Role of MicroRNAs in the Development of Hepatocellular Carcinoma in Nonalcoholic Fatty Liver Disease

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ABSTRACT

Hepatocellular carcinoma (HCC) is a prevalent liver malignancy that can be developed from nonalcoholic fatty liver disease (NAFLD). Numerous pathophysiological alterations, including insulin resistance, specific cytokine release, oxidative stress, and mitochondrial damage, are involved in the transition of NAFLD to cirrhosis and HCC. MicroRNAs, as post-transcriptional modulators, play a critical role in the pathogenesis of NAFLD-related HCC by regulating lipid metabolism, glucose homeostasis, cell proliferation, apoptosis, migration, and differentiation. This review summarizes the current progress of microRNAs in the risk and prognosis of NAFLD-related HCC.

Key words: nonalcoholic fatty liver disease; hepatocellular carcinoma; microRNA; therapy

Hepatocellular carcinoma (HCC) is the most prevalent type of liver cancer and the fifth leading cause of cancer-related mortality in men in the United States (Siegel et al., 2017). Although the recent progress in early diagnosis, detection techniques, surgical treatments, and drug therapies have increased patient’s survival time, HCC remains a major clinical challenge due to its high prevalence, poor prognosis and limited treatment options (Fujisawa et al., 2017). The development of HCC is caused by the interaction of environmental, genetic, and epigenetic factors (Tian et al., 2013; Riordan et al., 2018), including aflatoxin exposure, alcohol consumption, hepatitis virus infection, and familial tendency (Arzumanyan et al., 2013; Suh et al., 2015; Zheng et al., 2017).

ABBREVIATIONS: AKT = serine/threonine kinase; BMI = body mass index; CDA = choline-deficient and amino acid-defined; HBP1 = HMG-box transcription factor 1; ECM = extracellular matrix; ERK = extracellular signal-regulated kinase; Glu/Ins = glucose and insulin; HCC = hepatocellular carcinoma; HFD = high-fat diet.; HOXD10 = Homeobox D10; HSCs = hepatic stellate cells; IKKβ = inhibitor of kappa-B kinase β; IL-6 = interleukin-6; IR = insulin resistance; JNK = c-Jun N-terminal kinase; MMP = matrix metalloproteinase; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; NF-κB = nuclear factor-κB; PI3K = phosphatidylinositol-3-kinase; RhoC = Ras homolog family member C; RLPP = reactive lipid peroxidation products; RNS = reactive nitrogen species; ROS = reactive oxygen species; SIRT1 = sirtuin 1; STAT3 = signal transducer and activator of transcription 3; TNF-α = tumor necrosis factor-α; uPAR = plasminogen activator, urokinase receptor; VLDL = very low-density lipoprotein.

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However, the quantitative proportion of HCC associated with nonalcoholic fatty liver disease (NAFLD) is rapidly increasing in the Western countries (Dyson et al., 2014), and socioeconomic transformations toward Westernized diet also render Asian populations highly susceptible to these rising epidemics (Fan et al., 2017).

NAFLD is characterized by hepatic fat accumulation, which is tightly associated with over-nutrition, central obesity, insulin resistance and other features of the metabolic syndrome (Gluchowski et al., 2017; Tilg et al., 2017). Although most NAFLD patients remain asymptomatic, the strongest predictor of fibrotic progression in NAFLD is nonalcoholic steatohepatitis (NASH), which may further develop into cirrhosis and HCC (Eslam et al., 2017). Since obesity and type 2 diabetes mellitus are growing at alarming rates globally, there is a compelling need to understand the molecular pathways that contribute to liver cancer arising from metabolic syndromes.

MicroRNAs are small noncoding RNAs of 19–24 nucleotides in length that often bind to the 3'-untranslated regions (UTRs) of messenger RNAs to regulate the expression of their target genes (Anastasiadou et al., 2017; Chou et al., 2018). The binding of their target mRNAs can induce cleavage and degradation of mRNAs, contributing to the silencing of mRNA species and the repression of protein synthesis (Nishimura and Fabian, 2016). Biological functions of microRNAs affect many cellular processes, such as metabolism, proliferation, apoptosis, metastasis, and differentiation (Di Leva et al., 2014). Recent studies have demonstrated that microRNAs may contribute to hepatocarcinogenesis of NAFLD (Guo et al., 2016; Tessitore et al., 2016). In this review, we summarized the current studies on microRNAs in NAFLD-related HCC and discussed its biological effects and clinical implications.

