Hepatitis B virus pathogenesis: Fresh insights into hepatitis B virus RNA

Kazuma Sekiba, Motoyuki Otsuka, Motoko Ohno, Mari Yamagami, Takahiro Kishikawa, Tatsunori Suzuki, Rei Ishibashi, Takahiro Seimiya, Eri Tanaka, Kazuhiko Koike

Kazuma Sekiba, Motoyuki Otsuka, Motoko Ohno, Mari Yamagami, Takahiro Kishikawa, Tatsunori Suzuki, Rei Ishibashi, Takahiro Seimiya, Eri Tanaka, Kazuhiko Koike, Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo 113-8655, Japan

ORCID number: Kazuma Sekiba (0000-0002-9429-1974); Motoyuki Otsuka (0000-0003-2869-2881); Motoko Ohno (0000-0002-3437-3468); Mari Yamagami (0000-0002-1533 -8245); Takahiro Kishikawa (0000-0002-8602-5916); Tatsunori Suzuki (0000-0002-2344-7732); Rei Ishibashi (0000-0002 -9649-6471); Takahiro Seimiya (0000-0003-1698-030); Eri Tanaka (0000-0001-5823-0472); Kazuhiko Koike (0000-0002-9739-9243).

Author contributions: Sekiba K, Otsuka M and Ohno M wrote the manuscript; Yamagami M, Kishikawa T, Suzuki T, Ishibashi R, Seimiya T, and Tanaka E prepared the figures; Koike K supervised the entire project.

Supported by the Research Program on Hepatitis from Japan Agency for Medical Research and Development, AMED to Otsuka M, No. JP18fk0210214; and the Project for Cancer Research and Therapeutic Evolution (P-CREATE) from AMED to Otsuka M, No. JP19cm0106602.

Conflict-of-interest statement: No potential conflicts of interest. No financial support.

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/

Manuscript source: Invited manuscript

Correspondence to: Motoyuki Otsuka, MD, PhD, Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. otsukamo-tky@umin.ac.jp Telephone: +81-3-58008812

Fax: +81-3-38140021

Received: March 27, 2018
Peer-review started: March 28, 2018
First decision: April 19, 2018
Revised: April 24, 2018
Accepted: April 26, 2018
Article in press: April 26, 2018
Published online: June 7, 2018

Abstract

Hepatitis B virus (HBV) is still a worldwide health concern. While divergent factors are involved in its pathogenesis, it is now clear that HBV RNAs, principally templates for viral proteins and viral DNAs, have diverse biological functions involved in HBV pathogenesis. These functions include viral replication, hepatic fibrosis and hepatocarcinogenesis. Depending on the sequence similarities, HBV RNAs may act as sponges for host miRNAs and may deregulate miRNA functions, possibly leading to pathological consequences. Some parts of the HBV RNA molecule may function as viral-derived miRNA, which regulates viral replication. HBV DNA can integrate into the host genomic DNA and produce novel viral-host fusion RNA, which may have pathological functions. To date, elimination of HBV-derived covalently closed circular DNA has not been achieved. However, RNA transcription silencing may be an alternative practical approach to treat HBV-induced pathogenesis. A full understanding of HBV RNA transcription and the biological functions of HBV RNA may open a new avenue for the development of novel HBV therapeutics.

Key words: Hepatitis B virus; Hepatitis B virus RNA; MicroRNA; Smc5/6; Viral replication; Hepatic fibrosis; Genome integration; Hepatocellular carcinoma

© The Author(s) 2018. Published by Baishideng Publishing
Core tip: Recently, it has been shown that hepatitis B virus (HBV) RNAs have diverse biological functions in the pathogenesis of HBV. HBV RNAs may work as sponges for host miRNAs and deregulate miRNA functions. Novel viral-host fusion RNA may be produced from HBV-DNA integration sites, which may also have pathological functions. Understanding HBV RNA transcription and the biological functions of HBV-related RNAs may open a new avenue for the development of novel HBV therapeutics that target HBV RNAs.

