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Importance of MicroRNAs in Hepatitis B and C Diagnostics and Treatment

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Abstract

MicroRNAs (miRNAs) are small-sized RNAs with ability to regulate gene expression and have been recently discovered as promising diagnostic and therapeutic biomarkers in the field of clinical medicine and microbiology, specifically in viral diseases. Infections with hepatitis B virus (HBV) or hepatitis C virus (HCV) often lead to chronic infections and development of liver hepatocellular carcinoma (HCC). Challenges in early diagnosis of HCC and rapid development of novel HCV antivirals call for identification of novel miRNA biomarkers. An extensive selection of single miRNAs and miRNA panels has been provided by accumulating studies, discovering miRNA potentials in HBV and HCV diagnostics and treatment. Currently, the diagnostic potential of miRNAs in HBV and HCV has not been established yet. However, a promising HCV treatment drug Miravirsen, a locked nucleic acid, complementary to miRNA-122, has entered a human clinical trial recently. In this review, we outline the role of miRNAs in HBV and HCV pathogenesis and differences in up- and downregulation of miRNAs upon HBV and HCV infection and HCC development.

Keywords: microRNA, hepatitis B virus, hepatitis C virus, diagnosis, treatment

1. Introduction

Hepatitis B and C viruses (HBV and HCV) are globally spread hepatotropic pathogens and major etiological factors of liver cirrhosis and hepatocellular carcinoma (HCC), infecting millions of people worldwide. HBV and HCV significantly differ in structure, genomic characteristics, and pathogenesis [1, 2].

MicroRNAs (miRNAs) are small noncoding RNAs that control gene expression and participate in complex cellular pathways and pathogenesis of various viral infections and cancers [3–5].

Despite the availability of prophylactic HBV vaccine and recently improved HCV therapy strategies, early diagnosis of HBV/HCV-related HCC, viral reactivation, resistance, drug interactions, and viral interferences in HBV/HCV co-infected patients remain major obstacles in currently available HBV and HCV diagnostics and treatments [6, 7].

Multiple studies have proposed specific miRNA or miRNA panels to be used as biomarkers for HBV/HCV-related liver disease, staging of liver disease progression, and anti-HBV/HCV therapeutic options.

In this chapter, we briefly outline the HBV and HCV biology and basis of miRNA expression in liver and HCC. Subsequently, we summarize recently described and proposed miRNAs for HBV- and HCV-associated diagnostics, particularly HBV/HCV-related HCC.

2. Hepatitis B virus

Hepatitis B virus is an enveloped, partially double-stranded DNA virus, classified in the *Hepadnaviridae* family. HBV genome is approximately 3.2 kb long and contains four RNA transcripts (P, S, C, and X), of which the S transcript encodes the surface antigen HBsAg, the main indicator of HBV infection [1]. Upon infection, cellular polymerase converts HBV DNA into covalently closed circular DNA (cccDNA), which represents a constant template for pregenomic RNA and mRNA transcription. Due to the stable state of HBV cccDNA in hepatocytes, HBV can reactivate after immunosuppression [1, 8].

HBV is classified into eight genotypes (designated A through H). Most chronic infections are related to infection with HBV genotypes B and C; however, in Europe, genotype D has been shown to be more often associated with active liver disease [1].

Globally, two billion people are infected with HBV, with majority of HBV infections occurring in South-Eastern Asia and sub-Saharan Africa [9, 10]. Approximately, one-fourth of HBV-infected individuals suffer from liver cirrhosis and 70–90% of HCC develop from cirrhotic liver [1, 10]. In developing countries, HBV infection accounts for about 60% of the total liver cancer and in developed countries for about 23% [11]. In endemic areas, HBV infections occur mostly by vertical and perinatal transmission and in more than 90% lead to chronic HBV infections. In low prevalence countries, HBV infections occur mostly through a horizontal transfer (sexual transmission), which in more than 90% lead to acute HBV infections with spontaneous viral clearance [10]. Despite improved HBV antiviral therapy over the past two decades, and the prophylactic anti-HBV vaccine, available since 1981, prenatal maternal HBeAg seropositivity in endemic countries remains significantly connected to HCC and elimination of cccDNA remains the major challenge in HBV-related treatment strategies [1, 12, 13].

3. Hepatitis C virus

Hepatitis C virus has a positive-sense single-stranded RNA genome of approximate length of 9.6 kb and is classified in the family *Flaviviridae*. The HCV genome encodes a polyprotein that is cleaved into three structural and seven nonstructural proteins. Based on HCV genomic sequence diversity, HCV is classified into seven genotypes and more than 60 subtypes are identified [2, 14]. Genotypes 1, 2, and 3 are globally distributed and cause most of HCV infections in North America and Europe. In Middle East and North and Central Africa, genotype 4 prevails generally, in South Africa genotype 5, in Asia genotype 6, while a recently discovered genotype 7 originates from Central Africa [2, 15].

Currently, no vaccine is available for the prevention of HCV infection. Most HCV transmissions occur by intravenous drug abuse, sexual transmission, and occupational exposure to HCV-infected blood [2]. Potential long-term outcomes in chronically HCV-infected people are liver cirrhosis and HCC, which remain the leading cause for liver transplantation. HCV is globally infecting over 150 million individuals [16–18]. Recent estimate on global anti-HCV prevalence is 115 million-infected individuals, of whom 80 million are actively viremic [19].

A recent study by Sibley et al. [20] forecasted changes in HCV-related disease up to 2030 and concluded that HCV-related morbidity and mortality are estimated to increase due to an aging of the HCV-infected population and currently available treatment will be inadequate if reductions in HCV-related disease of this magnitude are to be achieved [20].

4. MicroRNAs

Micro-ribonucleic acids (microRNAs/miRNAs) are noncoding RNAs of 18–25 nucleotides in length that complementarily target the 3'-untranslated regions (3' UTRs), or less commonly 5'-untranslated regions (5' UTRs) of messenger RNAs (mRNAs) [3]. Genes encoding miRNAs are located in intragenic regions or introns of mRNAs or noncoding RNAs [21]. MiRNAs are transcribed from the genome by the RNA polymerase II into primary-miRNA (pri-miRNA) hairpins, which are processed by Drosha (class III RNase) into pre-miRNAs. Pre-miRNAs are exported from the nucleus to the cytoplasm, where they are processed by a second RNaseIII Dicer into short double-stranded mature miRNAs, consisting of 5' and 3' arms. Finally, single-stranded miRNAs are assembled with specific proteins and form a RNA-induced-silencing complex (RISC). At least two to seven nucleotide complementarities with the target sequence are required for RISC-mediated target silencing [22–24].

The binding of miRNAs in posttranscriptional or translational level provides a rapid and sensitive mechanism of gene expression regulation, either by suppressing the translation of mRNA or by promoting mRNA degradation [25]. Gene silencing by a full complementary miRNA sequence directs cleavage of the target mRNA, while partial complementary miRNA sequence suppresses mRNA translation [26, 27]. Currently, more than 2,588 mature miRNAs are reported in a human genome [28, 29] and due to sufficient partial complementarity to

the target sequence it has been shown that one type of miRNA could affect up to 200 genes, and over a 100 different targets can be involved in approximately 100 different biochemical pathways [30, 31].

4.1. MiRNAs mechanism

MiRNAs are participating in various cellular processes such as cell development, differentiation, proliferation, metabolism, immune responses, apoptosis, and oncogenesis [32, 33]. Estimates in humans suggest that 60–70% of all genes are regulated by miRNAs [4]. Being involved in numerous biological pathways, their expression and regulation reflect in various diseases, stages of the particular disease, especially in cancer development [34, 35].