EPIDEMIOLOGY OF NAFLD-RELATED HCC

NAFLD, the most common chronic hepatic disorder, refers to accumulation of hepatic steatosis for more than 5%–10% of the overall weight of the liver tissue or macrosteatosis of the same extent, which is not induced by excessive alcohol consumption (women ≤20 g/day, men ≤30 g/day; Weiss et al., 2014). NAFLD has two principal clinical-histological phenotypes: nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH), and the latter is characterized by inflammation of the liver tissue within fat deposition. NAFLD is increasingly becoming the most common liver disease that affects a high proportion of the world's population. The prevalence of NAFLD in adults and children are approximately 30% and 10% in Western countries, respectively (Sanyal et al., 2015). In China, the community prevalence of NAFLD is about 15% and has doubled in the past decade approximately (Fan, 2013; Wang et al., 2014).

NAFLD is a hepatic manifestation of metabolic syndrome which defines a series of complications including obesity, diabetes, hyperlipidemia, iron deposition, hypertension, and inflammation (Younossi et al., 2016). Some epidemiological studies indicate that overweight and obesity are associated with higher incidence and mortality rate of HCC (Kerr et al., 2017). In a population-based cohort study, high BMI and obesity have been shown to be associated with increased risk of HCC (Hagstrom et al., 2017). NAFLD might affect 20%–25% of diabetes mellitus patients, in which the prevalence of NASH might be more than 30%–40% (Okanoue et al., 2011). Diabetes is considered as the risk factor for HCC, especially in males (Hagstrom et al., 2017; Simon et al., 2017). In randomly selected cases from the Veterans Health Administration databases (2005–2015), the incidences of NAFLD, fibrosis, and HCC among diabetic patients were significantly higher than those without diabetes (Patel et al., 2018). Besides, hyperlipidemia is also a common metabolic risk factor for NAFLD. About 66.8% of NAFLD patients have dyslipidemia, and hepatic triglyceride accumulation is probably a consequence of saturation of fatty acid oxidation and VLDL secretion (Cotrim et al., 2011; Gaggini et al., 2013).

NAFLD comprises a spectrum of disorders from simple steatosis to NASH, and the fibrotic form of NAFLD may progress to cirrhosis and even HCC (Santhekadur et al., 2018). In general, 57.5%–74% of obese population may suffer from hepatic steatosis, which could develop into NASH (19%), cirrhosis (3%–5%), and HCC (0.3%–2%) (Kawamura et al., 2012; McPherson et al., 2015; Cholankeril et al., 2016; Rinella and Sanyal, 2016). NAFLD is a major risk factor for HCC in the United States and the proportion of HCC induced by NAFLD has risen to 14.1%. In addition, NAFLD-related HCC is relevant to shorter survival time, more advanced tumor stage and lower possibility of receiving a liver transplant (Younossi et al., 2015).

PATHOGENESIS OF NAFLD-RELATED HCC

HCC develops from NAFLD through a multistep process that results from the progressive accumulation of numerous alterations, such as activation of insulin-mediated proliferative pathways, specific cytokine release, oxidative stress, and mitochondrial damage (Fig. 1; Zhang et al., 2014; Ma et al., 2016; Inoue-Yamauchi et al., 2017).

Mechanisms of insulin-mediated proliferative pathways in NAFLD-related HCC involve the epigenetic regulation of histone deacetylase HDACs which promotes insulin resistance and stimulates β-catenin-dependent cell proliferation to drive hepatocarcinogenesis (Tian et al., 2015). What’s more, glucose and insulin (Glu/Ins) significantly stimulate proliferation, adhesion, invasion, and extracellular matrix (ECM) production in hepatic stellate cells (HSCs). Tetramethylpyrazine could inhibit Glu/Ins-stimulated HSCs activation and ECM production by inhibiting insulin receptor-mediated PI3K/AKT and ERK pathways (Zhang et al., 2014).

Cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), play an important role in the carcinogenesis of NAFLD (Duan et al., 2014). TNF-α is highly significant in NAFLD and induces inflammation and insulin resistance through JNK and IKKβ/NF-κB pathways (Chen et al., 2015; Wu et al., 2017a). Moreover, TNF-α can increase expression of IL-6 which involves a complex network of signaling pathways including phosphoinositide 3-kinase/protein kinase B, mitogen-activated protein kinase and activator of transcription 3 pathways, thus regulating cell proliferation, apoptosis and metastasis (Miller et al., 2011; Yang et al., 2013; Duan et al., 2014; Hassan et al., 2014; Wu et al., 2017a).

Oxidative stress may participate in the pathogenesis of HCC from NAFLD and cirrhosis. It can stimulate the
production of reactive oxygen species (ROS) causing increased hepatic oxidative DNA damage in NASH patients who may develop HCC (Tanaka et al., 2013). Oxidative stress can promote pathological polyploidization which is an early event in the pathogenesis of NAFLD-related HCC (Gentric et al., 2015). In addition, accumulation of oxidative DNA damage can induce DNA methylation of tumor suppressor genes responsible for hepatocarcinogenesis (Nishida et al., 2016).

Mitochondrial dysfunction induced by TNF-α, ROS, reactive lipid peroxidation products (RLPP) and reactive nitrogen species (RNS) can promote free radical production, block respiratory chain components, and alter mitochondrial DNA, leading to mitochondrial ROS formation and causing the “second hit” which may further cause inflammatory response in liver tissues and progression of NAFLD to cirrhosis and HCC (Satapati et al., 2015; Ma et al., 2016; Sunny et al., 2017). In particular, the permeabilization of mitochondrial membranes can be induced by TNF-α-mediated apoptosis, which may contribute to fibrosis and increase the risk of HCC through the stimulation of cell proliferation and the selection of apoptosis-resistant clones (Pagadala et al., 2012).

**MICRONAS IN NAFLD-RELATED HCC**

The process of pathogenesis from NAFLD to HCC includes the regulation of microRNAs which can modulate transcription and translation of target genes (Afonso et al., 2016; Guo et al., 2016; Tessitore et al., 2016). MicroRNAs play an important role in lipid metabolism, glucose homeostasis, cell proliferation, apoptosis, migration, and differentiation to promote the progression of NAFLD (Fig. 2, Table 1; Benhamouche-Trouillet and Postic, 2016; Wu et al., 2016; Wu et al., 2017b).

**MicroRNAs in Lipid Metabolism**

MicroRNAs play a key role in cholesterol and fatty acid metabolism, and the serum levels of microRNAs, such as microRNA-122, microRNA-21, microRNA-34a, and microRNA-451, have been shown to be higher in NAFLD patients (Yamada et al., 2013). Inhibition of microRNA-122 can lead to reduce plasma cholesterol levels, decrease synthesis rates of hepatic fatty acid and cholesterol, and increase hepatic fatty-acid oxidation (Clarke et al., 2014). NASH is associated with altered hepatic microRNA expression. Repression of microRNA-122 potentially alters lipid metabolism and contributes to the pathogenesis of NASH (Thakral and Ghoshal, 2015). MicroRNA-21 has been demonstrated to be overexpressed in livers of mice on a high-fat diet and HepG2 cells incubated with high levels of fatty acid. Knockdown of microRNA-21 can lead to reduce lipogenesis and inhibit the growth of xenograft tumor. Furthermore, microRNA-21 contributes to hepatic lipid accumulation and hepatocarcinogenesis through the Hbp1-p53-Srebp1c pathway (Wu et al., 2016).

**MicroRNAs in Glucose Homeostasis**

MicroRNA-375, a novel evolutionarily conserved microRNA, can inhibit glucose-induced insulin secretion, while suppression of microRNA-375 can enhance insulin secretion. The secretion mechanism modified by microRNA-375 is independent of glucose metabolism but associated with insulin exocytosis, indicating that microRNA-375 may be a new pharmacological target for metabolic diseases, such as diabetes and NAFLD (Poy et al., 2004). MicroRNA-23a was up-regulated in NASH-related HCC mouse model fed with a choline-deficient diet, and overexpression of microRNA-23a was activated by the IL-6/STAT3 signaling pathway leading to a decrease in glucose
In addition, obesity-induced overexpression of microRNA-143 which is associated with insulin resistance can inhibit insulin-stimulated AKT activation and impair glucose metabolism (Jordan et al., 2011). Wu et al. (2017b) showed that microRNA-206 could reduce levels of lipid and glucose in human hepatic cells and livers of dietary obese mice through regulation of lipogenesis and insulin signaling, indicating that microRNA-206 had diagnostic and therapeutic potential for NAFLD and hyperglycemia.

