Name of journal: *World Journal of Hepatology*

ESPS Manuscript NO: 14086

Columns: MINIREVIEWS

**Host cellular MicroRNA involvement in the control of *HBV* gene expression and replication**

Mizuguchi Y *et al*. MicroRNA in *HBV* gene expression and replication

Yoshiaki Mizuguchi, Toshihiro Takizawa, Eiji Uchida

**Yoshiaki Mizuguchi, Eiji Uchida,** Department of Surgery, Nippon Medical School Hospital, Bunkyo-Ku, Tokyo 113-8603, Japan

**Toshihiro Takizawa,** Department of Anatomy, Nippon Medical School, Bunkyo-Ku, Tokyo 113-8603, Japan

**Author contributions:** Takizawa T and Uchida E contributed equally to this work for generating the figures and revising the manuscript; Mizuguchi Y contributed to the writing of the manuscript.

**Conflict-of-interest:** The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers’ bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to: Yoshiaki Mizuguchi MD, PhD,** Department of Surgery, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-Ku, Tokyo 113-8603, Japan. yoshi1224@gmail.com

**Telephone:** +81-3-38222131

**Fax:** + 81-3-58146135

**Received:** September 17, 2014

**Peer-review started:** September 20, 2014

**First decision:** October 28, 2014

**Revised:** November 28, 2014

**Accepted:** Janurary 15, 2015

**Article in press:**

**Published online:**

**Abstract**

A large number of studies have demonstrated that the synergistic collaboration of a number of microRNAs, their growth factors and their downstream agents is required for the initiation and completion of pathogenesis in the liver. MicroRNAs are thought to exert a profound effect on almost every aspect of liver biology and pathology. Accumulating evidence indicates that several miRNAs are involved in the hepatitis B virus (HBV) life cycle and infectivity, in addition to HBV-associated liver diseases including fibrosis, cirrhosis and hepatocellular carcinoma (HCC). In turn, HBV can modulate the expression of several cellular miRNAs, thus promoting a favorable environment for its replication and survival. In this review, we focused on the involvement of host cellular microRNAs that are directly and indirectly associated with HBV RNA or HBV associated transcription factors. Exploring different facets of the interactions among miRNA, HBV and hepatitis C virus infections, and the carcinogenesis and progress of HCC, could facilitate the development of novel and effective treatment approaches for liver disease.

**Key words:** Hepatitis B virus; MicroRNA; Gene expression; Gene replication; Transcription

© The Author(s) 2015. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** A large number of studies have demonstrated that the synergistic collaboration of a number of microRNAs, their growth factors and their downstream agents is required for the initiation and completion of pathogenesis in the liver. MicroRNAs are thought to exert a profound effect on almost every aspect of biology and pathology. In this review, we focused on the microRNAs that play an important role in hepatitis B virus replication and gene expression, and summarized the involvement of host cellular microRNAs that are directly and indirectly associated with hepatitis B virus (HBV) RNA or HBV associated transcription factors.

Mizuguchi Y*,* Takizawa T, Uchida E. Host cellular microRNA involvement in the control of *HBV* gene expression and replication. *World J Hepatol* 2015; In press

**INTRODUCTION**

MicroRNAs (miRNAs) participate in crucial biological processes, including development, differentiation, apoptosis, and proliferation[[1](#_ENREF_1),[2](#_ENREF_2)], by either inhibiting target mRNA translation or inducing its degradation by pairing with complementary sequences within the 3'-untranslated regions (UTRs) of targeted transcripts at the post-transcriptional and/or translational level[3]. There are more than 2500 homo sapiens miRNAs (1881 precursors, 2588 mature; data taken from miRbase 21; <http://www.mirbase.org/>), each of which can influence hundreds of gene transcripts[4]. Several miRNAs can regulate a specific mRNA, engendering substantial complexity with respect to their capacity to modulate fundamental biological processes.

 MiRNAs are crucial in normal liver development[5] and biological processes, including liver differentiation, hepatocyte development, and many metabolic functions.

Recent studies have demonstrated that the profiling of miRNAs facilitates the classification of various tumors (whereas mRNA profiles are relatively inaccurate), which suggests that miRNAs possess a high potential for facilitating cancer diagnosis[6]. Another study using sequencing demonstrated that a set of miRNAs account for the majority of the differences in miRNA profiles among cell lineages and tissues[[7](#_ENREF_7" \o "Landgraf, 2007 #130)]. A large number of miRNAs are expected to be involved in critical aspects of liver physiology, because the liver serves as an endocrine and exocrine organ with numerous functions, including carbohydrate, lipid and amino-acid metabolism, urea synthesis, detoxification of drugs and toxic endogenous compounds, bile production and plasma protein secretion[8]. The dysregulation of tissue and serum microRNA expression has also been observed in the context of specific liver pathologies, including hepatitis, cirrhosis, and liver cancers, as well as chronic cholestasis[9].

