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**Micro-RNAs in hepatitis B virus-related chronic liver diseases and hepatocellular carcinoma**

Sagnelli E *et al.* Micro-RNAs in HBV-related CLD and HCC

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**Abstract**

MicroRNAs (miRNAs) are small non-coding RNAs that modulate gene expression at the post-transcriptional level by affecting both the stability and translation of complementary mRNAs. Several studies have shown that miRNAs are important regulators in the conflicting efforts between the virus (to manipulate the host for its successful propagation) and the host (to inhibit the virus), culminating in either the elimination of the virus or its persistence. An increasing number of studies report a role of miRNAs in hepatitis B virus (HBV) replication and pathogenesis. In fact, HBV is able to modulate different host miRNAs, particularly through the transcriptional transactivator HBx protein and, conversely, different cellular miRNAs can regulate HBV gene expression and replication by a direct binding to HBV transcripts or indirectly targeting host factors. The present review will discuss the role of miRNAs in the pathogenesis of HBV-related diseases and their role as a biomarker in the management of patients with HBV-related disease and as therapeutic targets.

**Key word:** Hepatitis B virus infection; MicroRNAs; Hepatitis B virus pathogenesis; Molecular mechanisms

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**Core tip:** This review article will focus on the emerging puzzle of hepatitis B virus (HBV)-hepatocyte interaction via miRNAs, indirectly or directly modulating HBV replication and pathogenesis, and thus on the role of microRNAs in the natural history of HBV infection. We evaluated the literature on their possible future role as a biomarker in the management of patients with HBV-related disease and as therapeutic targets.

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**INTRODUCTION**

MicroRNAs (miRNAs) are small non-coding RNAs that modulate gene expression at the post-transcriptional level by affecting both the stability and translation of complementary mRNAs[1]. MiRNAs play crucial roles in a variety of physiological processes, such as cell development and differentiation[2,3]. miRNA mutations, dysregulation of their expression or dysfunction of miRNA biogenesis lead to an interference with biological pathways involved in the development and evolution of human diseases, including cancer, cardiovascular diseases and infectious diseases[4-8].

About the host-virus interplay, various studies have shown that miRNAs are important regulators in the conflicting efforts between the virus (to manipulate the host for its successful propagation) and the host (to inhibit the virus), culminating in either the elimination of the virus or its persistence. In fact, different viruses encode miRNAs that modulate not only the viral mRNA expression to regulate its own lifecycle, but also the host mRNA expression to establish a cellular environment resulting favorable to their replication; on the other hand, host cells encode miRNAs counteracting viral replication[8-10]. An increasing number of studies report a role of miRNAs in hepatitis B virus (HBV) replication and pathogenesis. HBV is a non-cytopathic virus belonging to the Hepadnaviridae family. It has a 3.2 kb partially double-stranded DNA, showing 4 known open reading frames (ORFs): The S region, which contains three in-frame initiator codons, codes for the small, medium and large surface antigen (HBsAg) proteins; the C region, with two initiator codons for the core and “e” antigen (HBcAg, HBeAg); the P frame, coding for an RNA-dependent DNA polymerase; and the X region, coding for a protein regulating the transcription of both viral and cellular genes[11].

The present review will discuss the emerging puzzle of HBV-hepatocyte interaction via miRNAs, indirectly or directly modulating HBV replication and pathogenesis, and thus will focus on the role of microRNAs in the natural history of HBV infection and on their possible future role as biomarkers in the management of patients with an HBV-related disease and as therapeutic targets.

**HBV INFECTION**

Despite the universal vaccination campaigns against HBV infection undertaken in many countries over the last two decades, this infection remains a global health problem. In 2015, the WHO estimated that nearly 3.5% of the world population live with chronic HBV infection[12], with about 800000 deaths per year (460000 for complications of liver cirrhosis and 340000 for hepatocellular carcinoma-HCC)[13]. For non-immune/non-infected subjects any parenteral or mucosal exposure to blood, blood products or blood-contaminated material should be considered a risk for acquiring HBV infection[14]. In addition, being present in semen and cervical secretions at infectious concentrations, HBV is also transmitted by sexual and vertical routes[15]. The age at the time of infection strongly modulates the progression to chronicity, which occurs in around 90% of subjects infected at birth, a rate progressively decreasing with the increase in age at infection, up to 2%-5% in the adult population[16].

The geographical distribution of HBV chronic carriers is highly variable, ranging from 0.7%-1% in developed western countries to 8% or more in some countries in sub Saharan Africa and South-East Asia, depending on environmental factors, earning potential, educational levels and lifestyles. In countries with an intermediate-high level of endemicity, HBV infection is most frequently acquired at birth from an HBeAg-positive mother or through horizontal transmission in early childhood by household contacts (most frequently between siblings); in these cases the rate of progression to chronicity is high, which keeps the prevalence of infection in these geographical areas intermediate or high. Intravenous drug addiction is the major risk factors for acquiring HBV infection in countries with a low HBV endemicity like Western Europe[15] and North America[17], whereas promiscuous unprotected sexual activity is a main risk factor worldwide.

The clinical presentation of chronic HBV infection is variable, ranging from asymptomatic carriage of the virus to liver cirrhosis with or without HCC[18]. Patients with chronic hepatitis develop liver cirrhosis with an incidence of 1-5 per 100 persons/year, with a 5-year cumulative probability of progression ranging from 8% to 20%, depending on the degree of disease activity, HBeAg/anti-HBe status, the HBV load and co-morbidities[19]. The incidence of HCC in patients with HBV-related liver cirrhosis is estimated around 3.7 persons/year[12]. HCC may develop, but with a lower frequency also in patients with chronic HBV infection without cirrhosis, depending on demographic (male sex, older age), viral (higher levels of HBV replication; co-infections) and environmental (alcohol abuse) factors[20].

**MIRNAS ASSOCIATED WITH HBV INFECTION**

So far, there is no experimental evidence confirming the synthesis of miRNAs by HBV, though a computational analysis suggested one HBV pre-miRNA candidate[21]; however, HBV is able to modulate different host miRNAs, particularly through the transcriptional transactivator HBx protein. Conversely, different cellular miRNAs can regulate HBV gene expression and replication by a direct binding to HBV transcripts or indirectly targeting host factors, which in turn modulate viral replication.

***Cellular miRNAs directly targeting HBV transcripts***

The first miRNAs found to bind viral transcripts and repress HBV gene expression and replication were miR-210, miR-199-3p and miR-125a-5p. They were identified by two different experimental approaches (Table 1). In one procedure, Zhang *et al*[22] systematically screened for cellular miRNAs affecting HBV replication by a loss-of-function approach: Antagomirs targeting 328 miRNAs were transfected into a HepG2.2.15 cell model supporting full HBV replication, and then HBV surface antigen (HBsAg) expression was measured. Among the six miRNAs whose antagomirs caused an increase in HBsAg expression, miR-199a-3p and miR-210 were predicted to bind the HBsAg coding region and the HBV pre-S1 region, respectively; the direct effect of miRNAs on viral transcripts were further validated by GFP reporter assay[22]. In a different approach, Potenza *et al*[23] first predicted the potential targets of human hepatic miRNAs in different HBV sequence subtypes. The most promising targets were then subjected to a validation test based on cultured hepatic cells and luciferase reporter genes, demonstrating that miR-125a-5p was able to bind viral sequences. In particular, miR-125a was shown to be able to interfere with the HBsAg expression, since the transfection of miR-125a mimic or inhibitor into PLC/PRF/5 cell line that secretes HBsAg induced a marked decrease or enhancement in the amount of secreted HBsAg, respectively[23]. Two independent studies then confirmed the ability of miR-125a to inhibit HBsAg translation: in a screening of HBV replication-related miRNAs, a pri-miR-125a expression vector could repress HBsAg synthesis in HepG2 cells[24]; again, miR-125a mimic and inhibitor transfection in HepG2.2.15 cells resulted in an increase or decrease in HBV replication, respectively. The expression analysis of a panel of 814 miRNAs revealed that iron or TGF-α treatments, which increased or decreased HBV replication, respectively, had opposite effects on the expression of miR-125a, supporting its ability to interfere with HBV replication[25,26].

Later, another study reported an inhibitory effect of a cellular miRNA on HBV replication: Chen *et al*[27] focused on the liver-specific microRNA, miR-122, first demonstrating its inhibitory effect on HBV gene expression and replication in cultured cells and then validating its target sequence located at the coding region of the mRNA for the viral polymerase and the 3’ untranslated region of the mRNA for the core protein[27]. In a similar approach, two other studies found that miR-15a and miR-16-1 (differing by only one base outside the seed region) target HBx transcript and miR-20a/miR-92a-1 (belonging to miR-17-92 polycistron) and may inhibit HBV replication by targeting the viral transcripts[28,29].

More recently, miR-1231 has also been shown to suppress HBV replication by targeting the HBV core (HBc) protein. In HBV-transfected HepG2 cells, over-expression of hsa-miR-1231 resulted in the suppression of HBV replication by targeting the HBV core protein[30]. Also miR-205 was found to target a viral transcript, in particular HBx mRNA[31]. miR-125a, miR-205 and miR-15/miR-16-1 expression were found to be modulated by HBx protein, resulting in an upregulation for miR-125a and downregulation for the others[31-33]. These regulatory feedback loops may have an impact on the development of liver disease progressing to HCC, given the crucial role of HBx in hepatocarcinogenesis[34].

***Cellular miRNAs targeting regulators of HBV infection***

HBV transcripts are under the control of four promoters and two enhancers (enhancer I and II), interacting with cellular factors that regulate HBV gene expression. Thus, miRNA regulation of these factors results in the modulation of HBV transcription and replication. Some miRNAs suppress HBV replication by targeting positive regulators of HBV (Table 1). One example is miR-155, which suppresses HBV transcription and replication, given its ability to target CAAT enhancer-binding protein (C/EBP), which is a positive regulator of HBV transcription through its binding to HBV enhancer II, core promoter and S promoter[35-38]. miR-155 suppresses HBV infection also by modulating the host immune system (see below)[39]. Also miR-141 is able to suppress HBV replication, since it represses at both the transcriptional and translational levels the peroxisome proliferator-activated receptor alpha (PPARα), a transactivator of HBV promoters, with a critical role in HBV replication[24,40]. Similarly, miR-130a reduced HBV replication by targeting two major metabolic regulators PGC1α and PPARγ, both of which can potently stimulate HBV replication[41].

Other miRNAs promote HBV replication by targeting negative regulators of HBV activity or by enhancing a positive regulator. miR-501 promotes HBV replication by targeting HBXIP, a negative modulator of HBV replication, because of its binding to the transactivation domain of HBx protein[42]. A positive effect on HBV replication has also been described for miR-122, in contrast with that reported above. In particular, miR-122 can promote HBV replication in two ways: It prevents cyclin G1 from interacting with p53, which has a suppressive effect on HBV replication, and it targets heme oxygenase-1 (HO-1), an anti-HBV enzyme[43,44]; miR-122 targets heme oxygenase-1 (HO-1), whose anti-HBV activity has been shown in HBV-transfected hepatoma cells and in persistently HBV replicating transgenic mice. HO-1 acts by decreasing stability of HBV core protein, thus blocking refill of nuclear HBV covalently closed circular (ccc)DNA[45,46]. According to these mechanisms, the liver-rich miR-122 may have a similar role in stimulating the replication and gene expression of the two hepatotropic viruses, HCV and HBV[47]. microRNA-372/373 promote the expression of HBV by targeting the nuclear factor I/B (NFIB), a transcription factor able to reduce viral HBsAg and HBeAg protein levels and viral core-associated DNA levels, due to its binding to enhancer I and core promoter of HBV[48]. A similar mechanism has been described for miR-370, which suppresses HBV transcription and replication by targeting nuclear factor IA (NFIA)[49]. Also miR-15b is able to target a negative regulator of HBV Enhancer I, *i.e*., hepatocyte nuclear factor 1α (HNF1α) mRNA, thus resulting in the transactivation of HBV Enhancer I, in turn causing the enhancement of HBV transcription and replication[50].