INTRODUCTION
Hepatitis B virus (HBV) is a small enveloped DNA virus that belongs to the Hepadnaviridae virus family. HBV may establish a chronic infection in the liver, which can, in turn, lead to cirrhosis and hepatocellular carcinoma (HCC). Although HBV has infected humans for at least 500 years[1], the virus was not discovered until 1966[2], and in 1970 Dane et al[3] identified the virus particle by electron microscopy. Since then, an antiviral therapy has been developed; these anti-HBV drugs are nucleos(t)ide analogs that can sufficiently suppress viral DNA load in most cases[4-9]. Moreover, vaccination programs have already been established to prevent HBV infection[10]. However, these are not sufficient to eradicate HBV. In fact, an estimated 257 million people are still chronically infected, and 887 thousand people die annually, primarily from the complications of HBV, which include cirrhosis and HCC[11-13].

Recently, RNAs, especially non-coding RNAs, have been revealed to have diverse functions[14]. We and others previously reported that viral RNAs not only work as templates for protein synthesis and viral DNA replication in the case of HBV but also exhibit biological functions involved in its pathogenesis[15,16]. In this context, even when HBV DNA is maintained at a relatively low level by nucleos(t)ide analogs, viral RNAs alone may harm the host, leading to cirrhosis or HCC. Thus, understanding the functions of HBV RNAs may act as a platform for the future development of HBV therapeutics. In this paper, we review current knowledge on the biological impact of HBV RNAs on host cells.

THE PROCESS OF HBV-RNA TRANSCRIPTION
The HBV genome has four overlapping open reading frames: 3.5 kb pre-C/C or pre-genomic RNA (pgRNA), 2.4 kb pre-S, and 0.7 kb X mRNA (Figure 1). Viral particles with a 3.2-kb-long partially double-stranded relaxed circular DNA (rcDNA) genome invade the cell through the sodium taurocholate co-transporting polypeptide (NTCP) receptor. After un-coating of surface antigen, the core particles transport the genome to the hepatocyte nucleus. Then, covalently closed circular DNA (cccDNA) is molded from rcDNA. The cccDNA plays a role as a template in the transcription of HBV RNA (Figure 2)[17].

The viral genes are transcribed by the cellular RNA polymerase II from cccDNA. Two enhancers designated enhancer I (EnhI) and enhancer II (EnhII) have been identified in the HBV genome, which drive and regulate the expression of the complete viral transcripts[18]. Moreover, recently, various host proteins were revealed to be involved in the process of HBV RNA transcription from cccDNA, and the most representative host proteins are structural maintenance of chromosomes (Smc) proteins Smc5 and Smc6. Because Smc5/6 inhibit HBV RNA transcription from cccDNA, the efficient transcription of HBV RNA from cccDNA requires the degradation of Smc5/6. HBV regulatory protein X (HBx) hijacks the host Cullin 4-ROC1 RING E3 ubiquitin ligase (CRL4) complex to target Smc5/6 co-localized with nuclear domain 10 (ND10) for ubiquitination, which, in turn, promotes HBV transcription[19-21]. Thus, the existence of HBV RNAs means the degradation of Smc5/6. Because Smc5/6 is related to DNA repair[22], this degradation may eventually lead to carcinogenesis. Therefore, this ubiquitination pathway has strong potential as a novel therapeutic target in interventions for HBV pathogenesis.

HBV RNAS MAY DEREGLULATE THE FUNCTION OF HOST MICRO RNAS
MicroRNAs (miRNAs) are short, single-stranded, non-coding RNAs. Mature miRNAs are recruited into the Ago2-related RNA-induced silencing complex (RISC) and act to suppress the gene expression of target mRNAs. Depending on the target mRNA, miRNAs are responsible for various biological functions[23]. Recent studies have shown that HBV RNAs have several regions complementary to miRNAs, and act as miRNA sponges to upregulate the expression of miRNA targets; this results in the induction of HBV pathogenesis[15,24]. A list of miRNAs that could be trapped by HBV RNAs and may be involved in HBV pathogenesis is shown in Figure 3. In the following paragraphs, we discuss the potential biological roles of miRNAs in HBV pathogenesis.