MiRNAs are typically transcribed from genes in the nucleus by RNA polymerase II (Pol II) into initial transcripts of either monocistronic or polycistronic primary-miRNAs (pri-miRNAs; Figure 1). These pri-miRNAs are further processed *via* the canonical pathway (by Drosha-DGCR8)[[10](#_ENREF_9)] into hairpin-shaped precursor miRNAs (pre-miRNAs). These pre-miRNAs are exported *via* Exportin-5/RanGTP[[11](#_ENREF_11" \o "Lund, 2004 #66)] from the nucleus into the cytoplasm, where they undergo cleavage by the RNase III enzyme known as Dicer, as well as TBRP[[12](#_ENREF_11)], which produces an imperfect miRNA duplex. This duplex then splits, thereby generating a single-stranded mature miRNA that is loaded onto the RNA-induced silencing complex (RISC)[[12](#_ENREF_11)], which is then guided to the target mRNA through interactions with members of the Argonaute family. Due to complementarity with its target gene sequence, mature miRNA principally induces either translational repression or mRNA degradation[[13](#_ENREF_11" \o "Lund, 2004 #66)]. However, several reports have indicated that microRNAs and their associated protein complexes (micro-ribonucleoproteins or microRNPs) can also post-transcriptionally stimulate gene expression *via* direct and indirect mechanisms[[14,15](#_ENREF_11" \o "Lund, 2004 #66)]. miRNAs primarily regulate mRNAs by interacting with their 5' end (5p) and 3'-untranslated region (3'-UTR), although it has recently been suggested that the miRNA target sites may be located in the 5'-UTR or even at simultaneous 5'-UTR and 3'-UTR interaction sites[[16](#_ENREF_11)].

In the following section, we review recent advances in the understanding of the involvement of miRNA in the control of *HBV* gene expression and replication.

**HEPATITIS B VIRUS INFECTION**

The estimated number of new hepatocellular carcinoma (HCC) cases rose to 564300, with 548600 of these patients dying (*i.e.,* 97.2% of those receiving a diagnosis of HCC). The WHO estimates that 2 billion people worldwide have been infected with the hepatitis B virus (HBV), which represents the most common cause of HCC and, furthermore, that 350 million people have chronic-type HBV infection[[9](#_ENREF_11" \o "Lund, 2004 #66)]. Because the current therapeutic options for HCC patients are limited, there is an urgent need to analyze the molecular oncogenic mechanisms of HCC, to determine novel targets for specific systemic therapy and to detect novel biomarkers for early diagnosis. The biology and lifecycle of the HBV remains to be elucidated, but it is presumed to involve the following processes[[17](#_ENREF_11" \o "Lund, 2004 #66)]: (1) entry into hepatocytes *via* the fusion of viral and cellular membranes, such that the viral capsid is transported into the nucleus; (2) the HBV-relaxed circular genome is released into the nucleus and converted to 1-50 covalently closed circular DNA (cccDNA); (3) 3.2 kb cccDNA contains four major open reading frames of the pre-core/core gene, the polymerase gene, the *preS1/L-*, *preS2/M-*, and *Surface/S-* gene, and the *X* gene. The genes are transcribed into subgenomic RNA (sg RNA) and pregenomic RNA (pg RNA), hepatitis B virus surface antigen, hepatitis B virus core protein, viral reverse DNA polymerase, and X protein, as well as pregenomic RNA; (4) following nuclear export, the pgRNA is translated into the core protein and viral polymerase. The sgRNA is translated into regulatory X-protein and three envelope proteins; and (5) complex formation and reverse transcription of pgRNA produces an RNA-containing nucleocapsid. RNA-containing nucleocapsids mature into DNA-containing nucleocapsids within the cytoplasm.