Another two miRNAs have a role in HBV transcription and replication by modulating epigenetic modifications such as histone modification and methylation. In particular**,** miR-1**,** by targeting histone deacetylase 4 (HDAC4) changes the expression of different genes, including an upregulation of farnesoid X receptor a, which enhances HBV transcription and replication by binding to the HBV core promoter[51-53]. miR-152 has been shown to target DNA methyltransferase (DNMT-1), eventually resulting in a reduced methylation of covalently closed circular DNA (cccDNA), with an impact on the replicative activity of HBV[54,55]. Consistently, miR-152 expression was also shown to be downregulated in the livers of HBx transgenic mice and inversely correlated with DNMT1 expression in HBV-related HCC patients[56,57].

Some miRNAs may also have an indirect effect on HBV infection because of their role in the modulation of the host immune system. From the point of view of HBV, they represent a strategy to suppress antiviral immune responses, thus facilitating viral replication. This is the case of miR-146a and miR-548a, which promote HBV infection by suppressing T cell function through targeting Stat1 and by binding the 3’UTR of IFN-λ1, respectively[58,59]. Conversely, miR-34a and miR-155 suppress HBV infection by inhibiting Treg cell recruitment *via* chemokine CCL22 and augmenting the IFN signaling pathway, respectively[39,60].

Overall, it is clear that the host-virus interaction in HBV infection is mediated not only by immune responses but also by miRNAs; during the co-evolution and adaptation between HBV and humans, complex miRNA-based networks have been established; this complexity may partly explain the opposing effects on HBV transcription and replication reported for some miRNAs (*e.g.*, miR-122).

**MICRORNA DYSREGULATION IN HBV-RELATED HEPATOCELLULAR CARCINOMA**

An aberrant expression of miRNAs has a causative effect on several pathological conditions, including cancer[5]. In this field, several studies indicate that miRNAs can act as either tumor suppressors by downregulating the expression of oncogenes, or tumor promoters (oncomirs) by limiting the expression of oncosuppressor proteins[61-63]. Cancer cell downregulation of Dicer, an enzyme playing a critical role in the biosynthesis of miRNAs, or mutations in its structure suppress miRNA biogenesis, leading to increased tumor progression[64-67]. This implies that the oncosuppressive effect of miRNAs generally overcomes their oncogenic potential. It is also known that cell differentiation is often accompanied by increased Dicer expression[68-70].

In 2011, Hou *et al*[71] performed an extensive study of the miRNomes of healthy human liver and HCC. In this paragraph, their work will be discussed in some detail since it provides a good example of the experimental procedures currently employed to study the miRNAs with oncogenic or oncosuppressive roles. The authors used a next-generation sequencing (NGS) technique to analyze human liver miRNome and found that 9 miRNAs accounted for about 90% of the liver miRNA content, with miR-122 being the most represented (52%). Other highly expressed miRNAs included miR-192 (16.9%), miR-199a/b-3p (4.9%), miR-101 (3.7%), let-7a (3.3%), miR-99a (2.2%), let-7c (2.1%), let-7b (1.7%), and let-7f (1.5%). MicroRNAs -199a-3p and -199b-3p have an identical nucleotide sequence but are transcribed from three genes, a-1, a-2, and b, with a2 being the most expressed in the liver. The authors then analyzed liver biopsies from patients with hepatocellular carcinoma by comparing tumor samples with adjacent non-cancer tissues. In HBV-related HCCs, a remarkable decrease in 199a-3p was observed. This result was then validated by qRT-PCR in a cohort of 40 HBV-related HCCs. This analysis showed that the miRNA was downregulated in 100% of the patients, with a mean decrease of 8.3-fold. Other miRNAs markedly downregulated were miR-99a and miR-125b, both decreased by about 7-fold in the majority of the patients. MicroRNA-122 and miR-125a were also downregulated (Table 2). On the other hand, miR-21 was upregulated by 4.8-fold in half of the tumor samples. In the same study, genomic analyses of DNA samples from liver biopsies indicated that miR-199a-3p downregulation was not due to gene deletion or promoter DNA methylation but to histone modification and subsequent repression of transcription. Biological assays were then performed showing that transfection of a synthetic miR-199a-3p mimic in cultured HCC cell lines repressed cell proliferation and induced apoptosis, thus suggesting a tumor suppressive role *in vitro*. Experiments *in vivo* were then performed by monitoring human HCC cell growth in nude mice. Intra-tumoral injection of cholesterol-conjugated miR-199a-3p or its over-expression with a recombinant adeno-associated virus system markedly decreased tumor growth, further supporting its tumor suppressive role. The mechanism of action of miR-199a-3p was then studied. A computational analysis with TargetScan was used to identify the human genes whose mRNA sequence may allow binding of miR-199a-3p, possibly leading to gene silencing. Most of them were associated with the mitogen-activated protein kinase (MAPK) pathway, thus providing a possible explanation for the anti-proliferative activity of the miRNA. Among them, PAK4 was downregulated by miR-199a-3p transfection in HCC cells, and this effect was found to be due to a direct interaction with the gene transcript using a luciferase-based reporter assay. Finally, measurement of the miR-199a-3p content in the liver biopsies from two other cohorts of 142 and 152 patients showed that a markedly decreased HCC level of the miRNA correlated with poor survival. It should be noted that a downregulation of miR-199a-3p in HCC had already been observed in 2006 by Murakami *et al*[72] employing much less powerful miRNA detection techniques based on microarrays and Northern blotting analyses. The same study had also shown a tumor downregulation of miR-125a. Since then, other studies have confirmed the tumor-suppressive role of miR-199a-3p in HCC and identified CD44, CD51, c-MET, mTOR, YAP1, and ZHX1 as other direct miRNA targets contributing to its anti-proliferative and pro-apoptotic effects[73-78] (Table 2). The ability of miR-199a-3p to downregulate several target proteins should not surprise given the pleiotropic effects of microRNAs that are able to interact with several mRNAs provided with the same or similar binding sites located in their 3’-UTR. Therefore, a single microRNA often silences several genes with related functions, thus showing a marked effect on the cell physiology.

Other studies conducted with similar experimental approaches, profiling microRNAs by qPCR-arrays on multi-well plates, have confirmed the HCC downregulation of miR-99a, -122, -125a, -125b, and the upregulation of miR-21, also identifying their molecular targets (Table 2). Other miRNAs consistently downregulated in HCC included the let-7 family of microRNAs and miR-29; other examples of upregulated miRNAs were miR-155 and -221 (Table 2). These data have raised substantial interest in microRNAs in the field of molecular and cellular oncology, leading to the publication of several interesting reviews[79-84].

As regards the reasons for deregulated miRNA expression in HCC, it should be noted that the viral HBx protein plays a primary role in HBV cancerogenesis[85]. It is a transcriptional trans-activator lacking a DNA-binding domain but able to interact with several transcription factors[86,87]. Therefore, it is not surprising that HBx can modify the hepatic miRNA expression[88]. It is noteworthy that HBx downregulates the expression of oncosuppressive miRNAs let-7 and miR-122, whereas it upregulates oncomirs -21 and -221. MicroRNA-125a is induced by HBx[32,89] but is downregulated by its carboxyl-terminal truncated variant that is frequently found in HBV-related HCC[90]. These data indicate that the tumorigenic effect of HBx is partially mediated by microRNAs. Besides HBx, dysregulation of microRNAs in cancer cells may be determined by other genetic or epigenetic factors[91]. Chromosomal abnormalities, deletions, and mutations can downregulate cellular miRNA expression[92,93], and several miRNA genes associated with CpG islands are also transcriptionally repressed by promoter DNA methylation[94].

**MICRORNAS AS BIOMARKERS OF HBV-RELATED LIVER DISEASES**

***Biomarkers of liver damage and treatment response in chronic hepatitis B***

As mentioned above, chronic HBV infection is associated with a wide spectrum of clinical manifestations: An inactive carrier state, chronic hepatitis of different grade of activity and liver cirrhosis in different stages of compensation, with or without HCC. The mechanisms of virus/host interactions leading to different outcomes have been only partially clarified and a substantial contribution to their knowledge is expected from the studies on the expression profile of microRNAs and their role in liver fibrogenesis. To this regard, one of the most interesting microRNAs is miR-122, which accounts for 50%-70% of all miRNAs expressed in the human liver. Several investigations[90-97] have shown a higher miR-122 serum concentration in patients with chronic HBV infection than in normal subjects and a correlation between its serum levels and HBV load, HBsAg titers and liver biochemistry. However, the interpretation of these data as a consequence of an upregulation of microRNA requires the exclusion of the possibility that they could be a consequence of an increased release of miR-122 in the blood due to concomitant hepatic cytolysis. To this regard, Wang *et al*[44] showed that the miR-122 expression in the liver was significantly downregulated in 41 Chinese patients with HBV infection compared with 10 healthy controls, and that the miR-122 levels negatively correlated with the intrahepatic viral load and with the degree of necroinflammation, confirming *in vivo* theinhibitory activity of miR-122 on HBV replication.

Interesting information on the correlation between microRNAs and HBV-related liver damage comes from some investigations on miR-29. A study[98] performed on serum samples of 91 HBV-infected patients and 12 healthy controls demonstrated a downregulation of this miRNA in patients with more advanced liver fibrosis. In addition, the serum levels of three microRNAs (miR-29a, miR-143, miR-223) predicted the progression of liver fibrosis better than APRI or FIB-4 tests in 123 Chinese patients with chronic HBV infection[99].

In 2013, we identified the miR-125a-5p as an independent predictor of more severe liver lesions (necroinflammation and fibrosis) in a cohort of 27 treatment-naïve patients with HBeAg-negative chronic hepatitis B[100], findings confirmed by the data of a study by Zheng *et al*[102] on 91 HBV-infected patients. More recently, a Chinese study performed on 211 patients with chronic hepatitis B demonstrated that serum concentrations of miR-125b, a microRNA classified in the same family, correlate with the histological activity and HBV load[102].

The clinical use of microRNAs in chronic HBV infection may go beyond the assessment of liver damage. For example, Brunetto *et al*[103] reported that the use of a serum six miRNAs signature (MiR-B-Index) correctly discriminated 61 HBV subjects in a naturally inactive stage and 84 in a stage of treatment-induced immune-control. More recently, pre-treatment serum levels of two microRNAs predicted the off-treatment biochemical and virological response after a 48-week combination therapy with Peg-IFN and adefovir, the miR-301a-3p in 41 HBeAg-positive patients and the miR-145-5p in 45 HBeAg-negative patients[104].