MiRNAs are cell-free-circulating molecules that can be detected in almost every body fluid. Their high stability and accessibility make them ideal noninvasive markers for the early diagnosis of different pathophysiological processes. Indeed, a large amount of evidence suggests that miRNA profiles could provide a classification system for various tumors, as well as an important tool for the diagnosis and treatment of cancer and viral diseases [36–41]. Accordingly, cellular miRNAs have an ability to regulate pathogenesis of viral infections, and at the same time viruses manipulate with host cellular machinery, including miRNAs [42, 43].

Increased interest in hepatitis B and hepatitis C disease pathogenesis and diagnostics has lead to the emergence of various studies over the last 15 years that have tried to evaluate plasma and tissue levels of miRNAs in order to provide or improve the diagnosis of HBV and HCV infections as well as HBV- and HCV-related HCC [5].

5. MiRNAs in normal liver

Liver consists of various cell types, mainly divided in the parenchymal cells (hepatocytes) and non-parenchymal cells (biliary epithelial cells and lymphoid cells, etc.). Each cell type expresses its unique miRNA profile. While miRNAs are up- or downregulated in almost every stage of hepatic development, they accelerate or inhibit liver proliferation and play the major role in regulation of diverse liver functions [44]. It has been shown that a total of 277 miRNAs are expressed in the liver, with miR-122 being one of the most abundant and liver-specific miRNAs [45, 46]. Besides miR-192, miR-199a/b-3p, miR-101, miR-99a, and let-7a/b/c/f (let-7 family), which account for 80% of the total miRNA in liver, miR-122 accounts for 70% of the total liver miRNAs [45, 47]. Expression of miRNAs in the normal liver has been established by microarray systems and library sequencing [47–49].

5.1. Function of miRNAs in normal liver

The function of miR-122 has been explored in a variety of *in vivo* studies, including the miR-122 gene knockdown or silencing of miR-122 with antagomirs [50–52]. In the miR-122 gene knockdown mice, it has been shown that the deletion of the miR-122 gene resulted in hepatosteatosis, hepatitis, and the development of liver tumors [52]. Beyond that, studies implicate miR-122

as a key regulator of cholesterol and fatty-acid metabolism [50]. However, results of studies evaluating up- and downregulated miRNA profiles in differentiating liver cells are not consistent. Besides various technical issues, including differences in clinical samples and different miRNA matrices in miRNA assays, different degrees of miRNA expression among studies suggest that the miRNA profile is also influenced by the origin of the progenitor cell and that it is difficult to compare miRNA profiles in different cellular developmental stages [53].

However, it has been shown in two studies by Liu et al. [54] and Tzur et al. [55] that embryonic liver mainly contains miRNAs-122, -192, -194, -451, and -483-3p, whereas miR-122 can be detected in embryonic stem cells as well as in hepatocytes and continues to be expressed in the adulthood. Studies of hepatic malignancy pathologies have shown that miRNAs have specific targets in specific disease states [44, 53, 56]. Interestingly, a biphasic pattern of miRNA expression was observed in rats after liver surgical resection [57]. In the first 18 h after hepatectomy, about 40% of miRNAs were upregulated, whereas by 24 h there was a negative feedback mechanism which downregulated about 70% of all miRNAs [57]. These negative feedback loops are postulated to play an important role in liver regeneration processes, required for recovery of liver cells after injury; however, the abundance of miRNAs does not directly correlate with the predictable role of specific cells.

Since essential knowledge on liver regeneration processes has been delivered from rodent model studies, further studies are warranted to confirm post-hepatectomy miRNA level changes in humans [4, 57]. Investigation of miRNA levels in specific stages of liver organogenesis may reveal potential biomarkers for liver disease states.

6. miRNA and HBV- and HCV-related liver disease

HCC is the fifth most common cancer worldwide. Deregulation of miRNA expression could be one of the key factors in the development of liver pathology, including viral hepatitis and HCC [11]. Evidence is rapidly growing that specific miRNAs could be used as potential biomarkers for HCC, tumor progression, and response to therapeutic targets [4].

Infection with HBV and HCV can lead to chronic hepatitis, liver cirrhosis, or even HCC. Approximately 50–80% of HCC cases are associated with chronic HBV or HCV infection [10]. In the past several years, the involvement of miRNA in the pathogenesis of HBV-/HCV-related liver diseases has been well documented [53]. Since miRNAs can be directly involved in antiviral immune-pathological events, it is inevitable that miRNA target sequences in viral populations remained conserved, providing relevant evidence of the biological significance of potential miRNA-based antiviral interventions [58]. For the time being, no HBV- or HCV-encoded miRNAs have been reported. Using computational approaches, one candidate HBV miRNA has been found but its function remains undetermined [59].

It should be noted that miRNA dysregulation has been studied in various experimental settings, mainly involving *in vitro* systems, HBV-/HCV-replication-supporting cell lines, transgenic mice, cultured hepatocytes (mouse/rat/human), circulating blood cells or serum

of HBV-/HCV-infected individuals, and liver tissue samples. MiRNA expression in model systems has been measured mainly with qualitative real time-polymerase chain reactions (RT-PCRs) and/or miRNA microarrays and less frequently with next-generation sequencing (NGS) (for review, see [23, 60]).

6.1. MiRNAs and HBV infection

It is well known that numerous cellular miRNAs are able to promote or repress the HBV lifecycle, either by directly targeting HBV transcripts or by indirectly targeting cellular mediators, involved in the HBV pathogenesis. Alternatively, HBV infection dysregulates cellular miRNAs and in this manner controls the host gene expression to promote its replication [60].

A variety of miRNAs have been reported in regulation of HBV replication namely miR-122, let-7 family, miR-199 family, miR-15 family, miR-125 family, and miR-17-92 cluster (extensively reviewed in [60]). Reported effects of the most abundant liver miRNA, miR-122, on HBV lifecycle remain mixed. Whereas some studies reported miRNAs as inhibitors of HBV replication [61–64], others have failed to even identify miR-122 as a regulator of the HBV lifecycle [65]. For example, in a study by Qiu et al. [63], in comparison to the control system, co-transfection of Huh7 cells with miR-122 inhibitor, and a plasmid encoding the HBV genome, the production of HBsAg and HBeAg increased, suggesting a negative regulatory effect of miR-122 on HBV lifecycle.

The study of Wang et al. [64] reported downregulation of miR-122 expression in liver of patients with HBV infection, in comparison to healthy controls, and showed that the miR-122 levels were negatively correlated with intrahepatic viral load and hepatic inflammation. Researchers concluded that HBV-induced miR-122 downregulation enhances HBV replication through cyclin G1-modulated P53 activity and that HBV mRNAs harboring complementary sites to miR-122 sequester miR-122 and contribute to viral persistence and carcinogenesis [61, 64].

Guo et al. [66] showed that members of the miRs-371-372-373 (miRs-371-3) gene cluster were co-upregulated in HBV-producing HepG2.2.15 cells and revealed that miRs-372/373 promotes HBV expression by targeting the transcription factor NFIB. Furthermore, Zhang et al. [65] reported that miR-1 promotes HBV replication, transcription, and antigen expression by indirect modulation of host genes expression in HCC cell line. In addition, they have shown that miR-1 arrested cell cycle, inhibited proliferation, and therefore reversed the cancer cell phenotype, which is in contradiction with HBV-induced carcinogenesis, since HBV infection promotes hepatocellular proliferation [65].

Jin et al. [67] have shown that downregulation of miR-501 in HepG2.2.15 cells could significantly inhibit HBV replication, thus representing a potential therapeutic target. They suggested that miR-501 promotes HBV replication through inhibition of the HBXIP, which interacts with HBx protein and normally represses HBV replication [67]. In a mouse model, Dai et al. [68] reported that miR-15b promoted HBV replication by direct inhibition of hepatocyte nuclear factor HNF1α.

