miR-29a promotes hepatitis B virus replication and expression by targeting SMARCE1 in hepatoma carcinoma

Hong-Jie Wu, Ya Zhuo, Yan-Cai Zhou, Xin-Wei Wang, Yan-Ping Wang, Chang-Yun Si, Xin-Hong Wang

Abstract

**AIM**
To investigate the functional role and underlying molecular mechanism of miR-29a in hepatitis B virus (HBV) expression and replication.

**METHODS**
The levels of miR-29a and SMARCE1 in HBV-infected HepG2.2.15 cells were measured by quantitative real-time PCR and western blot analysis. HBV DNA replication was measured by quantitative PCR and Southern blot analysis. The relative levels of hepatitis B surface antigen and hepatitis B e antigen were detected by enzyme-linked immunosorbent assay. The Cell Counting Kit-8 (CCK-8) was used to detect the viability of HepG2.2.15 cells. The relationship between miR-29a and SMARCE1 were identified by target prediction and luciferase reporter analysis.

**RESULTS**
miR-29a promoted HBV replication and expression, while SMARCE1 repressed HBV replication and expression. Cell viability detection indicated that miR-29a transfection had no adverse effect on the host cells. Moreover, SMARCE1 was identified and validated to be a functional target of miR-29a. Furthermore, restored expression of SMARCE1 could relieve the increased HBV replication and expression caused by miR-29a overexpression.

**CONCLUSION**
miR-29a promotes HBV replication and expression through regulating SMARCE1. As a potential regulator of HBV replication and expression, miR-29a could be a promising therapeutic target for patients with HBV infection.

**Key words:** miR-29a; SMARCE1; Hepatitis B surface antigen; Hepatitis B virus replication; Hepatitis B e antigen
INTRODUCTION

Hepatitis B virus (HBV) infection is a global public health problem[9]. HBV is a small encircled DNA virus belonging to the Hepadnaviridae family. HBV infection could cause either acute or chronic hepatitis, even leading to liver cirrhosis and hepatocellular carcinoma (HCC)[2]. A previous study reported that 2 billion people were infected with the HBV virus worldwide, and more than 350 million were chronic HBV carriers[2]. Additionally, over 600000 deaths have been triggered by HBV virus infection[4]. Although some clinical drugs, such as α-interferon and nucleoside analogs, could inhibit HBV replication to some extent for treatment of HBV infection, they could not induce persistent HBV virus infection, its underlying mechanism remains unclear. In this study, we analyzed the expression levels of miR-29a in HBV-infected HepG2.2.15 cells. Our results indicated that miR-29a expression was up-regulated in HBV-associated hepatocellular carcinoma cells. In addition, SMARCE1 was confirmed to be a functional target of miR-29a. miR-29a promoted HBV replication and expression through directly regulating SMARCE1. These findings provide potential therapeutic targets for patients with HBV infection.

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Although aberrant miR-29a expression has been found to be involved in the process of hepatitis B virus (HBV) infection, its underlying mechanism remains unclear. In this study, we analyzed the expression levels of miR-29a in HBV-infected HepG2.2.15 cells. Our results indicated that miR-29a expression was up-regulated in HBV-associated hepatocellular carcinoma cells. In addition, SMARCE1 was confirmed to be a functional target of miR-29a. miR-29a promoted HBV replication and expression through directly regulating SMARCE1. These findings provide potential therapeutic targets for patients with HBV infection.
human HCC cell line HepG2.2.15 with HBV expression and HEK293T cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, United States). These cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Grand Island, NY, United States) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, United States) at 37 °C in a humidified atmosphere containing 5% CO2.

miR-control, miR-29a mimics, anti-miR-29a, pcDNA-control, pcDNA-SMARCE1, si-control or si-SMARCE1 was transfected into cells at the indicated concentrations using Lipofectamine 2000 (Invitrogen) in accordance with the manufacturer’s protocol.