***Biomarkers and therapeutic targets in HBV-related HCC***

HCC is the fifth most common cancer in men and the ninth in women, with respectively 554000 and 228000 new cases per year worldwide[105]. In addition, HCC is the second most common cause of death for cancer worldwide, responsible for nearly 750000 deaths per year, half of which in HBV-infected patients. Despite the great efforts of the scientific communities and Healthcare Authorities, the mortality rate of HCC has not significantly decreased in the last decade, mainly because the diagnosis is very late in most cases. In fact, the level of serum alpha-fetoprotein (-FP) lacks sensitivity and is no longer indicated for screening[106,107] and the imaging diagnostic techniques require quality of equipment and considerable experience by the radiologists. In addition, the use of sorafenib, the only treatment shown to improve the overall survival of patients in the advanced stages[108] is limited by the high rates of adverse reactions and treatment failures[109].

In this context, many microRNAs have been found dysregulated in serum and liver of HCC patients and therefore considered as possible diagnostic biomarkers and therapeutic targets[84,110-113]. In 2011, Zhou *et al*[111] screened for 723 microRNAs the serum samples of 934 Chinese patients with HBV-related chronic hepatitis, cirrhosis or HCC and identified and validated a panel of 7 miRNAs providing a high diagnostic accuracy for HCC, regardless of cancer stage. Subsequently, the serum level of miR-21 was proposed as a novel biomarker of HCC[112]. The diagnostic accuracy of miR-21 serum level has been further investigated in several subsequent studies[113-115] and in a meta-analysis[116] including 677 patients of different etiologies, with 81.2% sensitivity and 84.8% specificity in the diagnosis of HCC.

In 2010, Li *et al*[117] identified a panel of 13 miRNAs differentially present in serum samples of 120 HBV-related HCC, 135 HBV-infected patients and 210 healthy controls. Using a panel with miR-25, miR-375, and let-7f, they obtained a 97.9% sensitivity and 99.1% specificity in HCC prediction. Furthermore, the miR-375 proved to be of high diagnostic accuracy in two prospective Chinese cohorts[118].

The tissue expression of several miRNAs in HCC tissue has been investigated to identify therapeutic targets. Gao *et al*[119] analyzed the expression profile of 7 miRNAs in 24 dysplastic nodules, 29 HCC tissues and 40 non-tumoral liver tissues surrounding HCC from HBV-infected patients and found a downregulation of miR-145 and miR-199b and an upregulation of miR-224. They also demonstrated that the restoration of miR-145 in both HepG2 and Hep3B HCC cells significantly inhibited cell proliferation and reduced cell migration and invasion. As mentioned above, miR-122 is downregulated in liver tissue of patients with chronic hepatitis B, and its concentration is inversely correlated with the degree of liver fibrosis. This downregulation was reported also in 19 HBV-related HCC tissues by Li *et al*[120] in 2013; they also demonstrated that the pituitary tumor-transforming gene 1 (PTTG1) binding factor (PBF), a validated molecular target of miR-122, enhances the proliferation and invasion of HCC cells, while its silencing induced a significant reduction in tumor growth in a murine HCC model. It has also been demonstrated that the deletion of mouse Mir122 resulted in hepatosteatosis, hepatitis, and the development of tumors resembling HCC[121], while its re-expression reduced disease manifestations and tumor incidence[122]. We recently reported a lower expression of miR-125a-5p in HCC tissues compared with non-tumor tissue in 55 patients with hepatocellular cancer of different etiologies[123]. In addition, we found a significant upregulation of three oncogenes in HCC tissue, MMP-11, c-Raf and Sirt-7, already validated as molecular targets of miR-125a-5p, an observation that provides an explanation for the tumor suppressor activity exerted by this microRNA.

**FUTURE PERSPECTIVES: RNA-INTERFERENCE IN THE TREATMENT OF CHRONIC HEPATITIS B AND HEPATOCELLULAR CARCINOMA**

Some literature data suggest that microRNA-interference might be useful in the treatment of HBV-related chronic hepatitis and HCC. The RNA-interference directed to inhibit HBV replication has been investigated in several animal models and, more recently, in a clinical study[124]. In a phase 2 clinical trial enrolling entecavir- naïve or -exposed HBsAg-positive patients with chronic hepatitis[125], ARC-520, a mixture of small-interfering RNAs (siRNAs) targeting all viral transcripts, induced HBsAg reduction up to 1.5 log10 in HBeAg-positive and up to 0.5 log10 in HBeAg-negative subjects with a single intravenous administration of up to 4 mg/kg. The reasons for the limited efficacy in HBeAg-negative patients have been more recently investigated in a preclinical study on chimpanzees[126]. The authors demonstrated a lack of target sites for the siRNAs in the HBV DNA integrated in the host genome, which represents the dominant source of viral transcripts in HBeAg-negative patients. These findings highlight a novel issue that should be addressed by future research on HBV treatment. Other two RNAi-based therapies (TKM-HBV and ALN-HBV) are currently investigated in chimpanzees and mice with promising preliminary results[127].

There is some evidence of the efficacy of miRNAs mimics or inhibitors both in preclinical studies and in a recent phase I clinical trial regarding the treatment of hepatocellular cancer. In an HCC murine model, the systemic administration of miR-26a using an adeno-associated virus resulted in an inhibition of cancer cell proliferation, induction of tumor-specific apoptosis, and protection from disease progression[127-129]; a cholesterol-modified isoform of anti-miR-221 can reduce tumor cell proliferation and increase the tumor doubling time and the survival in mice with hepatocellular cancer. An miR-375 mimic delivered in gold nanoparticles has shown therapeutic efficacy without significant toxicity in primary and xenograft tumor mouse models[130]. Finally, MRX34, a liposomal miR-34a mimic, showed anti-tumor activity in a phase I clinical trial enrolling patients with refractory advanced primary liver cancers or other solid neoplasms[131], but this trial has been stopped because of serious adverse events.

**REFERENCES**

1 **Ambros V**. The functions of animal microRNAs. *Nature* 2004; **431**: 350-355 [PMID: 15372042 DOI: 10.1038/nature02871]

2 **Bartel DP**. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297 [PMID: 14744438 DOI: 10.1016/S0092-8674(04)00045-5]

3 **Wienholds E**, Plasterk RH. MicroRNA function in animal development. *FEBS Lett* 2005; **579**: 5911-5922 [PMID: 16111679 DOI: 10.1016/j.febslet.2005.07.070]

4 **Alvarez-Garcia I**, Miska EA. MicroRNA functions in animal development and human disease. *Development* 2005; **132**: 4653-4662 [PMID: 16224045 DOI: 10.1242/dev.02073]

5 **Calin GA**, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006; **6**: 857-866 [PMID: 17060945 DOI: 10.1038/nrc1997]

6 **Mosca N**, Starega-Roslan J, Castiello F, Russo A, Krzyzosiak WJ, Potenza N. Characterization of a naturally occurring truncated Dicer. *Mol Biol Rep* 2015; **42**: 1333-1340 [PMID: 25911188 DOI: 10.1007/s11033-015-3878-6]

7 **Marfella R**, Di Filippo C, Potenza N, Sardu C, Rizzo MR, Siniscalchi M, Musacchio E, Barbieri M, Mauro C, Mosca N, Solimene F, Mottola MT, Russo A, Rossi F, Paolisso G, D'Amico M. Circulating microRNA changes in heart failure patients treated with cardiac resynchronization therapy: responders vs. non-responders. *Eur J Heart Fail* 2013; **15**: 1277-1288 [PMID: 23736534 DOI: 10.1093/eurjhf/hft088]

8 **Russo A**, Potenza N. Antiviral effects of human microRNAs and conservation of their target sites. *FEBS Lett* 2011; **585**: 2551-2555 [PMID: 21784072 DOI: 10.1016/j.febslet.2011.07.015]

9 **Cullen BR**. Viruses and microRNAs. *Nat Genet* 2006; **38** Suppl: S25-S30 [PMID: 16736021 DOI: 10.1038/ng1793]

10 **Berkhout B**, Jeang KT. MicroRNAs in viral gene regulation. *Biochim Biophys Acta* 2011; **1809**: 587 [PMID: 22055652 DOI: 10.1016/j.bbagrm.2011.10.009]

11 Bennett JE, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 8th edition. Churchill Livingstone: Elsevier, 2015: 2514

12 **World Health Organization**. Global Hepatitis Report, 2017. Geneva: World Health Organization, 2017

13 **World Health Organization.** Global Health Estimates 2015: deaths by cause, age, sex, by country and by region, 2000–2015. Geneva: World Health Organization, 2016

14 **Liaw YF**, Chu CM. Hepatitis B virus infection. *Lancet* 2009; **373**: 582-592 [PMID: 19217993 DOI: 10.1016/S0140-6736(09)60207-5]

15 **Tosti ME**, Alfonsi V, Lacorte E, Mele A, Galli C, Zanetti AR, Romanò L; SEIEVA Collaborating Group. Acute Hepatitis B After the Implementation of Universal Vaccination in Italy: Results From 22 Years of Surveillance (1993-2014). *Clin Infect Dis* 2016; **62**: 1412-1418 [PMID: 27009250 DOI: 10.1093/cid/ciw162]

16 **Sundaram V**, Kowdley K. Management of chronic hepatitis B infection. *BMJ* 2015; **351**: h4263 [PMID: 26491030 DOI: 10.1136/bmj.h4263]

17 **Daniels D**, Grytdal S, Wasley A; Centers for Disease Control and Prevention (CDC). Surveillance for acute viral hepatitis - United States, 2007. *MMWR Surveill Summ* 2009; **58**: 1-27 [PMID: 19478727]

18 **McMahon BJ**. The natural history of chronic hepatitis B virus infection. *Hepatology* 2009; **49**: S45-S55 [PMID: 19399792 DOI: 10.1002/hep.22898]

19 **Iloeje UH**, Yang HI, Su J, Jen CL, You SL, Chen CJ; Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-In HBV (the REVEAL-HBV) Study Group. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; **130**: 678-686 [PMID: 16530509 DOI: 10.1053/j.gastro.2005.11.016]

20 **El-Serag HB**. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273.e1 [PMID: 22537432 DOI: 10.1053/j.gastro.2011.12.061]

21 **Jin WB**, Wu FL, Kong D, Guo AG. HBV-encoded microRNA candidate and its target. *Comput Biol Chem* 2007; **31**: 124-126 [PMID: 17350341 DOI: 10.1016/j.compbiolchem.2007.01.005]

22 **Zhang GL**, Li YX, Zheng SQ, Liu M, Li X, Tang H. Suppression of hepatitis B virus replication by microRNA-199a-3p and microRNA-210. *Antiviral Res* 2010; **88**: 169-175 [PMID: 20728471 DOI: 10.1016/j.antiviral.2010.08.008]

23 **Potenza N**, Papa U, Mosca N, Zerbini F, Nobile V, Russo A. Human microRNA hsa-miR-125a-5p interferes with expression of hepatitis B virus surface antigen. *Nucleic Acids Res* 2011; **39**: 5157-5163 [PMID: 21317190 DOI: 10.1093/nar/gkr067]