On the other hand, miR-125 family members, miR-125a and miR-125b, have been reported to suppress the HBV lifecycle. In the PLC/PRF/5 cell line, miR-125a-5p was identified as a downregulator of HBsAg expression by directly targeting HBV RNAs [69], while miR-125b inhibited HBV in HepG2.2.15 cells [70]. The miR-22 has been reported as a regulatory molecule which inhibits HBV infection [71]. Furthermore, miR-199a-3p and miR-210 were shown to repress the HBV replication in HepG2.2.15 cells by directly targeting the HBV S protein-coding region [72], while inhibition of miR-20 and miR-92a-1 increased levels of HBV RNAs in HepAD38 hepatoma cells [73]. Hu et al. [74] demonstrated that miR-141 suppresses HBV replication by reducing HBV promoter activities and two separate studies suggested upregulation of miR-181a in HBV-infected hepatoma cells, implying an important role in the development of HCC [75, 76].

6.2. MiRNAs and HCV infection

HCV lifecycle is influenced by host miRNAs in all stages: entry, translation, replication, and assembly [43]. As the HCV genome is single-stranded RNA, it serves as a template for its replication and direct binding site for host miRNAs. Among high number of miRNAs reported to be involved in the regulation of HCV infection and replication, most miRNAs have been documented to directly target the HCV genome: miR-1, miR-30, miR-122, miR-128, miR-196, miR-296, miR-351, miR-431, and miR-448 [77, 78].

Microarray analysis on human hepatoma cells has revealed changed expression profiles of 108 human miRNAs after HCV infection [79]. Furthermore, Liu et al. [79] showed that after acute HCV infection, miR-122 was downregulated, whereas miR-296 and miR-351 were significantly upregulated. In addition, it has been shown that HCV infection upregulated the expression of miR-192, miR-194, and miR-215, whereas the expression of miR-320 and miR-491 was downregulated [80]. It was reported that miR-192/miR-215 and miR-491 could enhance HCV replication [80].

For the most abundant miRNA in the liver, miR-122, it has been demonstrated that it promotes HCV replication by direct binding to the less commonly used UTR-binding site, the 5′ UTR site of the HCV RNA, which leads to Argonaute (Ago) protein complex recruitment, stabilization of the viral RNA, and activation of the RNA translation [77, 81, 82]. *In vitro* studies have shown that miR-122 is essential for HCV replication [81].

On the other hand, it has been shown that miR-122 exhibits anti-inflammatory and antitumorigenic properties in mice knockdown studies [52]. Mixed results exist on expression levels of miR-122 and development of HCC- or HCV-induced HCC. Coulouarn et al. [83] have shown that the loss of miR-122 expression in liver cancer correlated with HCC progression, whereas in another study, the upregulation of miR-122 promoted the HCV-related HCC [84].

The increased expression of miR-155 in HCV-infected patients promotes hepatocarcinogenesis and inhibits apoptosis of hepatocytes [85]. Furthermore, the direct effect on HCV replication cycle has been determined in the cell culture system for the miR-196b, which is complementary to the NS5A region of the HCV genome and is downregulated in HCV-infected patients. MiR-196b inhibits HCV replication directly by targeting HCV RNA or indirectly by increasing the expression of HMOX1. It has anti-inflammatory, antioxygenic, and hepatoprotective properties [86].

Some miRNAs can facilitate HCV lifecycle by targeting host proteins involved in innate immunity-signaling pathways. For example, HCV induced upregulation of miR-130 blocks expression of interferon stimulatory gene IFITM1, which promotes HCV entry into host cells [87]. Furthermore, miR-491 promotes HCV replication through inhibition of the PI3 kinase/Akt pathway, one of the pathways leading to cancerous properties [80]. Studies analyzing circulating miRNA profiles in serum provide novel insights on miRNA expression in HCV pathogenesis [88, 89]. In a study by Shwetha et al. [89], it has been shown that the expression of miR-134, miR-198, miR-320c, and miR-483-5p was upregulated in patients infected with HCV 1 and HCV 3 genotypes.

Complex correlation between hepatic expression of abundant liver miRNAs, miR-122, miR-126, miR-136, and miR-181a, and histopathological and clinical characteristic of HCV-infected patients has been reported by Boštjančič et al. [31]. The study included liver biopsies of patients infected with different genotypes (1, 1a, 1b, 3, and 4) and provided an important insight into miRNA expression patterns in different stages and grades of liver disease and revealed association among specific miRNA deregulation and patient gender, serum HCV viral load, presence of steatosis, and mode of HCV transmission [31].

By contrast, another study by Elhelw et al. [90] has demonstrated upregulation of miR-181a in serum samples of HCV genotype 4-infected individuals and downregulation in HCV-infected Huh7 cells. In addition, the inverse correlation between miR-181a serum levels, viral load, and liver enzymes was observed. Due to complexity of viral and host factors involved in HCV infection and progression to HCV-induced HCC, multiple clinical parameters should be considered and controlled for in the future studies. A systematic approach was recently reported by Oliveira et al. [91]. The study evaluated liver and serum expression of miR-122 in patients infected with HCV genotypes 1 and 3 to identify possible associations between miR-122 expression and lipid profiles, HCV viral load, apolipoproteins, and liver enzymes [91].

7. MiRNAs as biomarkers in diagnostics and treatment of HBV and HCV

Early diagnosis and treatment of HCC remain challenging due to the lack of early detection methods, limited access to diagnosis, expensive medications and the presence of comorbidities, coinfections, and contraindications due to different host and viral factors. Most of HBV-/HCV-infected individuals remain undiagnosed before they seek medical help due to advanced HCC [10, 92].

The gold standard for the etiology of liver diseases is liver biopsy, an invasive method being replaced recently by serological methods and imaging technologies. Current diagnostic techniques for HCC, which are generally divided into radiological (first-line diagnostic method is ultrasound) and serological methods (serological marker alpha-fetoprotein, AFP, and des-gamma-carboxy prothrombin), provide limited reliability [93, 94].

7.1. MiRNAs as biomarkers in body fluids

MiRNAs can be detected in various body fluids, such as plasma, serum, urine, and infected or diseased tissue and may exhibit host responses to the pathogen or other inflammatory

processes; however, miRNA levels in body fluids may not necessarily reflect the miRNA level in the infected/diseased tissue, and second, the same miRNA may be upregulated in one state of disease and downregulated in another. To provide optimal management of HBV- and HCV-related diseases, novel surrogate miRNA biomarkers should be considered [41]. A great amount of studies provide promising information on miRNAs as potential diagnostic biomarkers of HBV or HCV infection as well as potential diagnostic and treatment tool in HBV-/HCV-related HCC. According to specific miRNA targets and up- or downregulation of miRNA expression, potential imitating or antagonistic characteristics of miRNAs could be used in HBV-/HCV-related therapy.

7.2. HBV miRNA biomarkers

Currently used markers in the diagnostics of HBV can serve as indicators of specific HBV infection phases; however, none of them can be used to predict the HBV infection outcome [41].

Several studies suggest that the use of miRNA panels in serum or liver tissue could improve the specificity of HBV diagnostics. For example, Li et al. [41] have reported that their 13-miRNA-based biomarker panel could accurately discriminate between HBV cases from healthy controls and HCV cases, as well as HBV-positive HCC cases from healthy controls and HBV-infected patients. The panel of 13 miRNA consisted of the following miRNAs: miR-375, miR-92a, miR-10a, miR-223, miR-423, miR-23b/a, miR-342-3p, miR-99a, miR-122a, miR-125b, miR-150, and let-7c [41] (**Table 1**).