Quantitative real-time PCR
Total RNA was isolated from HepG2 or HepG2.2.15 cells by using TRIzol Reagent (Invitrogen) following the manufacturer’s instructions. cDNA was synthesized by using a ImProm-II™ Reverse Transcription System (Promega, Madison, WI, United States) according to the manufacturer’s instructions. The expression of miR-29a was detected by TaqMan miRNA assay (Ambion, Austin, TX, United States), and U6 snRNA was used as the internal control. The expression of SMARCE1 mRNA was quantified by PrimeScript RT-PCR Kit (TaKaRa, Shiga, Japan), and β-actin served as the internal control. The primer sequences were as follows: for miR-29a 5'-ACAGGATATCGCATTTGAGG-3' (forward) and 5'-TATACACATGCATTAGCAAG-3' (reverse); for SMARCE1 5'-CGGGTTATCTGTGAGCTTCT-3' (forward) and 5'-GCGAGGTCGACCACTACAAAC-3' (reverse); for U6 5'-CTGCCTCGCAGAGCAC-3' (forward) and 5'-AACGCTTACCAATCCGCGTGC-3' (reverse); for β-actin 5'-TGAGAGGGAAATCGTGCGTGAC-3' (forward) and 5'-GAATAAGTTTGCCTGTTTGTGCTGAGACTG-5' (reverse). The fragment was cloned into the pcDNA3.1 expression vector using the EcoRI and Xho I restriction sites in the multiple cloning site. The SMARCE1 expression construct was generated by PCR amplification of SMARCE1 from HepG2 cDNA using the following primers: forward, 5'-GTACGCTAGCGGCACCATCATCTGCTTG-3'; reverse, 3'-GAATAAGTTTGCCTGTTTGTGCTGAGACTG-5'. The predicted miR-29a target site was amplified by PCR amplification of SMARCE1 from HepG2 cDNA and then cloned into the pcDNA3.1 expression vector using the Nhe I and Xho I restriction sites in the multiple cloning site.

Luciferase reporter assays
HEK293T cells were co-transfected with 50 nmol/L miR-29a mimics or control miRNA and 0.4 µg of pGL3 reporter vectors containing wild-type or mutant 3'-UTR of SMARCE1 using Lipofectamine 2000 (Invitrogen). pRL-CMV (Promega) was co-transfected to control firefly luciferase expression. The luciferase activities were measured 48 h post-transfection using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer’s protocol.

Statistical analysis
All data were presented as mean ± SD and analyzed using SPSS 19.0 software (IBM Corp, Armonk, NY, United States). The statistical significance was calculated with the Student’s t-test or one-way ANOVA. A P value of less than 0.05 was considered statistically significant.

RESULTS

miR-29a is up-regulated and SMARCE1 is down-regulated in HBV-infected HepG2.2.15 cells
The levels of miR-29a and SMARCE1 in HepG2 (NC) were determined by real-time PCR as described previously. The levels of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) were determined using enzyme-linked immunosorbent assay (ELISA) kits (Kehua Biotech, Shanghai, China).

Cell viability assays
HepG2.2.15 cells transfected with miR-29a mimics or anti-miR-29a were cultured for 48 h, and then cell viability was determined using the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan).

Plasmid construction
The SMARCE1 expression construct was generated by PCR amplification of SMARCE1 from HepG2 cDNA using the following primers: forward, 5'-GTACGCTAGCGGCACCATCATCTGCTTG-3'; reverse, 3'-GAATAAGTTTGCCTGTTTGTGCTGAGACTG-5'. The fragment was cloned into the pcDNA3.1 expression vector using the EcoRI I and Xho I restriction sites in the multiple cloning site and sequenced verified. For 3'-UTR reporter plasmids, the 3'-UTR of SMARCE1 containing the predicted miR-29a target site was amplified by the primers (forward, 5'-GTACGCTAGCGGCACCATCATCTGCTTG-3'; reverse, 3'-ATCTGGTCTCGGGTGAAACTCGAGACTG-5'), and then cloned into a pGL3 reporter vector using the Nhe I and Xho I restriction sites in the multiple cloning site.