24 **Hu W**, Wang X, Ding X, Li Y, Zhang X, Xie P, Yang J, Wang S. MicroRNA-141 represses HBV replication by targeting PPARA. *PLoS One* 2012; **7**: e34165 [PMID: 22479552 DOI: 10.1371/journal.pone.0034165]

25 **Park SO**, Kumar M, Gupta S. TGF-β and iron differently alter HBV replication in human hepatocytes through TGF-β/BMP signaling and cellular microRNA expression. *PLoS One* 2012; **7**: e39276 [PMID: 22723983 DOI: 10.1371/journal.pone.0039276]

26 **Potenza N**, Russo A. Biogenesis, evolution and functional targets of microRNA-125a. *Mol Genet Genomics* 2013; **288**: 381-389 [PMID: 23783428 DOI: 10.1007/s00438-013-0757-5]

27 **Chen Y**, Shen A, Rider PJ, Yu Y, Wu K, Mu Y, Hao Q, Liu Y, Gong H, Zhu Y, Liu F, Wu J. A liver-specific microRNA binds to a highly conserved RNA sequence of hepatitis B virus and negatively regulates viral gene expression and replication. *FASEB J* 2011; **25**: 4511-4521 [PMID: 21903935 DOI: 10.1096/fj.11-187781]

28 **Liu N**, Zhang J, Jiao T, Li Z, Peng J, Cui Z, Ye X. Hepatitis B virus inhibits apoptosis of hepatoma cells by sponging the MicroRNA 15a/16 cluster. *J Virol* 2013; **87**: 13370-13378 [PMID: 24089558 DOI: 10.1128/JVI.02130-13]

29 **Jung YJ**, Kim JW, Park SJ, Min BY, Jang ES, Kim NY, Jeong SH, Shin CM, Lee SH, Park YS, Hwang JH, Kim N, Lee DH. c-Myc-mediated overexpression of miR-17-92 suppresses replication of hepatitis B virus in human hepatoma cells. *J Med Virol* 2013; **85**: 969-978 [PMID: 23532756 DOI: 10.1002/jmv.23534]

30 **Kohno T**, Tsuge M, Murakami E, Hiraga N, Abe H, Miki D, Imamura M, Ochi H, Hayes CN, Chayama K. Human microRNA hsa-miR-1231 suppresses hepatitis B virus replication by targeting core mRNA. *J Viral Hepat* 2014; **21**: e89-e97 [PMID: 24835118 DOI: 10.1111/jvh.12240]

31 **Zhang T**, Zhang J, Cui M, Liu F, You X, Du Y, Gao Y, Zhang S, Lu Z, Ye L, Zhang X. Hepatitis B virus X protein inhibits tumor suppressor miR-205 through inducing hypermethylation of miR-205 promoter to enhance carcinogenesis. *Neoplasia* 2013; **15**: 1282-1291 [PMID: 24339740 DOI: 10.1593/neo.131362]

32 **Mosca N**, Castiello F, Coppola N, Trotta MC, Sagnelli C, Pisaturo M, Sagnelli E, Russo A, Potenza N. Functional interplay between hepatitis B virus X protein and human miR-125a in HBV infection. *Biochem Biophys Res Commun* 2014; **449**: 141-145 [PMID: 24824183 DOI: 10.1016/j.bbrc.2014.05.009]

33 **Wang Y**, Jiang L, Ji X, Yang B, Zhang Y, Fu XD. Hepatitis B viral RNA directly mediates down-regulation of the tumor suppressor microRNA miR-15a/miR-16-1 in hepatocytes. *J Biol Chem* 2013; **288**: 18484-18493 [PMID: 23649629 DOI: 10.1074/jbc.M113.458158]

34 **Kim CM**, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature* 1991; **351**: 317-320 [PMID: 2034275 DOI: 10.1038/351317a0]

35 **López-Cabrera M**, Letovsky J, Hu KQ, Siddiqui A. Transcriptional factor C/EBP binds to and transactivates the enhancer element II of the hepatitis B virus. *Virology* 1991; **183**: 825-829 [PMID: 1853580 DOI: 10.1016/0042-6822(91)91019-D]

36 **López-Cabrera M**, Letovsky J, Hu KQ, Siddiqui A. Multiple liver-specific factors bind to the hepatitis B virus core/pregenomic promoter: trans-activation and repression by CCAAT/enhancer binding protein. *Proc Natl Acad Sci USA* 1990; **87**: 5069-5073 [PMID: 2367525 DOI: 10.1073/pnas.87.13.5069]

37 **Bock CT**, Kubicka S, Manns MP, Trautwein C. Two control elements in the hepatitis B virus S-promoter are important for full promoter activity mediated by CCAAT-binding factor. *Hepatology* 1999; **29**: 1236-1247 [PMID: 10094970 DOI: 10.1002/hep.510290426]

38 **Wang B**, Majumder S, Nuovo G, Kutay H, Volinia S, Patel T, Schmittgen TD, Croce C, Ghoshal K, Jacob ST. Role of microRNA-155 at early stages of hepatocarcinogenesis induced by choline-deficient and amino acid-defined diet in C57BL/6 mice. *Hepatology* 2009; **50**: 1152-1161 [PMID: 19711427 DOI: 10.1002/hep.23100]

39 **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. *Virol J* 2011; **8**: 354 [PMID: 21762537 DOI: 10.1186/1743-422X-8-354]

40 **Raney AK**, Johnson JL, Palmer CN, McLachlan A. Members of the nuclear receptor superfamily regulate transcription from the hepatitis B virus nucleocapsid promoter. *J Virol* 1997; **71**: 1058-1071 [PMID: 8995626]

41 **Huang JY**, Chou SF, Lee JW, Chen HL, Chen CM, Tao MH, Shih C. MicroRNA-130a can inhibit hepatitis B virus replication via targeting PGC1α and PPARγ. *RNA* 2015; **21**: 385-400 [PMID: 25595716 DOI: 10.1261/rna.048744.114]

42 **Jin J**, Tang S, Xia L, Du R, Xie H, Song J, Fan R, Bi Q, Chen Z, Yang G, Liu J, Shi Y, Fan D. MicroRNA-501 promotes HBV replication by targeting HBXIP. *Biochem Biophys Res Commun* 2013; **430**: 1228-1233 [PMID: 23266610 DOI: 10.1016/j.bbrc.2012.12.071]

43 **Ori A**, Zauberman A, Doitsh G, Paran N, Oren M, Shaul Y. p53 binds and represses the HBV enhancer: an adjacent enhancer element can reverse the transcription effect of p53. *EMBO J* 1998; **17**: 544-553 [PMID: 9430645 DOI: 10.1093/emboj/17.2.544]

44 **Wang S**, Qiu L, Yan X, Jin W, Wang Y, Chen L, Wu E, Ye X, Gao GF, Wang F, Chen Y, Duan Z, Meng S. Loss of microRNA 122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G(1) -modulated P53 activity. *Hepatology* 2012; **55**: 730-741 [PMID: 22105316 DOI: 10.1002/hep.24809]

45 **Qiu L**, Fan H, Jin W, Zhao B, Wang Y, Ju Y, Chen L, Chen Y, Duan Z, Meng S. miR-122-induced down-regulation of HO-1 negatively affects miR-122-mediated suppression of HBV. *Biochem Biophys Res Commun* 2010; **398**: 771-777 [PMID: 20633528 DOI: 10.1016/j.bbrc.2010.07.021]

46 **Protzer U**, Seyfried S, Quasdorff M, Sass G, Svorcova M, Webb D, Bohne F, Hösel M, Schirmacher P, Tiegs G. Antiviral activity and hepatoprotection by heme oxygenase-1 in hepatitis B virus infection. *Gastroenterology* 2007; **133**: 1156-1165 [PMID: 17919491 DOI: 10.1053/j.gastro.2007.07.021]

47 **Jopling CL**, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science* 2005; **309**: 1577-1581 [PMID: 16141076 DOI: 10.1126/science.1113329]

48 **Guo H**, Liu H, Mitchelson K, Rao H, Luo M, Xie L, Sun Y, Zhang L, Lu Y, Liu R, Ren A, Liu S, Zhou S, Zhu J, Zhou Y, Huang A, Wei L, Guo Y, Cheng J. MicroRNAs-372/373 promote the expression of hepatitis B virus through the targeting of nuclear factor I/B. *Hepatology* 2011; **54**: 808-819 [PMID: 21608007 DOI: 10.1002/hep.24441]

49 **Fan H**, Lv P, Lv J, Zhao X, Liu M, Zhang G, Tang H. miR-370 suppresses HBV gene expression and replication by targeting nuclear factor IA. *J Med Virol* 2017; **89**: 834-844 [PMID: 27664977 DOI: 10.1002/jmv.24695]

50 **Dai X**, Zhang W, Zhang H, Sun S, Yu H, Guo Y, Kou Z, Zhao G, Du L, Jiang S, Zhang J, Li J, Zhou Y. Modulation of HBV replication by microRNA-15b through targeting hepatocyte nuclear factor 1α. *Nucleic Acids Res* 2014; **42**: 6578-6590 [PMID: 24705650 DOI: 10.1093/nar/gku260]

51 **Zhang X**, Zhang E, Ma Z, Pei R, Jiang M, Schlaak JF, Roggendorf M, Lu M. Modulation of hepatitis B virus replication and hepatocyte differentiation by MicroRNA-1. *Hepatology* 2011; **53**: 1476-1485 [PMID: 21520166 DOI: 10.1002/hep.24195]

52 **Datta J**, Kutay H, Nasser MW, Nuovo GJ, Wang B, Majumder S, Liu CG, Volinia S, Croce CM, Schmittgen TD, Ghoshal K, Jacob ST. Methylation mediated silencing of MicroRNA-1 gene and its role in hepatocellular carcinogenesis. *Cancer Res* 2008; **68**: 5049-5058 [PMID: 18593903 DOI: 10.1158/0008-5472.CAN-07-6655]

53 **Reese V**, Ondracek C, Rushing C, Li L, Oropeza CE, McLachlan A. Multiple nuclear receptors may regulate hepatitis B virus biosynthesis during development. *Int J Biochem Cell Biol* 2011; **43**: 230-237 [PMID: 19941970 DOI: 10.1016/j.biocel.2009.11.016]

54 **Huang J**, Wang Y, Guo Y, Sun S. Down-regulated microRNA-152 induces aberrant DNA methylation in hepatitis B virus-related hepatocellular carcinoma by targeting DNA methyltransferase 1. *Hepatology* 2010; **52**: 60-70 [PMID: 20578129 DOI: 10.1002/hep.23660]

55 **Kim JW**, Lee SH, Park YS, Hwang JH, Jeong SH, Kim N, Lee DH. Replicative activity of hepatitis B virus is negatively associated with methylation of covalently closed circular DNA in advanced hepatitis B virus infection. *Intervirology* 2011; **54**: 316-325 [PMID: 21242658 DOI: 10.1159/000321450]

56 **Zhang X**, Liu S, Hu T, Liu S, He Y, Sun S. Up-regulated microRNA-143 transcribed by nuclear factor kappa B enhances hepatocarcinoma metastasis by repressing fibronectin expression. *Hepatology* 2009; **50**: 490-499 [PMID: 19472311 DOI: 10.1002/hep.23008]