In order to reliably differentiate HCC from chronic HBV infection, cirrhosis, and healthy subjects, plasma panel of seven miRNAs (miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a, and miR-801) has been investigated by Zhou et al. [104]. Mizuguchi et al. [107] employed sequencing-based miRNA clustering and showed that the panel of miRNAs was more effective for the detection of high-risk patients for HBV-related HCC recurrence after liver surgery, in comparison to investigation of a single miRNA. MiR-122, miR-21, and miR-34a were identified as potential biomarkers for the prediction of HBV-related HCC.

The miR-34a is a direct target of the P53 and it has been shown that the expression of miR-34a is downregulated in several human cancers, and when overexpressed, miR-34a can repress several oncogenes and induces apoptosis and arrest of the cell cycle. Deletion of gene region encoding miR-34a has been well detected in breast, lung, cervical, and prostate cancers (reviewed by Agostini and Knight [111]). A mimic miR-34a (MRX34) became a promising therapeutic tool for HCC and has reached a clinical trial, phase 1 in 2013 [111].

Individual miRNAs or combination of miRNA and serological markers for HCC have been examined and proposed. While an upregulated oncogenic miRNA-27a has been suggested as a therapeutic target in HBV-related HCC patients [108] and miR-101 as a potential noninvasive biomarker to differentiate HBV-related HCC from HBV liver cirrhosis [97, 112], a combination of circulating miR-126 and AFP has been proposed as a promising noninvasive-specific diagnostic biomarker for HBV-related HCC. Furthermore, combinations of miR-126/AFP AFP and miR-142-3p/AFP showed higher efficiency rather than AFP alone in discriminating HCC from non-HCC patients [99].

miRNA	Deregulation	Type of sample	Type of method	Clinical relevance	Reference
miR-101	Up/down	HepG2 and HepG2.2.15, HBV-HCC tissue, Hep3B, and L02, HBVHCC-related serum	Microarray, qRT-PCR	Biomarker for monitoring the progression of tumor development in HBV-related HCC	Zhang et al. [95], Wei et al. [96], Fu et al. [97]
miR-106b-25 cluster (miR-106b, miR-93, miR-25)	Up	HBV-infected HCC patients	Microarray, qRT-PCR,	Decrease in survival time, increase in the recurrence rate and HCC differentiation in HBV-related HCC	Yen et al. [98]
miR-122a	Up	HBV-infected serum, liver tissue of HBV-related HCC	qRT-PCR, NGS	HCC differentiation from healthy, chronic hepatitis and cirrhosis	Li et al. [41]
miR-126	Up	Plasma of HBV HCC, liver tissue of HCC	Microarray	Potential biomarker for HBV-related HCC	Ghosh et al. [99]
miR-132-3p	Up	Plasma of HBV-related HCC patients	qRT-PCR	Potential biomarker for HBV-related HCC	Wen et al. [100]
miR-141-3p	Up	Serum of HBV-related HCC patients	NGS, qRT-PCR	Clinical value in HBV-related HCC diagnostics	Tan et al. [101]
miR-142-3p	Up	Plasma and liver tissue of HBV-related HCC	Microarray	Potential biomarker for HBV-related HCC	Ghosh et al. [99]
miR-146a	Up/down	HepG2 and HepG2.2.15, HBV-related HCC and serum	Microarray and Northern blotting	Involved in chronicity of HBV infection	Liu et al. [75], Gui et al. [102], Zhang et al. [95]
miR-146b-5p	Up/down	HepG2 and HepG2.2.15, HBV-related HCC, HBV-infected serum	Microarray, qRT-PCR, NGS	Potential research interest in chronic HBV infections and HBV-induced HCC	Hou et al. [47], Zhang et al. [95]
miR-155	Up	HepG2, H7402	qRT-PCR	Inhibits HBV infection in human hepatoma cells	Su et al. [103]
miR-181a/b	Up	HepG2 and HepG2.2.15	Microarray, Northern blotting, qRT-PCR	Important role in HBV-induced HCC development	Liu et al. [75], Zou et al. [76]
miR-185-5p	Up	Plasma of HBV-related HCC patients	qRT-PCR	Potential biomarker for HBV-related HCC	Wen et al. [100]
miR-192	Up	HBV-related HCC plasma	qRT-PCR, microarray	HCC differentiation from healthy, chronic hepatitis and cirrhosis	Zhou et al. [104]

miRNA	Deregulation	Type of sample	Type of method	Clinical relevance	Reference
miR-1228-5p	Up	Serum of HBV-related HCC patients	NGS, qRT-PCR	Clinical value in HBV-related HCC diagnostics	Tan et al. [101]
miR-20a/20a-5p	Up/down	HepG2 and HepG2.2.15, plasma of HBV-related HCC	Microarray, qRT-PCR	Potential research interest in chronic HBV infections and HBV-induced HCC	Wen et al. [100], Zhang et al. [95]
miR-21	Up/down	HepG2, HepG2.2.15, Hep3B, HBV-related HCC, plasma of HBV HCC, liver tissue of HCC	qRT-PCR, stem-loop RT-qPCR, microarray, NGS, clone count	HCC differentiation from healthy, chronic hepatitis and cirrhosis	Hou et al. [47], Zhou et al. [104], Gao et al. [105], Bandopadhyay et al. [106], Ghosh et al. [99], Mizuguchi et al. [107]
miR-23a/b	Up	HBV-infected serum	qRT-PCR, NGS	Biomarker to differentiate HBV-related HCC from controls	Li et al. [41]
miR-25/25-3p	Up	HBV-infected serum, HepG2 and HepG2.2.15, plasma of HBV-related HCC	qRT-PCR, microarray, NGS	Biomarker to differentiate HBV-related HCC from controls	Li et al. [41], Zhang et al. [95], Wen et al. [100]
miR-27a	Up/down	HBV-related HCC, HepG2 and Huh7, HBV-related HCC plasma	qRT-PCR, microarray	HCC differentiation from healthy, chronic hepatitis and cirrhosis	Zhou et al. [104], Wu et al. [108]
miR-200b	Up	HepG2, HepG2.2.15	microarray, Northern blotting	involved in chronicity of HBV infection	Liu et al. [75]
miR-206	Up	Serum of HBV-related HCC patients	NGS, qRT-PCR	Clinical value in HBV-related HCC diagnostics	Tan et al. [101]
miR-221	Up/down	HepG2, HepG2.2.15, Hep3B, HBV-related HCC	Microarray, Stem-loop RT-qPCR	Potential research interest in chronic HBV infections and HBV-induced HCC	Gao et al. [105], Zhang et al. [95]
miR-222	Up/down	HepG2 and HepG2.2.15	Microarray, qRT-PCR	Promotes cell growth and migration	Bandopadhyay et al. [106], Zhang et al., 2011 [95]
miR-223	Up/down	HBV-infected serum, plasma	qRT-PCR, microarray, NGS	HCC differentiation from healthy, chronic hepatitis and cirrhosis	Zhou et al. [104], Li et al. [41]
miR-224	Up/down	Hep3B and HepG2	Stem-loop RT-qPCR	Significantly upregulated in HCC	Gao et al. [105], Zhang 2011 [95]