PROMOTING VIRAL REPLICATION BY HBV RNAS
Although our knowledge of the direct relationship between HBV RNAs and viral replication are limited, HBV RNAs may promote viral replication via sequestering
cellular miRNAs, such as miR-122 and miR-15 family\textsuperscript{[25,26]}.  

\textbf{miR-122}  

miR-122 is highly and specifically expressed in hepatocytes. It plays multiple roles in the control of lipid metabolism, iron homeostasis, and the circadian rhythm and has anti-inflammatory and anti-tumorigenic functions\textsuperscript{[27-31]}. The expression level of miR-122 is decreased in HBV-producing cells and in liver tissue from chronic hepatitis B patients\textsuperscript{[25,32,33]}. Furthermore, there is an inverse correlation between miR-122 expression level and HBV replication\textsuperscript{[32]}. Previously, it was found that the expression levels of pri-miR-122 and pre-miR122, the precursors of miR-122, were not decreased in HBV-positive HCC tissues and cells compared to normal liver tissue and cells\textsuperscript{[25,34,35]}. Therefore, the downregulation of mature miR-122 expression is thought to be the result of binding to a conserved sequence at the 3’ end of all HBV transcripts following degradation. Although the precise mechanisms remain to be clarified, viral non-coding RNAs may play a critical role modulating the turnover of host miRNAs through the degradation of target miRNAs\textsuperscript{[36-40]}. miR-122 negatively regulates HBV replication. It has been reported that one possible mechanism mediating the negative regulation of HBV replication by miR-122 depends on the expression level of cyclin G1, a target of miR-122. Decreased expression or function of miR-122 would result in the suppression of p53 through upregulation of cyclin G1 expression, which further increases HBV transcription by blocking specific binding of p53 to HBV enhancer elements\textsuperscript{[33]}.

\textbf{miR-15 family}  

The miR-15 family is also reported to regulate HBV replication. For instance, HBV RNA can sequester miR-15a and miR-16-1, and overexpression of these miRNAs decreases viral replication. Although the direct molecular mechanism of miR-15 family members has not been fully elucidated, among the multiple targets of miR-15a and miR16-1, cyclin D1 is thought to be involved in the regulation. Specifically, the up-regulation of cyclin D1 was demonstrated to be required for HBV replication\textsuperscript{[36]}.  

\textbf{HBV-encoded miRNA (HBV-miR-3)}  

Yang \textit{et al}\textsuperscript{[41]} recently showed that HBV-encoded HBV-
miR-3 was expressed in HBV-infected tissues and cells. The viral-derived miRNA targeted the 3.5-kb HBV transcript to reduce HBc protein and pgRNA/HBV-RI production. The inhibition of HBV replication was suggested to contribute to the development of persistent infection in chronic hepatitis B patients. However, there is insufficient direct evidence for this mechanism, and, therefore, further studies are warranted.

**PROMOTING HEPATIC FIBROSIS BY HBV RNAs**

Liver fibrosis underlies the majority of chronic liver diseases and is a precursor to cirrhosis and HCC. The cycle of liver damage and repair leads to the deposition of extracellular matrix proteins and the development of fibrosis. Some miRNAs, such as miR-21, miR-221/222 and miR-181b, cause liver fibrosis through deregulation of the transforming growth factor-β (TGF-β) or nuclear factor-κB (NF-κB) pathways. On the other hand, miR-29b, miR-101, miR-122, and miR-214-3p inhibit fibrosis by blocking collagen synthesis or the TGF-β pathway. Among these miRNAs, miR-122 was reported to have complementary lesion(s) in HBV RNAs. As previously mentioned, miR-122 is highly expressed in the healthy liver, but is downregulated in HBV-infected livers via sequestration by HBV RNA. This change in miR-122 expression leads to the development of liver fibrosis through the activation of collagen synthesis via the TGF-β pathway.

