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Correlation between hepatitis B virus protein and microRNA processor Drosha in cells expressing HBV

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ABSTRACT

Drosha regulates the biogenesis of microRNAs (miRNAs) and plays an essential role in the regulation of gene expression. Infection with hepatitis B virus (HBV) causes chronic hepatitis and liver cirrhosis. It is also a major risk factor for hepatocellular carcinoma. Emerging evidence suggests that HBV alters miRNA expression profiles, but the mechanisms underlying this process have not yet been fully elucidated. We therefore examined how HBV affected the production of miRNAs. We found that Drosha mRNA and protein expression were downregulated in cells expressing the HBV genome. This was associated with a reduction in the activity of the Drosha gene promoter. Gene silencing of HBx by RNA interference significantly restored the expression of Drosha. In conclusion, our data show that HBV could downregulate Drosha expression by inhibiting promoter activity, and the transcription factors SP1 and AP-2α may be involved in this process. This provides a new understanding of the mechanism of HBV-induced miRNAs dysregulation.

1. Introduction

MicroRNAs (miRNAs) are small, endogenous, non-coding RNAs of approximately 22 nucleotides (nts). MiRNAs modulate serials of fundamental cellular processes, including development, signal transduction, cell proliferation, differentiation, apoptosis, and tumorigenesis (Bartel, 2004; Bushati and Cohen, 2007). Mature miRNAs are first transcribed as pri-miRNA in the nucleus, which is subsequently cropped by the microprocessor consisted of the RNase Drosha and DGCR8 (DiGeorge syndrome critical region gene 8), resulting in the release of precursor miRNA (pre-miRNA), which is 60–70 nt in length characterized by a stem-loop structure (Lee et al., 2003). Pre-miRNA is exported by the nuclear export receptor exportin-5 into the cytoplasm, where it is processed by Dicer, another cellular RNase III enzyme, generating the mature miRNA (Yi et al., 2003; Bernstein et al., 2001; Khvorova et al., 2003). Mature miRNAs play important regulatory roles in gene expression at post-transcriptional levels by incomplete or complete complementation with the target mRNAs, causing translational repression or mRNA cleavage (Bartel, 2004).

The dysregulation of miRNA expression has been associated with the pathophysiology of a variety of human diseases, including those resulting from viral infection. Accumulated evidence has suggested that host-cellular miRNAs modulate the expression of various viral genes and play crucial roles in the host–virus interactions (Ghosh et al., 2009; Skalsky and Cullen, 2010). On the other hand, viruses also encode viral miRNAs that can alter host physiology and influence replication. Over 200 viral miRNAs have now been identified (Skalsky and Cullen, 2010; Gottwein and Cullen, 2008). Viruses tend to subvert the host-cellular miRNAs for the benefit of their replication (Pfeffer and Baumert, 2010; Sarnow et al., 2006).

HBV, a hepatotropic DNA virus from the *Hepadnaviridae* family, is a major cause of liver diseases, including chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). Emerging evidence indicates that HBV can alter the expression patterns of cellular miRNAs (Liu et al., 2009; Ji et al., 2011). However, the mechanisms underlying the miRNA dysregulation caused by HBV are not yet fully understood. Here we showed that HBV might impinge on the biogenesis of host miRNAs by modulating the expression of Drosha.

2. Materials and methods

2.1. Cell culture and transfection

HepG2 and HepG2.2.15 were cultured in minimum essential medium (MEM) with 10% fetal bovine serum (FBS) and maintained in a humidified incubator with 5% CO_2 at 37 °C. Transfections were performed with a Lipofectamine 2000 kit (Invitrogen) according to the manufacturer's instructions. Cells were harvested at 48 h post-transfection for analysis.

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Table 1
Primer sequences used for PCR or constructions of various plasmids.

Amplifier primer	Primer sequence (5′–3′)
Real-time PCR primer	
Drosha-F	GCAAGGGCATTCACATTGAGA
Drosha-R	TGATTGTGGCCTAGGGTCAGA
β-actin-F	CCTGGCACCCAGCACAAT
β-actin-R	GCCGATCCACACGGAGTA
Drosha promoter primer	
Drosha-P-F	TATGAGCTCACAGGGCTCTGCAGCCAGAC
Drosha-P-R	GCGAAGCTTGCTCCACAAA TGAATGAAAG
Transcription factor primer	
SP1-F	TTGTCGACCGCCACCATGGGCATGAGCGACCAAGA TCACTCCAT
SP1-R	TTAAGCTTTCAGAAGCCATTGCCACTG
AP-2α-F	ATGTCGACCGCCACCATGCTTTGGAAACTGACGGA
AP-2α-R	CCCAAGCTTTCACTTTCTGTGTTTCTCTTC

Restriction enzyme cutting located within PCR primers is underlined. *Sac* I site: GAGCTC; *Hin*d III site: AAGCTT; *Sal* I site: GTCGAC.

