells (99). However, since miR-4454 and miR-425 increase the proliferation, migration, and invasion ability of liver cancer cells (through pathways such as Wnt/β-catenin/EMT signaling pathway), inhibition of these miRNAs can be used as an effective therapeutic tool. However, this requires further research and clinical studies (94,95). Some of the miRNAs involved in hepatocellular carcinoma are shown in Figure 3. Table 6 briefly mentions the etiology, complications, and regulatory, diagnostic and therapeutic microRNAs of HCC.

Regulatory, diagnostic, and therapeutic miRNAs in the mentioned liver diseases are listed in table 1. Considering some similar regulatory and therapeutic pathways and overlap between effective elements, it is acceptable that some miRNAs are common between different liver diseases.

Conclusion

Liver diseases are highly prevalent worldwide, and due to modern lifestyle factors, such as alcohol consumption, consumption of sugars and fats in prepared foods, lack of physical activity and exercise, jobs with low physical activity, their prevalence is increasing. Despite the availability of various diagnostic and treatment methods, there is a continuous search for new and improved diagnostic and treatment methods, as many existing methods are invasive and have limited success.

MiRNAs are small non-coding RNAs that affect gene expression and are involved in multiple cellular pathways. Recently, there has been increased interest in the potential use of miRNAs as diagnostic and treatment tools for liver diseases. In this article, six common liver diseases are discussed: cirrhosis, alcoholic fatty liver, non-alcoholic fatty liver, non-alcoholic steatohepatitis, liver carcinoma, and viral hepatitis. In each case, we investigated the diagnostic, regulatory, and therapeutic roles of miRNAs by reviewing valid studies and discussing the possible mechanisms involved and any differences in opinions among the studies reviewed.

Owing to the high prevalence of liver disease and the low efficiency of current diagnostic and treatment methods, many researchers are looking for new diagnostic techniques and effective treatments. Understanding and investigating the cell signaling pathways involved can be very useful.

Our suggestion for researchers who are interested in our study, the topic of liver diseases, and the use of miRNAs is to focus more on the discovery and recognition of cellular and molecular pathways involved.

Author Contribution

All authors contributed to the study conception and design, and all authors have read and agreed to the published version of the manuscript. FS, RK and MKh were responsible for the design, draft and writing of the manuscript, FS, SAM and AK provided Conceptualization, critical review and revision of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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