Chapter

miRNAs in Liver Cancer

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Abstract

miRNAs are small noncoding RNAs, which are involved in epigenetic regulation of gene expression. Hepatocellular carcinoma (HCC), although not being at the top of most widespread cancers, nevertheless, remains among cancers with the most lethal cases. The chapter is dedicated to the epigenetic aspect of HCC development, namely the role of miRNA in this process. Being small and noncoding RNAs, they have a huge and significant function in gene regulation. This chapter will briefly cover following questions: miRNA biogenesis and function, metabolic and signaling pathways disrupted in HCC with a significant miRNA involvement, and main miRNAs contributing to HCC development and their targets.

Keywords: miRNA, hepatocellular carcinoma, target gene, gene expression, miRNA expression, diagnostic marker

1. Introduction

miRNAs are small noncoding RNAs ~20–25 nt long, which are involved in epigenetic regulation of gene expression. miRNAs were firstly discovered in 1993, when research groups led by Victor Ambros and Gary Ruvkun published two side-by-side papers in the journal Cell, describing the regulatory effects of tiny RNA discovered in Caenorhabditis elegans. Years later the term "microRNA" (miRNA, miR) was introduced [1]. Nowadays, more than 2600 miRNAs have been predicted to be encoded by the human genome, with the ability to modulate more than 15,000 genes [2]. Being small and noncoding RNAs, they have a huge and significant function in gene regulation and cancer development. Liver cancer, although not being at the top of most common cancers, remains among cancers with high mortality. Hepatocellular carcinoma (HCC) accounts for approximately 80% of all liver cancers and is a main cause of cancer mortality [3]. Hereinafter, when using the term liver cancer, it is meant to indicate hepatocellular carcinoma. The most obvious and significant reason for this high mortality rate in HCC patients is the late diagnosis of HCC. Against this background, every aspect of molecular pathogenesis becomes a valuable detail, which may aid in understanding HCC development. Some of the main miRNAs involved in the development of liver cancer will be discussed further. Changes in their expression levels were detected in comparable conditions: mostly in human liver tissue samples or human blood samples (plasma or serum) by qRT-PCR.

2. miRNA biogenesis and regulation

Despite the simple structure of a mature miRNA molecule—single-stranded RNA molecule of 20–25 nt—its biogenesis, like almost any process relating to nucleic acids, is multistage and multifactorial: It takes place both in the nucleus and in the cell cytoplasm, involves protein complexes for processing (miRNA maturation), may be performed *via* canonical and non-canonical pathways, and includes following transformations: primary miRNA (pri-miRNA), preliminary miRNA (pre-miRNA), miRNA duplex, and mature miRNA. Both canonical and non-canonical processing start in the nucleus.

miRNA processing may occur post- or co-transcriptionally [4]. miRNA biogenesis starts in the nucleus and requires RNA polymerase II/III; Drosha (an RNase III-like enzyme) with its cofactor, the RNA binding protein DGCR8 (DiGeorge Syndrome Critical Region 8), forms a microprocessor complex and functions in the nucleus, an exportin (frequently Exportin5 in the canonical pathway and Exportin1 in the non-canonical pathway) functioning as the transporter to the cytoplasm. Dicer, another RNase III-like endonuclease, RISC (RNA-induced silencing complex) functioning in the cytoplasm with Argonaute (AGO) as a core component. miRNA biogenesis starts with the processing of RNA polymerase II/III and forming pri-miRNAs, which are 5′ capped and 3′ polyadenylated, approximately several kilobases [5, 6]. In the canonical pathway, pri-miRNAs then are processed in the microprocessor complex of Drosha and DGCR8. The resulting ~70 nucleotide RNAs with 2 nt 3′ overhang are known as precursor (pre-) miRNAs, which fold into mini-helical structures [7]. Pre-miRNAs are transported from the nucleus to the cytoplasm with Exportin 5/RanGTP complex, where they undergo processing with Dicer, which recognizes the pre-miRNA hairpin and cuts it at the loop end, resulting in the removal of the terminal loop and creating a ~22 nt RNA duplex [8]. The final step of the miRNA biogenesis is processing the duplex miRNA into mature single-stranded miRNA by loading it onto an Argonaute (Ago) protein, which is the core protein in this final effector complex—RNA-induced silencing complex (RISC). The mature miRNA may be derived from both the 5' and 3' arms of the precursor duplex and are called the miRNA-5p and -3p, respectively [9].

As for regulation of miRNA expression with some miRNAs having their own promotors and some being regulated by other gene promotors, besides methylation, different endogenous factors, and hypoxia, different transcription factors (TF) also participate in miRNAs expression regulation and this is a double-edged process: TFs influence miRNA expression and miRNAs may repress TF expression [7, 10]. miRNA, TFs, and target genes form a complex relationship known as feedback loops (FBLs) and feed-forward loops (FFLs) [11]. Typically, FBLs occur when a TF activates or represses a miRNA, which in turn represses the TF; the miRNA and TF each regulate independent sets of TGs. FFLs are those where a regulator, such as a TF, controls the expression of a specific TG both directly, through promoting or enhancing its transcription, and indirectly, through another regulator, such as an miRNA that also regulates the TG [12].

mRNA and miRNA interaction implies binding of the last to the 3′ untranslated region (3′ UTR) of mRNA through base-pairing of the seed region of target mRNA, mainly at position 2–7 from the 5′ end of the miRNA; beyond the seed region, the binding between the whole mature miRNA sequence and the target mRNA is not perfectly complementary [13]. However, the interaction of miRNAs with other regions, including the 5′ UTR, coding sequence, and gene promoters, has also been reported.

In general, there is no direct correlation between miRNA and target mRNA expression levels. Multiple miRNAs can regulate a single gene/mRNA, and some miRNAs can target many mRNAs (up to more than 100 mRNAs) and from 1 to 2% of human transcripts interact with nine or more miRNAs, thus displaying sponge-like activity [14]. Furthermore, miRNAs have been shown to activate gene expression under certain conditions [5]. Along with mRNAs differing in their miRNA-binding capacity, binding activity of some highly expressed miRNAs may be weakened by either a high target-to-miRNA ratio or the relocation of this miRNA to the nucleus. Some miRNAs might be expressed at relatively low levels and interact with many mRNAs and, oppositely, some miRNAs might be expressed at a very high level and interact with only a few mRNAs [15].

Considering all of the above, it makes identifying the specific miRNA-target gene or transcription factor-target gene interactions difficult, and possibly unwarranted [12].

3. miRNA in liver functions

Apparently, miRNAs are involved in all processes underlying normal liver functioning, so main pathologic processes, such as nonalcoholic fatty liver disease (NAFLD), fibrosis at the background of various diseases, and cancer are associated with significant changes in miRNA expression profiles, although these changes are not always associated with target mRNA expression changes and the mechanism of miRNA participation in these processes is not clear. A significant role of some miRNAs was shown for main liver functions such as lipid metabolism and all the steps of glucose metabolism, including lipogenesis.

3.1 miRNAs and the regulation of lipid metabolism

Pivotal role of miR-34a in PPARα (the peroxisome proliferator-activated receptor alpha) pathway, which is a direct target of miR-34a and a master regulator of lipid metabolism, was shown in cultured cells transfected with miR-34a inhibitor and simultaneously consequences of miR-34a inhibition were shown in C57BL/6 mice injected with the miR-34a inhibitor [16]. The upregulation of miR-34a resulted in the downregulation of hepatic PPARα and SIRT1 (silent mating type information regulation 2 homolog 1), silencing miR-34a led to an initially increased expression of PPARα, SIRT1, and PPARα's downstream genes, and activation of the central metabolic sensor AMPK was also increased. In the mouse model, the miR-34a inhibitor suppressed lipid accumulation and improved the degree of steatosis, which is assumed to be regular as far as its level was significantly upregulated in liver tissues of high-fat diet-fed mice [17].

miR-122 is among those playing a crucial role in lipid metabolism in the liver: miR-122 expression in mice liver was increased by free fatty acids (FFAs) *via* activating the retinoic acid-related orphan receptor-alpha, inducing secretion of miR-122 to blood, entering muscle and adipose tissues of mice, reducing mRNA levels of genes involved in triglyceride synthesis, mainly, Agpat1 and Dgat1. It also led to the attenuated triglyceride synthesis and elevated β-oxidation pathway [18]. Before it was shown that cholesterol biosynthesis genes would be affected by miR-122, plasma cholesterol levels were reduced in antagomir-122-treated mice, thus illustrating attenuation of the cholesterol biosynthesis when silencing hepatic miR-122 [19]. There are another data,

also proving the meaning of miR-122 in lipid metabolism, its inhibition in normal mice resulted in reduced plasma cholesterol levels, increased hepatic fatty-acid oxidation, and a decrease in hepatic fatty-acid and cholesterol synthesis rates. Simultaneously, miR-122 inhibition in a diet-induced obesity mouse model also resulted in decreased plasma cholesterol levels and a significant improvement in liver steatosis, accompanied by reductions in several lipogenic genes [20]. An increase in miR-122 expression in obese subjects may appear to be the compensated mechanism to maintain lipid metabolism. Besides miRNAs, hepatic lipid accumulation recruits inflammatory response, impairing some signaling pathways involved in lipid metabolism, including the AMPK signaling pathway, the role of miR-122 in energy metabolism in the liver, skeletal muscle, and adipose tissues requires more evidence to evaluate [21].

Among other miRNAs, possibly acting as modulators of lipid and cholesterol levels in the maintenance of cholesterol and fatty acid metabolism, are miR-33, miR-103, miR-104, and miR-307 [22, 23]. Obviously, some miRNAs may be involved in both lipid and glucose metabolisms, as far as one miRNA may have multiple mRNA targets. One of these miRNAs is miR-33a and miR-33b, intronic miRNAs located within the sterol regulatory element-binding protein (SREBP) genes, working in concert with its host gene to ensure a fine-tuned regulation of lipid and glucose homeostasis. miR33b also cooperates with SREBP1, having an impact on key regulatory enzymes of hepatic gluconeogenesis glucose metabolism—phosphoenolpyruvate carboxykinase (PCK1) and glucose-6-phosphatase (G6PC). Overexpression of miR-33b in human hepatic cells leads to a significant reduction of glucose production *via* inhibition of PCK1 and G6PC expression [24].

miR-206 was shown as a potent lipid and glucose production inhibitor by simultaneously facilitating insulin signaling and impairing hepatic lipogenesis due to promoting phosphorylation of INSR (insulin receptor) and impaired hepatic lipogenesis by inhibiting Srebp1 (sterol regulatory element-binding transcription factor 1) transcription and inhibition of PTPN1 (protein tyrosine phosphatase, non-receptor type 1) *via* interaction with its 3′ untranslated region and following degradation. miR-206 reduced lipid and glucose production in human hepatocytes and livers of dietary obese mice [25].