2.2. Semiquantitative real-time PCR

Total cellular RNAs were extracted using Trizol (Invitrogen) and treated with DNase (Promega). The first-strand complementary DNA was generated with a Reverse Transcription System kit (Promega) according to manufacturer's instructions. Semiquantitative real-time PCR was performed using SYBR Green Supermix (Bio-Rad). A cycle threshold (CT) was assigned at the beginning of the logarithmic phase of PCR amplification and duplicate CT values were analyzed by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). β-Actin was used as an endogenous control to normalize the amount of total cDNA in each sample. The forward and reverse primers of semiquantitative real-time PCR were given in Table 1.

2.3. Western blot analysis

Cellular proteins were extracted using RIPA buffer supplemented with 1 mM PMSF. Protein concentrations were determined using a BCA protein concentration determination kit (Beyotime Institute of Biotechnology). Equal amounts of sample lysate were separated using 8% SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes. After incubation with primary antibodies including rabbit polyclonal anti-Drosha (Abcam) and mouse monoclonal anti-β-actin (Beijing ComWin Biotech Co. Ltd., China), blots were incubated with goat anti-rabbit or anti-mouse secondary antibody (Beijing Zhong Shan Golden Bridge Biological Technology Co. Ltd., China). Finally, signals were detected using a chemiluminescence ECL™ detection system (Pierce).

2.4. Construction of vectors

The sequence of primers for cloning was illustrated in Table 1. An approximately 1 kb DNA fragment of the Drosha promoter region, designed using Transcriptional Regulatory Element Database (TRED) (http://rulai.cshl.edu/cgi-bin/TRED/tred.cgi?process=home), was amplified by PCR using the genomic DNA of LO2 cells as template. pGL3-Drosha-P was generated by cloning the PCR product into a pGL3-Basic vector (Promega) immediately upstream of the luciferase gene. The HBV expression plasmid pCH9/3091, constructed by Michael et al. (Heidelberg University, Germany), was given to us by Dr. Lan Lin (Southwest Hospital affiliated with the Third Military Medical University, China). The pCMV-Sport6 plasmid was purchased from American Type Culture Collection (ATCC). The pCMV-Sport6-HBc, pCMV-Sport6-HBp, pCMV-Sport6-HBs, and pCMV-Sport6-HBx plasmids were constructed as reported previously (Tian et al., 2011). Their mRNA expression levels in HepG2 cells were confirmed with RT-PCR analysis, and HBc protein expression level in HepG2 cells was confirmed with Western blotting analysis (Supplement Figs. 3–5). SP1 and AP-2α cDNA were obtained using PCR and cloned into the pCMV-Sport6 plasmid.

2.5. Luciferase reporter assay

Cells were seeded at a density of 2×10^5 cells/well in 24-wells plates and cotransfected with 0.5 μg pGL3-Drosha-P, 0.5 μg of pCH9/3091 or pCMV-Sport6-HBc, -HBp, -HBs, -HBx, and 0.2 μg of control reporter vector pRL-TK (Promega). A luciferase activity assay was performed 48 h after transfection with Dual-Luciferase Reporter Assay System (Promega). The relative luciferase activity was normalized with renilla luciferase activity. Each experiment was repeated at least three times.

2.6. Analysis of RNA interference (RNAi)

The target sequences against mRNA of X gene, SP1, and AP-2α were as follows: HBx-1: GCAATGTCAACGACCGACC, HBx-2: GCACT TCGCTTCACCTCTG for HBx gene (Wu et al., 2005); SP1-1: GATCAC TCCATGGATGAAA (Youn et al., 2005), SP1-2: AGCGCTTCATGAGGA GTGA (Liu et al., 2006), SP1-3: TGGCAGTGAGTCTTCCAAG (Maor et al., 2007) for SP1 gene; AP-2α-1: CCGAATTTCCTGCCAAAGC (Allouche et al., 2008). AP-2α-2: AAGCAGTAGCTGAATTTCTCA (Orso et al., 2008). AP-2α-3: CCGTCTCCGCCATCCCTATTAACAA (Koinuma et al., 2009) for AP-2α gene. The scrambled sequence GCTTCATAAGGCGCATAGC was used as the control. These sequences were synthesized by Invitrogen, annealed and cloned into the *Bam*H I and *Hin*d III sites of pGenesil-1.0 vector (Wuhan Genesil Biotechnology Co., Ltd., China), containing the EGFP gene, kanr gene, and U6 promoter.