Western blot analysis
Anti-SMARCE1 (Bethyl, Montgomery, TX, United States) and anti-β-actin (Sigma-Aldrich, St Louis, MO, United States) antibodies were used as monoclonal rabbit antibodies. Cells were harvested and total proteins were isolated with RIPA reagents (Thermo Scientific, Rockford, IL, United States) following the manufacturer’s instructions. Subsequently, protein concentrations were quantified by using the BCA protein assay kit (Thermo Scientific, Rockford, IL, United States) following the manufacturer’s instructions. The expression of miR-29a was detected by TaqMan miRNA assay (Ambion, Austin, TX, United States), and U6 snRNA was used as the internal control. The expression of SMARCE1 mRNA was quantified by PrimeScript RT-PCR Kit (TaKaRa, Shiga, Japan), and β-actin served as the internal control. The primer sequences were as follows: for miR-29a 5'-ACAGGATATCGCATTTGAGG-3' (forward) and 5'-TATACACATGCATTAGCAAG-3' (reverse); for SMARCE1 5'-CGGGTTATCTGTGAGCTTCT-3' (forward) and 5'-GCGAGGTCGACCACTACAAAC-3' (reverse); for U6 5'-CTGCCTCGCAGAGCAC-3' (forward) and 5'-AACGCTTACCAATCCGCGTGC-3' (reverse); for β-actin 5'-TGAGAGGGAAATCGTGCGTGAC-3' (forward) and 5'-GAATAAGTTTGCCTGTTTGTGCTGAGACTG-5' (reverse). The relative expression of RNAs was calculated and normalized using the ∆∆Ct method, relative to the control gene. Each test was performed in triplicate.

HBV replication and gene expression analysis
HBV replicative intermediates were extracted from hepatoma cell lines and detected in Southern blotting, according to the previously published protocols. HBV DNA was extracted using the Column Viral DNAout kit (TIANDZ, Beijing, China) following the manufacturer’s protocol, and quantified by real-time PCR as described previously. The levels of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) were determined using enzyme-linked immunosorbent assay (ELISA) kits (Kehua Biotech, Shanghai, China).

Cell viability assays
HepG2.2.15 cells transfected with miR-29a mimics or anti-miR-29a were cultured for 48 h, and then cell viability was determined using the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan).

Plasmid construction
The SMARCE1 expression construct was generated by PCR amplification of SMARCE1 from HepG2 cDNA using the following primers: forward, 5'-GTACGCTAGCGGCACCATCATCTGCTTG-3'; reverse, 3'-ATCTGGTCTCGGGTGAAACTCGAGACTG-5'. The fragment was cloned into the pcDNA3.1 expression vector using the EcoRI I and Xho I restriction sites in the multiple cloning site and sequenced verified. For 3'-UTR reporter plasmids, the 3'-UTR of SMARCE1 containing the predicted miR-29a target site was amplified by the primers (forward, 5'-GTACGCTAGCGGCACCATCATCTGCTTG-3'; reverse, 3'-ATCTGGTCTCGGGTGAAACTCGAGACTG-5'), and then cloned into a pGL3 reporter vector using the Nhe I and Xho I restriction sites in the multiple cloning site.

Luciferase reporter assays
HEK293T cells were co-transfected with 50 nmol/L miR-29a mimics or control miRNA and 0.4 µg of pGL3 reporter vectors containing wild-type or mutant 3'-UTR of SMARCE1 using Lipofectamine 2000 (Invitrogen). pRL-CMV (Promega) was co-transfected to control firefly luciferase expression. The luciferase activities were measured 48 h post-transfection using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer’s protocol.
miR-29a promotes HBV replication and expression in HepG2.2.15 cells

To examine whether miR-29a affected HBV replication and expression, HepG2.2.15 cells were transfected with miR-control, miR-29a mimics or anti-miR-29a, respectively. The influence of miR-29a on HBV DNA replication was measured by quantitative PCR (qPCR) and Southern blot, while the expression levels of HBsAg and HBeAg were detected by ELISA. The qPCR and Southern blot analysis indicated that the overexpression of miR-29a significantly increased the HBV DNA replication, while the endogenous miR-29a inhibition by anti-miR-29a obviously decreased its replication compared with the controls (Figure 2A and B). Moreover, ELISA assay demonstrated that miR-29a overexpression significantly elevated the expression levels of HBsAg and HBeAg, while the endogenous miR-29a inhibition by anti-miR-29a markedly reduced their expression levels compared with the controls (Figure 2C). Together, these results implied that miR-29a promoted HBV replication and expression in HepG2.2.15 cells.

miR-29a has no effect on HepG2.2.15 cell viability

To further investigate whether miR-29a transfection had effects on host cells, CCK-8 was used to detect the cell viability of HepG2.2.15 cells transfected with miR-control, miR-29a mimics or anti-miR-29a. The CCK-8 assay showed that miR-29a mimics, as well as anti-miR-29a, had no significant effect on HepG2.2.15 cell viability compared to respective controls (Figure 3A and B). These results indicated that miR-29a transfection did not damage the host cells.