miRNA	Deregulation	Type of sample	Type of method	Clinical relevance	Reference
miR-30a-5p	Up	Plasma of HBV-related HCC	qRT-PCR	Potential biomarker for HBV-related HCC	Wen et al. [100]
miR-34a	Up	HBV-related liver samples	NGS, clone count	Expressed aberrantly in liver cancer	Mizuguchi et al. [107]
miR-320a	Up	Plasma of HBV HCC	qRT-PCR	Potential biomarker for HBV-related HCC	Wen et al. [100]
miR-324-3p	Up	Plasma of HBV HCC	qRT-PCR	Potential biomarker for HBV-related HCC	Wen et al. [100]
miR-342-3p	Up	HBV-infected serum	qRT-PCR, NGS	Biomarker for differentiation of HBV-positive HCC from controls	Li et al. [41]
miR-375	Up	HBV-infected serum	qRT-PCR, NGS	Biomarker for differentiation of HBV-positive HCC from controls	Li et al. [41]
miR-423	Up	HBV-infected serum	qRT-PCR, NGS	Biomarker for differentiation of HBV-positive HCC from controls	Li et al. [41]
miR-433-3p	Up	Serum of HBV-related HCC patients	NGS, qRT-PCR	Clinical value in HBV-related HCC diagnostics	Tan et al. [101]
miR-801	Up	HBV-related HCC plasma	qRT-PCR, microarray	HCC differentiation from healthy, chronic hepatitis and cirrhosis	Zhou et al. [104]
miR-92a/92a-3p	Up	HBV-infected serum, plasma of HBV HCC	qRT-PCR, microarray, NGS	Potential biomarker for HBV-related HCC	Wen et al. [100], Hou et al. [47], Li et al. [41]
let-7f	Up	HBV-infected serum	qRT-PCR, NGS	Biomarker for differentiation of HBV-positive HCC from controls	Li et al. [41]
miR-15a	Up/down	HepG2 and HepG2.2.15, plasma of HBV-related HCC, liver tissue of HCC	Microarray and Northern blotting	Involved in chronicity of HBV infection	Liu et al. [75], Ghosh et al. [99]
miR-18a/b	Up/down	HepG2 and HepG2.2.15, HBV-related HCC serum	Microarray, NGS, Northern blotting, qRT-PCR	Potential research interest in chronic HBV infections and HBV-induced HCC	Zhang et al. [95], Li et al. [109]
miR-100	Down	HBV-related HCC	qRT-PCR	Important deregulated miRNA in HCC	Hou et al. [47]
miR-106a	Down	HepG2 and HepG2.2.15	Microarray	Potential research interest in chronic HBV infections and HBV-induced HCC	Zhang et al. [95]

miRNA	Deregulation	Type of sample	Type of method	Clinical relevance	Reference
miR-122	Down	HepG2.2.15, HBV infected HCC tissue, HBV-related HCC plasma	Microarray, qRT-PCR, NGS, clone count	Potential biomarker for HCC detection	Zhou et al. [104], Li et al. [61], Fan et al. [62], Mizuguchi et al. [107]
miR-122-5p	Down	Serum of HBV-related HCC patients	NGS, qRT-PCR	Clinical value in HBV-related HCC diagnostics	Tan et al. [101]
miR-125b-5p	Up/down	HBV-related HCC, HBV positive plasma	qRT-PCR, microarray, NGS	Potential biomarker for HBV-related HCC	Hou et al. [47], Giray et al. [110]
miR-143	Down	HepG2 and HepG2.2.15	qRT-PCR, microarray, NGS	Potential research interest in chronic HBV infections and HBV-induced HCC	Hou et al. [47], Zhang et al. [95]
miR-145	Down	HepG2, Hep3B. HBV-related HCC, HBV-infected serum	Stem-loop RT-qPCR	Candidate tumor-suppressor miRNA	Gao et al. [105], Bandopadhyay et al. [106]
miR-192-5p	Down	Serum of HBV-related HCC patients	NGS, qRT-PCR	Clinical value in HBV-related HCC diagnostics	Tan et al. [101]
miR-199a-5p	Down	Serum of HBV-related HCC patients	NGS, qRT-PCR	Clinical value in HBV-related HCC diagnostics	Tan et al. [101], Zhang et al. [95]
miR-199a/b-3p	Down	HBV-related HCC	qRT-PCR, microarray, NGS	Important deregulated miRNA in HCC	Hou et al. [47]
miR-199b-5p	Down	HepG2, HepG2.2.15, Hep3B. HBV-related HCC	miRNA microarray, Stem-loop RT-qPCR	Potential research interest in chronic HBV infections and HBV-induced HCC	Gao et al. [105], Zhang et al. [95]
miR-22	Down	HepG2 and HBV-related HCC	qRT-PCR	Inhibits viral gene expression	Shi et al. [71]
miR-26a	Down	HBV-related HCC plasma	qRT-PCR, microarray	HCC differentiation from healthy, chronic hepatitis and cirrhosis	Zhou et al. [104]
miR-26a-5p	Down	Serum of HBV-related HCC patients	NGS, qRT-PCR	Clinical value in HBV-related HCC diagnostics	Tan et al. [101]
miR-29c	Down	HBV-related HCC	qRT-PCR, microarray, NGS	Tumor- suppressive miRNA	Hou et al. [47]
let-7a/b/c/d	Down	HBV-related HCC	qRT-PCR, microarray, NGS	Important deregulated miRNA in HCC	Hou et al. [47]

Abbreviations: HCC, hepatocellular carcinoma; NGS, next-generation sequencing; ND, no data available; qRT-PCR, quantitative real-time polymerase chain reaction.

Table 1. Studies reporting on miRNA deregulation in HBV-infected patients with HCC or in HBV-expressing cell lines.

Serum miRNAs could serve as biomarkers for the detection of liver pathologies [102]. Serum HBV-related miRNAs for HBV-related HCC diagnosis have been investigated by Tan et al. [101]. The study identified eight miRNAs (miR-206, miR-141-3p, miR-433-3p, miR-1228-5p, miR-199a-5p, miR-122-5p, miR-192-5p, and miR-26a-5p) and constructed a miRNA set that provided high diagnostic accuracy for HBV-related HCC [101]. The study published by Winther et al. [113] presented a panel of circulating plasma miRNAs that are differentially expressed in immunological phases of chronically HBV-infected children and positively correlated with the quantity of HBsAg.

A multicenter study was conducted by Wen et al. [100] to discover a panel of plasma miRNAs to discriminate HBV-related HCC patients from healthy controls. The study revealed that four miRNAs (miR-20a-5p, miR-320a, miR-324-3p, and miR-375) (alone or combined with AFP) could be used as preclinical biomarkers in HCC screening, while the expression profile of eight miRNAs (miR-20a-5p, miR-25-3p, miR-30a-5p, miR-92a-3p, miR-132-3p, miR-185-5p, miR-320a, and miR-324-3p) can discriminate HCC patients from noncancerous controls [100]. Some of the described miRNAs were studied as well by Zhang et al. [95]. MiR-18a, miR-125b-5p, and miR-223-3p were well described as potential biomarkers for HBV-related HCC [109, 110].

Hou et al. [47] performed an extensive study of miRNomes in human normal liver, hepatitis liver, and HCC. Researchers presented 15 deregulated miRNAs in 40 HBV-related HCC samples in comparison to healthy controls. Additionally, the consistent decline of miR-199a/b-3p in HCC and its significant correlation with poor prognosis of HCC patients has been elucidated, suggesting miR-199a/b-3p as a potential HBV therapeutic target. Gao et al. [105] investigated the expression of cancer-related miRNA profiles in early stages of HBV-related HCC development and observed altered miRNA expression at various pre-malignant stages of HCC and persistent downregulation of miR-145 and miR-199b and upregulation of miR-244 throughout the HCC development. The miR-145 has been suggested as a candidate tumor-suppressive miRNA due to suppression of cell proliferation caused by overexpression of miR-145 precursor in HepG2 cell lines and abundant expression of miR-145 in non-tumorous livers as well as pre-malignant low-grade dysplastic nodules.