2.7. Statistical analysis

Data were expressed as the means and standard deviations of at least three independent experiments. Statistical significance was determined by student's *t* test. Differences were deemed statistically significant at $P \leqslant 0.05$.

3. Results

3.1. Downregulation of Drosha expression by HBV

To determine whether expression of HBV influences Drosha, we compared Drosha mRNA and protein expression between parental HepG2 cells and HepG2.2.15 cells containing an integrated HBV genome. We performed a real-time PCR analysis. Our data showed that Drosha mRNA was expressed at a lower level in HepG2.2.15 than in HepG2 cells (Fig. 1A). The amount of Drosha protein was next estimated by Western blot analysis, and less endogenous Drosha protein appeared in HepG2.2.15 cells than in HepG2 cells (Fig. 1B). To further substantiate the association between HBV transcription and Drosha, we determined the Drosha expression in HepG2-H7, another HBV expressing HepG2 cell line that was generated by stable expression of a vector (pCH9/3091) containing 1.1-fold-overlength HBV genome driven by a CMV promoter. Consistently, the expression of Drosha was downregulated in HepG2-H7 as compared with parent HepG2 cells (Supplement Fig. 1). We also analyzed Drosha mRNA level in HepG2 cells transiently transfected with pCH9/3091. As expected, the expression of HBV also downregulated Drosha mRNA expression (Supplement

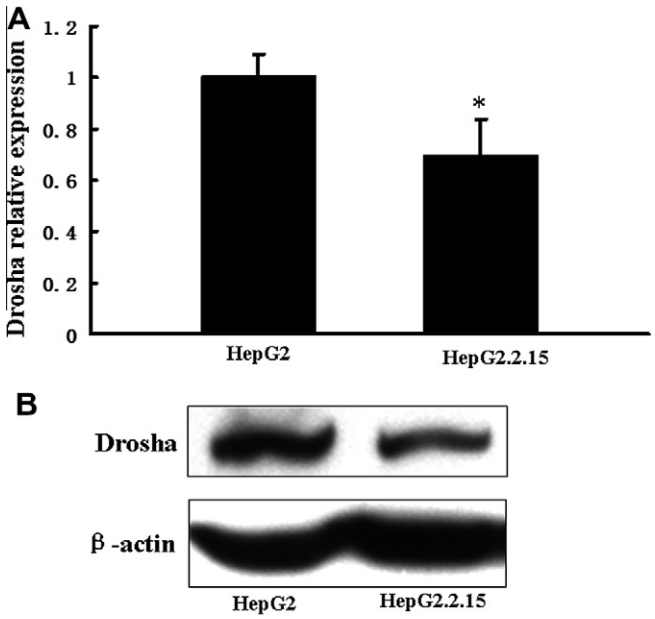

Fig. 1. Effects of HBV on the expression of Drosha (A) Drosha mRNA expression in HepG2 and HepG2.2.15 cells were analyzed with semiquantitative real-time PCR. β-actin was used as control. $^{*}P \leqslant 0.05$. (B) Drosha protein expression in HepG2 and HepG2.2.15 cells was analyzed with Western blot. β-actin was used as control.

Fig. 2). Together, these data show that the presence of HBV regulated Drosha mRNA and protein expression.

3.2. Modulation of Drosha promoter activity by HBV

To determine the mechanism by which HBV modulates Drosha expression, we conducted a luciferase assay to study the effects of HBV on Drosha promoter activity. We first generated the pGL3-Drosha-P plasmid, which contains a luciferase gene under the control of a 1 kb DNA fragment in the proximal promoter region. We confirmed that pGL3-Drosha-P conferred luciferase expression and that pGL3-Basic, the luciferase vector lacking the promoter, did not (Fig. 2A). Next, pGL3-Drosha-P and pCH9/3091 expressing HBV under the control of CMV promoter were cotransfected into HepG2 cells. pGL3-Basic was transfected as a control of pGL3-Drosha-P. pCMV-Sport6 was transfected as an irrelevant plasmid control of pCH9/3091. Transient expression of HBV repressed Drosha promoter activity by approximately 2.5-fold (Fig. 2B, groups 3 and group 4). Then we transfected HepG2 cells with Drosha promoter and increasing concentrations of the pCH9/3091 plasmid and found that HBV inhibited the Drosha promoter activity in a dose-dependent manner (Fig. 2C). These results were derived from HBV transient expression cells. We finally detected the activity of the Drosha promoter in HepG2.2.15 cells. Drosha promoter activity was significantly lower in HepG2.2.15 cells than in HepG2 cells (Fig. 2D). In conclusion, these findings suggest that HBV downregulates Drosha expression by inhibiting promoter activity.