SMARCE1 suppresses HBV replication and expression in HepG2.2.15 cells

To confirm the effect of SMARCE1 on HBV replication and expression, HepG2.2.15 cells were transfected with pcDNA-control, pcDNA-SMARCE1, si-control or si-SMARCE1, respectively. Western blot analysis indicated that SMARCE1 was overexpressed or down-regulated after pcDNA-SMARCE1 or si-SMARCE1 transfection (Figure 4A). The effect of SMARCE1 on HBV DNA replication was measured by quantitative (q)PCR and Southern blot, while the expression levels of HBsAg and HBeAg were detected by ELISA.

The results of qPCR and Southern blot analysis indicated that SMARCE1 overexpression significantly reduced the HBV DNA replication (Figure 4B and E),
Figure 2  Effect of miR-29a on hepatitis B virus replication and expression (n = 3). HepG2.2.15 cells were transfected with miR-control, miR-29a mimics or anti-miR-29a for 24 h. A: Relative HBV DNA copies were measured by qPCR analysis; B: HBV replication was detected by Southern blotting. The positions of relaxed circular (RC) and single-stranded (SS) DNAs are indicated 24 h post transfection. The ELISA assay was conducted to detect the levels of (C) HBsAg and (D) HBeAg 24 h post transfection. Data represent the mean ± SD. *P < 0.05 vs miR-control. HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen.
Wu HJ et al. miR-29a promotes HBV replication and expression

Figure 3  Effect of miR-29a on HepG2.2.15 cell viability (n = 3). HepG2.2.15 cells were transfected with miR-control, miR-29a mimics or anti-miR-29a for 48 h. HepG2 cell viability transfected with (A) miR-29a mimics and (B) anti-miR-29a was determined using the CCK-8 assay. Data represent the mean ± SD.

Figure 4  Effect of SMARCE1 on hepatitis B virus replication and expression (n = 3). A: HepG2.2.15 cells were transfected with pcDNA-control, pcDNA-SMARCE1, si-control, or si-SMARCE1 for 24 h; B and E: Western blotting was performed to detect the level of SMARCE1 after pcDNA-SMARCE1 or si-SMARCE1 transfection; C and D: qPCR and Southern blotting analysis showed that SMARCE1 overexpression significantly reduced the HBV DNA replication and SMARCE1 knockdown significantly increased the HBV DNA replication; F and G: ELISA assay showed that SMARCE1 overexpression significantly decreased the levels of HBsAg and HBeAg; H and I: Endogenous SMARCE1 inhibition by si-SMARCE1 significantly promoted the levels of HBsAg and HBeAg. *P < 0.05 vs controls. Data represent the mean ± SD.
while SMARCE1 knockdown dramatically increased its replication (Figure 4C and D). Moreover, ELISA assay revealed that SMARCE1 overexpression significantly decreased the expression levels of HBsAg and HBeAg (Figure 4F and G), while the si-SMARCE1 knockdown notably enhanced their expression levels (Figure 4H and I). These results indicated that SMARCE1 suppressed HBV replication and expression.

SMARCE1 is directly targeted by miR-29a in HepG2.2.15 cells

Considering that the expression and function of miR-29a and SMARCE1 were inverse and that miRNAs play their roles by targeting specific target, we speculated that miR-29a might promote HBV replication and expression by regulating the expression of SMARCE1. Therefore, miRNA target analysis tools TargetScan and PicTar were used to predict the potential targets of miR-29a. The results showed that 3’-UTR of SMARCE1 contained the binding sequence of miR-29a (Figure 5A). Then, dual-luciferase reporter assay was used to confirm that SMARCE1 was directly targeted by miR-29a. Results showed that miR-29a overexpression significantly decreased the luciferase activity of the wild-type reporter gene (WT), but not the mutant reporter gene (MUT) (Figure 5B).

To further determine whether miR-29a can indeed repress the expression of SMARCE1, HepG2.2.15 cells were transfected with miR-control, miR-29a mimics or anti-miR-29a, and then the mRNA and protein levels of SMARCE1 were detected by qRT-PCR and western blot, respectively. Results displayed that miR-29a overexpression significantly lowered the protein level of SMARCE1 and miR-29a inhibition significantly promoted the protein level of SMARCE1 (Figure 5D and E), and that miR-29a overexpression or inhibition had no obvious effects on the mRNA level of SMARCE1, which indicated that miR-29a suppressed SMARCE1 expression at the post-transcriptional level. Taken together, these results illuminated that miR-29a directly targeted 3’-UTR of SMARCE1 to regulate its expression in HepG2.2.15 cells.