57 **Braconi C**, Huang N, Patel T. MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes. *Hepatology* 2010; **51**: 881-890 [PMID: 20146264 DOI: 10.1002/hep.23381]

58 **Wang S**, Zhang X, Ju Y, Zhao B, Yan X, Hu J, Shi L, Yang L, Ma Z, Chen L, Liu Y, Duan Z, Chen X, Meng S. MicroRNA-146a feedback suppresses T cell immune function by targeting Stat1 in patients with chronic hepatitis B. *J Immunol* 2013; **191**: 293-301 [PMID: 23698745 DOI: 10.4049/jimmunol.1202100]

59 **Li Y**, Xie J, Xu X, Wang J, Ao F, Wan Y, Zhu Y. MicroRNA-548 down-regulates host antiviral response via direct targeting of IFN-λ1. *Protein Cell* 2013; **4**: 130-141 [PMID: 23150165 DOI: 10.1007/s13238-012-2081-y]

60 **Yang P**, Li QJ, Feng Y, Zhang Y, Markowitz GJ, Ning S, Deng Y, Zhao J, Jiang S, Yuan Y, Wang HY, Cheng SQ, Xie D, Wang XF. TGF-β-miR-34a-CCL22 signaling-induced Treg cell recruitment promotes venous metastases of HBV-positive hepatocellular carcinoma. *Cancer Cell* 2012; **22**: 291-303 [PMID: 22975373 DOI: 10.1016/j.ccr.2012.07.023]

61 **Costinean S**, Zanesi N, Pekarsky Y, Tili E, Volinia S, Heerema N, Croce CM. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. *Proc Natl Acad Sci USA* 2006; **103**: 7024-7029 [PMID: 16641092 DOI: 10.1073/pnas.0602266103]

62 **Negrini M**, Ferracin M, Sabbioni S, Croce CM. MicroRNAs in human cancer: from research to therapy. *J Cell Sci* 2007; **120**: 1833-1840 [PMID: 17515481 DOI: 10.1242/jcs.03450]

63 **Di Leva G**, Garofalo M, Croce CM. MicroRNAs in cancer. *Annu Rev Pathol* 2014; **9**: 287-314 [PMID: 24079833 DOI: 10.1146/annurev-pathol-012513-104715]

64 **Potenza N**, Papa U, Scaruffi P, Mosca N, Tonini GP, Russo A. A novel splice variant of the human dicer gene is expressed in neuroblastoma cells. *FEBS Lett* 2010; **584**: 3452-3457 [PMID: 20615407 DOI: 10.1016/j.febslet.2010.06.045]

65 **Kitagawa N**, Ojima H, Shirakihara T, Shimizu H, Kokubu A, Urushidate T, Totoki Y, Kosuge T, Miyagawa S, Shibata T. Downregulation of the microRNA biogenesis components and its association with poor prognosis in hepatocellular carcinoma. *Cancer Sci* 2013; **104**: 543-551 [PMID: 23398123 DOI: 10.1111/cas.12126]

66 **Rupaimoole R**, Wu SY, Pradeep S, Ivan C, Pecot CV, Gharpure KM, Nagaraja AS, Armaiz-Pena GN, McGuire M, Zand B, Dalton HJ, Filant J, Miller JB, Lu C, Sadaoui NC, Mangala LS, Taylor M, van den Beucken T, Koch E, Rodriguez-Aguayo C, Huang L, Bar-Eli M, Wouters BG, Radovich M, Ivan M, Calin GA, Zhang W, Lopez-Berestein G, Sood AK. Hypoxia-mediated downregulation of miRNA biogenesis promotes tumour progression. *Nat Commun* 2014; **5**: 5202 [PMID: 25351346 DOI: 10.1038/ncomms6202]

67 **Foulkes WD**, Priest JR, Duchaine TF. DICER1: mutations, microRNAs and mechanisms. *Nat Rev Cancer* 2014; **14**: 662-672 [PMID: 25176334 DOI: 10.1038/nrc3802]

68 **O'Rourke JR**, Georges SA, Seay HR, Tapscott SJ, McManus MT, Goldhamer DJ, Swanson MS, Harfe BD. Essential role for Dicer during skeletal muscle development. *Dev Biol* 2007; **311**: 359-368 [PMID: 17936265 DOI: 10.1016/j.ydbio.2007.08.032]

69 **Kawase-Koga Y**, Otaegi G, Sun T. Different timings of Dicer deletion affect neurogenesis and gliogenesis in the developing mouse central nervous system. *Dev Dyn* 2009; **238**: 2800-2812 [PMID: 19806666 DOI: 10.1002/dvdy.22109]

70 **Potenza N**, Papa U, Russo A. Differential expression of Dicer and Argonaute genes during the differentiation of human neuroblastoma cells. *Cell Biol Int* 2009; **33**: 734-738 [PMID: 19393748 DOI: 10.1016/j.cellbi.2009.04.002]

71 **Hou J**, Lin L, Zhou W, Wang Z, Ding G, Dong Q, Qin L, Wu X, Zheng Y, Yang Y, Tian W, Zhang Q, Wang C, Zhang Q, Zhuang SM, Zheng L, Liang A, Tao W, Cao X. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. *Cancer Cell* 2011; **19**: 232-243 [PMID: 21316602 DOI: 10.1016/j.ccr.2011.01.001]

72 **Murakami Y**, Yasuda T, Saigo K, Urashima T, Toyoda H, Okanoue T, Shimotohno K. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. *Oncogene* 2006; **25**: 2537-2545 [PMID: 16331254 DOI: 10.1038/sj.onc.1209283]

73 **Fornari F**, Milazzo M, Chieco P, Negrini M, Calin GA, Grazi GL, Pollutri D, Croce CM, Bolondi L, Gramantieri L. MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res* 2010; **70**: 5184-5193 [PMID: 20501828 DOI: 10.1158/0008-5472.CAN-10-0145]

74 **Henry JC**, Park JK, Jiang J, Kim JH, Nagorney DM, Roberts LR, Banerjee S, Schmittgen TD. miR-199a-3p targets CD44 and reduces proliferation of CD44 positive hepatocellular carcinoma cell lines. *Biochem Biophys Res Commun* 2010; **403**: 120-125 [PMID: 21055388 DOI: 10.1016/j.bbrc.2010.10.130]

75 **Jia XQ**, Cheng HQ, Qian X, Bian CX, Shi ZM, Zhang JP, Jiang BH, Feng ZQ. Lentivirus-mediated overexpression of microRNA-199a inhibits cell proliferation of human hepatocellular carcinoma. *Cell Biochem Biophys* 2012; **62**: 237-244 [PMID: 21847633 DOI: 10.1007/s12013-011-9263-8]

76 **Kim JH**, Badawi M, Park JK, Jiang J, Mo X, Roberts LR, Schmittgen TD. Anti-invasion and anti-migration effects of miR-199a-3p in hepatocellular carcinoma are due in part to targeting CD151. *Int J Oncol* 2016; **49**: 2037-2045 [PMID: 27599545 DOI: 10.3892/ijo.2016.3677]

77 **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. *J Biomed Sci* 2016; **23**: 79 [PMID: 27832779 DOI: 10.1186/s12929-016-0295-7]

78 **Guan J**, Liu Z, Xiao M, Hao F, Wang C, Chen Y, Lu Y, Liang J. MicroRNA-199a-3p inhibits tumorigenesis of hepatocellular carcinoma cells by targeting ZHX1/PUMA signal. *Am J Transl Res* 2017; **9**: 2457-2465 [PMID: 28559996]

79 **Negrini M**, Gramantieri L, Sabbioni S, Croce CM. microRNA involvement in hepatocellular carcinoma. *Anticancer Agents Med Chem* 2011; **11**: 500-521 [PMID: 21554203 DOI: 10.2174/187152011796011037]

80 **Braconi C**, Henry JC, Kogure T, Schmittgen T, Patel T. The role of microRNAs in human liver cancers. *Semin Oncol* 2011; **38**: 752-763 [PMID: 22082761 DOI: 10.1053/j.seminoncol.2011.08.001]

81 **Callegari E**, Elamin BK, Sabbioni S, Gramantieri L, Negrini M. Role of microRNAs in hepatocellular carcinoma: a clinical perspective. *Onco Targets Ther* 2013; **6**: 1167-1178 [PMID: 24039437 DOI: 10.2147/OTT.S36161]

82 **Xie KL**, Zhang YG, Liu J, Zeng Y, Wu H. MicroRNAs associated with HBV infection and HBV-related HCC. *Theranostics* 2014; **4**: 1176-1192 [PMID: 25285167 DOI: 10.7150/thno.8715]

83 **Ghidini M**, Braconi C. Non-Coding RNAs in Primary Liver Cancer. *Front Med* (Lausanne) 2015; **2**: 36 [PMID: 26131450 DOI: 10.3389/fmed.2015.00036]

84 **Klingenberg M**, Matsuda A, Diederichs S, Patel T. Non-coding RNA in hepatocellular carcinoma: Mechanisms, biomarkers and therapeutic targets. *J Hepatol* 2017; **67**: 603-618 [PMID: 28438689 DOI: 10.1016/j.jhep.2017.04.009]

85 **Kew MC**. Hepatitis B virus x protein in the pathogenesis of hepatitis B virus-induced hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 144-152 [PMID: 21199526 DOI: 10.1111/j.1440-1746.2010.06546]

86 **Qadri I**, Maguire HF, Siddiqui A. Hepatitis B virus transactivator protein X interacts with the TATA-binding protein. *Proc Natl Acad Sci USA* 1995; **92**: 1003-1007 [PMID: 7862623 DOI: 10.1073/pnas.92.4.1003]

87 **Cougot D**, Wu Y, Cairo S, Caramel J, Renard CA, Lévy L, Buendia MA, Neuveut C. The hepatitis B virus X protein functionally interacts with CREB-binding protein/p300 in the regulation of CREB-mediated transcription. *J Biol Chem* 2007; **282**: 4277-4287 [PMID: 17158882 DOI: 10.1074/jbc.M606774200]

88 **Zhang B**, Han S, Feng B, Chu X, Chen L, Wang R. Hepatitis B virus X protein-mediated non-coding RNA aberrations in the development of human hepatocellular carcinoma. *Exp Mol Med* 2017; **49**: e293 [PMID: 28186085 DOI: 10.1038/emm.2016.177]

89 **Wang Y**, Lu Y, Toh ST, Sung WK, Tan P, Chow P, Chung AY, Jooi LL, Lee CG. Lethal-7 is down-regulated by the hepatitis B virus x protein and targets signal transducer and activator of transcription 3. *J Hepatol* 2010; **53**: 57-66 [PMID: 20447714 DOI: 10.1016/j.jhep.2009.12.043]

90 **Yip WK**, Cheng AS, Zhu R, Lung RW, Tsang DP, Lau SS, Chen Y, Sung JG, Lai PB, Ng EK, Yu J, Wong N, To KF, Wong VW, Sung JJ, Chan HL. Carboxyl-terminal truncated HBx regulates a distinct microRNA transcription program in hepatocellular carcinoma development. *PLoS One* 2011; **6**: e22888 [PMID: 21829663 DOI: 10.1371/journal.pone.0022888]

91 **Iorio MV**, Croce CM. Causes and consequences of microRNA dysregulation. *Cancer J* 2012; **18**: 215-222 [PMID: 22647357 DOI: 10.1097/PPO.0b013e318250c001]