3.3. Role of HBx in HBV-induced downregulation of Drosha expression

HBV encodes several proteins from four known genes (P, S, X, and C). To determine which of these proteins correlates with the repression of Drosha promoter activity, we cotransfected pGL3-

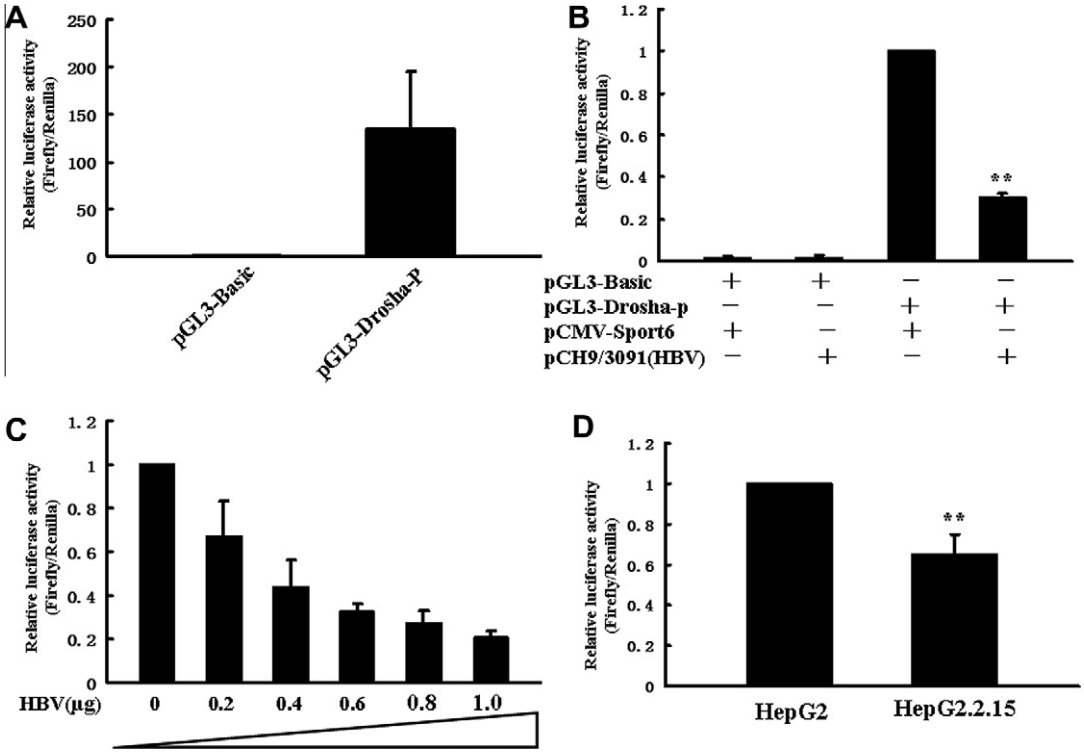

Fig. 2. Mechanism of the effects of HBV on the expression of Drosha (A) The activity of Drosha promoter-luciferase reporter constructs was here confirmed (pGL3-Drosha-P). pGL3-basic was used as a negative control. (B) The activity of the Drosha promoter was inhibited in cells transiently transfected with HBV. Then 0.5 μg of pCH9/3091 was cotransfected with 0.5 μg of pGL3-Drosha-P, and 0.2 μg of reporter renilla luciferase plasmid (pRL-TK) into HepG2 cells. A luciferase assay was performed. Luciferase activity was normalized with the renilla luciferase activity in cell lysate. $^{**}P \leqslant 0.01$. (C) The activity of Drosha promoter was inhibited by HBV in a dose-dependent manner. HepG2 cells were transiently transfected with different amounts of pCH9/3091 (0 μg, 0.2 μg, 0.4 μg, 0.6 μg, 0.8 μg, 1.0 μg), 0.5 μg pGL3-Drosha-P and 0.2 μg pRL-TK. (D) The activity of the Drosha promoter was inhibited in HepG2.2.15 cells (stable HBV-expressing cells). Then 0.5 μg of pGL3-Drosha-P and 0.2 μg of pRL-TK were cotransfected into HepG2 and HepG2.2.15 cells. A luciferase assay was performed. Error bars indicate standard deviations (SD) obtained from three different experiments prepared in triplicate. $^{**}P \leqslant 0.01$.