3.2 miRNAs and insulin signaling

miR-103 and miR-107 were the first two miRNAs shown to regulate insulin sensitivity in liver and adipose tissue in mice: Their overexpression in these mouse models led to downregulation of caveolin-1 expression, a component of caveolae lipid raft required for insulin receptor signaling. miR-802 was also shown to be involved in the regulation of insulin sensitivity and glucose transport: elevated miR-802 decreased expression of HNF1β while increasing expression of the insulin suppressors, SOCS1 and SOCS3. Increased expression of both SOCS1 and SOCS2, in turn, desensitizes insulin signaling, resulting in increased hepatic glucose production in these mouse models [26]. It is worth noting that miR-23a was first reported as a regulator of gluconeogenesis through direct binding at the 3'-UTRs of both G6Pase and PGC-1α mRNAs, and later its expression was found to be elevated in hepatocytes of hepatocellular carcinoma mice where gluconeogenesis is attenuated [27].

3.3 Circular RNAs

In general, when discussing miRNA functions and interactions, it should be noted, besides its elusive relations with mRNA, that they are not limited to mRNA

and involve other RNAs as well, that may influence its activity and function, for example, circRNAs have been validated as microRNA (miRNA) sponges, which have complementary sequences binding to their target miRNAs, thereby inhibiting the function of those miRNAs and abolishing the inhibition of target gene expression [27]. Circ-0000092 with such miRNA sponging activity and miR-338-3p as a target miRNA was shown to be elevated in HCC tissue (40 patients, RT-qPCR, GAPDH) and cell lines, while miR-338-3p was shown to be decreased (40 patients, RT-qPCR, U6 as an internal control) [28]. Simultaneously, miRNA-338-3p target, HN1, shown to be overexpressed by Liu et al. in liver cancer and known to be involved in metastasis and invasion development in breast and prostate cancer partly due to negative impact on the b-catenin/E-cadherin interaction, was shown to be elevated along with circ-0000092 [29]. These data along with the effects of delivery of a series of mimic, inhibitor, or siRNA plasmids into HCC cells on cell proliferation, migration, invasion, and angiogenesis *in vitro* may allow to assume that circ-0000092, absorbing miRNA-338-3p and positively influencing HN1 expression, and promote cancer cell proliferation and invasion. A possible mechanism for maintaining CSC (cancer stem cell) self-renewal with HN1 involvement is enhancing of oncogenic factor MYC, and the LEPR–STAT3 pathway [29]. Another circRNA with specific sponging activity for miRNA-338-3p is circMAT2Bm, which also negatively influences miRNA-338-3p and, as a result, positively one of its targets—PKM2, which encodes one of the key enzymes in the process of glycolysis [30]. CircASAP1 in liver cancer acts as a competing endogenous RNA for miRNA-326 and miRNA-532-5p, which play a tumor suppressor role in liver cancer, regulating MAPK1 and CSF1. CircUHRF1, which is predominantly secreted into plasma in exosomes by HCC cells, inhibits the activity of miRNA-449, upregulating the expression of TIM3 and inhibiting NK cell function. The expression of circUHRF1 is higher in HCC tissues than in corresponding adjacent nontumor tissues [31]. There are other circRNAs, whose expression level is decreased in HCC tissues compared with noncancerous tissues, and that were shown to have miRNAs among their targets, such as circTRIM33-12 and miRNA-191, circHIAT1 and miRNA-3171, circLARP4 and miRNA-761, and circMTO1 and miRNA9. Decreased expression of these circRNAs in HCC resulted in elevated expression level of the corresponding miRNAs and sustaining of proliferation, invasion, and metastasis of cancer cells [31].

4. miRNA expression patterns in HCC

It is evident that most of the processes taking place in the liver in normal condition require different miRNAs and assumably healthy hepatocyte should have its "normal" miRNA profile with wide ranges, which make it possible to suggest—there are strong pieces of evidence that will be discussed further—those different pathologic processes, forming the diseases, are accompanied with different changes in miRNA expression levels. With regard to hepatocellular carcinoma, it could be speculated that in the very initial stages, particularly at preclinical stages when it is favorable for cancer background but still no clinical manifestation of cancer, liver cancer may have different miRNA expression profiles within the same cancer type. Further will be discussed the liver cancer development and partly the background of viral hepatitis B, viral hepatitis C, nonalcoholic fatty liver disease (NAFLD), and alcohol-related liver disease (ARLD).

miRNAs along with other nucleic acids have a significant impact on cancer development, where they may have both the role of cancer promotion and cancer

suppression; therefore, miRNAs with increased expression in tumors are thought to function as oncogenes and are termed as oncomirs. On the contrary, miRNAs with decreased expression in cancer cells are considered tumor suppressor genes, presumably preventing tumor development by negatively inhibiting oncogenes and/or genes that control cell differentiation or apoptosis [32]. miRNAs are known to be involved in most signaling pathways, and in the liver cancer development, the same signaling pathways are involved, like in most other cancer types, such as TGF-β, Wnt/B-catenin, Hh, Notch, EGF, HGF, VEFG, JAK/STAT, Hippo, and HIF, which lead to uncontrolled cell division and metastasis [32].

Main miRNAs, involved in TGF-β regulation, are miR-200, miR-21, miR-211, miR-17/92, miR-106b/25, and miR-182 [33]. miR-200 and miR-21 are one of the main players among noncoding RNAs in interaction with TGF-β signaling in the process of EMT. miR-200 forming a double-negative feedback loop with ZEB factors (zinc finger E-box-binding homeobox) plays a significant role in EMT (epithelial-mesenchymal transition): miR-200 is downregulated because of reversible DNA methylation of the miR-200 loci as a result of prolonged autocrine TGF-β signaling, driving a sustained ZEB expression, and thus maintaining a stable mesenchymal phenotype. miR-200 is known to interact with both ZEB factors—(ZEB1; also known as deltaEF1) and SIP1 (also known as ZEB2) [34]. miR-200a is responsible for significant inhibition of cell proliferation and colony formation rate in HCCLM3 and HepG2 cell lines, while knocking out miR-200a restores the rate of proliferation and colony formation of cancer cells [35]. miRNA expression levels of miR-200 family tend to be decreased in individuals with liver cancer (plasma and tissue), compared with healthy individuals, and have a prognostic value for patients with HCC: microRNA-200a and miR-200c were independent prognostic factors for hepatocellular carcinoma and induced cell cycle arrest by targeting CDK6 or MAD2L1, respectively [36]. Opposite to miR-200 family, expression of miR-21 is induced in response to TGF-β signaling and is associated with tumor invasion and chemoresistance *in vitro*. Besides this, Wang Z et al. mention, indicating this is unpublished data, that Notch-1 could be one of miR-200b targets because overexpression of miR-200b significantly inhibited Notch-1 expression [37]. Moreover, miR-21 is able to directly interact with TGF-beta receptors: Mishra S. et al. revealed that miR-21 suppresses a tumor-suppressor gene TGFBR2 (transforming growth factor-beta receptor II) levels by binding to its 3 0-UTR, hence inhibiting the tumor-suppressive activity of TGFβ pathway [38]. Reported target genes for miR-21 in HCC are the following: FASLG, PTEN, HBP1, IL-12, RECK, and TIMP-3; some of these genes were shown simultaneously to be miR-21 targets in other liver diseases, such as ALD (FASLG), NAFLD (HBP1), and liver fibrosis (TIMP3) [39]. There are plenty of data showing an increase in miR-21 expression level in the background of liver cancer development or the chronic liver diseases, which are the risk factors for liver cancer development.

Members of the miR-17-92 and the miR-106b-25 clusters have been implicated in the progression of liver fibrosis through the influence on the expression of TGF-β receptor II (TGF-βRII), having opposite effects on this expression. miR-19b has been shown to play an inhibitory role in hepatic stem cell-mediated fibrogenesis and to be decreased in fibrotic rats and human livers. Overexpression of miR-19b inhibited the expression of TGF-βRII, which in turn inhibited SMAD3 expression and, as a result, reduced type-1 collagen production. Unlike miR-19b, miR-93 and miR-106b were observed to be consistently upregulated during the development of cirrhosis, and miR-106b along with miR-181was shown to have a diagnostic value for liver cirrhosis irrespective of the etiology [40].

miR-211, which is known to be involved in TGF-β interaction in prostate cancer cells, was shown to be involved in WNT-β signaling regulation via SATB2. In prostate cancer cells, increased expression of miR-211 inhibited expression of TGF-β1, TGF-β2, smad2, smad3, phosphorylated smad2, and smad3, and stem cell markers and *in vitro* resulted in reductions in the proliferation, invasion, colony-forming ability, sphere-forming ability, and stemness of prostate cancer stem cells, *in vivo* in decreased tumor growth, and cell apoptosis [41]. In HCC cells, miR-211 is supposed to suppress cancer cell proliferation *via* WNT-β and SATB2 downregulation. 3′-UTR of SATB2 was shown to be the direct target of miR-211, it contains a conserved target site for miR-211, and *in vitro* miR-211 mimics repressed the luciferase activity of the luciferase gene with inserted 3′-UTR of SATB2 in the pGL3-control vector. miR-211 expression in HCC tissues and cells is inversely correlated with SATB2, when in HCC tissues miR-211 expression was decreased, SATB2 expression was upregulated.