Several studies have suggested the role of HBx protein in miRNA expression during HBV infection [67, 96, 98, 106]. For example, the recent study, conducted on 120 patients with HBV-related HCC, has shown that the expression of miR-106b was significantly higher in HBV-related HCC patients in comparison to non-HBV/non-HCV-related HCC patients and suggested that HBx enhances miR-106b transcription and therefore promotes tumor progression in HBV-related HCC [98]. Transfection with the HBx expression plasmid has been recently used in an additional study by Yu et al. [114] to investigate HBx-related regulation of miR-19a, miR-122, and miR-223 in malignant hepatocytes. The study has shown that the expression of miRNAs was regulated by HBx protein, which enhanced the proliferation of HBx-transfected HepG2 cells.

7.3. HCV miRNA biomarkers

Apart from a variety of published studies focusing on the identification of HCV infection miRNA biomarkers [88], our review focused more on difference in miRNAs profiles

between HCV-infected cancerous liver cells and HCV-infected cells without progression to HCC. Despite the fact that treatment for most common HCV genotype 1 has been evolving rapidly in the past 10 years, no effective and safe anti-HCV vaccine is available and only approximately 50% of patients, infected with HCV genotype 1 (the more common genotype in USA and Western Europe), reach sustained viral response (SVR) while treated with most accessible treatment, the interferon. Therefore, the management of HCV-induced liver disease remains problematic [115].

Antagonism of miR-122 by locked nucleic acid is a promising tool for the treatment of HCV. The current most advanced research on miR-122 antagonist Miravirsen is discussed in the following section. Likewise, the therapeutic potential of mimic miR-196b is presented by Kaluzna [30], reviewing studies which confirmed the ability of miR-196b to inhibit HCV replication and revealed that interferon-induced overexpression of miR-196b decreases inflammation and leads to a better response in interferon-based HCV therapy.

Circulating serum levels of miR-122 and miR-222 have been shown to be useful potential diagnostic biomarkers for chronic HCV infection in Egyptian patients [116–118], whereas in another study miR-122, miR-199a, and miR-16 have been implicated as potential early diagnostic biomarkers for HCC in Egyptian patients, chronically infected with HCV [118] (**Table 2**).

Of note, miR-222 and miR-224 have been reported to be upregulated in both, HBV and HCV infections [95, 105, 116, 121]. However, Bandopadhyay et al. [106] and Zhang et al. [95] reported downregulation of miR-222 and miR-224 in HBV-infected patients, respectively. As expression profiles of circulating serum biomarkers became a subject of interest in different disease studies, serum miRNAs possibly involved in HCV-related HCC have been investigated in several studies. Oksuz et al. [119] examined HCV-infected patients with chronic infection, cirrhosis, and HCC and compared them with control group samples. When all groups of samples were compared, the study revealed deregulation of miR-30c-5p, miR-223-3p, miR-302c-3p, and miR-17-5p in cirrhosis and HCC, suggesting possible novel noninvasive biomarkers for HCC.

Using whole-genome expression profiling, Abdalla and Haj-Ahmad [120] identified 10 potential HCV-induced HCC biomarker candidates in urine; five of which were upregulated in HCC: miR-335, miR-618, miR-625, miR-532, and miR-7, and five downregulated: miR-323, miR-449, miR-520d, miR-516-5p, and miR-650. The proposed tandem signature of downregulated miR-650 and upregulated miR-618 showed improved sensitivity and specificity for HCC detection, in comparison to the traditional AFP-level-based detection method.

Increased expression of miR-155 in hepatocytes of patients infected with HCV has been confirmed *in vitro* and *in vivo* by Zhang et al. [85]. In addition, the study revealed that overexpression of miR-155 inhibits apoptosis, and promotes hepatocyte proliferation and tumorigenesis, which suggested miR-155 could be a negative prognostic biomarker for HCC (reviewed by Kaluzna [30]). In addition, miR-155 has been shown to be upregulated in HBV-infected human hepatoma cells, where it inhibits HBV infection [103] (**Figure 1**).

MiRNA	Deregulation	HCV genotype	Type of sample	Method	Clinical relevance	Reference
miR-10a	Up	ND	HCV-related HCC	qRT-PCR	Increased expression in HCV-related HCC	Varnholt et al. [84]
miR-15a	Up	ND	HCV-related HCC	qRT-PCR	Increased expression in HCV-related HCC	Varnholt et al. [84]
miR-16	Up/down	ND	HCV-related HCC, serum of HCV chronic infections	qRT-PCR, stem loop qRT-PCR	Associated with progression of HCC	Varnholt et al. [84], El-Abd et al. [118]
miR-17-5p	Up	ND	Plasma of HCV-related HCC	qRT-PCR	Noninvasive biomarker of HCV-positive HCC	Oksuz et al. [119]
miR-100	Up	ND	HCV-related HCC	qRT-PCR	Increased expression in HCV-related HCC	Varnholt et al. [84]
miR-122	Up/down	ND	Serum of HCV patients serum of patients with chronic HCV infection, HCV-related HCC	RT-PCR, qRT-PCR, stem loop qRT-PCR, microarray	Detection of HCC in HCV-chronic patients	El-Garem et al. [117], Motawi et al. [116], El-Abd et al. [118], Varnholt et al. [84]
miR-125b	Up	ND	HCV-related HCC	qRT-PCR	Increased expression in HCV-related HCC	Varnholt et al. [84]
miR-155	Up	ND	HCV-infected patients	Stem loop qRT-PCR	Promotes hepatocarcinogenesis	Zhang et al. [85]
miR-192	Up	Chimera of 1a/2	Huh7, Huh-RepSI	Microarray	Relevant to carcinogenesis	Ishida et al. [80]
miR-194	Up	Chimera of 1a/2	Huh7, Huh-RepSI	Microarray	Relevant to carcinogenesis	Ishida et al. [80]
miR-200a	Up	4	Urine of HCV-related HCC	Microarray, qRT-PCR	Potential HCC biomarker	Abdalla et al. [120]
miR-215	Up	Chimera of 1a/2	Huh7, Huh-RepSI	Microarray	Relevant to carcinogenesis	Ishida et al. [80]
miR-221	Up	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-222-3p	Up	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-224-3p	Up	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]

MiRNA	Deregulation	HCV genotype	Type of sample	Method	Clinical relevance	Reference
miR-224-5p	Up	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-299	Up	ND	HCV-related HCC	qRT-PCR	Increased expression in HCV-related HCC	Varnholt et al. [84]
miR-30c-5p	Up	ND	Plasma of HCV-related HCC patients	qRT-PCR	Noninvasive biomarker of HCV-positive HCC	Oksuz et al. [119]
miR-302c-3p	Up	ND	Plasma of HCV-related HCC patients	qRT-PCR	Noninvasive biomarker of HCV-positive HCC	Oksuz et al. [119]
miR-326	Up	ND	HCV-related HCC tumors	qRT-PCR	Increased expression in HCV-related HCC	Varnholt et al. [84]
miR-335	Up	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	Potential HCC biomarker	Abdalla et al. [120]
miR-370	Up	ND	HCV-related HCC	qRT-PCR	Increased expression in HCV-related HCC	Varnholt et al. [84]
miR-452	Up	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-520a*	Up	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	Potential HCC biomarker	Abdalla et al. [120]
miR-521	Up	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	Potential HCC biomarker	Abdalla et al. [120]
miR-522-3p	Up	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-532	Up	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	Potential HCC biomarker	Abdalla et al. [120]
miR-618	Up	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	miR-618/650 in tandem to detect HCC among HCV-positive patients	Abdalla et al. [120]
miR-625	Up	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	Potential HCC biomarker	Abdalla et al. [120]