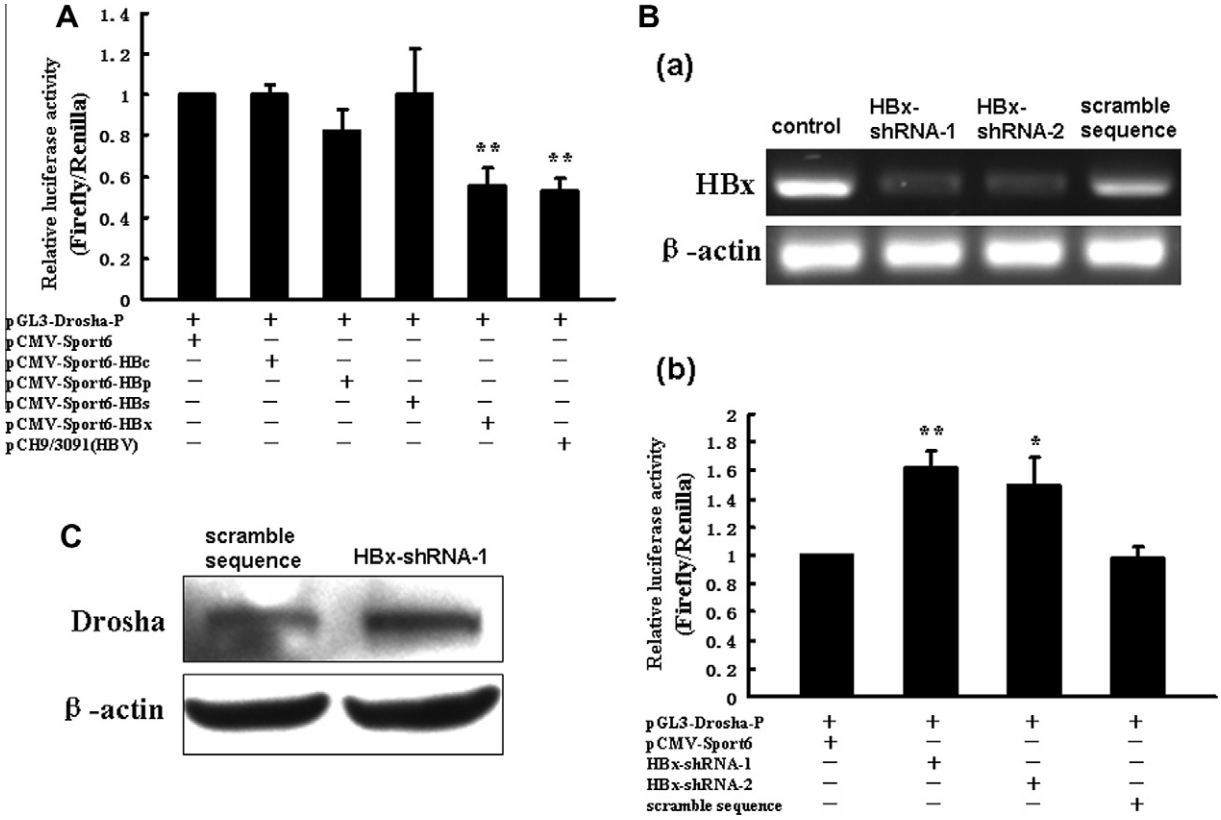

Fig. 3. Effects of HBV and HBx on the expression of Drosha (A) The activity of the Drosha promoter was suppressed in the presence of HBx. 0.5 μg of pGL3-Drosha-P, and 0.2 μg of pRL-TK were cotransfected with 0.5 μg of pCMV-Sport6 (negative control), pCMV-Sport6-HBc, -HBp, -HBs, -HBx, pCH9/3091 (positive control), respectively, into HepG2 cells. (B) Inhibition of HBx expression increased Drosha promoter activity in HepG2.2.15 cells. (a) HBx expression was detected using RT-PCR in HepG2.2.15 which were transiently transfected with the indicated amount (0.5 μg) of HBx-shRNA plasmids. pCMV-Sport6, an irrelevant plasmid, and scramble sequence were used as negative controls. (b) Drosha promoter activity was enhanced when HBx expression inhibited in HepG2.2.15 cells. $^{**}P \leqslant 0.01$ $^{*}P \leqslant 0.05$. (C) Drosha protein expression in the absence of HBx was tested by Western blotting. Three micrograms of HBx-siRNA plasmids was transfected into HepG2.2.15 in 6-wells plates.