**PROMOTION OF CARCINOGENESIS BY HBV RNAs**

HBV is the leading risk factor for the development of HCC worldwide. Many mechanisms have been reported to lead to the development of HCC, and one such mechanism involves the sequestration of host miRNAs by HBV RNA.

**miR-122**

Decreased miR-122 levels resulted in increased pituitary...
tumor transforming gene 1 (PTTG1)-binding factor (PBF) expression, which enhanced the proliferation and invasiveness of HCC in vitro and tumorigenicity in vivo, through PBF-mediated activation of the PTTG1 transcription factor. The possible contribution of these mechanisms to HBV-related carcinogenesis should be further examined in studies on human samples.

let-7 family
miRNAs in the let-7 family are classified as putative tumor suppressor miRNAs. The expression level of this family of miRNAs is often decreased in human cancers, including HCC, and promotes transformation by suppressing oncogenic targets, such as LIN28B, HMGA2 and c-Myc. Studies conducted by our group and others found that let-7 family miRNAs (e.g., let-7g and let-7a) could be sequestered by HBV-RNA. Furthermore, we demonstrated that this functional downregulation could lead to the promotion of tumorigenesis.

miR-199a-3p
miR-199a-3p is also involved in carcinogenesis and contributes to the malignant potential of HCC. Indeed, downregulation of miR-199a-3p correlated with poor HCC patient survival. This miRNA targets mammalian target of rapamycin (mTOR) and c-Met in HCC cells. The restoration of miR-199a-3p levels in HCC cells resulted in G(1)-phase cell cycle arrest, decreased invasive capability, enhanced susceptibility to hypoxia, and increased sensitivity to doxorubicin-induced apoptosis.

miR-15a
miR-15a can be sponged off by HBV miRNAs. One of the proposed targets of miR-15a is Smad7, an inhibitor of the TGF-β pathway. Thus, HBV mRNA can interfere with TGF-β signaling by upregulating Smad7 expression, which obstructs TGF-β-induced apoptosis and promotes tumor development.

HBV DNA may promote carcinogenesis
HBV DNA can integrate into host chromosomes at various locations. Integrated HBV DNA lacks the ability to transcribe pgRNA because HBV double-stranded linear DNA is only ~16 nt longer than the length of the genome, making it too short to transcribe pgRNA. Despite this, integrated HBV DNA levels correlate with the development of HCC. Indeed, the majority of HBV-related HCCs contain at least one HBV genome integration site. While the mechanism of carcinogenesis induced by the integration of the HBV genome has been explained in several ways, virus-related RNAs from the integration sites are definitely involved.

HBx-long interspersed nuclear element 1
HBV DNA integration often occurs within or near repetitive, non-coding sequences, such as long interspersed nuclear element 1 (LINEs) and short interspersed nuclear elements (SINES). By applying Viral-Fusion-Seq to detect possible fusions between viral and human sequences, a viral-human hybrid RNA transcript called HBx-LINE1 was identified in HBV-related HCCs. The presence of this long non-coding RNA, a fusion of the...
human LINE1 and HBx genes, was correlated with poor prognosis in HCC patients\(^{54}\).

HBx-LINE1 contains six binding sites for miR-122, which enable the chimeric HBx-LINE1 transcript to act as a molecular sponge for miR-122. This sequestration leads to an increase in hepatic cell β-catenin signaling, a decrease in E-cadherin levels, increased cell migration, and significant mouse liver injury, leading to HCC\(^{35}\). Therefore, HBx-LINE1 is a potential therapeutic target and prognostic biomarker for HCC. While this is an interesting result, further studies are needed to uncover the precise mechanism of oncogenesis.