Figure 5  SMARCE1 is a target of miR-29a (n = 3). A: Wild-type (WT) and mutant (MUT) 3’-UTR binding sites are shown. The mutated bases are labelled with a horizontal line; B: Relative luciferase activity was measured in HEK293 cells co-transfected WT or MUT SMARCE1 3’-UTR with miR-17 mimic or miR-control; C-E: The relative mRNA (C) and protein (D and E) levels of SMARCE1 were measured in HepG2.2.15 cells transfected with miR-29a mimics or anti-miR-29a. Data represent the mean ± SD. *P < 0.05 vs miR-control.
miR-29a promotes HBV replication and expression by regulating SMARCE1

To clarify whether miR-29a promoted HBV replication and expression through regulating SMARCE1, HepG2.2.15 cells were transfected with miR-control or miR-29a mimics or co-transfected miR-29a mimics with pcDNA-control or pcDNA-SMARCE1. The results showed that miR-29a overexpression strikingly augmented HBV replication, whereas co-transfection of pcDNA-SMARCE1 attenuated the effect of miR-29a on HepG2.2.15 cells (Figure 6A and B). As shown in Figure 6C and D, miR-29a overexpression evidently enhanced HBsAg and HBeAg expression, whereas co-transfection of pcDNA-SMARCE1 significantly restored the effect of miR-29a on HBsAg and HBeAg expression in HepG2.2.15 cells. These results demonstrated that miR-29a promoted HBV replication and expression through targeting SMARCE1 in HepG2.2.15 cells.

DISCUSSION

To improve the treatment of HBV infection, new therapeutic targets and strategies must be identified and developed. Recently, studies on miRNAs have provided novel insights into the potential cure of HBV infection. Moreover, a growing number of works have revealed that miRNAs play critical roles in the HBV replication and expression processes. However, their underlying molecular mechanisms remain to be further elucidated.

In our study, we explored the role of miR-29a in the regulation of HBV replication and expression. We found that the expression of miR-29a was significantly up-regulated in the HBV-infected HCC cell line HepG2.2.15. Overexpression of miR-29a increased the HBV replication and expression, while miR-29a knockdown decreased the replication and expression of HBV. These results suggested that miR-29a could be a promising diagnostic biomarker and therapeutic target for HBV infection.

Recent studies indicated that the host cellular miRNAs could regulate the HBV replication and expression by directly targeting HBV transcripts or indirectly targeting HBV transcription regulatory factors.
needed for HBV transcription\(^{[31]}\). For instance, Zhang et al.\(^{[37]}\) showed that miR-199a-3p targeted the coding regions of HBsAg and miR-210 specifically bound to the preS1 region of HBV, and they were further confirmed to inhibit HBV replication and expression through directly targeting HBV RNA. Potenza et al.\(^{[45]}\) also found that miR-125a-5p interacted with the RNA encoded by HBV surface antigen, eventually reducing the secretion of HBsAg. Wu et al.\(^{[32]}\) reported that miR-7, miR-196, miR-433 and miR-511 might directly target HBV DNA polymerase and surface antigen genes, and miR-205 and miR-345 could target HBx and the pre-core gene, respectively. In addition, Wang et al.\(^{[33]}\) demonstrated that miR-155 could promote HBV enhancer II activity in a dose-dependent manner by directly targeting CEBP-β. Zhang et al.\(^{[30]}\) confirmed that miR-1 improved farnesoid X receptor alpha (FXRA) expression and enhanced HBV core promoter transcription activity. Furthermore, HCC cell proliferation and cell cycle arrest modulated by miR-29a may also influence HBV replication and gene expression\(^{[34]}\). In the present study, we found that miR-29a was up-regulated in the HCC cell line HepG2.2.15, consistent with findings from a previous study\(^{[27]}\). However, the possible influencing mechanism of miR-29a on HBV replication and expression was not illustrated. Our study enriches the miRNAs and HBV-host interaction theory.