MiR-125b is known to interact with the Hh pathway, which is a well-known factor regulating liver reconstitution. miR-125b, produced by CP-MSCs (chorionic platelet-derived mesenchymal stem cells), attenuates Hh activation partly due to Smo expression inhibition and the consequence of this regulation is the promotion of the regression of fibrosis, contributing to liver regeneration [42]. It was demonstrated that another target of miR-125b in HCC cells is LIN28B, and simultaneously miR-125b may increase p21Cip1/Waf1 expression and arrest cell cycle at G_1 to S transition, which may contribute to suppression of HCC cell migration, invasion, and growth *in vitro* and *in vivo* [43]. With regard to this data, Liu W. et al. demonstrated that the expression level of serum exosomal miR-125b in patients with HCC (158 samples, qRT-PCR, normalization normalized to caenorhabditis elegans miRNA (Cel-miR-39)) was decreased in comparison with the expression level of serum exosomal miR-125b in patients with chronic hepatitis B (n=30) and liver cirrhosis (n=30). Moreover, the exosomal serum miR-125b level was shown to have a prognostic value for HCC patients: It predicted the recurrence and survival of HCC patients with an area under the ROC curve of 0.739 (83.0% sensitivity and 67.9% specificity) and 0.702 (82.5% sensitivity and 53.4% specificity) [44]. Moreover, inhibition of miR-125b suppressed the expression of profibrogenic genes in culture-activated primary HSCs and reduced the basal and transforming growth factor β (TGF-β)-induced alpha-smooth muscle actin (α-SMA) expression and cell contraction of the immortalized HSC cell line [45].

miRNA-199a-3p, being one of the putative therapeutic tools in liver cancer, may perform its anticancer effect through involvement in NOTCH signaling. miRNA-199a-3p is downregulated in liver cancer tissues and most liver cancer cell lines; in liver cancer cell lines (MHCC97H, Hep3B, SMMC-7721, Huh7, and HepG2), its expression was significantly lower than in normal liver cell lines; simultaneously, mRNA YAP1 expression was significantly higher than in normal liver cell lines. It was shown that miRNA-199a-3p targets YAP1, downregulates Jagged1, and suppresses the Notch signaling, which results in HCC cell proliferation inhibition and apoptosis promotion [46]. In a mouse model with induced HCC treatment with miRNA-199a-3p showed regression of hepatocellular carcinoma with the restoration of normal architecture on histopathological examination of liver specimens [47].

In liver cancer, HGF, ERBB3, and NF-κB form a positive feedback loop: higher expression of ERBB3 makes liver cancer cells more sensitive to HGF stimulation; moreover, HGF enhances ERBB3 expression by NF-κB transcriptional activity. miR-17-5p and miR-20a-5p in liver cancer cell lines and mice xenograft models were shown to suppress liver cancer cell proliferation after hepatectomy *via* blocking

HGF, ERBB3, and NF-κB positive feedback loop. HCC patients with lower levels of miR-17-5p and miR-20a-5p or higher levels of ERBB3 had significantly shorter OS and PFS survivals after surgical resection [48]. Simultaneous deregulation of VEFG and miRNA expression was shown in tissue samples of patients with liver cirrhosis, while VEGF did not show a significant difference in expression level in HCC samples compared to control (non-cancer and non-cirrhotic) samples. Expression level of VEGF was 12.97-fold higher in cirrhotic patients compared to liver cancer samples; concurrently, miR-206 and miR-637 (RT-qPCR, U6, RNU44, and RNU48 were used as reference genes) were down-expressed in LC samples. miR-637 was downregulated in HCC samples too [49]. Before it was shown that in HCC cells, miR-637 is responsible for suppressing autocrine leukemia inhibitory factor (LIF) expression and exogenous LIF-triggered activation of the transcription factor Stat3, which regulates several growth factors, including the VEGFA gene [50]. miR-146a indirectly influences VEGF expression in HCC cells through upregulating APC, which inhibits β-catenin accumulation in nucleus, and downregulating NF-κB p65 by targeting HAb18G [51]. An increase in expression of mRNA Jak2 and Stat3 along with reduced expression of miRNA-409 and reduction of Jak2 and Stat3 protein in response to miRNA-409 overexpression in liver cancer cells may allow to assume miRNA-409 in liver cancer playing an antitumor function through interaction with Jak2 and Stat3. Increased expression of miRNA-409 in liver cancer cells led to a decrease in cell viability and increased apoptosis. This miRNA expression level was significantly decreased in liver cancer tissues compared with paracancerous and normal liver tissues and was negatively correlated with tumor stage, tumor size, and overall survival time of patients with liver cancer [52]. Another miRNA, which is putatively involved in interaction with Jak2 and Stat3 in liver cancer, is miRNA-543, whose expression level was also shown to be decreased in liver cancer tissues. Like miRNA-409, it has a protective role in liver cancer and OS in patients with liver cancer and increased miRNA-543 is longer than in patients with decreased miRNA-543. Inhibition of miRNA-543 expression resulted in liver cancer cells with exactly the same consequences like inhibition of miRNA-409: increased cancer cell proliferation and decreased apoptosis. It also activated the protein expression of phosphorylated JAK2, phosphorylated STAT3, c-Myc, and B-cell lymphoma 2 (Bcl-2) in liver cancer cells [53]. miRNA-3662, which downregulated in HCC tissues and cell lines, may be involved in reprogramming cancer cells' glucose metabolism and forming of Warburg effect while having hypoxia-inducible factor-1α (HIF-1α) as one of the direct targets. Gain-of-function and loss-of-function assays showed that miR-3662 dampened glycolysis by reducing lactate production, glucose consumption, cellular glucose-6-phosphate level, ATP generation, and extracellular acidification rate, and increasing oxygen consumption rate in HCC cells [54]. Another putative target of miRNA-3662 in HCC cells, which allows its regulation of glucose metabolism, is hexokinase 2 (HK2). miR-3662 expression was decreased in liver cancer tissues and cells, while overexpression of miR-3662 or knockdown of HK2 inhibited cell proliferation, invasion, and glucose metabolism in cancer liver cells, which could be reversed by upregulating HK2 [55].

Taking into account the ambiguous relation between miRNA and mRNA expression levels and other factors, including circRNAs and proteins, associated with miRNA biogenesis and those involved in miRNA and mRNA interactions, prediction of changes in miRNA expression levels in any cancer, including liver cancer, becomes not that obvious task.

5. miRNAs and cancer cell metabolism

Along with changes on the genetic level, metabolic changes accompany cancer development in order to provide cells functioning in changing conditions, mainly hypoxia and glucose insufficiency due to intensive cell proliferation and clonal expansion and lagging in blood vessel formation. One of the main such metabolic reorganizations is Warburg effect, firstly reported in rat liver carcinoma in the 1920s and defined as an increase in the rate of glucose uptake and preferential production of lactate even in the presence of oxygen [56]. Main miRNAs, which are involved in Warburg effect realization in HCC cells, are miR-1, miR-122, and miR-338-3p [57]. The expression of the miR-1 targets G6PD and is mediated by NRF2, which, besides activating the transcription of genes encoding glycolytic enzymes, inhibits the conversion of pyruvate to acetyl-CoA by directly activating pyruvate dehydrogenase kinase 1 (PDK1) and leads to inhibition of tricarboxylic acid (TCA) cycle, promoting Warburg effect [58]. At the background of these interactions, it may appear to be regular that a higher expression of miR-1 showed a significant positive prognostic meaning for patients with HCC: Individuals with higher miR-1 serum levels showed longer OS than those with lower miR-1 serum concentrations (195 sera of HCC patients and 54 patients with liver cirrhosis; HR 0.451, 95% CI 0.228–0.856, P = 0.015). At the same time, serum miR-1 and miR-122 concentrations did not differ significantly between patients with HCC and liver cirrhosis [58].

miR-122 is another element of the processes that are assumed to restrain Warburg effect promotion, as far as among its main targets are Agpat 1 and Dgat 1 mRNAs, involved in triglyceride synthesis. One of the most important direct targets of miR-122 in HCC cells is PKM2, which is the most abundant pyruvate kinase iso-enzyme in liver tumors and a co-activator of several transcription factors, such as HIF-1α, β-catenin/c-Myc, NF-κB, and STAT3. Once in the nucleus, PKM2 can promote the transcription of target genes, such as HIF-1α targeted expression of GLUTs, PKM2, LDH-A, and VEGF-A, leading to the promotion of growth, positive feedback regulated glycolysis, and angiogenesis in cancer cells [59], and all these allow miR-122 to promote a decrease in lactate production and increase in oxygen consumption, thus reversing oxygen-independent glycolytic metabolism. Along with this, Yang G. et al. showed that miR-122 is downregulated in tissue samples from patients with HCC and also participates in ADAM17 regulation, which makes upregulation of miR-122 to inhibit proliferation of HCC cells *in vitro* [60]. In general, miR-122 is putatively one of the first examples of a tissue-specific miRNA and is highly expressed in the liver, where it constitutes 70% of the total miRNA pool [61]. Moreover, miR-122 has a prognostic role in HCC patients, and its downregulation is associated with poor prognosis: The overall survival time of the patients with low and high miR-122 expression in HCC was 30.3±8.0 and 83.7±10.3 months, respectively ($P<0.001$, tissue samples from 64 HCC patients and 28 matched non-neoplastic surrounding liver tissues) [62].

miR-338-3p has the same impact on Warburg effect in cancer liver cells such as miR-1 and miR-122, inhibiting it through decreasing expression of liver and red blood cell pyruvate kinase isoform (PKLR). miR-338-3p may be inactivated in HCC due to upregulation of circMAT2B, sequestering miR-338-3p due to its sponging activity, and disabling the regulation of its target gene PKM2 leading to increased proliferation, invasion, spheroid formation, and organoid dimensions, especially in hypoxic

conditions [59]. Expression of miR-338-3p in tissue samples from patients with HCC was also shown to be decreased [33].

miR-23 was shown to be involved in gluconeogenesis regulation in liver cancer developed in the mouse model. Reduction in serum glucose in tumor-bearing mice correlated with a reduction in the expressions of G6pc, Pepck, and Fbp1 encoding the key gluconeogenic enzymes glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, fructose-1,6-phosphatase, respectively, and the transcription factor Pgc-1α along with upregulation of miR-23a expression. mRNA levels of these genes were reduced to ≈80% in the majority of primary human HCC tissue samples compared with matching peritumoral liver samples and miR-23a was also upregulated in human liver cancer samples. Moreover, PGC-1α and G6PC expression negatively correlated with miR-23a expression in human HCCs [63]. miR-23a has a significant diagnostic value as far as it may distinguish cirrhotic liver samples from cancer liver samples, being higher in the HCC group than cirrhotic. miR-23a was significantly higher in HCC patients with focal lesion size equal or more than 5 cm, patients with multiple focal lesions, and Okuda stage III. At cutoff value ≥ 210, miR-23a showed accuracy of 79.3% to diagnose HCC patients with sensitivity of 89.47% and specificity of about 64.91% ((57 patients with HCC, 57 patients with liver cirrhosis (LC), and 57 healthy subjects as control group) and serum alpha-fetoprotein at cut off level ≥ 200 ng/mL had 73.68% sensitivity and 52.63% specificity for diagnosis of HCC [64].