**HBV-cyclin A2**

Cyclin A2 (CCNA2) is a cell cycle regulatory protein that acts as a regulatory subunit of cyclin-dependent kinase\(^{55}\). Integration of HBV into the CCNA2 gene has been observed in HBV-positive HCCs\(^{56}\). The integration site is intron 2 of CCNA2, which results in the formation of a new splice site in the pre-mRNA. This new splice site leads to the formation of a 177-bp in-frame pseudo-exon and produces a novel and recurrent HBV-CCNA2 fusion transcript, A2S\(^{56}\). Disruption of the destruction box of A2S causes A2S to become non-degradable; however, the function enhancing cell cycle progression of CCNA2 is retained, which demonstrates its potential role in hepatocarcinogenesis.

**CONCLUSION**

HBV RNAs are not only templates for protein synthesis and viral DNA replication but also exhibit biological functions that play a role in pathogenesis. Because current therapies are unable to solve this problem, novel therapeutic agents that target the cccDNA itself, or inhibit its transcription, are strongly warranted.

**REFERENCES**

1. Patterson Ross Z, Klunk J, Fornaciari G, Giuffra V, Duchêne S, Duggan AT, Poinar D, Douglas MW, Eden JS, Holmes EC, Poinar HN. The paradox of HBV evolution as revealed from a 16th century mummy. *PLoS Pathog* 2018; 14: e1006750 [PMID: 29300782 DOI: 10.1371/journal.ppat.1006750]
2. Alter HJ, Blumberg BS. Further studies on a "new" human isoprecipitin system (Australia antigen). *Blood* 1966; 27: 297-309 [PMID: 5930797]
3. Dane DS, Cameron CH, Briggs M. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. *Lancet* 1970; 1: 695-698 [PMID: 4190997 DOI: 10.1016/S0140-6736(70)90926-8]
4. Kim GA, Lim YS, An J, Lee D, Shim JH, Kim KM, Lee HC, Chung YH, Lee YS, Suh DJ. HBsAg seroclearence after nucleoside analogue therapy in patients with chronic hepatitis B: clinical outcomes and durability. *Gut* 2014; 63: 1325-1332 [PMID: 2416293] DOI: 10.1136/gutjnl-2013-305517
5. Gao Y, Feng J, Yang G, Zhang S, Liu Y, Bu Y, Sun M, Zhao M, Chen F, Zhang W, Ye L, Zhang X. Hepatitis B virus X protein- elevated MSL2 modulates hepatitis B virus covalently closed circular DNA by inducing degradation of APOBEC3B to enhance hepatocarcinogenesis. *Hepatology* 2017; 66: 1413-1429 [PMID: 28608964 DOI: 10.1002/hep.29316]
6. Ono A, Suzuki F, Kawamura Y, Sezaki H, Hosaka T, Akuta N, Kobayashi M, Suzuki Y, Saitou S, Arase Y, Ikeda K, Kobayashi M, Watabaki S, Mineta R, Kumada H. Long-term continuous entecavir therapy in nucleus(t)ide-naïve chronic hepatitis B patients. *J Hepatol* 2012; 57: 508-514 [PMID: 22659518 DOI: 10.1016/j.jhep.2012.04.037]
7. Chang TT, Lai KL, Kew Yoon S, Lee SS, Coelho HS, Carrilho FJ, Poordad F, Halota W, Horsmans Y, Tsai N, Zhang H, Tenney DJ, Tamez R, Iloeje U. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. *Hepatology* 2010; 51: 422-430 [PMID: 20497553 DOI: 10.1002/hep.23327]
8. Heathcote EJ, Marcellin P, Buti M, Gane E, De Man RA, Krastev Z, Germandis G, Lee SS, Flisiak R, Kaita K, Manns M, Kotzev I, Tchernev K, Buggisch P, Weilert F, Shiffman ML, Trinh H, Garell S, Snow-Lampart A, Borroto-Esoda K, Mondou E, Anderson J, Sorbel J, Rousseau F. Three-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B. *Gastroenterology* 2011; 140: 132-143 [PMID: 20955704 DOI: 10.1053/j.gastro.2010.10.011]
9. Buti M, Gane E, Seto WK, Chan HL, Chuang WL, Stepanova T, Hui AJ, Lim YS, Mehta R, Janssen HL, Acharya SK, Flaherty KM, Massetto B, Cathcart AL, Kim K, Gagger K, Subramanian GM, McHutchison JG, Pan CQ, Brunetto M, Izumi N, Marcellin P, GS-US-320-0108 Investigators. Tenofovir alafenamide versus tenofovir disoproxil fumarate for tenofovir-naive chronic hepatitis B: a randomised, double-blind, phase 3, non-inferiority trial. *Lancet* 2014; 384: 196-206 [PMID: 25804092 DOI: 10.1016/S0140-6736(14)60107-8]
10. Fisman DN, Agravat D, Leder K. The effect of age on immunologic response to recombinant hepatitis B vaccine: a meta-analysis. *Clin Infect Dis* 2002; 35: 1368-1375 [PMID: 12439800 DOI: 10.1086/344271]
11. Nelson PK, Mathers BM, Cowie B, Hagan H, Des Jarlais D, Horyniak D, Degenhardt L. Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. *Lancet* 2011; 378: 571-583 [PMID: 21802134 DOI: 10.1016/S0140-6736(11)61097-0]
12. Degenhardt L, Charlson F, Stanaway J, Larney S, Alexander
September 1, 2018