Accumulating evidence indicates that several miRNAs are involved in the HBV life cycle and infectivity, in addition to HBV-associated liver diseases including fibrosis, cirrhosis and HCC. In turn, HBV can modulate the expression of several cellular miRNAs, thus promoting a favorable environment for its replication and survival. Several studies exploring the involvement of HBV in hepatocytes utilized HepG2.2.15 cells, derived from HepG2 and containing a stable transfected full-length HBV genome (ayw subtype), hepatitis B surface antigens (HBsAg) and hepatitis B e antigens (HBeAg), thereby supporting full HBV replication[[18](#_ENREF_11" \o "Lund, 2004 #66)].

**DIRECT INTERACTION BETWEEN HBV TRANSCRIPTS AND HOST miRNAs**

Although HBV is a DNA virus, its transcripts may be targeted and regulated by several cellular miRNAs similar to those of hepatitis C virus (HCV) (Table1). The direct interaction between HCV RNA and miR-122, which results in a stable heterotrimeric structure, enhances HCV translation and protects against HCV RNA degradation[[19](#_ENREF_11" \o "Lund, 2004 #66)]. Conversely, all of the interactions reported thus far between host cellular microRNAs, including miR-122, and HBV RNA transcripts inhibit HBV genome replication. Chen *et al*[[20](#_ENREF_11" \o "Lund, 2004 #66)] demonstrated that miR-122, a liver-specific miRNA, down-regulates *HBV* gene expression and replication, as determined using HBsAg and HBeAg. MiR-122 can inhibit *HBV* gene expression by interacting with the target sequence coding for nucleotides 2738-2760 and by targeting sequences located at the coding region of the mRNA for viral polymerase and the 3' UTR region for the core protein of the HBV genome, *via* base-pairing interactions. Chen *et al*[[20](#_ENREF_11" \o "Lund, 2004 #66)] also demonstrated an inverse linear relationship *in vivo* between miR-122 levels and viral load in the peripheral blood mononuclear cells of HBV-positive patients. Zhang *et al*[[21](#_ENREF_11" \o "Lund, 2004 #66)] demonstrated that miR-199a-3p and miR-210 effectively reduced HBsAg expression in HepG2 2.2.15 cells containing an integrated HBV genome. Bioinformatics analysis indicated a putative binding site for miR-199a-3p in the HBsAg coding region and a putative binding site for miR-210 in the HBV pre-S1 region21. Comparison of the expression levels of miR-199a-3p and miR-210 between HepG2 2.2.15 cells and the parent cell line (*i.e.*, HepG2 cells) revealed a 9-fold increase in miR-199a-3p and miR- 210 in HepG2 2.2.15 cells compared with HepG2 cells[[21](#_ENREF_11" \o "Lund, 2004 #66)]. Potenza *et al*[[22](#_ENREF_11" \o "Lund, 2004 #66)] reported that miR-125a-5p can interact with the HBV surface antigen and interfere with its expression, thus reducing the amount of HBsAg secreted. A recent study by Jung[[23](#_ENREF_11" \o "Lund, 2004 #66)] demonstrated that HBV infection transactivates c-Myc, after which it up-regulates the miR-17-92 cluster. Conversely, miR-20a and miR-92a can down-regulate HBV pregenomic RNA by directly targeting its *X* and polymerase gene, indicating that these miRNAs suppress HBV replication by creating a negative feedback loop. HBV X protein (HBx) plays a crucial role in the development of HCC by inducing epigenetic changes within host genetic and epigenetic architecture, including aberrations in DNA methylation, histone modifications, and microRNA expression[[24](#_ENREF_11" \o "Lund, 2004 #66)]. Zhang *et al*[[25](#_ENREF_11" \o "Lund, 2004 #66)] demonstrated that HBx can down-regulate miR-205, otherwise known as down-regulated miRNA in HCC, by inducing hypermethylation of the miR-205 promoter within cells. In turn, miR-205 suppresses HBx expression by directly targeting HBx mRNA. Kohno *et al*[[26](#_ENREF_11" \o "Lund, 2004 #66)] recently demonstrated that in HBV-transfected HepG2 cells, the overexpression of hsa-miR-1231 is associated with the suppression of HBV replication and HBV core reduction. The mechanism by which these interactions between host cellular miRNAs and HBV RNA transcripts affect infectivity could involve the maintenance of a suitable reduction in virus antigen level and virion production, thereby contributing to a persistent, chronic HBV infection or latent HBV state.

**CELLULAR miRNAs CONTROL HBV TRANSCRIPTION BY TARGETING TRANSCRIPTION FACTORS**

In addition to direct interactions, several miRNAs regulate HBV replication by targeting HBV-associated genes, including transcription factors (Table 2 and Figure2).