MiRNA	Deregulation	HCV genotype	Type of sample	Method	Clinical relevance	Reference
miR-640	Up	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	Potential HCC biomarker	Abdalla et al. [120]
miR-7	Up	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	Potential HCC biomarker	Abdalla et al. [120]
miR-765	Up	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	Potential HCC biomarker	Abdalla et al. [120]
miR-9	Up	ND	HCV-related HCC	qRT-PCR	Increased expression in HCV-related HCC	Varnholt et al. [84]
miR-1269	Up	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
let-7g	Up	ND	HCV-related HCC	qRT-PCR	Increased expression in HCV-related HCC	Varnholt et al. [84]
miR-16-5p	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-104	Down	ND	HCV-related HCC tumors	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-106a	Down	ND	HCV-related HCC tumors	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-122a	Down	ND	Mainly HCV-related HCC	Microarray	Deregulated in HCV-related HCC	Gramantieri et al. [122]
miR-125a-5p	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-125b-5p	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-130a	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-134	Down	ND	HCV-related HCC tumors	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-137	Down	ND	HCV-related HCC tumors	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]

MiRNA	Deregulation	HCV genotype	Type of sample	Method	Clinical relevance	Reference
miR-139-3p	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-139-5p	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-145	Down	ND	HCV-related HCC tumors	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-147	Down	ND	HCV-related HCC tumors	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-159a	Down	ND	HCV-related HCC tumors	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-181a	Up/down	4	Huh7, blood, serum liver biopsies from chronically infected HCV patients	RT-PCR	Disparity in expression between serum and liver tissue. Upregulation in serum-good prognosis, downregulation-progression to HCC	Elhelw et al. [90]
miR-185	Down	ND	HCV-related HCC	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-195	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-198	Down	ND	HCV-related HCC	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-199a/-3p	Down	ND	HCV-related HCC, serum of chronically HCV infected	Microarray, qRT-PCR, stem loop qRT-PCR	Exclusively expressed in HCV-associated HCC	Diaz et al. [121], El-Abd et al. [118]
miR-199a-5p	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-199b	Down	ND	HCV-related HCC	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-199b-3p	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]

MiRNA	Deregulation	HCV genotype	Type of sample	Method	Clinical relevance	Reference
miR-29c	Down	ND	HCV-related HCC	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-204	Down	ND	HCV-related HCC	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-214	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-218	Down	ND	HCV-related HCC	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84])
miR-221	Down	ND	Serum chronic HCV patients	RT-PCR, qRT-PCR	Potential noninvasive biomarker for HCV-related HCC	El-Garem et al. [117]
miR-223-3p	Down	ND	Plasma of HCV-related HCC	qRT-PCR	Noninvasive biomarker of HCV-positive HCC	Oksuz et al. [119]
miR-301a-3p	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-302b	Down	ND	HCV-related HCC	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-320	Down	Chimera of 1a/2	Huh7, Huh-RepSI	Microarray	Relevant to carcinogenesis	Ishida et al. [80]
miR-323	Down	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	Potential HCC biomarker	Abdalla et al. [120]
miR-330	Down	ND	HCV-related HCC	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-368	Down	ND	HCV-related HCC	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-424-3p	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-449	Down	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	Potential HCC biomarker	Abdalla et al. [120]
miR-491	Down	chimera of 1a/2	Huh7, Huh-RepSI	Microarray	Relevant to carcinogenesis	Ishida et al. [80]

MiRNA	Deregulation	HCV genotype	Type of sample	Method	Clinical relevance	Reference
miR-497	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-516-5p	Down	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	Potential HCC biomarker	Abdalla et al. [120]
miR-520d	Down	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	Potential HCC biomarker	Abdalla et al. [120]
miR-650	Down	4	Urine of HCV-related HCC patients	Microarray, qRT-PCR	miR-618/650 in tandem to detect HCC among HCV-positive patients	Abdalla et al. [120]
miR-761	Down	ND	HCV-related HCC	Microarray	Exclusively expressed in HCV-associated HCC	Diaz et al. [121]
miR-9*	Down	ND	HCV-related HCC	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
miR-95	Down	ND	HCV-related HCC	qRT-PCR	Decreased expression in HCV-related HCC	Varnholt et al. [84]
let-7 family	Down	ND	Mainly HCV-related HCC	Microarray	Deregulated in HCV-related HCC	Gramantieri et al. [122]

Abbreviations: HCC, hepatocellular carcinoma; NGS, next-generation sequencing; ND, no data available; qRT-PCR, quantitative real-time polymerase chain reaction.
* Different mature miRNA from the same stem loop.

Table 2. Studies reporting on miRNA deregulation in HCV-infected patients with HCC- or in HCV-expressing cell lines.
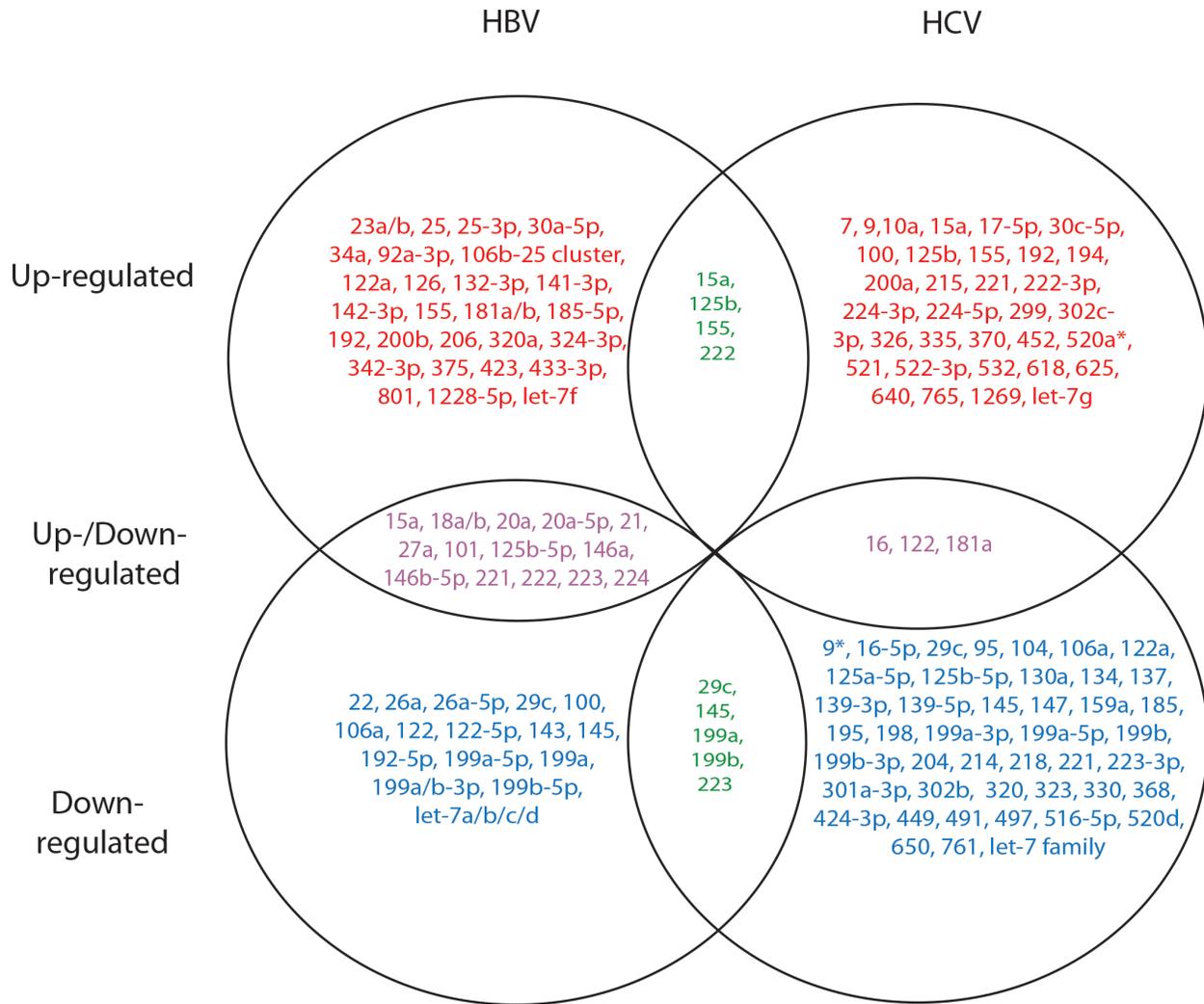
Figure 1. miRNAs deregulated in HBV- and HCV-related HCC or HBV/HCV-expression cell lines. miRNAs upregulated in HBV and HCV infections are presented in red color, miRNAs downregulated in HBV and HCV infections are presented in blue color, while miRNAs that were shown to be up- and downregulated in different studies are presented in violet color. MiRNAs reported to be up- or downregulated in HBV and HCV infections are presented in green color.