6. Differential expression of miRNAs in chronic liver disease

Pathological states in some cases underlying liver cancer development are also accompanied by changes in miRNA expression levels such as hepatitis B virus infection, hepatitis C virus infection, nonalcoholic fatty liver disease (NAFLD), and alcoholic liver disease. One of miRNAs, which is associated with hepatitis virus, is miR-23 [31]. Other miRNAs also may differentiate HCC samples at the background of viral hepatitis from others, such as miR-17-92. Together with miR-21, its expression level was increased in hepatitis B virus-positive human and woodchuck HCC samples. Possibly, hepatitis B virus X and miR-17-92 share common target gene, which is c-myc, which is activated by virus and is known to be the instrument of carcinogenesis promotion of miR-17-92 [65]. Unlike HCV infection, HBV is known to induce HCC development gapping cirrhosis stage, thus making molecular predictors of cancer in this case especially required. Besides miR-122 and miR-17-92, other miRNAs, involved in HBV pathogenesis following HCC development, are miR-184, miR-185, miR-196a, miR-199a-3p, miR-210, miR-217, and miR-34a, which are involved in HBV transcription process. The expression of the last is inhibited by HBV X protein (HBx) *via* p53 stimulation in hepatocytes, upregulating a macrophage-derived chemokine (CCL22), participating in regulatory T cells stimulation and effector T cells suppression, which results in increasing HBV genome transcription [66, 67]. Another miRNA, whose expression changes in HBV infection due to HBx, is miR-155. Upregulation of miR-155 leads to a reduction in the suppressor of cytokine signaling-1 (SOCS1) expression, increasing JAK/STAT signaling and suppressing HBV infection mediated by the induction of interferon (IFN) signaling [68]. Some of these miRNAs, which are involved in HBV transcription process, are also involved in the regulation of signaling pathways, disrupted in the cancer development. Among putative targets of miRNA-199a-3p are mTOR, c-Met, HIF-1α, CD44, ROCK1, and Axl, so this miRNA, being downregulated in

liver cancer samples, may inhibit cell proliferation, migration, and invasion [69]. miRNA-184 may function as an anti-apoptotic factor in liver cancer, and INPPL1 was identified as one of its targets. Through inhibition of the activities of caspases 3/7 and INPPL1 loss, it promotes cancer cell proliferation [70]. Similar function in HCC development through another target may play miRNA-196a. One of its direct targets is FOXO1, whose inhibition caused by miRNA-196a overexpression resulted in liver cancer cell migration and invasion *in vivo* [71]. miRNA-210 is known to be overexpressed in liver cancer samples and in HBV-associated liver cirrhosis; moreover, its expression was significantly higher in HepG2.2.15 cells than that in HepG2 cells. EGR3 was shown to be the target of miRNA-210, contrary to miRNA-210 the expression of EGR3 was downregulated in HBV-associated liver cirrhosis and liver cancer. In general, its role, like the role of miRNA-196a and miRNA-184, may be in proliferation promotion, and it is hard to assume whether the described mechanism with involvement in EGR3 regulation is specific for HBV-associated liver cancer, as far as EGR3 is known to be decreased in other cancers, like in head and neck cancers and gastric cancer [72]. miRNA-210 was also shown to be involved in bile acid-induced cholestatic liver injury through its direct target MLL4 (the histone methyltransferase mixed-lineage leukemia-4). miRNA-210 was the most highly elevated miR in mice with elevated hepatic BA levels and its expression was increased in patients with primary biliary cholangitis/cirrhosis (PBC) [73]. Opposite to the mentioned miRNAs, miRNA-217 inhibits liver cancer cell proliferation *via* targeting NAT2; moreover, its overexpression contributed to steatosis in hepatocytes as well as inflammation in mice, acting as a critical regulator in ethanol-induced hepatic inflammation [74]. miRNA-185 was shown to be involved in cholesterol metabolism in mouse model, and animals with knockout of this miRNA developed worsened hepatic steatosis upon high-fat high-cholesterol Western diet feeding with accumulation of triglyceride and cholesterol in the liver and developed hypercholesterolemia upon Western diet feeding. Treatment with miRNA-185 showed an improved accumulation of lipids in high-fat diet mouse model and insulin sensitivity *via* upregulation of the insulin-receptor substrate-2 [75, 76].

In case of HCV infection, there are miRNAs, which not just regulate expression of the genes, involved in virus replication, but are able to directly target the viral genome. Among these miRNAs are miR-196, miR-448, and miR-122, which stabilize the 5′ and 3′ UTRs of the HCV genome, so inhibition of this miRNA dramatically reduces the replication of HCV RNA [77, 78]. miRNAs, involved in viral replication *via* reducing tumor suppressor deleted in liver cancer 1 (DLC-1) and cell entry *via* the PI3K/AKT signaling pathway, are miR-141 and miR-491, respectively [76].

When it concerns alcoholic liver disease, miRNA expression profile changes especially due to increase in expression of inflammation-related miRNAs, such as miR-132, miR-155, miR-146, and miR-21, which influence alcohol/lipopolysaccharide (LPS)/TLR4 pathways, transmitting proinflammatory stimuli *via* a mitogen-activated protein kinases (MAPKs) or TIR domain-containing adaptor-inducing IFN-β (TRIF) [79, 80]. The imbalance between expression of let-7 and LIN28/28B has the crucial consequences, as far as regular alcohol overdose consumption diminishes let-7 expression, and loss of let-7 induces transformation of hepatic stellate cells (HSC) to mesenchymal phenotype, enhancing liver injury *via* inhibiting LIN28B, and thus promoting oncogenesis [81]. One of mechanisms underlying fibrosis formation in response to alcohol consumption is miR-34a, and its upregulation causes deregulation of its direct targets such as caspase-2, SIRT1, and matrix metallopeptidase (MMP) 1 and MMP2 [82].

Another risk factor for HCC development is NAFLD. It was revealed that miR-34a and miR-122 identified in blood serum are potential markers for discriminating NAFLD patients from healthy controls with an area under the curve (AUC) values of 0.781 and 0.858, respectively, along with miR-21, miR-125b, and miR-375 did not show significant difference in level expression between NAFLD patients and healthy controls. Serum levels of miR-34a and miR-122 were found to be significantly higher among NAFLD patients and were positively correlated with VLDL-C and triglyceride levels [83]. The other study showed the same tendency for miRNA-34a and miRNA-122, and also found a significant difference in level expression of following miRNA: miR-21and miR-451. Moreover, the serum level of miR-122 was correlated with the severity of liver steatosis; however, in the previous study, the expression levels of miR-34a and miR-122 did not correlate with the histological features of NAFLD [83]. There is at least one common signaling pathway, involving both miRNA-34a and miRNA-122, which is AMPK [21, 82]. It should be admitted that along with being possibly involved in the regulation of the same targets, miRNA-122 and miRNA-34 definitely should have similar effects as far as miRNA-122 expression is decreased in cancers including liver cancer, and miRNA-34 expression is decreased in liver cancer while being elevated in chronic hepatitis C patients. Targets and mechanisms of miRNA-34 involvement in the liver cancer development are known in less details than miRNA-122. It is supposed, that through involvement in the Sirt1/p53 pathway regulation, miRNA-34 promotes liver fibrosis in patients with HCV [84]. It is possible to speculate that with loss of the benign liver cells phenotype, malignant liver cells lose possibility of normal miRNA-34 expression as far as miRNA-34 expression is significantly decreased in liver cancer cells. One of the possible mechanisms of miRNA-34 involvement in the liver cancer development is glucose metabolism, in which LDHA, which is the target gene of miRNA-34, participates in glucose metabolism reprogramming with its switch to the increased glycolysis [85]. In its turn, miRNA-122 is also indirectly involved in glucose metabolism, having Igf1R as one of its targets [63]. The other putative intersection of these two miRNAs is p53 pathway, as far as both participate in its regulation. miRNA-34 in the context of HCV fibrosis via Sirt1 regulation and miRNA-122 through cyclin G1, which results in increased p53 protein stability activity and reduction in invasion capabilities of HCC cells with elevated miRNA-122 expression [86]. Another miRNA-132, which possibly shares with miRNA-34 SIRT1 as a common target, is miRNA-132. This miRNA is elevated in the response to alcohol consumption, while *in vivo* and *in vitro* studies suggest miR-132 targets SIRT1, being increased in HCC cells and associated with unfavorable survival in HCC patients [87].

With the cancer development, differences in miRNA expression profiles smooth out as far as cancer cell phenotype and functions despite its different background development including such common features as promoted proliferation, disrupted apoptosis, and increased migratory and invasion capabilities. miRNA expression during the process of malignization changes in conformity with these demands, so far miRNA-34, being elevated in HCV liver cirrhosis, is decreased in the liver cancer cells. Obviously, all changes in miRNA expression during cancer development tend to upregulation of oncogenic miRNAs and downregulation of tumor suppressor miRNAs, and with evolution of the stage of the tumor differences in miRNA expression associated with different background liver disease level out. However, different miRNAs may be involved in the same signaling pathways or share common target genes, which is allowed by the sequence and molecular nature of miRNA—mRNA interaction—and indirect influence of miRNA on the expression levels of the genes, which are not its direct targets.