92 **Calin GA**, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 2002; **99**: 15524-15529 [PMID: 12434020 DOI: 10.1073/pnas.242606799]

93 **Calin GA**, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci USA* 2004; **101**: 2999-3004 [PMID: 14973191 DOI: 10.1073/pnas.0307323101]

94 **Weber B**, Stresemann C, Brueckner B, Lyko F. Methylation of human microRNA genes in normal and neoplastic cells. *Cell Cycle* 2007; **6**: 1001-1005 [PMID: 17457051 DOI: 10.4161/cc.6.9.4209]

95 **Waidmann O**, Bihrer V, Pleli T, Farnik H, Berger A, Zeuzem S, Kronenberger B, Piiper A. Serum microRNA-122 levels in different groups of patients with chronic hepatitis B virus infection. *J Viral Hepat* 2012; **19**: e58-e65 [PMID: 22239527 DOI: 10.1111/j.1365-2893.2011.01536.x]

96 **Xing TJ**, Jiang DF, Huang JX, Xu ZL. Expression and clinical significance of miR-122 and miR-29 in hepatitis B virus-related liver disease. *Genet Mol Res* 2014; **13**: 7912-7918 [PMID: 25299106 DOI: 10.4238/2014]

97 **Nakamura M**, Kanda T, Jiang X, Haga Y, Takahashi K, Wu S, Yasui S, Nakamoto S, Yokosuka O. Serum microRNA-122 and Wisteria floribunda agglutinin-positive Mac-2 binding protein are useful tools for liquid biopsy of the patients with hepatitis B virus and advanced liver fibrosis. *PLoS One* 2017; **12**: e0177302 [PMID: 28475652 DOI: 10.1371/journal.pone.0177302]

98 **Huang C**, Zheng JM, Cheng Q, Yu KK, Ling QX, Chen MQ, Li N. Serum microRNA-29 levels correlate with disease progression in patients with chronic hepatitis B virus infection. *J Dig Dis* 2014; **15**: 614-621 [PMID: 25138057 DOI: 10.1111/1751-2980.12185]

99 **Bao S**, Zheng J, Li N, Huang C, Chen M, Cheng Q, Yu K, Chen S, Zhu M, Shi G. Serum MicroRNA Levels as a Noninvasive Diagnostic Biomarker for the Early Diagnosis of Hepatitis B Virus-Related Liver Fibrosis. *Gut Liver* 2017; **11**: 860-869 [PMID: 28750488 DOI: 10.5009/gnl16560]

100 **Coppola N**, Potenza N, Pisaturo M, Mosca N, Tonziello G, Signoriello G, Messina V, Sagnelli C, Russo A, Sagnelli E. Liver microRNA hsa-miR-125a-5p in HBV chronic infection: correlation with HBV replication and disease progression. *PLoS One* 2013; **8**: e65336 [PMID: 23843939 DOI: 10.1371/journal.pone.0065336]

101 **Zheng J**, Zhou Z, Xu Z, Li G, Dong P, Chen Z, Lin D, Chen B, Yu F. Serum microRNA-125a-5p, a useful biomarker in liver diseases, correlates with disease progression. *Mol Med Rep* 2015; **12**: 1584-1590 [PMID: 25815788 DOI: 10.3892/mmr.2015.3546]

102 **Li F**, Zhou P, Deng W, Wang J, Mao R, Zhang Y, Li J, Yu J, Yang F, Huang Y, Lu M, Zhang J. Serum microRNA-125b correlates with hepatitis B viral replication and liver necroinflammation. *Clin Microbiol Infect* 2016; **22**: 384.e1-384.e10 [PMID: 26802212 DOI: 10.1016/j.cmi.2015.12.024]

103 **Brunetto MR**, Cavallone D, Oliveri F, Moriconi F, Colombatto P, Coco B, Ciccorossi P, Rastelli C, Romagnoli V, Cherubini B, Teilum MW, Blondal T, Bonino F. A serum microRNA signature is associated with the immune control of chronic hepatitis B virus infection. *PLoS One* 2014; **9**: e110782 [PMID: 25350115 DOI: 10.1371/journal.pone.0110782]

104 **van der Ree MH**, Jansen L, Kruize Z, van Nuenen AC, van Dort KA, Takkenberg RB, Reesink HW, Kootstra NA. Plasma MicroRNA Levels Are Associated With Hepatitis B e Antigen Status and Treatment Response in Chronic Hepatitis B Patients. *J Infect Dis* 2017; **215**: 1421-1429 [PMID: 28368488 DOI: 10.1093/infdis/jix140]

105 **Fidler MM**, Gupta S, Soerjomataram I, Ferlay J, Steliarova-Foucher E, Bray F. Cancer incidence and mortality among young adults aged 20-39 years worldwide in 2012: a population-based study. *Lancet Oncol* 2017; **18**: 1579-1589 [PMID: 29111259 DOI: 10.1016/S1470-2045(17)30677-0]

106 **Heimbach JK**, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, Zhu AX, Murad MH, Marrero JA. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018; **67**: 358-380 [PMID: 28130846 DOI: 10.1002/hep.29086]

107 **European Association For The Study Of The Liver.**; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

108 **Finn RS**, Zhu AX, Farah W, Almasri J, Zaiem F, Prokop LJ, Murad MH, Mohammed K. Therapies for advanced stage hepatocellular carcinoma with macrovascular invasion or metastatic disease: A systematic review and meta-analysis. *Hepatology* 2018; **67**: 422-435 [PMID: 28881497 DOI: 10.1002/hep.29486]

109 **Bruix J**, Reig M, Sherman M. Evidence-Based Diagnosis, Staging, and Treatment of Patients With Hepatocellular Carcinoma. *Gastroenterology* 2016; **150**: 835-853 [PMID: 26795574 DOI: 10.1053/j.gastro.2015.12.041]

110 **Giordano S**, Columbano A. MicroRNAs: new tools for diagnosis, prognosis, and therapy in hepatocellular carcinoma? *Hepatology* 2013; **57**: 840-847 [PMID: 23081718 DOI: 10.1002/hep.26095]

111 **Zhou J**, Yu L, Gao X, Hu J, Wang J, Dai Z, Wang JF, Zhang Z, Lu S, Huang X, Wang Z, Qiu S, Wang X, Yang G, Sun H, Tang Z, Wu Y, Zhu H, Fan J. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. *J Clin Oncol* 2011; **29**: 4781-4788 [PMID: 22105822 DOI: 10.1200/JCO.2011.38.2697]

112 **Tomimaru Y**, Eguchi H, Nagano H, Wada H, Kobayashi S, Marubashi S, Tanemura M, Tomokuni A, Takemasa I, Umeshita K, Kanto T, Doki Y, Mori M. Circulating microRNA-21 as a novel biomarker for hepatocellular carcinoma. *J Hepatol* 2012; **56**: 167-175 [PMID: 21749846 DOI: 10.1016/j.jhep.2011.04.026]

113 **Liu AM**, Yao TJ, Wang W, Wong KF, Lee NP, Fan ST, Poon RT, Gao C, Luk JM. Circulating miR-15b and miR-130b in serum as potential markers for detecting hepatocellular carcinoma: a retrospective cohort study. *BMJ Open* 2012; **2**: e000825 [PMID: 22403344 DOI: 10.1136/bmjopen-2012-000825]

114 **Bandopadhyay M**, Banerjee A, Sarkar N, Panigrahi R, Datta S, Pal A, Singh SP, Biswas A, Chakrabarti S, Chakravarty R. Tumor suppressor micro RNA miR-145 and onco micro RNAs miR-21 and miR-222 expressions are differentially modulated by hepatitis B virus X protein in malignant hepatocytes. *BMC Cancer* 2014; **14**: 721 [PMID: 25260533 DOI: 10.1186/1471-2407-14-721]

115 **Guo X**, Lv X, Lv X, Ma Y, Chen L, Chen Y. Circulating miR-21 serves as a serum biomarker for hepatocellular carcinoma and correlated with distant metastasis. *Oncotarget* 2017; **8**: 44050-44058 [PMID: 28477010 DOI: 10.18632/oncotarget.17211]

116 **Liao Q**, Han P, Huang Y, Wu Z, Chen Q, Li S, Ye J, Wu X. Potential Role of Circulating microRNA-21 for Hepatocellular Carcinoma Diagnosis: A Meta-Analysis. *PLoS One* 2015; **10**: e0130677 [PMID: 26114756 DOI: 10.1371/journal.pone.0130677]

117 **Li LM**, Hu ZB, Zhou ZX, Chen X, Liu FY, Zhang JF, Shen HB, Zhang CY, Zen K. Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. *Cancer Res* 2010; **70**: 9798-9807 [PMID: 21098710 DOI: 10.1158/0008-5472.CAN-10-1001]

118 **Wen Y**, Han J, Chen J, Dong J, Xia Y, Liu J, Jiang Y, Dai J, Lu J, Jin G, Han J, Wei Q, Shen H, Sun B, Hu Z. Plasma miRNAs as early biomarkers for detecting hepatocellular carcinoma. *Int J Cancer* 2015; **137**: 1679-1690 [PMID: 25845839 DOI: 10.1002/ijc.29544]

119 **Gao P**, Wong CC, Tung EK, Lee JM, Wong CM, Ng IO. Deregulation of microRNA expression occurs early and accumulates in early stages of HBV-associated multistep hepatocarcinogenesis. *J Hepatol* 2011; **54**: 1177-1184 [PMID: 21145831 DOI: 10.1016/j.jhep.2010.09.023]

120 **Li C**, Wang Y, Wang S, Wu B, Hao J, Fan H, Ju Y, Ding Y, Chen L, Chu X, Liu W, Ye X, Meng S. Hepatitis B virus mRNA-mediated miR-122 inhibition upregulates PTTG1-binding protein, which promotes hepatocellular carcinoma tumor growth and cell invasion. *J Virol* 2013; **87**: 2193-2205 [PMID: 23221562 DOI: 10.1128/JVI.02831-12]

121 **Hsu SH**, Wang B, Kota J, Yu J, Costinean S, Kutay H, Yu L, Bai S, La Perle K, Chivukula RR, Mao H, Wei M, Clark KR, Mendell JR, Caligiuri MA, Jacob ST, Mendell JT, Ghoshal K. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. *J Clin Invest* 2012; **122**: 2871-2883 [PMID: 22820288 DOI: 10.1172/JCI63539]

122 **Tsai WC**, Hsu SD, Hsu CS, Lai TC, Chen SJ, Shen R, Huang Y, Chen HC, Lee CH, Tsai TF, Hsu MT, Wu JC, Huang HD, Shiao MS, Hsiao M, Tsou AP. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. *J Clin Invest* 2012; **122**: 2884-2897 [PMID: 22820290 DOI: 10.1172/JCI63455]

123 **Coppola N**, de Stefano G, Panella M, Onorato L, Iodice V, Minichini C, Mosca N, Desiato L, Farella N, Starace M, Liorre G, Potenza N, Sagnelli E, Russo A. Lowered expression of microRNA-125a-5p in human hepatocellular carcinoma and up-regulation of its oncogenic targets sirtuin-7, matrix metalloproteinase-11, and c-Raf. *Oncotarget* 2017; **8**: 25289-25299 [PMID: 28445974 DOI: 10.18632/oncotarget.15809]