1. LT, Hickman M, Cowie B, Hall WD, Strang J, Whiteford H, Vos T. Estimating the burden of disease attributable to injecting drug use as a risk factor for HIV, hepatitis C, and hepatitis B: findings from the Global Burden of Disease Study 2013. *Lancet Infect Dis* 2016; 16: 1385-1398 [PMID: 27665254 DOI: 10.1016/S1473-3099(16)30325-5]

2. World Health Organization. Global hepatitis report, 2017. Geneva. 2017: 1-68

3. Otsuka M, Kishikawa T, Yoshikawa T, Yamagami M, Ohno M, Takata A, Shibata C, Ishibashi R, Koike K. MicroRNAs and liver disease. *J Hum Genet* 2017; 62: 73-80 [PMID: 27225582 DOI: 10.1038/jhg.2016.53]

4. Takata A, Otsuka M, Ohno M, Kishikawa T, Yoshikawa T, Koike K. Novel therapeutic approaches for hepatitis B virus covalently closed circular DNA. *World J Gastroenterol* 2015; 21: 7084-7088 [PMID: 26197995 DOI: 10.3748/wjg.v21.i23.7084]

5. Doitsch G, Shad Y. Enhancer I predominance in hepatitis B virus gene expression. *Mol Cell Biol* 2004; 24: 1799-1808 [PMID: 14749394 DOI: 10.1128/MCB.24.4.1799-1808.2004]

6. Murphy CM, Xu Y, Li F, Nio K, Resaka-Blanco N, Li X, Wu Y, Yu Y, Xiong S. Hepatitis B Virus X Promote Proteins Degradation of SMCS-6 to Enhance HBV Replication. *Cell Rep* 2016; 16: 2846-2854 [PMID: 27626656 DOI: 10.1016/j.celrep.2016.08.026]

7. Decorsière A, Mueller H, van Breugel PC, Abdul F, Gerossier L, Beran RK, Livingston CM, Niu C, Fletcher SP, Hantz O, Strubin M. Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor. *Nature* 2016; 531: 386-389 [PMID: 26983541 DOI: 10.1038/nature17170]

8. Niu C, Livingston CM, Li L, Beran RK, Daffis S, Ramakrishnan D, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005; 120: 15-20 [PMID: 15652477 DOI: 10.1016/j.cell.2004.12.035]

9. Deng M, Hou J, Hu J, Wang S, Chen M, Chen L, Ju Y, Li C, Meng S. Hepatitis B virus microRNAs functionally sequester let-7a and enhance hepatocellular carcinoma. *Cancer Lett* 2016; 383: 62-72 [PMID: 27693636 DOI: 10.1016/j.canlet.2016.09.028]