HBV contains a 3.2-kb partially double-stranded DNA genome with four promoters (*i.e.,* core, pre-S1, pre-S2/S, and X promoters) and two enhancer regions (ENI and ENII), which are involved in viral transcription regulation and thus play a central role in the control of HBV replication[[27](#_ENREF_11" \o "Lund, 2004 #66)]. The transcription of HBV cccDNA is tightly regulated according to epigenetic mechanisms, such as DNA methylation, acetylation, and histone modifications[[28](#_ENREF_11" \o "Lund, 2004 #66)]. HBV utilizes a number of ubiquitous and liver-enriched transcription factors, in addition to nuclear receptors in hepatocytes, to cause the efficient transcription of the *HBV* gene by binding to HBV promoter/enhancer elements[27]. The control of HBV during transcription thus influences both *HBV* gene expression and replication. Lu *et al*[[29](#_ENREF_11" \o "Lund, 2004 #66)] demonstrated that miR-1 enhanced HBV core promoter transcription activity by augmenting the expression of farnesoid X receptor alpha (FXRα), a liver-enriched transcription factor activated by bile acids. FXRα can function as a transcription factor by binding to the HBV enhancer II and core promoter in heterodimers with RXRα. Lu *et al*[[29](#_ENREF_11" \o "Lund, 2004 #66)] also demonstrated that miR-1 targets deacetylase 4 and E2F transcription factor 5, thus suppressing HBV replication. Liver-specific miR-122 is part of a complicated signaling network for HBV infectivity, through which it can down-regulate HBV replication as discussed above. For instance, the inhibition of miR-122 causes an increase in cellular heme oxygenase-1, which can decrease HBV covalently closed circular DNA (cccDNA) levels[[30](#_ENREF_11" \o "Lund, 2004 #66)]. Cyclin G1 is one target of miR-122 and is involved in the regulation of HBV replication. Wang *et al*[[31](#_ENREF_11" \o "Lund, 2004 #66)] demonstrated that interactions between cyclin G1 and p53 block the specific binding of p53 to HBV enhancer elements and simultaneously abrogate p53-mediated inhibition of HBV transcription. Fan *et al*[[32](#_ENREF_11" \o "Lund, 2004 #66)] reported an inverse correlation between the expression levels of miR-122 and NDRG3 (a member of the N-myc downstream-regulated gene) in HBV-related HCC specimens, which might accelerate HBeAg and HBsAg secretion and HBV DNA replication. miR-372/373 represses PRKACB and NFIB by targeting its 3”-UTR, resulting in reduced expression of HBV[[33,34](#_ENREF_11)]. PPKACB induces the phosphorylation of cAMP-response element binding protein (CREB) and dissociates CREB from its promoter. CREB is required for the expression of all HBV transcription units in the binding of viral enhancer I[[35](#_ENREF_11" \o "Lund, 2004 #66)]. NFIB is significantly down-regulated in HBV-associated liver cirrhosis (LC), compared with non-LC tissues, whereas pre-miR-372 is increased significantly in liver cirrhosis. Together, these independent findings confirm that HBV genomic NFI sites play important and complex roles in the regulation of HBV expression[[36-38](#_ENREF_11" \o "Lund, 2004 #66)]. Conversely, a recent study has reported that PPAR-γ and the HBV X protein (HBx) down-regulate miR-122 transcription. Dai X reported that miR-15b promotes HBV replication by augmenting HBV enhancer I activity *via* direct targeting of HNF1α, while HBV replication and antigen expression, particularly of the HBx protein, repress the expression of miR-15b[[39](#_ENREF_11" \o "Lund, 2004 #66)].

The miR-29 family members miR-29a, miR-29b, and miR-29c exert a suppressive action on tumors and are downregulated in several types of cancer, in which miR-29 directly targets DNA methyltransferase (DNMT)3A and -3B, two key enzymes involved in DNA methylation, in addition to methylation-silencing tumor suppressor genes, such as FHIT and WWOX[[40](#_ENREF_11)]. Wang *et al*[[41](#_ENREF_11" \o "Lund, 2004 #66)] reported that miR-29c functions as a tumor-suppressive gene by targeting TNFAIP3, a key regulator in inflammation and immunity. Furthermore, this interaction results in the suppression of HBV replication, as indexed by HBsAg/HBeAg secretion and HBV DNA replication.