7. Differential expression of miRNAs in HCC

Concerning signaling pathways disrupted in HCC, almost all these pathways the regulation is double-sided: miRNA may regulate the expression of the genes and genes may regulate the expression of miRNAs. For example, TGF-β signaling may modulate miRNA expression level *via* canonical pathway, which is Smad-dependent requiring co-Smad Smad4, and non-canonical pathway, such as Smad4-independent. The Smad binding element found in the promoters of TGF-β/BMP-regulated genes contains a conserved sequence similar to the pre-miRNAs of TGF-β/BMP-regulated miRNAs, which is CAGAC [88]. miRNAs, containing this RNA-Smad binding element (R-SBE), are following: miR-105, miR-199a, miR-215, miR-421, and miR-529 [34]. The miRNAs upregulated by TGF-β signaling include miR-21, the miR-181 family, miR-10b, the miR-17/92 cluster, miR-155, miR-192, the miR-23/24/27 cluster, miR-216/217, miR-494, and miR-182. The miRNAs downregulated by TGF-β signaling include the miR-200 family, miR-203, let-7, miR-34a, and miR-584 [34]. Many miRNAs targeting different substrates of TGF-β pathway are frequently oncogenic, such as miR-200, having impact on the expression of TGF-β ligands and TGF-β receptors type I and II, miR-21, miR0211, miR-17/92, and miR-106b/206, mainly participating in regulation of expression TGF-β receptors type I and II and miR-182, involved in the regulation of R-Smad, co-Smad (I-Smad), and Smad7, and it modulates a negative feedback loop of TGF-β signaling as Smad7 is also induced by TGF-β. Besides TGF-β receptors type I and II, miR-17/92 and miR-106b/205 participate in the regulation of downstream targets of TGF-β pathway [34].

miR-141 and miR-200a expression levels were shown to be decreased and serum samples from patients with liver cancer (blood samples were taken from 30 patients with liver cancer and from 30 normal subjects, RNU6 or GAPDH as internal controls). The sensitivity and specificity of the investigated miRNAs for diagnosing tumor invasion in liver cancer and comparing metastasis of patients with liver cancer were higher in combination of miR-141 and miR-200a rather than alone, although the difference between AUC values in both cases—combination and one miR regimen—had no significant changes. The possible mechanism of cancer processes modulation was explained with E-cadherin and vimentin inhibition due to STAT4 inhibition, which was firstly reported as a target gene for these miRNAs [89, 90]. The study of Dhayat et al. also showed a significant negative correlation of miR-200a and miR-200b to the expression of the mesenchymal markers Vimentin and ZEB-1 and a significant positive correlation to the epithelial marker E-cadherin. Moreover, in this study, miR-200 family was significantly downregulated in HCC samples compared to liver cirrhosis and was shown to be able to distinguish between cirrhotic and HCC tissue [90].

miR-211-5p was significantly downregulated in patients with HCC (30 pairs of HCC tissues and matched adjacent tumor-free tissues, qRT-PCR, RNU6 (miRNA) as an endogenous control), although miR-211-5p expression in liver cancer samples was not significantly different from adjacent normal samples based on TCGA cohorts, it was considerably downregulated in 30 pairs of HCC tissues compared with matched adjacent tumor-free tissues from patients in clinics or real-world cohorts. It was also shown that miR-211-5p may have a prognostic role for HCC patients: Patients with a decreased expression of miR-211-5p had poor overall survival [91]. In another study, miR-211-5p was found to be decreased in 33 out of 40 HCC tissue samples compared with the corresponding non-tumor tissues; moreover, tissues from lymph node metastases also expressed lower levels of miR-211 compared with primary HCC tissues and the adjacent normal tissue (qRT-PCR, the

endogenous U6 snRNA or GAPDH as the internal control) [68]. Among miR-211-5p targets are STAB2, SPARC, ZEB2, and ACSL4, negatively regulating these genes, miR-211-5p participates in the suppression of cell proliferation, migration, and invasion in HCC tissues [92].

In contrast, miR-17/92 expression levels were shown to be highly expressed in HCC tissues compared to the non-tumor liver tissues (94 cases of HCC, 5 cases of cancer adjacent to normal hepatic tissue, and 5 cases of normal liver tissue; U6 small nuclear 2 (U6b) as an internal control, RT-PCR). In this study, it was shown that expression of miR-17-92 was negatively correlated with several target genes, including CREBL2, PRRG1, and NTN4, when analyzing the miRNA and mRNA sequencing data from the 312 hepatocellular cancer patients available from the TCGA database [68].

Expression level of miR-182-5p was also elevated in HCC tissues and its high expression level correlated with poor prognosis such as early recurrence in patients who underwent curative surgery (tissue samples from 119 patients; RT-PCR, U6 snRNA was probed as a loading control; the disease-free survival was calculated from the date of resection to the date of tumor recurrence). Promotion of HCC proliferation by miR-182-5p is partly possible due to the ability of the last to directly target 3′-UTR of FOXO3a and thus inhibits FOXO3a expression, activating AKT/FOXO3a pathway. MiR-182-5p interacts with 3`-UTR of FOXO3a by binding to the 72-79 site, but not the 914–921 site in the 3′-UTR of FOXO3a [93].

However, in almost all these pathways the regulation is double-sided: miRNA may regulate the expression of the genes, and genes may regulate the expression of miRNAs. TGF-β signaling may modulate miRNA expression level *via* the canonical pathway, which is Smad-dependent requiring co-Smad Smad4, and non-canonical pathway, such as Smad4-independent. The Smad binding element found in the promoters of TGF-β/BMP-regulated genes contains a conserved sequence similar to the pre-miRNAs of TGF-β/BMP-regulated miRNAs, which is CAGAC [34]. miRNAs, containing this RNA-Smad binding element (R-SBE), are following: miR-105, miR-199a, miR-215, miR-421, and miR-529 [34]. The miRNAs upregulated by TGF-β signaling include miR-21, the miR-181 family, miR-10b, the miR-17/92 cluster, miR-155, miR-192, the miR-23/24/27 cluster, miR-216/217, miR-494, and miR-182. The miRNAs downregulated by TGF-β signaling include the miR-200 family, miR-203, let-7, miR-34a, and miR-584 [34]. Many miRNAs targeting different substrates of TGF-β pathway are frequently oncogenic, such as miR-200, having an impact on the expression of TGF-β ligands and TGF-β receptors type I and II, miR-21, miR0211, miR-17/92, and miR-106b/206, mainly participating in regulation of expression TGF-β receptors type I and II and miR-182, involved in regulation of R-Smad and co-Smad (I-Smad) and Smad7, and it modulates a negative feedback loop of TGF-β signaling as Smad7 is also induced by TGF-β. Besides TGF-β receptors type I and II, miR-17/92 and miR-106b/205 participate in regulation of downstream targets of TGF-β pathway [34].

It may seem interesting that almost none of these miRNAs were included in the list of miRNAs, implying HCC signature based on TCGA database, which consists of 540 miRNA expression profiles from 348 HCC patients, of whom 248 had early-stage and 90 had advanced-stage HCC. SVM-HCC, based on an SVM29 incorporating the feature selection algorithm IBCGAa proposed method, used a feature selection algorithm (IBCGA) to select a significant miRNA signature associated with early and advanced stages of HCC. This signature contains 23 miRNAs: in order of decreasing MED (Main

Effect Difference) scores, miR-550a, miR-549, miR-518b, miR-512, miR-1179, miR-574, miR-424, miR-4286, let-7i, miR-320a, miR-17, miR-299, miR-3651, miR-2277, miR-621, miR-181c, miR-539, miR-106b, miR-1269, miR-139, miR-152, miR-2355, and miR-150. Moreover, the significance of 10 top-ranked miRNAs in distinguishing HCC tissue samples from normal tissue samples and their prognostic values were proved on different datasets [94]. For some of these miRNAs with the highest MED scores, there are known targets in the context of liver cancer development. Among direct targets of miRNA-320a, there are β-catenin, c-myc, cyclin D1, and dickkopf-1; functional studies have shown a significantly decreased capability of cell proliferation and G0/G1 growth arrest *in vitro* when miRNA-320 is overexpressed [95]. miRNA-424 demonstrated a strong prognostic value in liver cancer: Its expression level in HCC tissues was associated with a relapse after liver transplantation. The study involved samples from 121 patients, the median follow-up duration was 25.12 months, U6 snRNA was used as the endogenous control [96]. In liver cancer cell lines, it was shown that miRNA-574-3p has an ADAM28 as a direct target and binds 3′-untranslated region of the ADAM28 mRNA leading to reduced cell proliferation and migration and promoted cell apoptosis [97]. Like miRNA-200 and miRNA-211-5p, which are mentioned above in the context of HCC development and which are not listed in this miRNA list, miRNA-1179 directly interacts with zinc-finger E-box-binding homeobox 2 (ZEB2) and has antitumor function, leading to attenuated proliferation and migration of HCC cells. Its expression was decreased in HCC tissues compared with corresponding noncancerous tissues (40 paired HCC samples with matched normal tissues, U6 were used as internal reference) [98]. miRNA-550a plays a controversial role in HCC development, promoting HCC cell migration and invasion, and cytoplasmic polyadenylation element-binding protein 4 (CPEB4) is one of its potential targets, which is commonly decreased in liver cancer. The function of CPEB4, which is from the gene family CPEB involved in regulation of translation by controlling the polyadenylation of target genes, is yet to be elucidated. There are contradictional data of its function in cancer development: In pancreatic ductal cancer and neuroblastoma, its expression is upregulated, driving the growth and invasion of cancer cells. In HCC, it was shown that CPEB4 siRNA could promote the migration and invasion of HCC cells. This contradiction may be explained by different downstream targets regulated by CPEB4 in different cells because CPEB4 along with involvement in polyadenylation can control the translation by binding to the CPE sequence in 3′ UTR of the corresponding genes [99]. miRNA-512 may play one of the crucial roles in HCC progression, being significantly upregulated in human HCC samples and HCC cell lines. Along with miRNA-519, it targets tumor suppressors MAP3K2 and MAP2K4, and the integration of these two miRNAs into the AJCC staging system significantly improved the accuracy of the prediction of HCC recurrence [100]. Some of these miRNAs, such as miRNA-518b, miRNA-549, miRNA-2277, and miRNA-2355, are rarely mentioned in the context of liver cancer development, and their role in this process is yet to be elucidated.

This emphasizes the importance of working with customized algorithms and validated big datasets, when many of the aspects of the process you are studying—like miRNA involvement in carcinogenesis—stay unclear, making identification of the most promising diagnostic and/or prognostic and/or therapeutic molecules *via* analyzing only mRNA-miRNA interactions or miRNAs in signaling or metabolic pathways not very effective.

In **Table 1**, there are listed miRNAs, whose expression levels are changed in the HCC development in order of mention in the text.