Fig. 2. Role of microRNAs in the pathogenesis of NAFLD-related HCC.

| MicroRNA   | Expression            | Biological functions                                           |
|------------|-----------------------|----------------------------------------------------------------|
| microRNA-10b | Up-regulation/down-regulation | Lipid metabolism and migration                                  |
| microRNA-21  | Up-regulation         | Lipid metabolism and proliferation                              |
| microRNA-23a | Up-regulation         | Glucose homeostasis                                              |
| microRNA-26a | Down-regulation       | Proliferation and apoptosis                                      |
| microRNA-34a | Up-regulation         | Apoptosis                                                        |
| microRNA-122 | Down-regulation       | Lipid metabolism, proliferation, and differentiation             |
| microRNA-130a-3p | Down-regulation      | Proliferation and apoptosis                                      |
| microRNA-143 | Up-regulation         | Glucose homeostasis, lipid metabolism, migration, and differentiation |
| microRNA-155 | Up-regulation         | Proliferation                                                    |
| microRNA-206 | Down-regulation       | Glucose homeostasis and lipid metabolism                         |
| microRNA-296-5p | Up-regulation       | Apoptosis                                                        |
| microRNA-375 | Up-regulation         | Glucose homeostasis                                              |
MicroRNAs in Proliferation

MicroRNA-155 has an important function at early stages of choline-deficient and amino acid-defined (CDAO) diet-induced hepatocarcinogenesis. Activation of NF-κB induced by CDAO diet can upregulate hepatic microRNA-155 and overexpression of microRNA-155 can accelerate the growth of Hep3B and HepG2 cells, but depletion of endogenous microRNA-155 can inhibit the growth of SNU-182 cells (Wang et al., 2009). MicroRNA-21 is highly expressed in hepatocytes, and can lead to reduced proliferation, delayed G1/S transition and repressed growth of xenograft tumor. HBPI is a direct target of microRNA-21, and microRNA-21 knockdown by targeting HBPI can prevent hepatocarcinogenesis (Wu et al., 2016). In addition, microRNA-122 is associated with not only lipid metabolism but also hepatocyte proliferation. Overexpression of microRNA-122 can reduce cell proliferation, while deletion of microRNA-122 may lead to hepatosteatosis, hepatic fibrosis, and HCC (He et al., 2015; Jin et al., 2017).

MicroRNAs in Apoptosis

MicroRNA-34a is a putative mediator in apoptosis and its expression in severe NAFLD is more than mild one (Castro et al., 2013; Shan et al., 2015). The expression of microRNA-34a can be activated by p53 to induce hepatocyte apoptosis through the microRNA-34a/SIRT1/p53 signaling pathway (Castro et al., 2013; Ferreira et al., 2014). MicroRNA-296-5p is considered as a negative regulator of PUMA (a proapoptotic protein) expression during hepatocyte lipoprotein metabolism, and increasing microRNA-296-5p expression may become a novel strategy for inducing apoptosis in NAFLD (Cazanave et al., 2011). It has been shown that adeno-associated virus-mediated microRNA-26a could induce apoptosis specifically in HCC cells without causing the death of normal hepatocytes (Kota et al., 2009). Wang et al. showed that the expression of microRNA-130a-3p was decreased in the mice and patients with nonalcoholic fibrosing steatohepatitis. In addition, microRNA-130a-3p could inhibit proliferation and promote caspase-mediated apoptosis of HSCs to contribute to the development of nonalcoholic fibrosing steatohepatitis (Wang et al., 2017).