10. Li C, Wang Y, Wang S, Wu B, Yao J, Fan H, Ju Y, Ding Y, Chen L, Chu X, Liu W, Ye X, Meng S. Hepatitis B virus microRNA-mediated miR-122 inhibition upregulates PTTG1-binding protein, which promotes hepatocellular carcinoma tumor growth and cell invasion. *J Viral* 2013; 87: 2193-2205 [PMID: 23221562 DOI: 10.1128/JVI.02881-12]

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

12. Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Peer M, Watts L, Button SL, Graham M, McKay R, Subramaniam A, Propp S, Lollo BA, Freier S, Bennett CF, Bhanoit S, Monia BP. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metab* 2006; 3: 87-98 [PMID: 16459310 DOI: 10.1016/j.cmet.2006.01.005]

13. Castoldi M, Vasquez J, Altamura S, Elnem J, Lindow M, Kiss J, Stolte J, Srapla R, D’Alessandro L, Klimm, Müller E, Fleming ER, Longerich T, Gröne HJ, Benes V, Kauppinen S, Hentze MW, Muckenthaler MU. The liver-specific microRNA miR-122 controls systemic iron homeostasis in mice. *J Clin Invest* 2011; 121: 1386-1396 [PMID: 21364282 DOI: 10.1172/JCI44883]

14. Galiffi D, Ferretti A, Maesani M, Gialinto F, Cavallini G, Grey F, Tollervey D, Buck AH. Murine cytomegalovirus encodes a miR-27 inhibitor disguised as a target. *PLoS Pathog* 2012; 8: e1002510 [PMID: 22346748 DOI: 10.1371/journal.ppat.1002510]

15. Lee S, Song J, Kim S, Kim J, Hong Y, Kim Y, Kim D, Baek D, Ahn K. Selective degradation of host MicroRNAs by an intergenic HCMV noncoding RNA accelerates virus production. *Cell Host Microbe* 2013; 13: 678-690 [PMID: 23768492 DOI: 10.1016/j.chom.2013.05.007]

16. Yang X, Li H, Sun H, Fan H, Yu Y, Liu M, Li X, Tang H, Hepatitis B
Sekiba K et al. Fresh insights into HBV RNA

Virus-Encoded MicroRNA Controls Viral Replication. J Virol 2017; 91: pii: e01919-16 [PMID: 28148795 DOI: 10.1128/JVI.01919-16]

42 Zhang J, Jiao J, Cermelli S, Muir K, Jung KH, Zou R, Rashid A, Gagea M, Zabludoff S, Kalluri R, Beretta L. mir-21 Inhibition Reduces Liver Fibrosis and Prevents Tumor Development by Inducing Apoptosis of CD24+ Progenitor Cells. Cancer Res 2015; 75: 1859-1867 [PMID: 25769721 DOI: 10.1158/0008-5472. CAN-14-1254]

43 Ogawa T, Enomoto M, Fuji H, Sekiya Y, Yoshizato K, Ikeda K, Kawada N. MicroRNA-221-222 upregulation indicates the activation of stellate cells and the progression of liver fibrosis. Gut 2012; 61: 1600-1609 [PMID: 22267590 DOI: 10.1136/gutjnl-2011-300717]

44 Wang B, Li W, Guo K, Xiao Y, Wang Y, Fan J. mir-181b promotes hepatic stellate cells proliferation by targeting p27 and is elevated in the serum of cirrhosis patients. Biochem Biophys Res Commun 2012; 421: 4-8 [PMID: 22446332 DOI: 10.1016/j.bbrc.2012.03.025]

45 Wang J, Chu ES, Chen HY, Man K, Go MY, Huang XR, Deng Y, Sun T, Ching AK, He M, Li JW, Wong AM, Co NN, Chan TF, Wong N. ViralFusionSeq: accurately discover viral integration events and reconstruct fusion transcripts at single-base resolution. Bioinformatics 2013; 29: 649-651 [PMID: 23143233 DOI: 10.1093/bioinformatics/btt011]