No.	miRNA	Decreased or increased compared with normal tissue	Possible targets	Effects of overexpression	References
1	miRNA-200a	Decreased	ZEB1, ZEB2, TGF-β	Inhibition of cell proliferation and colony formation rate	[34, 35]
2	miRNA-21	Increased	TGFBR2 FASLG, PTEN, HBP1, IL-12, RECK, and TIMP-3	Promotion of tumor invasion and metastasis	[37, 39]
3	miR-211	Decreased	WNT-β signaling regulation *via* SATB2	Inhibition of cancer cells proliferation	[41]
4	miR-125b	Decreased	Hh	Arrest cell cycle at G_1 to S transition	[43, 44]
5	miRNA-199a-3p	Decreased	NOTCH, YAP1	Inhibition of HCC cell proliferation and induction of HCC cell apoptosis	[46]
6	miRNA-17-5p	Decreased	ERBB3	Inhibition of cancer cell proliferation	[48]
7	miRNA-637	Decreased	Autocrine leukemia inhibitory factor (LIF), Stat 3	Inhibition of cancer proliferation	[50]
8	miRNA-409	Decreased	Jak2, STAT3	Decreased cancer cell viability and increased apoptosis	[52]
9	miRNA-543	Decreased	Jak2, Stat3	Decreased cancer cell viability and increased apoptosis	[53]
10	miRNA-3662	Decreased	HIF-1α, HK2	Glucose metabolism transformation to Warburg effect, decreased glycolysis	[54, 55]
11	miRNA-122	Decreased	Agpat 1, Dgat1, PKM2, ADAM17	Inhibition of cancer cells proliferation	[59, 60]
12	miRNA-338-3p	Decreased	PKLR	Inhibition of Warburg effect	[30, 33]
13	miRNA-23	Increased	G6pc, Pepck, and Fbp1	Increased gluconeogenesis	[63]
14	miRNA-184	Increased	INPPL1	Promoting cancer cells proliferation	[70]
15	miRNA-196a	Increased	FOXO1	Promotion of liver cancer cell migration and invasion	[71]
16	miRNA-210	Increased	EGR3, MLL4	Promotion of liver cancer cell proliferation	[72, 73]
17	miRNA-217	Decreased	NAT2	Inhibition of cancer cell proliferation	[74]

No.	miRNA	Decreased or increased compared with normal tissue	Possible targets	Effects of overexpression	References
18	miRNA-34	Decreased	AMPK, Sirt1/p53, LDHA	Inhibition of gluconeogenesis	[85]
19	miRNA-122	Decreased	AMPK, Cyclin G1	Promotion of cancer cells proliferation	[86]
20	miRNA-132	Increased	SIRT1	Promotion of cancer cell proliferation	[87]
21	miRNA-141	Decreased	STAT4	Promotion of cancer cell proliferation	[89]
22	miRNA-17/92	Increased	CREBL2, PRRG1, NTN4	Promotion of cancer cell proliferation and invasion	[68]
23	miRNA-182-5p	Increased	FOXO3a	Promotion of cancer cell proliferation and invasion	[93]
24	miRNA-320a	Decreased	β-catenin, c-myc, cyclin D1 and dickkopf-1	Decreased capability of cell proliferation and G0/G1 growth arrest	[95]
25	miRNA-574-3p	Decreased	ADAM28	Reduced cancer cell proliferation and migration	[97]
26	miRNA-1179	Decreased	ZEB2	Reduced cancer cell proliferation and migration	[98]
27	miRNA-550a	Increased	CPEB4	Promotion of cancer cell proliferation	[99]
28	miRNA-512	Increased	MAP3K2, MAP2K4	Increased cancer cell proliferation rate and migration capability	[100]

Table 1.
miRNAs in liver cancer development.

8. Conclusion

miRNAs play a pivotal role in liver cancer development; presumably in the early stages of HCC development, its multiple miRNA expression profiles may be distinguished depending on the etiological factor, such as HBV, HCV, NAFLD, or ALD. miRNAs are biomarkers with a huge potency as far as they are small, stable, and protected from RNases *via* protein binding or exosome membrane and their expression level change at the background of metabolism and signaling pathways modification during malignancy in the liver. Biogenesis of miRNA and its interaction with mRNA are not clear and involve different proteins and other noncoding miRNAs, which make it difficult to predict miRNAs expression level change in the specific cancer type and require special algorithm and big data sets. It should be noted that changes

in expression levels of almost all miRNAs listed above are not specific to liver cancer and are identified in prostate cancer, gastric cancer, colon cancer, and other cancer types. In this context, a multiple miRNA panel is a promising option for diagnostic purposes.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Leitão AL, Enguita FJ. A structural view of miRNA biogenesis and function. Noncoding RNA. 2022;**8**(1):10. DOI: 10.3390/ncrna8010010

[2] Chou CH, Shrestha S, Yang CD, Chang NW, Lin YL, Liao KW, et al. miRTarBase update 2018: A resource for experimentally validated microRNA-target interactions. Nucleic Acids Research. 2018;**46**(D1):D296-D302. DOI: 10.1093/nar/gkx1067

[3] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A, et al. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2018;**2018**(68):394-424. DOI: 10.3322/caac.21492

[4] O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol (Lausanne). 2018;**9**:402. DOI: 10.3389/fendo.2018.00402

[5] Ha M, Kim VN. Regulation of microRNA biogenesis. Nature Reviews. Molecular Cell Biology. 2014;**15**:509-524. DOI: 10.1038/nrm3838

[6] Adams L. Non-coding RNA: Pri-miRNA processing: Structure is key. Nature Reviews. Genetics. 2017;**18**(3):145. DOI: 10.1038/nrg.2017.6

[7] Michlewski G, Guil S, Semple CA, Cáceres JF. Posttranscriptional regulation of miRNAs harboring conserved terminal loops. Molecular Cell. 2008;**32**(3):383-393. DOI: 10.1016/j.molcel.2008.10.013

[8] Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes & Development. 2003;**17**:3011-3016. DOI: 10.1101/gad.1158803

[9] Olena AF, Patton JG. Genomic organization of microRNAs. Journal of Cellular Physiology. 2010;**222**(3):540-545. DOI: 10.1002/jcp.21993

[10] Stavast CJ, Erkeland SJ. The non-canonical aspects of MicroRNAs: Many roads to gene regulation. Cell. 2019;**8**(11):1465. DOI: 10.3390/cells8111465

[11] Arora S, Rana R, Chhabra A, Jaiswal A, Rani V. miRNA-transcription factor interactions: A combinatorial regulation of gene expression. Molecular Genetics and Genomics. 2013;**288**(3-4):77-87. DOI: 10.1007/s00438-013-0734-z

[12] Martinez NJ, Walhout AJ. The interplay between transcription factors and microRNAs in genome-scale regulatory networks. BioEssays. 2009;**31**(4):435-445. DOI: 10.1002/bies.200800212

[13] Mullany LE, Herrick JS, Wolff RK, Stevens JR, Samowitz W, Slattery ML. MicroRNA-transcription factor interactions and their combined effect on target gene expression in colon cancer cases. Genes, Chromosomes & Cancer. 2018;**57**(4):192-202. DOI: 10.1002/gcc.22520

[14] Zhou L, Miller C, Miraglia LJ, Romero A, Mure LS, Panda S, et al. A genome-wide microRNA screen identifies the microRNA-183/96/182 cluster as a modulator of circadian rhythms. Proceedings of the National Academy Science USA.

2021;**118**(1):e2020454118. DOI: 10.1073/pnas.2020454118

[15] Thomson DW, Dinger ME. Endogenous microRNA sponges: Evidence and controversy. Nature Reviews. Genetics. 2016;**17**(5):272-283. DOI: 10.1038/nrg.2016.20

[16] Plotnikova O, Baranova A, Skoblov M. Comprehensive analysis of human microRNA–mRNA interactome. Frontiers in Genetics. 2019;**10**:1-11. DOI: 10.3389/fgene.2019.00933

[17] Kersten S. Integrated physiology and systems biology of PPARα. Molecular Metabolism. 2014;**3**(4):354-371. DOI: 10.1016/j.molmet.2014.02.002

[18] Ding J, Li M, Wan X, Jin X, Chen S, Yu C, et al. Effect of miR-34a in regulating steatosis by targeting PPARα expression in nonalcoholic fatty liver disease. Scientific Reports. 2015;**5**:13729. DOI: 10.1038/srep13729

[19] Chai C, Rivkin M, Berkovits L, Simerzin A, Zorde-Khvalevsky E, Rosenberg N, et al. Metabolic circuit involving free fatty acids, microRNA 122, and triglyceride synthesis in liver and muscle tissues. Gastroenterology. 2017;**153**(5):1404-1415. DOI: 10.1053/j.gastro.2017.08.013

[20] Krützfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, et al. Silencing of microRNAs in vivo with 'antagomirs'. Nature. 2005;**438**(7068):685-689. DOI: 10.1038/nature04303

[21] Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, et al. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metabolism. 2006;**3**(2):87-98. DOI: 10.1016/j.cmet.2006.01.005

[22] Chung HH. New insights for controversial issues of miR-122 in hepatic lipid metabolism. Gastroenterology. 2018;**154**(5):1552-1553. DOI: 10.1053/j.gastro.2017.12.039

[23] Morishita A, Oura K, Tadokoro T, Fujita K, Tani J, Masaki T. MicroRNAs in the pathogenesis of hepatocellular carcinoma: A review. Cancers (Basel). 2021;**13**(3):514. DOI: 10.3390/cancers13030514

[24] Ramírez CM, Goedeke L, Rotllan N, Yoon JH, Cirera-Salinas D, Mattison JA, et al. MicroRNA 33 regulates glucose metabolism. Molecular and Cellular Biology. 2013;**33**(15):2891-2902. DOI: 10.1128/MCB.00016-13

[25] Wu H, Zhang T, Pan F, Steer CJ, Li Z, Chen X, et al. MicroRNA-206 prevents hepatosteatosis and hyperglycemia by facilitating insulin signaling and impairing lipogenesis. Journal of Hepatology. 2017;**66**(4):816-824. DOI: 10.1016/j.jhep.2016.12.016

[26] Suksangrat T, Phannasil P, Jitrapakdee S. miRNA regulation of glucose and lipid metabolism in relation to diabetes and non-alcoholic fatty liver disease. Advances in Experimental Medicine and Biology. 2019;**1134**:129-148. DOI: 10.1007/978-3-030-12668-1_7