124 **Gish RG**, Yuen MF, Chan HL, Given BD, Lai CL, Locarnini SA, Lau JY, Wooddell CI, Schluep T, Lewis DL. Synthetic RNAi triggers and their use in chronic hepatitis B therapies with curative intent. *Antiviral Res* 2015; **121**: 97-108 [PMID: 26129970 DOI: 10.1016/j.antiviral.2015.06.019]

125 **Yuen MF**, Chan HL, Liu S, Given BD, Sclhuep T, Hamilton J, Lai CL, Locarnini SA, Lau JY, Ferrari C, Gish R. ARC-520 produces deep and durable knockdown of viral antigens and DNA in a phase II study in patients with chronic hepatitis B. *Hepatology* 2015; **62**: 1385A

126 **Wooddell CI**, Yuen MF, Chan HL, Gish RG, Locarnini SA, Chavez D, Ferrari C, Given BD, Hamilton J, Kanner SB, Lai CL, Lau JYN, Schluep T, Xu Z, Lanford RE, Lewis DL. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. *Sci Transl Med* 2017; **9**: [PMID: 28954926 DOI: 10.1126/scitranslmed.aan0241]

127 **Durantel D**, Zoulim F. New antiviral targets for innovative treatment concepts for hepatitis B virus and hepatitis delta virus. *J Hepatol* 2016; **64**: S117-S131 [PMID: 27084032 DOI: 10.1016/j.jhep.2016.02.016]

128 **Kota J**, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, Hwang HW, Chang TC, Vivekanandan P, Torbenson M, Clark KR, Mendell JR, Mendell JT. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell* 2009; **137**: 1005-1017 [PMID: 19524505 DOI: 10.1016/j.cell.2009.04.021]

129 **Park JK**, Kogure T, Nuovo GJ, Jiang J, He L, Kim JH, Phelps MA, Papenfuss TL, Croce CM, Patel T, Schmittgen TD. miR-221 silencing blocks hepatocellular carcinoma and promotes survival. *Cancer Res* 2011; **71**: 7608-7616 [PMID: 22009537 DOI: 10.1158/0008-5472.CAN-11-1144]

130 **Xue HY**, Liu Y, Liao JZ, Lin JS, Li B, Yuan WG, Lee RJ, Li L, Xu CR, He XX. Gold nanoparticles delivered miR-375 for treatment of hepatocellular carcinoma. *Oncotarget* 2016; **7**: 86675-86686 [PMID: 27880727 DOI: 10.18632/oncotarget.13431]

131 **Beg MS**, Brenner AJ, Sachdev J, Borad M, Kang YK, Stoudemire J, Smith S, Bader AG, Kim S, Hong DS. Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. *Invest New Drugs* 2017; **35**: 180-188 [PMID: 27917453 DOI: 10.1007/s10637-016-0407-y]

132 **Ji J**, Zhao L, Budhu A, Forgues M, Jia HL, Qin LX, Ye QH, Yu J, Shi X, Tang ZY, Wang XW. Let-7g targets collagen type I alpha2 and inhibits cell migration in hepatocellular carcinoma. *J Hepatol* 2010; **52**: 690-697 [PMID: 20338660 DOI: 10.1016/j.jhep.2009.12.025]

133 **Johnson SM**, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. RAS is regulated by the let-7 microRNA family. *Cell* 2005; **120**: 635-647 [PMID: 15766527 DOI: 10.1016/j.cell.2005.01.014]

134 **Lan FF**, Wang H, Chen YC, Chan CY, Ng SS, Li K, Xie D, He ML, Lin MC, Kung HF. Hsa-let-7g inhibits proliferation of hepatocellular carcinoma cells by downregulation of c-Myc and upregulation of p16(INK4A). *Int J Cancer* 2011; **128**: 319-331 [PMID: 20309945 DOI: 10.1002/ijc.25336]

135 **Tian N**, Han Z, Li Z, Zhou M, Fan C. Lin28/let-7/Bcl-xL pathway: the underlying mechanism of drug resistance in Hep3B cells. *Oncol Rep* 2014; **32**: 1050-1056 [PMID: 24970027 DOI: 10.3892/or.2014.3292]

136 **Shimizu S**, Takehara T, Hikita H, Kodama T, Miyagi T, Hosui A, Tatsumi T, Ishida H, Noda T, Nagano H, Doki Y, Mori M, Hayashi N. The let-7 family of microRNAs inhibits Bcl-xL expression and potentiates sorafenib-induced apoptosis in human hepatocellular carcinoma. *J Hepatol* 2010; **52**: 698-704 [PMID: 20347499 DOI: 10.1016/j.jhep.2009.12.024]

137 **Qiu X**, Dong S, Qiao F, Lu S, Song Y, Lao Y, Li Y, Zeng T, Hu J, Zhang L, Zhang L, Fan H. HBx-mediated miR-21 upregulation represses tumor-suppressor function of PDCD4 in hepatocellular carcinoma. *Oncogene* 2013; **32**: 3296-3305 [PMID: 23604124 DOI: 10.1038/onc.2013.150]

138 **Chen M**, Liu Y, Varley P, Chang Y, He XX, Huang H, Tang D, Lotze MT, Lin J, Tsung A. High-Mobility Group Box 1 Promotes Hepatocellular Carcinoma Progression through miR-21-Mediated Matrix Metalloproteinase Activity. *Cancer Res* 2015; **75**: 1645-1656 [PMID: 25720799 DOI: 10.1158/0008-5472.CAN-14-2147]

139 **Meng F**, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology* 2007; **133**: 647-658 [PMID: 17681183 DOI: 10.1053/j.gastro.2007.05.022]

140 **Yin D**, Wang Y, Sai W, Zhang L, Miao Y, Cao L, Zhai X, Feng X, Yang L. HBx-induced miR-21 suppresses cell apoptosis in hepatocellular carcinoma by targeting interleukin-12. *Oncol Rep* 2016; **36**: 2305-2312 [PMID: 27571873 DOI: 10.3892/or.2016.5026]

141 **Wu H**, Ng R, Chen X, Steer CJ, Song G. MicroRNA-21 is a potential link between non-alcoholic fatty liver disease and hepatocellular carcinoma via modulation of the HBP1-p53-Srebp1c pathway. *Gut* 2016; **65**: 1850-1860 [PMID: 26282675 DOI: 10.1136/gutjnl-2014-308430]

142 **Xiong Y**, Fang JH, Yun JP, Yang J, Zhang Y, Jia WH, Zhuang SM. Effects of microRNA-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma. *Hepatology* 2010; **51**: 836-845 [PMID: 20041405 DOI: 10.1002/hep.23380]

143 **Bae HJ**, Noh JH, Kim JK, Eun JW, Jung KH, Kim MG, Chang YG, Shen Q, Kim SJ, Park WS, Lee JY, Nam SW. MicroRNA-29c functions as a tumor suppressor by direct targeting oncogenic SIRT1 in hepatocellular carcinoma. *Oncogene* 2014; **33**: 2557-2567 [PMID: 23728341 DOI: 10.1038/onc.2013.216]

144 **Zhang J**, Jin H, Liu H, Lv S, Wang B, Wang R, Liu H, Ding M, Yang Y, Li L, Zhang J, Fu S, Xie D, Wu M, Zhou W, Qian Q. MiRNA-99a directly regulates AGO2 through translational repression in hepatocellular carcinoma. *Oncogenesis* 2014; **3**: e97 [PMID: 24732044 DOI: 10.1038/oncsis.2014.11]

145 **Liu XS**, Fan BY, Pan WL, Li C, Levin AM, Wang X, Zhang RL, Zervos TM, Hu J, Zhang XM, Chopp M, Zhang ZG. Identification of miRNomes associated with adult neurogenesis after stroke using Argonaute 2-based RNA sequencing. *RNA Biol* 2017; **14**: 488-499 [PMID: 27315491 DOI: 10.1080/15476286.2016.1196320]

146 **Cheng H**, Xue J, Yang S, Chen Y, Wang Y, Zhu Y, Wang X, Kuang D, Ruan Q, Duan Y, Wang G. Co-targeting of IGF1R/mTOR pathway by miR-497 and miR-99a impairs hepatocellular carcinoma development. *Oncotarget* 2017; **8**: 47984-47997 [PMID: 28624790 DOI: 10.18632/oncotarget.18207]

147 **Gramantieri L**, Ferracin M, Fornari F, Veronese A, Sabbioni S, Liu CG, Calin GA, Giovannini C, Ferrazzi E, Grazi GL, Croce CM, Bolondi L, Negrini M. Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. *Cancer Res* 2007; **67**: 6092-6099 [PMID: 17616664 DOI: 10.1158/0008-5472.CAN-06-4607]

148 **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**: 3526-3536 [PMID: 19617899 DOI: 10.1038/onc.2009.211]

149 **Fornari F**, Gramantieri L, Giovannini C, Veronese A, Ferracin M, Sabbioni S, Calin GA, Grazi GL, Croce CM, Tavolari S, Chieco P, Negrini M, Bolondi L. MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res* 2009; **69**: 5761-5767 [PMID: 19584283 DOI: 10.1158/0008-5472.CAN-08-4797]

150 **Tsai WC**, Hsu PW, Lai TC, Chau GY, Lin CW, Chen CM, Lin CD, Liao YL, Wang JL, Chau YP, Hsu MT, Hsiao M, Huang HD, Tsou AP. MicroRNA-122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. *Hepatology* 2009; **49**: 1571-1582 [PMID: 19296470 DOI: 10.1002/hep.22806]

151 **Wang B**, Hsu SH, Wang X, Kutay H, Bid HK, Yu J, Ganju RK, Jacob ST, Yuneva M, Ghoshal K. Reciprocal regulation of microRNA-122 and c-Myc in hepatocellular cancer: role of E2F1 and transcription factor dimerization partner 2. *Hepatology* 2014; **59**: 555-566 [PMID: 24038073 DOI: 10.1002/hep.26712]

152 **Xu H**, He JH, Xiao ZD, Zhang QQ, Chen YQ, Zhou H, Qu LH. Liver-enriched transcription factors regulate microRNA-122 that targets CUTL1 during liver development. *Hepatology* 2010; **52**: 1431-1442 [PMID: 20842632 DOI: 10.1002/hep.23818]

153 **Ahsani Z**, Mohammadi-Yeganeh S, Kia V, Karimkhanloo H, Zarghami N, Paryan M. WNT1 Gene from WNT Signaling Pathway Is a Direct Target of miR-122 in Hepatocellular Carcinoma. *Appl Biochem Biotechnol* 2017; **181**: 884-897 [PMID: 27687586 DOI: 10.1007/s12010-016-2256-8]

154 **Bi Q**, Tang S, Xia L, Du R, Fan R, Gao L, Jin J, Liang S, Chen Z, Xu G, Nie Y, Wu K, Liu J, Shi Y, Ding J, Fan D. Ectopic expression of MiR-125a inhibits the proliferation and metastasis of hepatocellular carcinoma by targeting MMP11 and VEGF. *PLoS One* 2012; **7**: e40169 [PMID: 22768249 DOI: 10.1371/journal.pone.0040169]