Despite the fact that several studies examined the expression of miRNAs in HCC, discrepancies exist among published data. Diaz et al. [121] assumed that non-concordance may be the result of differences in selection of noncancerous control samples or commonly included HCC samples without prior confirmation of possible HBV or HCV infection. In some previous studies, Diaz et al. [121] investigated the expression of miRNAs in HCV-induced HCC, in comparison to a wide range of liver samples and identified 18 miRNAs exclusively expressed in HCV-induced HCC. Several other studies have as well examined subsets of miRNAs potentially involved in hepatocellular changes, advancing to HCC [80, 84, 122].

According to an increasing amount of miRNAs identified and examined throughout various stages Of HBV/HCV infection and HCC development (**Tables 1** and **2**), miRNAs not specifically presented in this review should be as well included as a subject of interest in future studies.

7.4. MiRNA-122 in HBV and HCV

The levels of liver-specific miRNA-122 (miR-122) are down- and upregulated in HBV and HCV, respectively. The miR-122 promotes the replication of HCV and blocks the replication of HBV. It has been shown recently that HBV inhibits the miR-122 expression, suggesting a possibility of miR-122 replacement therapy in HBV-infected individuals [61, 64]. The study by Fan et al. [62] showed that miR-122 inhibited the expression of the NDRG3 protein, which subsequently inhibited malignant cell transformation and presented the miR-122 and its target NDRG3 as key diagnostic markers and potential therapeutic targets in HBV-related HCC. On the other hand, in HCV infections, it has been shown *in vitro* and *in vivo* that antagonistic utility of miR-122 inhibits HCV replication cycle and reduces viral load, and thus represents an effective treatment of HCV infection. A model of Miravirsen interaction with miR-122 is shown in **Figure 2** [77, 123].
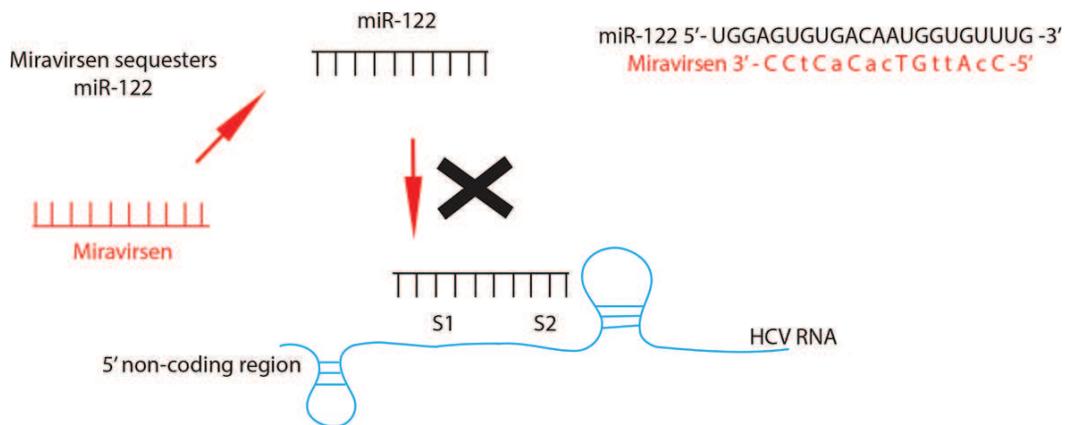
Figure 2. Miravirsen interaction with miR-122 inhibits binding between miR-122 and the 5′ UTR of the HCV RNA. The most abundant liver miRNA-miRNA-122 binds with two seed sites in the 5′ UTR of the HCV genome. Miravirsen sequesters mature miR-122 and suppresses HCV.

Recently, the safety and efficacy of the Miravirsen, a locked nucleic acid form of antisense-miR-122 that sequesters miR-122, has been evaluated in a phase 2a clinical study, which included 36 patients with chronic HCV genotype 1 infection from seven international sites [40]. The study observed that treatment with Miravirsen prolonged dose-dependent reductions in HCV RNA, without evidence of viral resistance [40]. Moreover, a recent study, conducted on 51 HCV-infected Japanese patients, treated with interferon, presented miR-122 as an independent predictor of SVR [124]. Unfortunately, no single miRNA representing a promising treatment option in HBV infections has been pointed out as yet.

Nevertheless, therapies that silence HBV RNAs are emerging. Multiple cell-line-based studies and *in vivo* studies on mouse models have evaluated synthetically engineered or chemically modified small RNAs that complementarily target HBV transcripts and lead to RNA degradation and thus to the inhibition of HBV replication. However, due to short duration of their activity, the lack of applicable animal model for testing in clinical trials, and subsequent

pharmacokinetic difficulties, further investigations are warranted to evaluate RNA-interference-based approaches to clinical practice (reviewed by Ivacik et al. [125]).

HBV/HCV dual infection is not an uncommon event, occurring in approximately 2–10% of chronically infected HCV patients and in 5–20% of chronically infected HBV patients [126]. Dual HBV/HCV infection has prognosis of a more aggressive clinical course of liver disease than either mono-infection. Despite the fact that compiling evidence exists on reciprocal inhibition between HBV and HCV and that miR-122 represents a crucial host gene involved in pathogenesis of both viruses, the role of miR-122 in HBV/HCV dual infection has not been defined so far [127].

8. Conclusion

Cellular miRNAs contribute to HBV and HCV pathogenesis by direct or indirect interactions with viral genome or proteins and molecules critical for regulation of the cell cycle. Regulation of miRNAs expression upon HBV and HCV infection significantly differs between both viruses. Reports summarized in this chapter indicate that miRNAs represent an effective, noninvasive biomarker tools for early diagnosis of HBV and HCV infection, early diagnosis of liver disease and its progressive stages, particularly HCC. Mimic and antagonistic effects of cellular miRNAs have been considered in diagnostic and treatment of HBV/HCV-related liver disease, with miR-122 representing a promising treatment option for chronic infection with HCV genotype 1. Because most studies identified and validated miRNAs in heterogenic tumors and because miRNA targets were validated mostly in the already-transformed cell culture systems, transfected with plasmids encoding HBV or HCV genome or parts of their genome, discrepancies exist in candidate biomarker miRNAs across published studies. Due to an extensive number of miRNA targets and other clinical factors considered in significant number of studies published in the last 10 years, efforts should be made to establish a specific, repetitive, and easy-to-operate method to identify reliable panels of miRNA biomarkers for early diagnosis and treatment of HBV-HCV-related diseases. Suitable reference miRNA targets and positive and negative controls should be included in such profiling applications. The application of novel techniques such as next-generation sequencing, development of synthetic small RNAs, and hepatoma cell lines will impact the subsequent advances in miRNA studies related to HBV and HCV pathogenesis as well as miRNA deregulation in other pathological conditions.

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