Drosha-P with one of four HBV protein expression plasmids, specifically pCMV-Sport6-HBc, pCMV-Sport6-HBp, pCMV-Sport6-HBs, and pCMV-Sport6-HBx, which expresses core protein, DNA polymerase, HBs of different sizes, and HBx protein, respectively (Tian et al., 2011). As shown in Fig. 3A, expression of the P, S, and core proteins did not affect Drosha promoter activity. Expression of HBx significantly reduced Drosha promoter activity. To determine whether depletion of HBx would restore Drosha promoter activity in cells expression HBV, we first confirmed by RT-PCR that shRNA against HBx reduces mRNA expression of the X gene in HepG2.2.15 cells (Fig. 3B upper panel). Also, HBx shRNA specific inhibition effects (do not inhibit other genes expression of HBV) was checked with RT-PCR analysis (Supplement Fig. 6). When a vector expressing HBx shRNA was cotransfected with pGL3-Drosha-P into HepG2.2.15 cells, the activity of Drosha promoter was partially restored (Fig. 3B lower panel). Expression of HBx shRNA expression vector partially restored the protein level of Drosha in HepG2.2.15 cells (Fig. 3C). These data showed that HBx negatively regulated Drosha promoter activity.

3.4. The transcription factor SP1 and AP-2α downregulated the expression of Drosha

It has been shown previously that HBx exerts multi-regulative functions through indirect interactions with host transcription factors, such as AP-1, AP-2, NF-κB, and ATF2, in the gene promoters (Feitelson and Lee, 2007). To determine which transcription factors could play regulatory roles in Drosha promoter activity, we performed bioinformatic analysis of the (1 kb) Drosha promoter (http://www.gene-regulation.com). We identified SP1 and AP-2α transcription factor-binding sites. To determine whether SP1 and AP-2α play a role in the regulation of Drosha promoter activities, pGL3-Drosha-P was cotransfected into the HepG2 cells with vectors expressing SP1 (pCMV-Sport6-SP1) and AP-2α (pCMV-Sport6-AP-2α). Overexpression of SP1 (Fig. 4A upper panel) or AP-2α (Fig. 4B upper panel) resulted in a significant suppression of Drosha promoter activity in HepG2 cells, respectively. In order to clarify these results, we tested Drosha mRNA expression levels in SP-1 or AP-2α overexpression cells and found that Drosha mRNA was decreased in the presence of ectopic expression of SP-1 or AP-2α (Fig. 4A lower panel; Fig. 4B lower panel). To provide a more direct indicator of the roles of these two transcription factors in the regulation of Drosha expression, we used shRNA to knock down the expression of SP1 and P-2α in HepG2.2.15 cells. The expression of SP1 and AP-2α was diminished by their respective shRNAs, as indicated by RT-PCR analysis (Fig. 4C upper panel; Fig. 4D upper panel). Gene knockdown of SP1 and AP2 significantly enhanced the activity of the Drosha promoter (Fig. 4C middle panel, Fig. 4D middle panel). We also directly assayed Drosha mRNA levels in HepG2.2.15 cells with knocked down SP1 and AP-2α expression. When SP1 or AP-2α expression was specifically inhibited by shRNA, Drosha mRNA expression increased (Fig. 4C lower panel, Fig. 4D lower panel). Collectively, these data suggest that transcription factor SP1 or AP-2α represses Drosha promoter activity.

4. Discussion

Recently, the interactions between miRNAs and viruses have attracted considerable attention. The main relationship between miRNAs and viruses could be summarized in as follows:

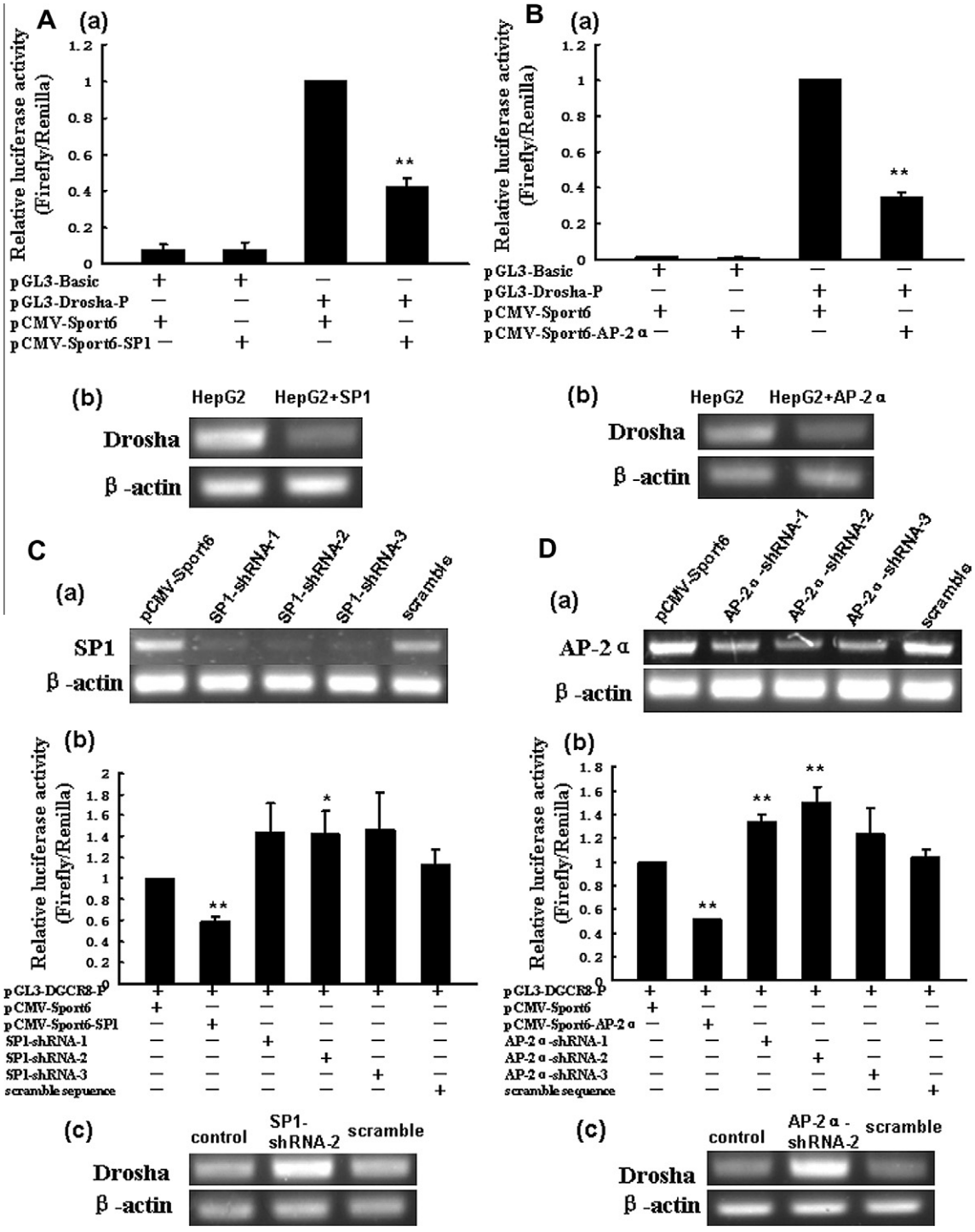

Fig. 4. Transcription factor SP1 and AP-2α downregulated the expression of Drosha (A) Overexpression of SP1 downregulated Drosha expression in HepG2 cells. (a) The activity of Drosha promoter was inhibited in HepG2 cells which were transfected with 0.5 μg SP1 into each well in 24-well plates. $^{**}P \leqslant 0.01$ (b) The expression of Drosha mRNA was inhibited in HepG2 cells transfected with SP1. (B) Overexpression of AP-2α downregulated Drosha expression in HepG2cells. (a) The activity of Drosha the promoter was inhibited in HepG2 cells transfected with 0.5 μg AP-2α into each well in 24-well plates. $^{**}P \leqslant 0.01$. (b) The expression of Drosha mRNA was inhibited in HepG2 cells transfected with AP-2α. (C) Inhibition of SP1 expression increased Drosha expression in HepG2.2.15 cells. (a) SP1 expression was detected with RT-PCR in HepG2.2.15 cells, which were transiently transfected with 0.5 μg SP1-shRNA-1, -2, or -3. pCMV-Sport6 and scramble sequence were used as negative controls. (b) HepG2.2.15 cells were cotransfected with 0.5 μg of SP1 or SP1-shRNA plasmid, 0.5 μg of pGL3-Drosha-P, and 0.2 μg of pRL-TK. Luciferase activity was confirmed by using the luciferase assay system. $^{*}P \leqslant 0.05$ $^{**}P \leqslant 0.01$ (c) The expression of Drosha mRNA was inhibited in HepG2.2.15 cells transfected with SP1-shRNA-2 plasmid. pCMV-Sport6, an irrelevant plasmid, and scramble sequence were used as negative controls. (D) Inhibition of AP-2α expression increased Drosha expression in HepG2.2.15 cells. (a) AP-2α expression was detected with RT-PCR in HepG2.2.15 cells transiently transfected with 0.5 μg AP-2α-shRNA-1, -2, or -3. pCMV-Sport6 and scramble sequences were used as negative controls. (b) HepG2.2.15 cells were cotransfected with 0.5 μg of AP-2α or AP-2α-shRNA plasmid, 0.5 μg of pGL3-Drosha-P and 0.2 μg of pRL-TK. Luciferase activity was confirmed using the luciferase assay system. $^{**}P \leqslant 0.01$. (c) The expression of Drosha mRNA was inhibited in HepG2.2.15 cells transfected with AP-2α-shRNA-2 plasmid. pCMV-Sport6, an irrelevant plasmid, and scramble sequence were used as negative controls.

