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MicroRNA: Role in hepatitis B and C virus infections

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MicroRNAs: Role in hepatitis B and C virus infection

Abstract

Around 257 million people are living with hepatitis B virus (HBV) chronic infection and71 million with hepatitis C virus (HCV) chronic infection. Both HBV and HCV infections can lead to liver complications such as cirrhosis and hepatocellular carcinoma (HCC). To take care of these chronically infected patients, one strategy is to diagnose the early stage of fibrosis in order to treat them as soon as possible to decrease the risk of HCC development. MicroRNAs (miRNAs) are small endogenous non-coding functional RNAs. They can play vital Roles in post-transcriptional regulating mRNAs transcripts in nearly all biological processes. More and more reports on miRNAs role in development, immunity, apoptosis, tumor and virus-host interaction were published. These recent findings provide new insights into the roles of miRNAs as well as their function. This review aims to summarize current knowledge of principal miRNAs (biogenesis, origin, evolution) and their modulation involved in fibrosis progression during chronic Hepatitis B/C infections. Furthermore, we also discuss the potential use of miRNAs as non-invasive biomarkers to diagnose fibrosis with the intention of prioritizing patients with advanced fibrosis for treatment and surveillance

Key words: miRNA, liver diseases, HCV, HBV

Introduction:

Hepatitis B and C viral infections are still major public health problems of the 21st century despite the implementation of different therapeutics. Around 257 million people are living with hepatitis B virus (HBV) and 71 million with hepatitis C virus (HCV) chronic infections [1]. Both HBV and HCV infections can induce liver complications such as cirrhosis and hepatocellular carcinoma (HCC).

MicroRNAs (miRNAs) are small non-coding RNAs which regulate many processes in metazoans [2]. miRNAs expressions are frequently modulated by up- or down-regulation during fibrosis progression and cirrhosis. This review aims to summarize current knowledge of viral hepatitis B/C and miRNAs in the development and the progression of fibrosis. This review will also discuss the potential of miRNAs as biomarkers to diagnose fibrosis, since patients with advanced fibrosis are prioritized for treatment and surveillance.

Historical perspective:

In the nineties, Victor Ambros and his team reported a microRNA (miRNA), a small non-coding RNA transcript from Lin-4 gene. This miRNA Lin-4 controls the post-embryonic development of C. elegans by interacting with Lin-4 mRNA to regulate its translation [23]. miRNAs are a class of endogenous single-stranded RNAs (approximately 20 nucleotides) which negatively regulate Metazoans genes by targeting mRNAs in their 3'-untranslated region (3'-UTR). The interaction with the 3'-UTR of targeted mRNA induces the silencing of the gene by mRNA translational repression or degradation [24]. Because miRNAs regulate diverse cellular pathways or activities, their dysregulation is involved in liver fibrosis and a number of

human cancers. The specific Differences of miRNAs expression during CHB and CHC leads the way to their potential use in the Diagnosis of fibrosis progression.

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 [4]. 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 [5–6]. 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 [7]. Gene silencing by a full complementary miRNA sequence directs cleavage of the target mRNA, while partial complementary miRNA sequence suppresses mRNA translation, figure 1 [8, 9].

Currently, more than 2,588 mature miRNAs are reported in a human genome [10, 11] 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 [12].