[27] Lotterman CD, Kent OA, Mendell JT. Functional integration of microRNAs into oncogenic and tumor suppressor pathways. Cell Cycle. 2008;**7**(16):2493-2499. DOI: 10.4161/cc.7.16.6452

[28] Pu J, Wang J, Li W, et al. Hsa_circ_0000092 promotes hepatocellular carcinoma progression through up-regulating HN1 expression by binding to microRNA-338-3p. Journal of Cellular and Molecular Medicine. 2020:1-13. DOI: 10.1111/jcmm.15010

[29] Liu Z, Yang D, Li Y, Jiao Y, Lv G. HN1 as a diagnostic and prognostic biomarker for liver cancer. Bioscience Reports. 2020;**40**(7):BSR20200316. DOI: 10.1042/BSR20200316

[30] Li Q, Pan X, Zhu D, Deng Z, Jiang R, Wang X. Circular RNA MAT2B promotes glycolysis and malignancy of hepatocellular carcinoma through the miR-338-3p/PKM2 axis under hypoxic stress. Hepatology. 2019;**70**(4):1298-1316. DOI: 10.1002/hep.30671

[31] Louis C, Leclerc D, Coulouarn C. Emerging roles of circular RNAs in liver cancer. JHEP Reports. 2021;**4**(2):100413. DOI: 10.1016/j.jhepr.2021.100413

[32] Liu J, Liu T, Wang X, He A. Circles reshaping the RNA world: From waste to treasure. Molecular Cancer. 2017;**16**(1):58. DOI: 10.1186/s12943-017-0630-y

[33] Suzuki HI. MicroRNA control of TGF-β signaling. International Journal of Molecular Sciences. 2018;**19**(7):1901. DOI: 10.3390/ijms19071901

[34] Gregory PA, Bracken CP, Smith E, et al. An autocrine TGF-beta/ZEB/miR-200 signaling network regulates establishment and maintenance of epithelial-mesenchymal transition. Molecular Biology of the Cell. 2011;**22**(10):1686-1698. DOI: 10.1091/mbc.E11-02-0103

[35] Feng J, Wang J, Chen M, et al. miR-200a suppresses cell growth and migration by targeting MACC1 and predicts prognosis in hepatocellular carcinoma. Oncology Reports. 2015;**33**:713-720. DOI: 10.3892/or.2014.3642

[36] Mao Y, Chen W, Wu H, Liu C, Zhang J, Chen S. Mechanisms and functions of MiR-200 family in hepatocellular carcinoma. Oncotargets and Therapy. 2021;**13**:13479-13490. DOI: 10.2147/OTT.S288791

[37] Wang Z, Li Y, Kong D, Ahmad A, Banerjee S, Sarkar FH. Cross-talk between miRNA and Notch signaling pathways in tumor development and progression. Cancer Letters. 2010;**292**(2):141-148. DOI: 10.1016/j.canlet.2009.11.012

[38] Mishra S, Deng JJ, Gowda PS, et al. Androgen receptor and microRNA-21 axis downregulates transforming growth factor beta receptor II (TGFBR2) expression in prostate cancer. Oncogene. 2014;**33**(31):4097-4106. DOI: 10.1038/onc.2013.374

[39] Zhang T, Yang Z, Kusumanchi P, Han S, Liangpunsakul S. Critical role of microRNA-21 in the pathogenesis of liver diseases. Front Med (Lausanne). 2020;**7**:7. DOI: 10.3389/fmed.2020.00007

[40] Tan W, Li Y, Lim SG, Tan TM. miR-106b-25/miR-17-92 clusters: Polycistrons with oncogenic roles in hepatocellular carcinoma. World Journal of Gastroenterology. 2014;**20**(20):5962-5972. DOI: 10.3748/wjg.v20.i20.5962

[41] Zhao Z, Wang K, Tan S. microRNA-211-mediated targeting of the INHBA-TGF-β axis suppresses prostate tumor formation and growth. Cancer Gene Therapy. 2021;**28**(5):514-528. DOI: 10.1038/s41417-020-00237-w

[42] Hyun J, Wang S, Kim J, et al. MicroRNA125b-mediated Hedgehog signaling influences liver regeneration by chorionic plate-derived mesenchymal stem cells. Scientific Reports. 2015;**5**:14135. DOI: 10.1038/srep14135

[43] Liang L, Wong CM, Ying Q, et al. MicroRNA-125b suppressesed human liver cancer cell proliferation and

metastasis by directly targeting oncogene LIN28B2. Hepatology. 2010;**52**(5): 1731-1740. DOI: 10.1002/hep.23904

[44] Liu W, Hu J, Zhou K, et al. Serum exosomal miR-125b is a novel prognostic marker for hepatocellular carcinoma. Oncotargets and Therapy. 2017;**10**:3843-3851. DOI: 10.2147/OTT.S140062

[45] You K, Li SY, Gong J, et al. MicroRNA-125b promotes hepatic stellate cell activation and liver fibrosis by activating RhoA signaling. Molecular Therapy--Nucleic Acids. 2018;**12**:57-66. DOI: 10.1016/j.omtn.2018.04.016

[46] Ren K, Li T, Zhang W, Ren J, Li Z, Wu G. miR-199a-3p inhibits cell proliferation and induces apoptosis by targeting YAP1, suppressing Jagged1-Notch signaling in human hepatocellular carcinoma. Journal of Biomedical Science. 2016;**23**(1):79. DOI: 10.1186/s12929-016-0295-7

[47] Atta S, Kramani NE, Mohamed SR, et al. MicroRNA-199: A potential therapeutic tool for hepatocellular carcinoma in an experimental model. Asian Pacific Journal of Cancer Prevention. 2021;**22**(9):2771-2779. DOI: 10.31557/APJCP.2021.22.9.2771

[48] Liu DL, Lu LL, Dong LL, et al. miR-17-5p and miR-20a-5p suppress postoperative metastasis of hepatocellular carcinoma via blocking HGF/ERBB3-NF-κB positive feedback loop. Theranostics. 2020;**10**(8):3668-3683. DOI: 10.7150/thno.41365

[49] de Oliveira ARCP, Castanhole-Nunes MMU, Biselli-Chicote PM, et al. Differential expression of angiogenesis-related miRNAs and VEGFA in cirrhosis and hepatocellular carcinoma. Archives of Medical Science. 2020;**16**(5):1150-1157. DOI: 10.5114/aoms.2020.97967

[50] Zhang JF, He ML, Fu WM, et al. Primate-specific microRNA-637 inhibits tumorigenesis in hepatocellular carcinoma by disrupting signal transducer and activator of transcription 3 signaling. Hepatology. 2011;**54**(6):2137-2148. DOI: 10.1002/hep.24595

[51] Zhang Z, Zhang Y, Sun XX, Ma X, Chen ZN. microRNA-146a inhibits cancer metastasis by downregulating VEGF through dual pathways in hepatocellular carcinoma. Molecular Cancer. 2015;**14**:5. DOI: 10.1186/1476-4598-14-5

[52] Zhang CS, Lin Y, Sun FB, Gao J, Han B, Li SJ. miR-409 down-regulates Jak-Stat pathway to inhibit progression of liver cancer. European Review for Medical and Pharmacological Sciences. 2019;**23**(1):146-154. DOI: 10.26355/eurrev_201901_16758

[53] Xiu D, Wang D, Wang J, Ji F, Zhang W. MicroRNA-543 suppresses liver cancer growth and induces apoptosis via the JAK2/STAT3 signaling pathway. Oncology Letters. 2019;**17**(2):2451-2456. DOI: 10.3892/ol.2018.9838

[54] Chen Z, Zuo X, Zhang Y, et al. MiR-3662 suppresses hepatocellular carcinoma growth through inhibition of HIF-1α-mediated Warburg effect. Cell Death & Disease. 2018;**9**(5):549. DOI: 10.1038/s41419-018-0616-8

[55] Ye J, Xiao X, Han Y, Fan D, Zhu Y, Yang L. MiR-3662 suppresses cell growth, invasion and glucose metabolism by targeting HK2 in hepatocellular carcinoma cells. Neoplasma. 2020;**67**(4):773-781. DOI: 10.4149/neo_2020_190730N689

[56] Farzaneh Z, Vosough M, Agarwal T, Farzaneh M. Critical signaling pathways governing hepatocellular carcinoma behavior; small molecule-based approaches. Cancer Cell International.

2021;**21**(1):208. DOI: 10.1186/s12935-021-01924-w

[57] Liberti MV, Locasale JW. The Warburg effect: How does it benefit cancer cells? Trends in Biochemical Sciences. 2016;**41**(3):211-218. DOI: 10.1016/j.tibs.2015.12.001

[58] Chang CW, Chen YS, Tsay YG, Han CL, Chen YJ, Yang CC, et al. ROS-independent ER stress-mediated NRF2 activation promotes warburg effect to maintain stemness-associated properties of cancer-initiating cells. Cell Death & Disease. 2018;**9**(2):194. DOI: 10.1038/s41419-017-0250-x

[59] Gramantieri L, Giovannini C, Piscaglia F, Fornari F. MicroRNAs as modulators of tumor metabolism, microenvironment, and immune response in hepatocellular carcinoma. Journal of Hepatocellular Carcinoma. 2021;**8**:369-385. DOI: 10.2147/JHC.S268292

[60] Köberle V, Kronenberger B, Pleli T, Trojan J, Imelmann E, Peveling-Oberhag J, et al. Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. European Journal of Cancer. 2013;**49**(16):3442-3449. DOI: 10.1016/j.ejca.2013.06.002

[61] Luo W, Hu H, Chang R, Zhong J, Knabel M, O'Meally R, et al. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell. 2011;**145**(5):732-744. DOI: 10.1016/j.cell.2011.03.054

[62] Yang G, Zhang M, Zhao Y, Pan Y, Kan M, Li J, et al. HNF-4α inhibits hepatocellular carcinoma cell proliferation through mir-122-adam17 pathway. PLoS One. 2020;**15**(3):e0230450. DOI: 10.1371/journal.pone.0230450

[63] Jopling C. Liver-specific microRNA-122: Biogenesis and function. RNA Biology. 2012;**9**(2):137-142. DOI: 10.4161/rna.18827

[64] Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. Oncogene. 2009;**28**(40):3526-3536. DOI: 10.1038/onc.2009.211