46 Xie Y, Yao Q, But AM, Guo J, Tian Z, Bao X, Li H, Meng Q, Lu J. Expression profiling of serum microRNA-101 in HBV-associated chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Cancer Biol Ther 2014; 15: 1248-1255 [PMID: 24971953 DOI: 10.4161/cbt.29688]

47 Zeng C, Wang YL, Xie C, Sang Y, Li TJ, Zhang M, Wang R, Zhang Q, Zheng L, Zhuang SM. Identification of a novel target of serum response factor signaling cascade and its implication in hepatic fibrogenesis. Oncotarget 2015; 6: 12224-12233 [PMID: 25909171 DOI: 10.18632/oncotarget.3652]

48 Chen R, Wu JC, Liu T, Qu Y, Lu LG, Xu MY. MicroRNA profile analysis in the liver fibrotic tissues of chronic hepatitis B patients. J Dig Dis 2017; 18: 115-124 [PMID: 28127890 DOI: 10.1111/j.1751-2980.12452]

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

50 Liu N, Jiao T, Huang Y, Liu W, Li Z, Ye X. Hepatitis B virus regulates apoptosis and tumorigenesis through the microRNA-15a-5p/Smad7-transforming growth factor beta pathway. J Virol 2015; 89: 2739-2749 [PMID: 25540364 DOI: 10.1128/JVI.02784-14]

51 Matsubara K, Tokino T. Integration of hepatitis B virus DNA and its implication in hepatocarcinogenesis. Mol Biol Med 1990; 7: 243-260 [PMID: 2170810]

52 Ding D, Lou X, Hua D, Yu W, Li L, Wang J, Gao F, Zhao N, Ren G, Li L, Lin B. Recurrent targeted genes of hepatitis B virus in the liver cancer genomes identified by a next-generation sequencing-based approach. PLoS Genet 2012; 8: e1003065 [PMID: 23236287 DOI: 10.1371/journal.pgen.1003065]

53 Li JW, Wan R, Yu CS, Co NN, Wong N, Chan TF. ViralFusionSeq: accurately discover viral integration events and reconstruct fusion transcripts at single-base resolution. Bioinformatics 2013; 29: 649-651 [PMID: 23143233 DOI: 10.1093/bioinformatics/btt011]

54 Lau CC, Sun T, Chang AK, He M, Li JW, Wong AM, Co NN, Chan AW, Li PS, Lung RW, Tong JH, Lai PB, Chan HL, To KP, Chan TF, Wong N. Viral-human chimeric transcript predisposes risk to liver cancer development and progression. Cancer Cell 2014; 25: 335-349 [PMID: 24582836 DOI: 10.1016/j.ccr.2014.01.030]

55 Pagano M, Pepperkok R, Verde F, Ansorge W, Draetta G. Cyclin A is required at two points in the human cell cycle. EMBO J 1992; 11: 961-971 [PMID: 1312467]

56 Chiu YT, Wong JK, Choi SW, Sze KM, Ho DW, Chan LK, Lee JM, Man K, Cherny S, Yang WL, Wong CM, Shan PC, Ng IO. Novel pre-mRNA splicing of intronically integrated HBV generates oncogenic chimera in hepatocellular carcinoma. J Hepatol 2016; 64: 1256-1264 [PMID: 26867494 DOI: 10.1016/j.jhep.2016.02.005]

57 Li H, Sheng C, Wang S, Yang L, Liang Y, Huang Y, Liu H, Li P, Yang C, Yang X, Liu J, Xie J, Wang L, Hao R, Du X, Xu D, Zhou J, Li M, Sun Y, Tong Y, Li Q, Qiu S, Song H. Removal of Integrated Hepatitis B Virus DNA Using CRISPR-Cas9. Front Cell Infect Microbiol 2017; 7: 91 [PMID: 28382278 DOI: 10.3389/fcimm.2017.00991]

P- Reviewer: Parvez MK S- Editor: Gong ZM L- Editor: Filippia E- Editor: Huang Y