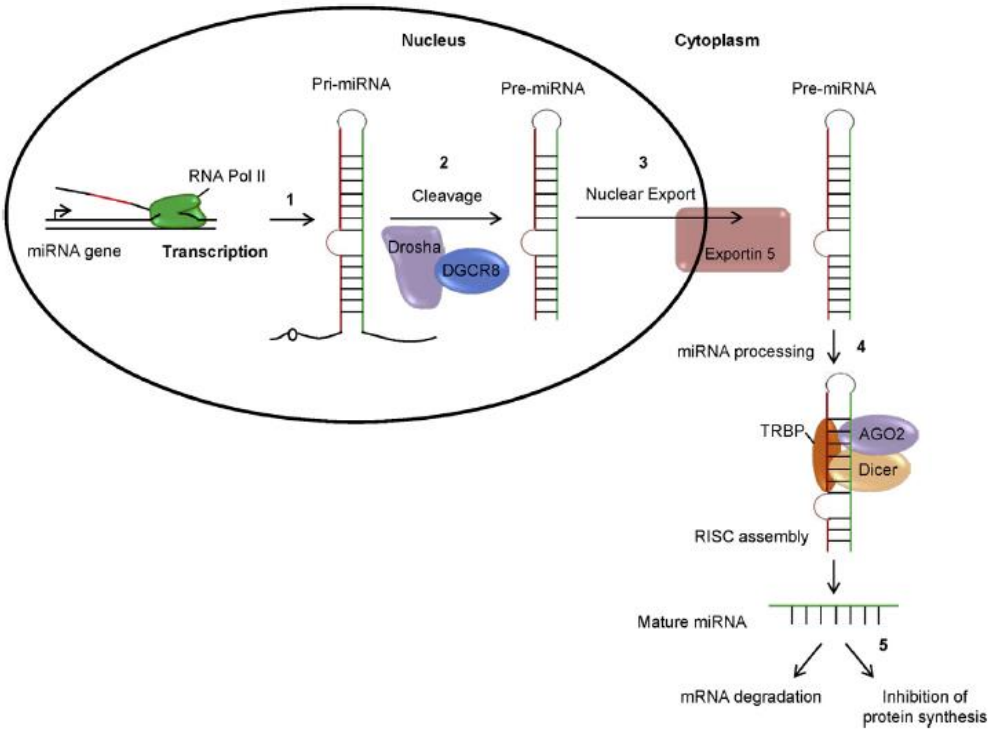

Figure 1: miRNA biogenesis and regulation of gene expression.

(1) First, the miRNA gene transcribed to a primary long transcript with stem loop structure as pri-miRNA. (2) This pri-miRNA is processed by RNase III family of enzymes, Drosha with the help of double stranded RNA binding protein, DGCR8 and produce smallw70-nucleotide precursor hairpin structure as precursor miRNA (pre-miRNA). (3) Pre-miRNA then transported to the cytoplasm with the help of exportin5 protein. (4) Pre-miRNA was further cleaved by Dicer together with transactivation-responsive (TAR) RNA-binding protein TRBP, in the cytoplasm and generate a w20-bp miRNA/miRNA* duplex. Following processing, one strand of the miRNA/miRNA*duplex (the guide strand) is preferentially loaded into the miRNA induced silencing complex (miRISC) containing argonaute 2 (AGO2) and form mature miRNA. (5) Then, the mature miRNA targets specific messenger RNA (mRNA) at the seed region that lead to either mRNA degradation or inhibition of [79].. Shrivastava et al. Genes & Diseases (2015) 2, 35e45

MicroRNAs mechanism

miRNAs are participating in various cellular processes such as cell development, differentiation, proliferation, metabolism, immune responses, apoptosis, and oncogenesis [13, 14]. Estimates in humans suggest that 60–70% of all genes are regulated by miRNAs [15]. Being involved in numerous

biological pathways, their expression and regulation reflect in various diseases, stages of the particular disease, especially in cancer development [16, 17]. 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 [18–19].

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 [20, 21]. Increased interest in hepatitis B and hepatitis C disease pathogenesis and diagnostics has led 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 [22].

miRNAs in normal Liver Tissues:

The liver consists of cell types (parenchymal hepatocytes, non-parenchymal biliary epithelial cells, lymphoid cells). Each cell sort communicates a special miRNA profile. While miRNAs are up or down regulated in almost every stage of hepatic development, they accelerate or inhibit liver proliferation and play a major role in the regulation of diverse liver functions. 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 [25,26]. Besides miR-192, miR-199a/b-3p, miR-101, miR-99a, and let-7a/b/c/f (let-7 family), are abundant in liver whose miR-122 accounts for 70% of total liver miRNAs. 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 antagonists, anti-inflammatory and anti-tumorigenic effector

in liver [27]. In the miR-122 gene knockdown mice, it has been shown that it acts as a key regulator of cholesterol and fatty-acid metabolism and its gene resulted in the development of liver tumors [26].