MicroRNAs in Migration

MicroRNAs are associated with metastasis of HCC and can become biomarkers for prediction of survival and recurrence of HCC (Zhou et al., 2016). MicroRNA-10b is an active regulator to induce liver steatosis by promoting the accumulation of intracellular lipids and triglycerides (Tsiloulis et al., 2017). Serum level of circulating microRNA-10b was decreased in NAFLD (Celikbilek et al., 2014), but was overexpressed in HCC to promote HCC cell migration and invasion through the HOXD10/RhoC/uPAR/MMPs pathway (Liao et al., 2014). Zhang et al. showed that microRNA-143 was dramatically increased in metastatic HCC patients and promoted HepG2 cells migration by transactivation of the NF-κB signaling pathway. Besides, blocking microRNA-143 could inhibit local liver metastasis and distant lung metastasis (Zhang et al., 2009, 2017).

MicroRNAs in Differentiation

MicroRNA-143 is an important regulator of adipocyte differentiation. Expression levels of microRNA-143 rise in differentiating adipocytes and inhibition of microRNA-143 can cause decrease in triglyceride accumulation, suggesting that microRNA-143 is a potential therapeutic target for obesity and NAFLD (Esau et al., 2004). MicroRNA-122a is also involved in the pathological process of steatohepatitis, fibrosis, and HCC by restoring hepatocyte differentiation. This microRNA may participate in the liver cancer stem cell self-renewal and adjust the balance between cell differentiation and proliferation. The re-expression of microRNA-122a can reduce hepatic disease manifestation and HCC incidence in Mirt122a−/− mice (Laudadio et al., 2012; Tsai et al., 2012).

MICRORNAS-BASED POTENTIAL THERAPY FOR NAFLD-RELATED HCC

Proper diet and exercise are the basic lifestyle interventions in the early stage of NAFLD patients. However, when NAFLD may deteriorate and even progress to cirrhosis and HCC, the effective pharmacological interventions are needed. Due to the effect of microRNAs on lipid metabolism and hepatocarcinogenesis, microRNAs have been considered as novel therapeutic targets for metabolic disorders and HCC. The functions of microRNAs can be modulated by antisense oligonucleotides (Biglino et al., 2017; Meng et al., 2017). Inhibition of microRNA-122 resulted in the decline of plasma cholesterol to improve liver steatosis, and down-regulation of microRNA-122 level in the liver did not produce any adverse effects (Clarke et al., 2014). In addition, the restoration of microRNA-122 can diminish the incidence of HCC, thus showing a new approach for the treatment of HCC by increasing the level of microRNA-122 (Tsai et al., 2012). On the other hand, decrease in the microRNA level also represents a potential therapeutic strategy for NAFLD-related HCC. For example, depletion of endogenous microRNA-155 can inhibit the growth of HCC cells to prevent NASH-induced hepatocarcinogenesis (Wang et al., 2009).

Although microRNAs highlight potential promise for therapeutic interventions, there are some obstacles to their clinical applications, such as low cellular uptake, instability of structure, and degradation by nucleases (Wang et al., 2015). In the future, more studies are needed to explore the mechanisms of microRNAs in NAFLD-related HCC to contribute to the development of microRNAs-based therapy.

CONCLUSIONS

NAFLD-related HCC is a liver malignancy derived from metabolic disorders. The molecular regulatory mechanisms are the complex multiple processes from NAFLD to HCC, attributing to the accumulation of pathophysiologic alterations, such as activation of insulin-mediated proliferative pathways, specific cytokine release, oxidative stress, and mitochondrial damage. MicroRNAs play a key role in the pathogenesis of NAFLD-related HCC by the regulation of lipid metabolism, glucose homeostasis, cell proliferation, apoptosis, migration, and differentiation. The effects of microRNAs on these biological functions
are diverse and interrelated, and numerous microRNAs form a complex signaling network. Metabolic disorders of lipid and glucose can induce hepatic cell proliferation, apoptosis, migration, and differentiation, so normal homeostasis of lipid and glucose can prevent the development of NAFLD-related HCC. However, there is still a long way to understand the comprehensive molecular mechanisms of NAFLD-related HCC pathogenesis and progression. The application of microRNAs for the diagnosis and therapy of NAFLD-related HCC is in the early stage and more research is needed to understand the function and significance of microRNAs in NAFLD-related HCC.

CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

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