SMARCE1 is a member of the SWI/SNF family of chromatin-remodeling complexes, playing key roles in transcriptional control. A previous study reported that SWI/SNF was recruited to neuronal genes by the CoREST corepressor, interacting with the DNA binding repressor\(^{[35]}\). In another study, the potential tumor suppressor, prohibitin, was implied to recruit SWI/SNF to a particular E2F-dependent promoter and inhibit E2F-mediated transcription\(^{[36]}\). However, the role of SMARCE1 in HBV replication and expression was largely unknown. In the present study, we found that overexpression of SMARCE1 decreased the HBV replication and expression, while inhibition of SMARCE1 increased the HBV replication and expression. SMARCE1 repressed HBV replication through binding to the mutant core promoter of HBV in HepG2 cells\(^{[28]}\), which also revealed that SMARCE1 could repress HBV replication; however, the functional mechanism of SMARCE1 involved in HBV replication may be different from that observed in our study. Furthermore, a dual-luciferase assay was performed and SMARCE1 was verified to be a target of miR-29a and was regulated by miR-29a at the protein levels. Restoration of SMARCE1 expression by pcDNA-SMARCE1 recuperated the promoting effect of miR-29a on the HBV replication and expression. These findings suggested that miR-29a promoted HBV replication and expression by targeted inhibition of SMARCE1 expression, providing a theoretical foundation for the clinical application of miRNAs in therapy of HBV infection.

In summary, the expression of miR-29 was up-regulated in the HCC cell line HepG2.2.15 infected with HBV. aberrantly expressed miR-29a affected the HBV replication and expression at least partially by targeted inhibition of SMARCE1 expression. Our findings suggest that the inhibition of miR-29a could be a promising therapeutic strategy for the treatment of patients with HBV infection.

**REFERENCES**

1. Belongia EA, Costa J, Gareen IF, Grem JL, Inadomi JM, Kern ER, McHugh HF, Petersen GM, Rein MF, Sorrell MF, Strader DB, Trotter HT. NIH consensus development statement on management of hepatitis B. NIH Consens State Sci Statements 2008; 25: 1-29 [PMID: 18949020]
2. McMahon BJ. The natural history of chronic hepatitis B virus infection. Hepatology 2009; 49: S45-S55 [PMID: 19399792 DOI: 10.1002/hep.22898]
3. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. J Viral Hepat 2004; 11: 97-107 [PMID: 14996343]
4. Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. Hepatology 2007; 45: 1056-1075 [PMID: 17393513 DOI: 10.1002/hep.21627]
5. Billoud G, Pichoud C, Puestringer G, Neys J, Zoulim F. The main hepatitis B virus (HBV) mutants resistant to nucleoside analogs are susceptible in vitro to non-nucleoside inhibitors of HBV replication. Antiviral Res 2011; 92: 271-276 [PMID: 21871497 DOI: 10.1016/j.antiviral.2011.08.012]
6. Daniels HJ, Meager A, Eddleston AL, Alexander GJ, Williams R. Spontaneous production of tumour necrosis factor alpha and interleukin-1 beta during interferon-alpha treatment of chronic HBV infection. Lancet 1990; 335: 875-877 [PMID: 1969983]
21876625 DOI: 10.3748/wjg.v17.i28.3353

Pan H, Niu DD, Feng H, Ng LF, Ren EC, Chen WN. Cellular transcription modulator SMARCE1 binds to HBV core promoter containing naturally occurring deletions and represses viral replication. Biochim Biophys Acta 2007; 1772: 1075-1084 DOI: 10.1016/j.bbadis.2007.03.004

Zhang ZZ, Liu X, Wang DQ, Teng MK, Niu LW, Huang AL, Liu S, Schwind S, Santhanam R, Hickey CJ, Becker H, Huang AL, Wang FS, Huang LT, Chuang JH, Kuo HC, Yang YL, Huang YH. MicroRNA-29a protects against acute liver injury in a mouse model of obstructive jaundice via inhibition of the extrinsic apoptosis pathway. Apoptosis 2014; 19: 30-41 DOI: 10.1007/s10495-013-0909-4

Matsumoto Y, Itami S, Kuroda M, Yoshizato K, Kawada N, Murakami Y. Micro-29a inhibits Akt phosphorylation and promotes the apoptosis pathway. J Pathol 2013; 228: 1-6 DOI: 10.1002/path.421876625