Zhang *et al*[[42](#_ENREF_11" \o "Lund, 2004 #66)] used a miRNA microarray to assess miRNA expression during HBV infection *in vitro*. miR-125b expression was decreased in both HepG2-HBV1.3 (a HepG2 cell line transiently transfected with an HBV expression plasmid) and HepG2.2.15 cells. Furthermore, the ectopic expression of miR-125b inhibited HBV DNA intermediates and the secretion of HBsAg and HBeAg. miR-125b also reduced SCNN1A mRNA and protein levels. Using a dual luciferase assay, SCNN1A was shown to be one of the targets of miR-125b, indicating that miR-125b inhibits HBV expression by targeting the *SCNN1A* gene. These results suggest a potential role of miRNA in HBV infection.

The core promoters pre-S1 promoter, X promoter, ENI and ENII all contain a PPARα binding site; these regions are transactivated in the presence of RXRα and PPARα[[27](#_ENREF_11" \o "Lund, 2004 #66)], suggesting that PPARα likely plays a critical role in HBV biogenesis. miRNA 141, a member of the miR-200 family, plays a central role in EMT[[43](#_ENREF_11)]. Hu *et al*[[44](#_ENREF_11" \o "Lund, 2004 #66)] demonstrated that miR-141 can repress HBV replication effectively, and further that miR-141 inhibitor transfection precipitates a marked increase in HBsAg/HBeAg expression, which had no significant effect on HBV DNA replication, through direct targeting of the PPARα mRNA 3’UTR.

MiR-501 expression was significantly up-regulated in hepatocellular carcinoma tissues, in which the level of HBV replication remained high[[45](#_ENREF_11" \o "Lund, 2004 #66)]. Down-regulating miR-501 significantly inhibited HBV replication but did not influence the growth of HepG2.2.15 cells[[45](#_ENREF_11" \o "Lund, 2004 #66)]. Luciferase reporter and western blot assays revealed that HBXIP, an HBV replication inhibitor, is a potential target of miR-501[[45](#_ENREF_11" \o "Lund, 2004 #66)].

Although the association between miR-155 and HBV replication remains to be demonstrated, it has been reported that CCAAT/enhancer binding protein beta (C/EBP), which can bind to HBV promoters and enhancers, is one target of miR-155[[46](#_ENREF_11" \o "Lund, 2004 #66)].

**CONCLUSION AND FUTURE DIRECTIONS**

In this review, we focused on the role of miRNAs in *HBV* gene expression and replication. The available evidence suggests that several microRNAs mediate HBV RNA accumulation. miRNAs might have other functions specific to individual miRNAs, cell types or tissue environments and may also play a suppressive role in multi-target gene expression, thereby suggesting that miRNAs may serve as novel targets for therapeutic interventions. Despite progress in drug discovery and development, as evidenced by novel protease inhibitors and polymerase inhibitors, the clinical use of direct-acting antivirals is limited due to poor compliance and rapid-onset drug resistance. The use of imatinib mesylate, a platelet-derived growth factor receptor, and other tyrosine kinase inhibitors, as molecular targets against HCC progression, has been proposed[[47,48](#_ENREF_11" \o "Lund, 2004 #66)]; however, clinical trials have indicated a lack of efficacy using this approach[[49](#_ENREF_11)]. The development of more effective, cost efficient, and better-tolerated novel treatments is crucial to control the development of hepatitis, cirrhosis, and hepatocellular carcinoma. Exploring different facets of the interactions among miRNA, HBV and HCV infections, and the carcinogenesis and progression of HCC could facilitate the development of novel and effective treatment approaches for liver disease.

Although relatively poorly understood in the context of human cancers, evidence continues to accumulate indicating that long ncRNAs play a crucial role in regulating numerous developmental and biological genomic pathways[[5](#_ENREF_11" \o "Lund, 2004 #66)0].

**REFERENCES**

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

2 **Harfe BD**. MicroRNAs in vertebrate development. *Curr Opin Genet Dev* 2005; **15**: 410-415 [PMID: 15979303 DOI: 10.1016/j.gde.2005.06.012]

3 **Rajewsky N**. microRNA target predictions in animals. *Nat Genet* 2006; **38** Suppl: S8-13 [PMID: 16736023 DOI: 10.1038/ng1798]

4 **Lim LP**, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 2005; **433**: 769-773 [PMID: 15685193 DOI: 10.1038/nature03315]

5 **Si-Tayeb K**, Lemaigre FP, Duncan SA. Organogenesis and development of the liver. *Dev Cell* 2010; **18**: 175-189 [PMID: 20159590 DOI: 10.1016/j.devcel.2010.01.011]