Fibrosis Progression:

Fibrosis is the consequence of chronic tissue injury and inflammation inflicted by various factors Such as viral hepatitis, alcohol consumption, and non-alcoholic steato-hepatitis [28]. Fibrosis process is characterized by an excessive and persistent accumulation of the extracellular matrix (ECM) as a consequence of activation of hepatic stellate cells, exaggerated expression of profibrogenic genes, and/or suppression of antifibrogenic genes [29]. The collagen deposits in the ECM and leads to the expansion of the portal zone with risk of cirrhosis and HCC development. Risk factors for fibrosis progression include host-related factors (advanced age, co-morbidities such as diabetes or obesity, etc.) and exogenous factors (HIV co-infections, medication, and alcohol for example) [30]. Fibrosis regression in Patients with HBV and HCV infection is achievable by antiviral treatments [31,32]. It is important to diagnose fibrosis and score its stage to prioritize patients for treatment. Two types of clinical tests are used to diagnose and determine the stage of fibrosis: non-invasive tests based on serological markers or on elasticity (fibroscan), despite their difficulties to differentiate mild from moderate fibrosis; and histological analysis after percutaneous liver biopsy. Among different scores Used for liver fibrosis, METAVIR score is based on necro-inflammation and fibrosis evaluation [33]. Necro-inflammation activity (A) is graded as A0 (absent), A1 (mild), A2 (moderate), or A3 (severe).

Fibrosis is staged as F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa without cirrhosis; F4, cirrhosis. Significant fibrosis is defined as METAVIR score F > 2 (F3 or F4)

[33]. Complications are associated with percutaneous liver biopsy such as pain and bleeding [34]. Therefore, new non-invasive biomarkers are needed to determine, with high precision, the stage of fibrosis to improve prognosis evaluation in patients with Chronic Hepatitis B (CHB) and C (CHC). In patients with CHB, plasma HBsAg is used as a biomarker to stratify the risk of disease progression [35,36]. It has been shown that HBsAg titer was negatively correlated with the stage of the fibrosis in HBeAg-positive patients [37]. Circulating miRNAs are deregulated in liver fibrosis and HCC and are candidate biomarkers for diagnosis [38,39]. Diagnosis based on plasma miRNAs is presumably an attractive non-invasive strategy because of their stability and because of their potential correlation with different stages of Fibrosis as reported in earlier studies [40].

HCV Infection

HCV is an enveloped virus with a positive single-stranded RNA belonging to the Flaviridae family [41]. In 2015, approximately 71 million people were living with HCV chronic infection worldwide, with 399,000 deaths due to liver complications (cirrhosis and HCC) [42].

The difference with HBV infection is that there is no HCV integration in the human genome and no viral reservoir because HCV replication is localized within the cytoplasm [43]. Drug discovery has allowed the development of HCV direct-acting antivirals with more than 95% of sustained virological response with complete eradication of HCV virus in infected patients and favorable tolerability [43]. The persisting problem is the access to diagnosis and to treatment, mainly in developing countries [44]. Moreover, no vaccine is available to prevent new infections and propagation of HCV [43].

HCV life cycle is influenced by host miRNAs in all stages: entry, translation, replication, and assembly [45]. 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 [46, 47].

Microarray analysis on human hepatoma cells has revealed changed expression profiles of 108 human miRNAs after HCV infection [48]. Furthermore, Liu et al. [48] 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 [9]. It was reported that miR-192/miR-215 and miR-491 could enhance HCV replication [49]. 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 [50, 51, 52].

In vitro studies have shown that miR-122 is essential for HCV replication [51]. On the other hand, it has been shown that miR-122 exhibits anti-inflammatory and anti-tumorigenic properties in mice knockdown studies [53]. Mixed results exist on expression levels of miR-122 and development of HCC- or HCV-induced HCC. Coulouarn et al. [54] 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 [55]. The increased expression of miR-155 in HCV-infected patients promotes hepatocarcinogenesis and inhibits apoptosis of hepatocytes [56]. 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 [57].

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 Furthermore, miR-491 promotes HCV replication through inhibition of the PI3 kinase/Akt pathway, one of the pathways leading to cancerous properties.

Studies analyzing circulating miRNA profiles in serum provide novel insights on miRNA expression in HCV pathogenesis. In a study by Shwetha *et al.*, 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.

HBV Infection

HBV is a small enveloped DNA virus belonging to the Hepadnaviridae family, figure 2 [58]. According to the World Health Organization (WHO), one third of people in the world have been exposed to HBV (antibodies to hepatitis B core antigen (anti-HBc)-positive) and around 257 million people are living with HBV chronic infection (Hepatitis B surface antigen [HBsAg]-positive) [59]. HBV is a Hepatotropic virus, able to persist in infected cells and no current treatment is able to eradicate the Virus from these cells [58]. An HBV infectious particle, which contains HBV genome (a partly double-stranded DNA in relaxed circular form called rcDNA), interacts with the human sodium taurocholate co-transporting polypeptide receptor (hNTCP or SLC10A1), the major HBV receptor described [60]. This interaction Involved the Large Hepatitis B surface antigens (L-HBsAg), one of the three HBsAg exposed at the virion surface [61]. Then, HBV rcDNA is release into the nucleus where it can be integrated into the human genome or repaired by different cellular mechanisms into covalently closed circular DNA (cccDNA) [62,63]. HBV

cccDNA is a mini chromosome and is the major impediment to achieving an HBV cure and complete eradication of the infection [64].