[65] Xiao G, Wang Q, Li B, Wu X, Liao H, Ren Y, et al. MicroRNA-338-3p Suppresses proliferation of human liver cancer cells by targeting SphK2. Oncology Research. 2018;**26**(8):1183-1189. DOI: 10.3727/096504018X15151495109394

[66] Wang B, Hsu SH, Frankel W, Ghoshal K, Jacob ST. Stat3-mediated activation of microRNA-23a suppresses gluconeogenesis in hepatocellular carcinoma by down-regulating glucose-6-phosphatase and peroxisome proliferator-activated receptor gamma, coactivator 1 alpha. Hepatology. 2012;**56**(1):186-197. DOI: 10.1002/hep.25632

[67] Mohamed AA, Ali-Eldin ZA, Elbedewy TA, El-Serafy M, Ali-Eldin FA, AbdelAziz H. MicroRNAs and clinical implications in hepatocellular carcinoma. World Journal of Hepatology. 2017;**9**(23):1001-1007. DOI: 10.4254/wjh.v9.i23.1001

[68] Zhu H, Han C, Wu T. MiR-17-92 cluster promotes hepatocarcinogenesis. Carcinogenesis. 2015;**36**(10):1213-1222. DOI: 10.1093/carcin/bgv112

[69] Mohr R, Özdirik B, Lambrecht J, et al. From liver cirrhosis to cancer: The role of micro-RNAs in hepatocarcinogenesis. International Journal of Molecular

Sciences. 2021;**22**(3):1492. DOI: 10.3390/ijms22031492

[70] Gao B, Gao K, Li L, Huang Z, Lin L. miR-184 functions as an oncogenic regulator in hepatocellular carcinoma (HCC). Biomedicine & Pharmacotherapy. 2014;**68**(2):143-148. DOI: 10.1016/j.biopha.2013.09.005

[71] Yang L, Peng F, Qin J, Zhou H, Wang B. Downregulation of microRNA-196a inhibits human liver cancer cell proliferation and invasion by targeting FOXO1. Oncology Reports. 2017;**38**(4):2148-2154. DOI: 10.3892/or.2017.5873

[72] Li X, Yuan M, Song L, Wang Y. Silencing of microRNA-210 inhibits the progression of liver cancer and hepatitis B virus-associated liver cancer via targeting EGR3. BMC Medical Genetics. 2020;**21**(1):48. DOI: 10.1186/s12881-020-0974-9

[73] Kim YC, Jung H, Seok S, et al. MicroRNA-210 promotes bile acid-induced cholestatic liver injury by targeting mixed-lineage leukemia-4 methyltransferase in mice. Hepatology. 2020;**71**(6):2118-2134. DOI: 10.1002/hep.30966

[74] Yang CL, Zheng XL, Ye K, et al. Effects of microRNA-217 on proliferation, apoptosis, and autophagy of hepatocytes in rat models of CCL4-induced liver injury by targeting NAT2. Journal of Cellular Physiology. 2019;**234**(4):3410-3424. DOI: 10.1002/jcp.26748

[75] Chen C, Matye D, Wang Y, Li T. Liver-specific microRNA-185 knockout promotes cholesterol dysregulation in mice. Liver Research. 2021;**5**(4):232-238

[76] Wang XC, Zhan XR, Li XY, Yu JJ, Liu XM. MicroRNA-185 regulates expression of lipid metabolism genes and improves insulin sensitivity in mice with non-alcoholic fatty liver disease. World Journal of Gastroenterology. 2014;**20**(47):17914-17923. DOI: 10.3748/wjg.v20.i47.17914

[77] Szabo G, Bala S. MicroRNAs in liver disease. Nature Reviews. Gastroenterology & Hepatology. 2013;**10**:542-552. DOI: 10.1038/nrgastro.2013.87

[78] Xie KL, Zhang YG, Liu J, Zeng Y, Wu H. MicroRNAs associated with HBV infection and HBV-related HCC. Theranostics. 2014;**4**:1176-1192. DOI: 10.7150/thno.8715

[79] Su C, Hou Z, Zhang C, Tian Z, Zhang J. Ectopic expression of microRNA-155 enhances innate antiviral immunity against HBV infection in human hepatoma cells. Virology Journal. 2011;**8**:354. DOI: 10.1186/1743-422X-8-354

[80] Yamada H, Suzuki K, Ichino N, Ando Y, Sawada A, Osakabe K, et al. Associations between circulating microRNAs (miR-21, miR-34a, miR-122 and miR-451) and non-alcoholic fatty liver. Clinica Chimica Acta. 2013;**424**:99-103. DOI: 10.1016/j.cca.2013.05.021

[81] McDaniel K, Huang L, Sato K, Wu N, Annable T, Zhou T, et al. The let-7/Lin28 axis regulates activation of hepatic stellate cells in alcoholic liver injury. The Journal of Biological Chemistry. 2017;**292**(27):11336-11347. DOI: 10.1074/jbc.M116.773291

[82] Wan Y, McDaniel K, Wu N, Ramos-Lorenzo S, Glaser T, Venter J, et al. Regulation of cellular senescence by miR-34a in alcoholic liver injury. The American Journal of Pathology. 2017;**187**(12):2788-2798. DOI: 10.1016/j.ajpath.2017.08.027

[83] Salvoza NC, Klinzing DC, Gopez-Cervantes J, Baclig MO. Association of circulating serum miR-34a and miR-122 with dyslipidemia among patients with non-alcoholic fatty liver disease. PLoS One. 2016;**11**(4):e0153497. DOI: 10.1371/journal.pone.0153497

[84] Li X, Zhang W, Xu K, Lu J. miR-34a promotes liver fibrosis in patients with chronic hepatitis via mediating Sirt1/p53 signaling pathway. Pathology, Research and Practice. 2020;**216**(5):152876. DOI: 10.1016/j.prp.2020.152876

[85] Zhang HF, Wang YC, Han YD. MicroRNA-34a inhibits liver cancer cell growth by reprogramming glucose metabolism. Molecular Medicine Reports. 2018;**17**(3):4483-4489. DOI: 10.3892/mmr.2018.8399

[86] Fornari F, Gramantieri L, Giovannini C, et al. MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Research. 2009;**69**(14):5761-5767. DOI: 10.1158/0008-5472.CAN-08-4797

[87] Momen-Heravi F, Catalano D, Talis A, Szabo G, Bala S. Protective effect of LNA-anti-miR-132 therapy on liver fibrosis in mice. Molecular Therapy--Nucleic Acids. 2021;**25**:155-167. DOI: 10.1016/j.omtn.2021.05.007

[88] Davis BN, Hilyard AC, Nguyen PH, Lagna G, Hata A. Smad proteins bind a conserved RNA sequence to promote microRNA maturation by Drosha. Molecular Cell. 2010;**39**(3):373-384. DOI: 10.1016/j.molcel.2010.07.011

[89] Chen S, Zhang J, Chen Q, Cheng J, Chen X, Mao Y, et al. MicroRNA-200a and microRNA-141 have a synergetic effect on the suppression of epithelial-mesenchymal transition in liver cancer by targeting STAT4. Oncology Letters. 2021;**21**(2):137. DOI: 10.3892/ol.2020.12398

[90] Dhayat SA, Mardin WA, Köhler G, Bahde R, Vowinkel T, Wolters H, et al. The microRNA-200 family--a potential diagnostic marker in hepatocellular carcinoma? Journal of Surgical Oncology. 2014;**110**(4):430-438. DOI: 10.1002/jso.23668

[91] Qin X, Zhang J, Lin Y, Sun XM, Zhang JN, Cheng ZQ. Identification of MiR-211-5p as a tumor suppressor by targeting ACSL4 in Hepatocellular Carcinoma. Journal of Translational Medicine. 2020;**18**(1):326. DOI: 10.1186/s12967-020-02494-7

[92] Jiang G, Cui Y, Yu X, Wu Z, Ding G, Cao L. miR-211 suppresses hepatocellular carcinoma by downregulating SATB2. Oncotarget. 2015;**6**(11):9457-9466. DOI: 10.18632/oncotarget.3265

[93] Cao MQ, You AB, Zhu XD, Zhang W, Zhang YY, Zhang SZ, et al. miR-182-5p promotes hepatocellular carcinoma progression by repressing FOXO3a. Journal of Hematology & Oncology. 2018;**11**(1):12. DOI: 10.1186/s13045-018-0555-y

[94] Yerukala-Sathipati S, Ho SY. Novel miRNA signature for predicting the stage of hepatocellular carcinoma. Scientific Reports. 2020;**10**(1):14452. DOI: 10.1038/s41598-020-71324-z

[95] Lu C, Liao Z, Cai M, Zhang G. MicroRNA-320a downregulation mediates human liver cancer cell proliferation through the Wnt/β-catenin signaling pathway. Oncology Letters. 2017;**13**(2):573-578. DOI: 10.3892/ol.2016.5479

[96] Wu L, Yang F, Lin B, et al. MicroRNA-424 expression predicts

tumor recurrence in patients with hepatocellular carcinoma following liver transplantation. Oncology Letters. 2018;**15**(6):9126-9132. DOI: 10.3892/ol.2018.8539

[97] Zha Z, Jia F, Hu P, Mai E, Lei T. MicroRNA-574-3p inhibits the malignant behavior of liver cancer cells by targeting ADAM28. Oncology Letters. 2020;**20**(3):3015-3023. DOI: 10.3892/ol.2020.11852

[98] Gao HB, Gao FZ, Chen XF. MiRNA-1179 suppresses the metastasis of hepatocellular carcinoma by interacting with ZEB2. European Review for Medical and Pharmacological Sciences. 2019;**23**(12):5149-5157. DOI: 10.26355/eurrev_201906_18179

[99] Tian Q, Liang L, Ding J, et al. MicroRNA-550a acts as a pro-metastatic gene and directly targets cytoplasmic polyadenylation element-binding protein 4 in hepatocellular carcinoma. PLoS One. 2012;7(11):e48958. DOI: 10.1371/journal.pone.0048958

[100] Rui T, Zhang X, Feng S, et al. The similar effects of miR-512-3p and miR-519a-2-5p on the promotion of hepatocellular carcinoma: Different Tunes Sung with equal skill. Frontiers in Oncology. 2020;**10**:1244