6 **Lu J**, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. *Nature* 2005; **435**: 834-838 [PMID: 15944708 DOI: 10.1038/nature03702]

7 **Landgraf P**, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, Lin C, Socci ND, Hermida L, Fulci V, Chiaretti S, Foà R, Schliwka J, Fuchs U, Novosel A, Müller RU, Schermer B, Bissels U, Inman J, Phan Q, Chien M, Weir DB, Choksi R, De Vita G, Frezzetti D, Trompeter HI, Hornung V, Teng G, Hartmann G, Palkovits M, Di Lauro R, Wernet P, Macino G, Rogler CE, Nagle JW, Ju J, Papavasiliou FN, Benzing T, Lichter P, Tam W, Brownstein MJ, Bosio A, Borkhardt A, Russo JJ, Sander C, Zavolan M, Tuschl T. A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell* 2007; **129**: 1401-1414 [PMID: 17604727 DOI: 10.1016/j.cell.2007.04.040]

8 **Chen Y**, Verfaillie CM. MicroRNAs: the fine modulators of liver development and function. *Liver Int* 2014; **34**: 976-990 [PMID: 24517588 DOI: 10.1111/liv.12496]

9 **Mizuguchi Y**, Mishima T, Yokomuro S, Arima Y, Kawahigashi Y, Shigehara K, Kanda T, Yoshida H, Uchida E, Tajiri T, Takizawa T. Sequencing and bioinformatics-based analyses of the microRNA transcriptome in hepatitis B-related hepatocellular carcinoma. *PLoS One* 2011; **6**: e15304 [PMID: 21283620 DOI: 10.1371/journal.pone.0015304]

10 **Lau NC**, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. *Science* 2001; **294**: 858-862 [PMID: 11679671 DOI: 10.1126/science.1065062]

11 **Lund E**, Güttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. *Science* 2004; **303**: 95-98 [PMID: 14631048 DOI: 10.1126/science.1090599]

12 **Hutvágner G**, Zamore PD. A microRNA in a multiple-turnover RNAi enzyme complex. *Science* 2002; **297**: 2056-2060 [PMID: 12154197 DOI: 10.1126/science.1073827]

13 **He L**, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet* 2004; **5**: 522-531 [PMID: 15211354 DOI: 10.1038/nrg1379]

14 **Vasudevan S**, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. *Science* 2007; **318**: 1931-1934 [PMID: 18048652 DOI: 10.1126/science.1149460]

15 **Vasudevan S**. Posttranscriptional upregulation by microRNAs. *Wiley Interdiscip Rev RNA* 2012; **3**: 311-330 [PMID: 22072587 DOI: 10.1002/wrna.121]

16 **Lee I**, Ajay SS, Yook JI, Kim HS, Hong SH, Kim NH, Dhanasekaran SM, Chinnaiyan AM, Athey BD. New class of microRNA targets containing simultaneous 5'-UTR and 3'-UTR interaction sites. *Genome Res* 2009; **19**: 1175-1183 [PMID: 19336450 DOI: 10.1101/gr.089367.108]

17 **Urban S**, Schulze A, Dandri M, Petersen J. The replication cycle of hepatitis B virus. *J Hepatol* 2010; **52**: 282-284 [PMID: 20056291 DOI: 10.1016/j.jhep.2009.10.031]

18 **Sells MA**, Chen ML, Acs G. Production of hepatitis B virus particles in Hep G2 cells transfected with cloned hepatitis B virus DNA. *Proc Natl Acad Sci U S A* 1987; **84**: 1005-1009 [PMID: 3029758 DOI: 10.1073/pnas.84.4.1005]

19 **Li X,** Yang W, Ye W, Jin L, He J, Lou L. microRNAs: Novel players in hepatitis C virus infection. *Clin Res Hepatol Gastroenterol* 2014; **38**: 664-675 [PMID: 24875730 DOI: 10.1016/j.clinre.2014.04.008]

20 **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]

21 **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]

22 **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]

23 **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]

24 **Tian Y**, Yang W, Song J, Wu Y, Ni B. Hepatitis B virus X protein-induced aberrant epigenetic modifications contributing to human hepatocellular carcinoma pathogenesis. *Mol Cell Biol* 2013; **33**: 2810-2816 [PMID: 23716588 DOI: 10.1128/MCB.00205-13]

25 **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]

26 **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]