Recent advances in treatment of CHB include nucleos(t)idic analogs with a high efficacy and favorable safety profile, but with long-life treatment duration [65]. However, while being able to control the viral replication, these antiviral therapies do not completely eliminate HBV in patients [65]. Furthermore, an effective prophylactic Vaccine is available, but campaigns are not well-implemented.

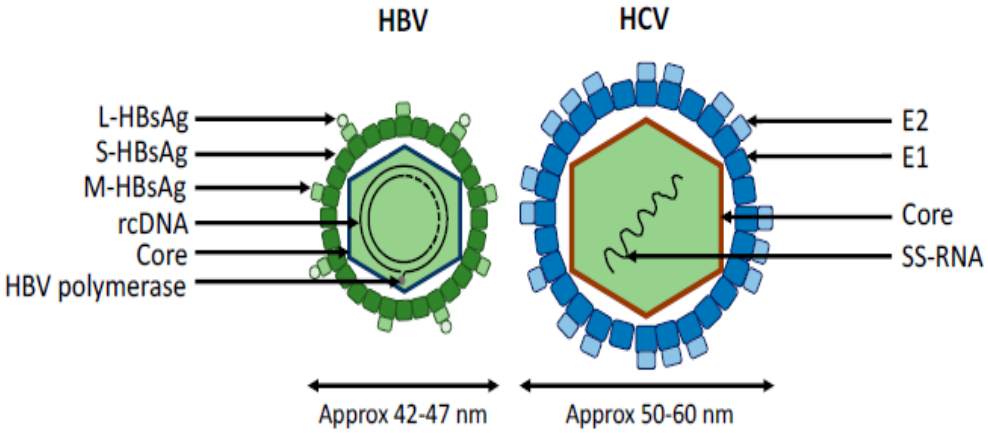

Figure 2. Comparison of hepatitis B virus (HBV) and hepatitis C virus (HCV) viral structures. The HCV virion is larger than the HBV virion by approximately 20 nm. HBV and HCV are two hepatotropic viruses which use different receptors for viral entry. Three different Hepatitis B surface antigens are exposed on HBV particles: the small (S-HBsAg), the medium (M-HBsAg) and the large (L-HBsAg) surface antigens. The HBV nucleocapsid is formed by dimers of hepatitis B core proteins (HBcAg) and contains a partly double-stranded DNA genome in relaxed conformation (3.2 kb in length). The HCV virion exposes two different viral envelope proteins on its surface: E1 and E2. HCV capsid is formed by HCV core proteins which contains a positive single-stranded RNA (9.6 kb in length). HBV = Hepatitis B virus; HCV = Hepatitis C virus; rcDNA = relaxed circular DNA; cccDNA = [78]. Lancet **2015**, 385, 1124–1135.

Hepatitis B Genome Encodes for Two Viral miRNAs: HBV-miR-2 and 3:

To promote their replication and gene transcription, and to control host genes expression, viruses have developed diverse strategies such as viral

miRNAs production [66]. The HBV 3.2 kp partly double-stranded DNA contains four overlapping open reading frames (ORFs) , Five viral transcripts are produced by the human RNA polymerase II which are translated into seven HBV proteins PreC mRNA (3.5 kb), pre-genomic RNA (pgRNAs, 3.5 kb), PreS1 mRNA (2.4 kb), PreS2 mRNA (2.1 kb), and X mRNA (0.7 kb) [69]. Additionally, recent studies highlighted that in addition to viral transcripts, HBV produced two different miRNAs: HBV-miR-2 and HBV-miR-3, figure 3 [67,68].