155 **Kim JK**, Noh JH, Jung KH, Eun JW, Bae HJ, Kim MG, Chang YG, Shen Q, Park WS, Lee JY, Borlak J, Nam SW. Sirtuin7 oncogenic potential in human hepatocellular carcinoma and its regulation by the tumor suppressors MiR-125a-5p and MiR-125b. *Hepatology* 2013; **57**: 1055-1067 [PMID: 23079745 DOI: 10.1002/hep.26101]

156 **Kong J**, Liu X, Li X, Wu J, Wu N, Chen J, Fang F. miR-125/Pokemon auto-circuit contributes to the progression of hepatocellular carcinoma. *Tumour Biol* 2016; **37**: 511-519 [PMID: 26227218 DOI: 10.1007/s13277-015-3596-7]

157 **Takashima Y**, Terada M, Udono M, Miura S, Yamamoto J, Suzuki A. Suppression of lethal-7b and miR-125a/b Maturation by Lin28b Enables Maintenance of Stem Cell Properties in Hepatoblasts. *Hepatology* 2016; **64**: 245-260 [PMID: 26990797 DOI: 10.1002/hep.28548]

158 **Potenza N**, Mosca N, Zappavigna S, Castiello F, Panella M, Ferri C, Vanacore D, Giordano A, Stiuso P, Caraglia M, Russo A. MicroRNA-125a-5p Is a Downstream Effector of Sorafenib in Its Antiproliferative Activity Toward Human Hepatocellular Carcinoma Cells. *J Cell Physiol* 2017; **232**: 1907-1913 [PMID: 27982429 DOI: 10.1002/jcp.25744]

159 **Potenza N**, Panella M, Castiello F, Mosca N, Amendola E, Russo A. Molecular mechanisms governing microRNA-125a expression in human hepatocellular carcinoma cells. *Sci Rep* 2017; **7**: 10712 [PMID: 28878257 DOI: 10.1038/s41598-017-11418-3]

160 **Liang L**, Wong CM, Ying Q, Fan DN, Huang S, Ding J, Yao J, Yan M, Li J, Yao M, Ng IO, He X. MicroRNA-125b suppressesed human liver cancer cell proliferation and metastasis by directly targeting oncogene LIN28B2. *Hepatology* 2010; **52**: 1731-1740 [PMID: 20827722 DOI: 10.1002/hep.23904]

161 **Zhao A**, Zeng Q, Xie X, Zhou J, Yue W, Li Y, Pei X. MicroRNA-125b induces cancer cell apoptosis through suppression of Bcl-2 expression. *J Genet Genomics* 2012; **39**: 29-35 [PMID: 22293115 DOI: 10.1016/j.jgg.2011.12.003]

162 **Zhao L**, Wang W. miR-125b suppresses the proliferation of hepatocellular carcinoma cells by targeting Sirtuin7. *Int J Clin Exp Med* 2015; **8**: 18469-18475 [PMID: 26770454]

163 **Fu X**, Wen H, Jing L, Yang Y, Wang W, Liang X, Nan K, Yao Y, Tian T. MicroRNA-155-5p promotes hepatocellular carcinoma progression by suppressing PTEN through the PI3K/Akt pathway. *Cancer Sci* 2017; **108**: 620-631 [PMID: 28132399 DOI: 10.1111/cas.13177]

164 **Yan XL**, Jia YL, Chen L, Zeng Q, Zhou JN, Fu CJ, Chen HX, Yuan HF, Li ZW, Shi L, Xu YC, Wang JX, Zhang XM, He LJ, Zhai C, Yue W, Pei XT. Hepatocellular carcinoma-associated mesenchymal stem cells promote hepatocarcinoma progression: role of the S100A4-miR155-SOCS1-MMP9 axis. *Hepatology* 2013; **57**: 2274-2286 [PMID: 23316018 DOI: 10.1002/hep.26257]

165 **Xie Q**, Chen X, Lu F, Zhang T, Hao M, Wang Y, Zhao J, McCrae MA, Zhuang H. Aberrant expression of microRNA 155 may accelerate cell proliferation by targeting sex-determining region Y box 6 in hepatocellular carcinoma. *Cancer* 2012; **118**: 2431-2442 [PMID: 21989846 DOI: 10.1002/cncr.26566]

166 **Gramantieri L**, Fornari F, Ferracin M, Veronese A, Sabbioni S, Calin GA, Grazi GL, Croce CM, Bolondi L, Negrini M. MicroRNA-221 targets Bmf in hepatocellular carcinoma and correlates with tumor multifocality. *Clin Cancer Res* 2009; **15**: 5073-5081 [PMID: 19671867 DOI: 10.1158/1078-0432.CCR-09-0092]

167 **Pineau P**, Volinia S, McJunkin K, Marchio A, Battiston C, Terris B, Mazzaferro V, Lowe SW, Croce CM, Dejean A. miR-221 overexpression contributes to liver tumorigenesis. *Proc Natl Acad Sci USA* 2010; **107**: 264-269 [PMID: 20018759 DOI: 10.1073/pnas.0907904107]

168 **Callegari E**, Elamin BK, Giannone F, Milazzo M, Altavilla G, Fornari F, Giacomelli L, D'Abundo L, Ferracin M, Bassi C, Zagatti B, Corrà F, Miotto E, Lupini L, Bolondi L, Gramantieri L, Croce CM, Sabbioni S, Negrini M. Liver tumorigenicity promoted by microRNA-221 in a mouse transgenic model. *Hepatology* 2012; **56**: 1025-1033 [PMID: 22473819 DOI: 10.1002/hep.25747]

169 **Fornari F**, Pollutri D, Patrizi C, La Bella T, Marinelli S, Casadei Gardini A, Marisi G, Baron Toaldo M, Baglioni M, Salvatore V, Callegari E, Baldassarre M, Galassi M, Giovannini C, Cescon M, Ravaioli M, Negrini M, Bolondi L, Gramantieri L. In Hepatocellular Carcinoma miR-221 Modulates Sorafenib Resistance through Inhibition of Caspase-3-Mediated Apoptosis. *Clin Cancer Res* 2017; **23**: 3953-3965 [PMID: 28096271 DOI: 10.1158/1078-0432.CCR-16-1464]

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**Table 1 miRNAs involved in hepatitis B virus infection**

|  |  |  |  |
| --- | --- | --- | --- |
| **miRNA** | **Validated target** | **Effect on HBV**  | **Ref.** |
| **miRNA targeting HBV transcripts** |  |  |  |
| **miR-15a/miR-16-1** | HBx | ↓ | [28,33] |
| **miR-20a/miR-92a-1** | Polymerase/HBx | ↓ | [29] |
| **miR-122** | Polymerase/HBc | ↓ | [27] |
| **miR-125a** | HBsAg | ↓ | [23-26,32] |
| **miR-199a-3p** | HBsAg | ↓ | [22] |
| **miR-205** | HBx | ↓ | [31] |
| **miR-210** | HBsAg pre-S1 region | ↓ | [22] |
| **miR-1231** | HBc | ↓ | [30] |
|  |  |  |  |
| **miRNAs targeting HBV regulators**  |  |  |  |
| **miR-1** | HDAC4 | ↑ | [51-53] |
| **miR-15b** | HNF1 | ↑ | [50] |
| **miR-34a** | CCL22 | ↓ | [60] |
| **miR-122** | Cyclin G1HO-1 | ↑ | [43-45][45,46] |
| **miR-130a** | PGC1PPAR | ↓ | [41] |
| **miR-141** | PPAR | ↓ | [24,40] |
| **miR-146a** | STAT1 | ↑ | [58] |
| **miR-152** | DNMT-1 | ↑ | [54-57] |
| **miR-155** | C/EBPSOCS1 | ↓↓ | [35-38][39] |
| **miR-370** | NFIA | ↑ | [49] |
| **miR-372/373** | NFIB | ↑ | [48] |
| **miR-501** | HBXIP | ↑ | [42] |
| **miR-548a** | IFNλ-1 | ↑ | [59] |

MiRNAs are listed according to their increasing number name. ↓: Suppress HBV infection; ↑: Promote HBV infection; HBV: Hepatitis B virus.

**Table 2 MicroRNAs playing oncogenic or oncosuppressive roles in hepatocellular carcinoma**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **MicroRNA** | **Expression in HCC** | **Cellular effects** | **Direct targets in HCC** | **Ref.** |
| **let-7 family** | Downregulated | Proliferation (-) migration (-)apoptosis (+) | Bcl-xL, c-Myc, collagen type 1α2, RAS, STAT3 | [89,132-136] |
| **miR-21** | Upregulated | Proliferation (+) migration (+) apoptosis (-) | IL-12, HBP1, PDCD4, PTEN, RECK, TIMP-3 | [72,137-141] |
| **miR-29** | Downregulated | Proliferation (-) apoptosis (+) | Bcl-2, lncRNA MEG3, Mcl-1, SIRT1 | [80,142,143] |
| **miR-99a** | Downregulated | Proliferation (-) | AGO2, IGF1R, mTOR | [144-147] |
| **miR-122** | Downregulated | Proliferation (-)migration (-) | ADAM17, c-Myc, CUTL1, CCNG1, WNT1  | [145,148-153] |
| **miR-125a** | Downregulated | Proliferation (-) migration (-) angiogenesis (-) | c-RAF, LIN28B, MMP11, SIRT7, VEGFA, Zbtb7a | [72,122,145,155-158] |
| **miR-125b** | Downregulated | Proliferation (-)apoptosis (+) | Bcl-2, LIN28B, SIRT7 | [72,145,157,160-162] |
| **miR-155** | Upregulated | Proliferation (+) migration (+) | ARID2, C/EBPbeta, PTEN, SOCS1, SOX6 | [38,162-165] |
| **miR-199a-3p** | Downregulated | Proliferation (-) apoptosis (+)  | CD44, CD51, c-MET, mTOR, PAK4, YAP1, ZHX1  | [71-78,154] |
| **miR-221** | Upregulated | Proliferation (+) apoptosis (-) | BMF, Caspase-3, CDKN1B, CDKN1C, DDIT4 | [166-169] |

ADAM17: Disintegrin and metalloprotease 17; AGO2: Argonaute-2 protein; Bcl-2: B-cell lymphoma 2 protein; ARID2: AT-rich interactive domain 2; BMF: Bcl-2-modifying factor; C/EBPbeta: CCAT/enhancer binding protein beta; CCNG1: Cyclin-G1; CDKN: Cyclin-dependent kinase inhibitor; DDIT4: DNA-damage inducible transcript 4; HBP1: HMG-box transcription factor 1; IGF1R: Insulin-like growth factor 1 receptor; IL-12: Interleukin-12; Mcl-1: Induced myeloid leukemia cell differentiation protein 1; PAK4: Serine/threonine-protein kinase 4; PDCD4: Programmed cell death protein 4; PTEN: Phosphatase and tensin homolog; RECK: Reversion-inducing-cysteine-rich protein with kazal motifs; SIRT: Sirtuin; SOCS1: Suppressor of cytokine signaling 1; STAT3: Signal transducer and activator of transcription 3; TIMP3: Metalloproteinase inhibitor 3; VEGFA: Vascular endothelial growth factor A; YAP1: Yes-associated protein 1; Zbtb7a: Zinc finger and BTB domain-containing protein 7A; ZHX1: Zinc-fingers and homeoboxes-1.