27 **Quasdorff M,** Protzer U. Control of hepatitis B virus at the level of transcription. *J Viral Hepat* 2010; **17**: 527-36 [PMID: 20546497 DOI: 10.1111/j.1365-2893.2010.01315.x]

28 **Liu WH**, Yeh SH, Chen PJ. Role of microRNAs in hepatitis B virus replication and pathogenesis. *Biochim Biophys Acta* 2011; **1809**: 678-685 [PMID: 21565290 DOI: 10.1016/j.bbagrm.2011.04.008]

29 **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]

30 **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]

31 **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]

32 **Fan CG**, Wang CM, Tian C, Wang Y, Li L, Sun WS, Li RF, Liu YG. miR-122 inhibits viral replication and cell proliferation in hepatitis B virus-related hepatocellular carcinoma and targets NDRG3. *Oncol Rep* 2011; **26**: 1281-1286 [PMID: 21725618 DOI: 10.3892/or.2011.1375]

33 **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]

34 **Wang J**, Liu X, Wu H, Ni P, Gu Z, Qiao Y, Chen N, Sun F, Fan Q. CREB up-regulates long non-coding RNA, HULC expression through interaction with microRNA-372 in liver cancer. *Nucleic Acids Res* 2010; **38**: 5366-5383 [PMID: 20423907 DOI: 10.1093/nar/gkq285]

35 **Kim BK**, Lim SO, Park YG. Requirement of the cyclic adenosine monophosphate response element-binding protein for hepatitis B virus replication. *Hepatology* 2008; **48**: 361-373 [PMID: 18615500 DOI: 10.1002/hep.22359]

36 **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 U S A* 2010; **107**: 264-269 [PMID: 20018759 DOI: 10.1073/pnas.0907904107]

37 **Shaul Y**, Ben-Levy R, De-Medina T. High affinity binding site for nuclear factor I next to the hepatitis B virus S gene promoter. *EMBO J* 1986; **5**: 1967-1971 [PMID: 3463507]

38 **Ben-Levy R**, Faktor O, Berger I, Shaul Y. Cellular factors that interact with the hepatitis B virus enhancer. *Mol Cell Biol* 1989; **9**: 1804-1809 [PMID: 2725524]

39 **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]

40 **Fabbri M**, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, Liu S, Alder H, Costinean S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chan KK, Marcucci G, Calin GA, Huebner K, Croce CM. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci U S A* 2007; **104**: 15805-15810 [PMID: 17890317 DOI: 10.1073/pnas.0707628104]

41 **Wang CM**, Wang Y, Fan CG, Xu FF, Sun WS, Liu YG, Jia JH. miR-29c targets TNFAIP3, inhibits cell proliferation and induces apoptosis in hepatitis B virus-related hepatocellular carcinoma. *Biochem Biophys Res Commun* 2011; **411**: 586-592 [PMID: 21763284 DOI: 10.1016/j.bbrc.2011.06.191]

42 **Zhang Z**, Chen J, He Y, Zhan X, Zhao R, Huang Y, Xu H, Zhu Z, Liu Q. miR-125b inhibits hepatitis B virus expression in vitro through targeting of the SCNN1A gene. *Arch Virol* 2014; **159**: 3335-3343 [PMID: 25173609 DOI: 10.1007/s00705-014-2208-y]

43 **Mizuguchi Y**, Isse K, Specht S, Lunz JG, Corbitt N, Takizawa T, Demetris AJ. Small proline rich protein 2a in benign and malignant liver disease. *Hepatology* 2014; **59**: 1130-1143 [PMID: 24123265 DOI: 10.1002/hep.26889]

44 **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/annotation/cbbe9454-0b72-44b3-a972-10dcaf22db68]

45 **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]

46 **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]

47 **Treiber G**, Wex T, Schleyer E, Troeger U, Hosius C, Malfertheiner P. Imatinib for hepatocellular cancer--focus on pharmacokinetic/pharmacodynamic modelling and liver function. *Cancer Lett* 2008; **260**: 146-154 [PMID: 18083304 DOI: 10.1016/j.canlet.2007.10.041]

48 **Höpfner M**, Schuppan D, Scherübl H. Growth factor receptors and related signalling pathways as targets for novel treatment strategies of hepatocellular cancer. *World J Gastroenterol* 2008; **14**: 1-14 [PMID: 18176955 DOI: 10.3748/wjg.14.1]