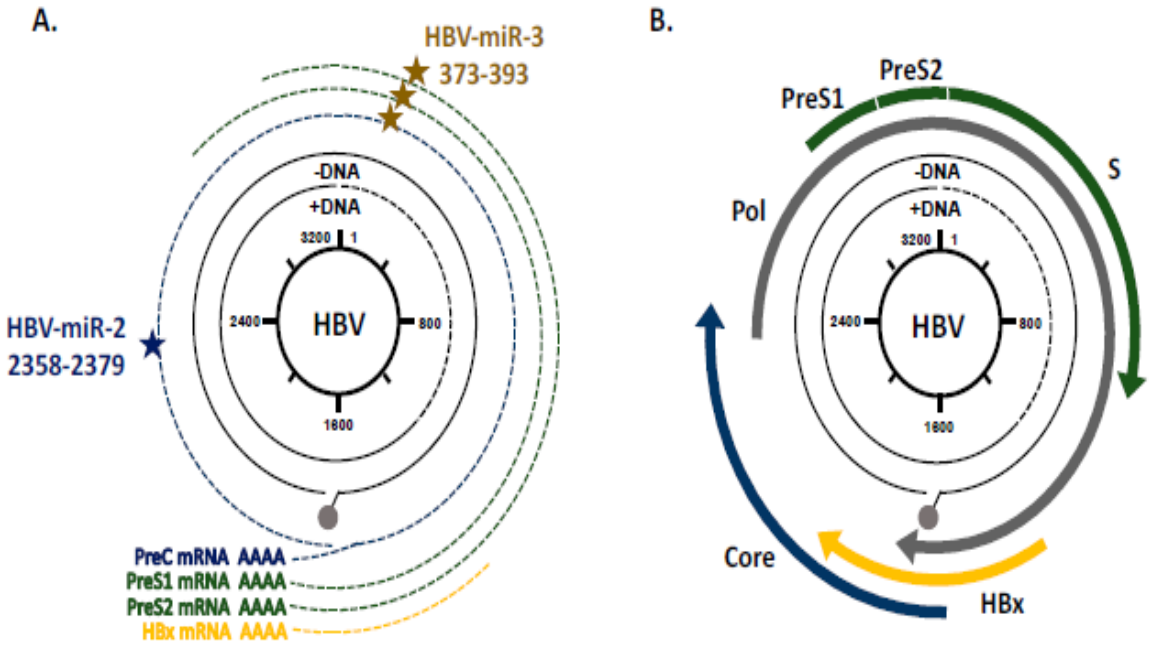

Figure 3: HBV genome organization. (**A**) Different HBV transcripts and positions of HBV sequences encoded for HBV-miR-2 and HBV-miR-3. HBV-miR-2 (blue star) extended from nucleotides 2358 to 2379 of HBV pgRNA. HBV-miR-3 (brown stars) is encoded in the HBV genome at the position 373 to 393 in PreC, PreS1, and PreS2 mRNAs. (**B**) The HBV genome contains four overlapping Open Reading Frames (ORFs): core proteins, HBV polymerase, Surface proteins, and Hepatitis B X protein (HBx) [77]. Virology 2015, 479–480, 672–686

HBV-miR-2

Yao et al. [70] identified HBV-miR-2 by deep sequencing. HBV-miRNA produced from the pgRNA is encoded by the sequence extended from the nucleotides 2358 to 2379 of HBV genome [70]. HBV-miR-2 is expressed in infected livers and secreted in serums of patients with HBV infection and in

those with HBV-related HCC. The HBV-miR-2 sequence is rather well preserved among different HBV subtypes, with only a single nucleotide change in the subtypes D, G and H. Few is known however its HBV 3,5 kb mRNA transcript with HBV-miR-3 to reduce the expression of HBV core proteins (HBc) and as an oncogene and promotes cell growth, migration, and invasion during HCC. HBV-miR-2 down-regulates tripartite motif-containing protein 35 (TRIM35) and up-regulates ras-related nuclear protein (RAN) expressions in vitro [70]. In ovarian cancer and HCC, modulation of RAN expression is correlated with cells proliferation, migration, and invasion, figure 3 [70,71].