Lam A, Espiritu C, Flores O, Hartmanet G, Klumpp K. P06640: Effect of the combination of the HBV core inhibitor NVR 3-778 with nucleoside analogs or other HBV core inhibitors on the inhibition of HBV DNA replication in HepG2. 2.15 cells. J Hepatol 2015; 62: 5559 DOI: 10.1016/j.jpANGES.2015.03.085-

Maillard ME, Gollan JL. Emerging therapeutics for chronic hepatitis B. Annu Rev Med 2006; 57: 155-166 DOI: 10.1146/annurev.med.57.121304.134222

Liu M, Lang N, Qiu M, Xu F, Li Q, Tang Q, Chen J, Chen X, Zhang S, Liu Z, Zhou J, Zhu Y, Deng Y, Zheng Y, Bi F. miR-137 targets Ccnd4 expression, induces cell cycle G1 arrest and inhibits invasion in colorectal cancer cells. Int J Cancer 2011; 128: 1269-1279 DOI: 20073490 DOI: 10.1016/jijic.2010.09.007

Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350-355 DOI: 15372048 DOI: 10.1038/nature02871

Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297 DOI: 14744438

Eiring AM, Harb JG, Neviani P, Garton C, Oakes JJ, Spizzo R, Liu S, Schwind S, Santhanam R, Hickey CJ, Becker H, Chandler JC, Andino R, Cortes J, Hokland P, Huettner CS, Bhatia R, Roy DC, Liebhaber SA, Caligiuri MA, Marucci G, Garzon R, Croce CM, Calin GA, Perrotti D. miR-328 functions as an RNA decoy to modulate hnrNP E2 regulation of mRNA translation in leukemic blasts. Cell 2010; 140: 652-665 DOI: 201016.2010.0007

Zsooldis GK, Kai ZS, Chang RK, Pasquinelli AE. Autoregulation of microRNA biogenesis by let-7 and Argonauta. Nature 2012; 486: 541-544 DOI: 10.1038/nature11134

Garzon R, Calin GA, Croce CM. MicroRNAs in Cancer. Annu Rev Med 2009; 60: 167-179 DOI: 19630570 DOI: 10.1146/annurev.med.59.030506.104707

Li C, Feng Y, Coukos G, Zhang L. Therapeutic microRNA stargazer causes regression of lung adenocarcinoma. Cell 2006; 126: 717-747 DOI: 19876744 DOI: 10.1016/s1228-0091-9459-5

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 DOI: 21317190 DOI: 10.1038/nar.kkit.067

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 DOI: 20728471 DOI: 10.1016/j.antiviral.2010.08.008

Zhang X, Hou J, Lu M. Regulation of hepatitis B virus replication by epigenetic mechanisms and microRNAs. Front Genet 2013; 4: 202 DOI: 24133502 DOI: 10.3389/fgene.2013.00202

Zhang X, Liu H, Xie Z, Deng W, Wu C, Qin B, Hou J, Lu M. Epigenetically regulated miR-449a enhances hepatitis B virus replication by targeting cAMP-responsive element binding protein 5 and modulating hepatocytes phenotype. Sci Rep 2016; 6: 25389 DOI: 27138288 DOI: 10.1038/srep25389

Gebsheuber CA, Zatoloulk K, Martinez J. miR-29a suppresses tristetraprolin, which is a regulator of epithelial polarity and metastasis. EMBO Rep 2009; 10: 400-405 DOI: 19247375 DOI: 10.1038/emboj.doi:2009.9

Han YC, Park CY, Bhagat G, Zhang J, Wang Y, Fan JH, Liu M, Zou Y, Weissman IL, Gu H. microRNA-29a induces aberrant self-renewal capacity in hematopoietic progenitors, biased myeloid differentiation, and acute myeloid leukemia. J Exp Med 2010; 207: 475-489 DOI: 20120666 DOI: 10.1084/jem.20090831

Fabhri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, Liu S, Alder H, Costinane S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chen KC, Marucci G, Calin GA, Lu M, Hou J, Faller DV. Prohibitin requires Brg-1 and Brm for the repression of E2F and cell growth. EMBO J 2002; 21: 3019-3028 DOI: 12605415 DOI: 10.1038/embj.20023002

P- Reviewer: AiZawa Y S- Editor: Ma YJ L- Editor: Filippida E- Editor: Wang CH