49 **Eckel F**, von Delius S, Mayr M, Dobritz M, Fend F, Hosius C, Schleyer E, Schulte-Frohlinde E, Schmid RM, Lersch C. Pharmacokinetic and clinical phase II trial of imatinib in patients with impaired liver function and advanced hepatocellular carcinoma. *Oncology* 2005; **69**: 363-371 [PMID: 16319507 DOI: 10.1159/000089990]

50 **Gupta RA**, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature* 2010; **464**: 1071-1076 [PMID: 20393566 DOI: 10.1038/nature08975]

**P-Reviewer:** De Minicis S, Sugimura H, Vespasiani-Gentilucci U

 **S-Editor:** Tian YL **L-Editor: E-Editor:**

**Table 1 Direct interactions of miRNAs with hepatitis C virus and hepatitis B virus RNA**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Host** **miRNA** | **Binding location** | **Function** | **Expression** | 　**Ref.** |
| miR-20a | X and polymerase | Inhibits HBV replication | up | [23](#_ENREF_23) |
| miR-92a-1 | X and polymerase | Inhibits HBV replication | up | [23](#_ENREF_23) |
| miR-122 | Core and DNA polymerase | Inhibits HBV replication | down | [20](#_ENREF_20) |
| miR-125a-5p | HBsAg | Inhibits HBV replication | up | [22](#_ENREF_22) |
| miR-199a- 3p | HBsAg | Inhibits HBV replication | up | 　[21](#_ENREF_21) |
| miR-205 | HBx | Suppresses HBx production | down | [25](#_ENREF_25) |
| miR-210 | PreS1 | Inhibits HBV replication | up | [21](#_ENREF_21) |
| miR-1231 | HBx/HB core  | Inhibits HBV replication | up | [26](#_ENREF_26) |

HBsAg: Hepatitis B surface antigens; HBeAg: Hepatitis B e antigens; HBV: Hepatitis B virus; HBx: HBV X.

**Table 2 miRNAs that control *HBV* gene replication and expression through cellular targets**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **MiRNAs** | **Cellular targets** | **Effects on HBV biology or pathogenesis** | **Expression** | **Ref.** |
| miR-1 | HDAC4/E2F5 | Increases HBV replication by augmenting FXRA activity | down | [29](#_ENREF_29) |
| miR-15b | HNF1α | Promotes HBV replication and expression of HBV antigens, including HBx protein | down | [39](#_ENREF_39) |
| miR-29c | TNFAIP3 | Suppresses HBV DNA replication and cell proliferation and inhibits apoptosis of HCC | down(HCC) | [41](#_ENREF_41) |
| miR-122 | Cyclin G1 | Suppresses HBV replication | down | [31](#_ENREF_31) |
|  | (HO-1) | Promotes HBV expression by inhibiting HO-1 expression |  | [30](#_ENREF_30) |
|  | NDRG3 | Inhibits viral replication and HBV HCC proliferation |  | [32](#_ENREF_32) |
| miR-125b | SCNN1A | Inhibits HBV expression | down | [42](#_ENREF_42) |
| miR-141 | PPARA | Suppresses HBV replication by inhibiting PPARA-HBV promoter interaction |  | [44](#_ENREF_49) |
| miR-155 | C/EBP | Elevates HCC levels/promotes HCC cell growth | up | [46](#_ENREF_44) |
| miR-372/373 | NFIB  | Promotes HBV replication | up | [33](#_ENREF_33) |
| upmiR-372 | CREB | Promotes HBV replication |  | [34](#_ENREF_34) |
| miR-501 | HBIP (HBx inhibitor) | Promotes HBV replication | up | [45](#_ENREF_50) |

HBV: Hepatitis B virus; HBx: HBV X; CREB: cAMP-response element binding protein; C/EBP: CCAAT/enhancer binding protein beta; HCC: Hepatocellular carcinoma; HNF1α: Human hepatocyte nuclear factor 1 α.

**Figure 1 Schematic illustration of microRNA biogenesis.** mRNA: Messenger RNA; RISC: RNA-induced silencing complex; AGO: Argonaute; Pre-miRNA: Premature-microRNA; Pri-miRNA: Primary-microRNA; RNA pol II: RNA polymerase II.



**Figure 2 Summary of the effect of miRNAs on *HBV* transcription.** Binding sites of ubiquitous and hepatocyte-enriched transcription factors within *HBV* promoter and enhancer regions as well as miRNAs that can modulate target transcription factors are indicated. The various *HBV* promoter and enhancer sites are schematically depicted as boxes.