HBV-miR-3

HBV-miR-3 is encoded from nucleotides 373 to 393 in the HBV genome and is generated from three HBV transcripts: PreC, PreS1, and PreS2 mRNAs [72]. During HBV infection, HBV-miR-3 is highly expressed and its expression correlates with HBV activity [73]. HBV-miR-3 enhances IFN production, activates JAK/STAT signaling, macrophages polymerization/ depolarization, and induces the production of interferons and IL-6 by repressing SOCS5/STAT1 pathway [74]. Moreover, HBV-miR-3 interacts directly with the Protein phosphatase 1A (PPM1A) and Phosphatase and TENsin homolog (PTEN), silences these human genes, and enhances cell invasion and proliferation in HCC development [73,75]. Furthermore, HBV attenuates its replication targeting its HBV 3,5 kb mRNA transcript with HBV-miR-3 to reduce the expression of HBV core proteins (HBc) and the level of pgRNA, figure 4 [72]. The role of HBV-miR-2 or HBV-miR-3 in liver fibrosis remains unclear. These two miRNAs encoded by HBV could be considered as potential targets to block HBV replication and thus the comprehension of their mechanisms of action needs to be further investigated.

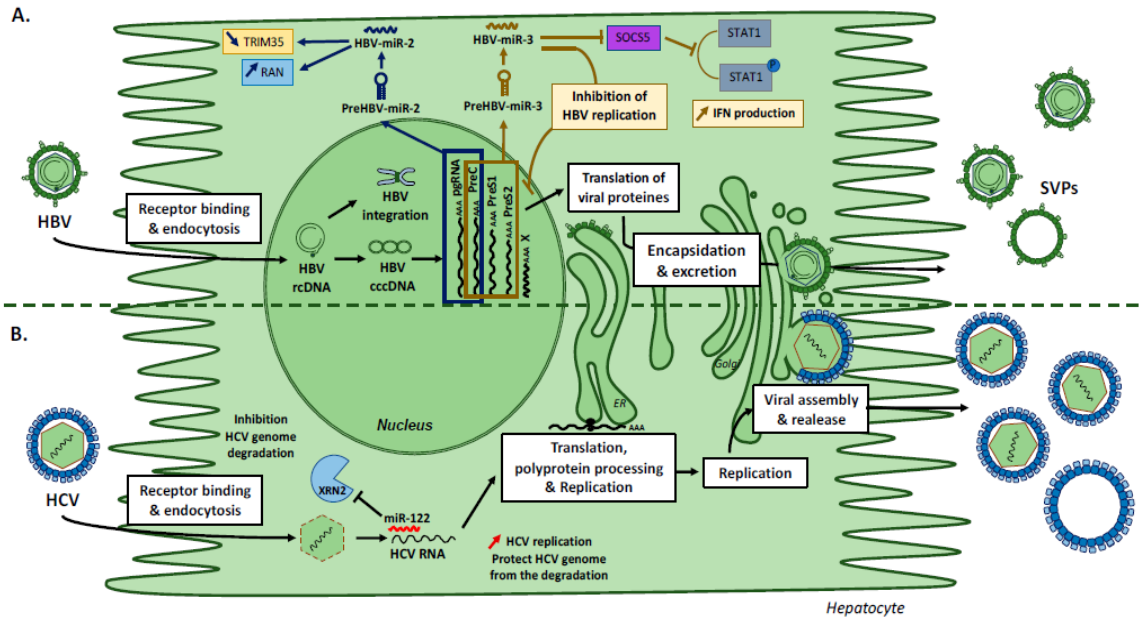

Figure 4:.

HBV-encoded miRNAs production and their targets (A), and modulation of HCV replication by miR-122 (B). (**A**) HBV encodes for two different miRNAs: HBV-miR-2 and HBV-miR-3. HBV-miR-2 is encoded from HBV pgRNA and HBV-miR-3 from PreC, PreS1 and PreS2 mRNAs. HBV-miR-2 down-regulates TRIM35 and up-regulates RAN expressions. HBV-miR-3 represses SOCS5 and PPM1A expression to increase interferons. Moreover, HBV-miR-3 regulates HBV core proteins (HBc) and the level of pgRNA. (**B**) Mode of actions of miR-122 on HCV replication and maintenance. miR-122 binds to HCV RNA genome and protect HCV against the degradation by exoribonucleases and promote HCV replication. HBV = Hepatitis B virus; HCV = Hepatitis C virus; TRIM35 = Tripartite motif-containing protein 35; PPM1A = Protein phosphatase 1A; RAN = Rasrelated Nuclear protein; rcDNA = relaxed circular DNA; cccDNA = covalently closed circular DNA; SOCS5 = Suppressor of cytokine signaling 5; STAT1 = Signal transducer and activator of transcription 1; SVPs = Subviral particles XRN2 = 5'- 3' exoribonuclease [76]. EBioMedicine **2019**, 48, 117–129

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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