(1) host-cellular miRNAs can variably influence viral replication by targeting cellular and viral mRNAs. For instance, miR-122 was found to indirectly modulate HCV abundance through the regulation of Heme oxygenase-1 (HO-1), an antioxidant defense and key cyto-protective enzyme (Shan et al., 2007). Additionally, miR-122 positively regulated HCV replication and translation in a manner dependent upon direct interactions between two binding sites in HCV 5′-UTR (Jopling et al., 2005; Niepmann, 2009; Jangra et al., 2010). (2) The miRNAs encoded by viruses play a role in modulating both cellular and viral gene expression. Viral miRNAs target cellular mRNAs involved in cellular survival, proliferation, differentiation, and immune responses in support of viral replication. Through these activities, viruses maintain a host cell environment beneficial to the progression of the viral life cycle. However, viral miRNAs also are perfectly or imperfectly complementary with their own genes, which may play roles in lytic transactivation and viral growth (Skalsky and Cullen, 2010; Sullivan et al., 2005). (3) Viruses can exert a profound impact on the expression profiles of cellular miRNAs to augment their replication potential and pathogenicity. For instance, HBV has been found to alter the expression of liver miRNAs, and the dysregulation of miRNAs in HBV infection was confirmed to be association with cell death, DNA damage, recombination, and signal transduction (Liu et al., 2009; Ji et al., 2011). HBx has been found to significantly down-regulated let-7, which negatively regulated cell proliferation by targeting STAT3 (Wang et al., 2010). However, the underlying mechanisms have not yet been established. In this study, we demonstrated a previously unrecognized mechanism of miRNA regulation, in which HBV changes the miRNA expression pattern by exerting a negative influence on the miRNA processor Drosha. To our knowledge, this is the first study to clarify the mechanism by which HBV dysregulates the miRNA processor Drosha.

Inhibition of Drosha promoter activity by HBV caused dysregulation of miRNAs. Alterations in miRNA expression may be involved in various human diseases, including cancer (Chang and Mendell, 2007). In 2005, Lu et al. first reported on miRNA expression profiles and found that the majority of differentially expressed miRNAs were generally downregulated in tumor tissue relative to healthy tissues (Lu et al., 2005). Aberrant miRNAs expressions have been detected in different types of human tumors, including lung cancer and colorectal neoplasm (Takamizawa et al., 2004; Michael et al., 2003). In hepatocellular carcinoma, the let-7 family, miR-122a, and miR-145 were found to be down-regulated. These had previously been found to be deregulated in other human cancers. The liver is abundant in miR-122a, which targets cyclin G1 to inhibit tumor growth. It has been found to be down-regulated in 70% of HCCs and in all HCC-derived cell lines (Gramantieri et al., 2007). However, the mechanism underlying the widespread deregulation of miRNAs observed in primary cancer remains unknown. It is possible that impaired miRNA maturation processing contributes to this deregulation. An analysis of gene expression in primary tumors has indicated that the widespread down-regulation of miRNAs was due to a defect in Drosha processing (Thomson et al., 2006). Impairment of miRNA processing has been found to accelerate cellular transformation and tumorigenesis (Kumar et al., 2007). We here propose that HBV induces HCC by changing the miRNAs expressed in hepatocytes by impinging on Drosha expression. This provides a novel mechanism for understanding the formation of HBV-induced HCC.

Although our reports describe the regulation of miRNAs by HBV protein via Drosha expression, there are other mechanisms of HBV-induced miRNA deregulation. P53, a tumor suppressor, has been reported to interact with the Drosha processing complex through the association with DDX5. It facilitates the processing of primary miRNAs to precursor miRNAs. siRNA-mediated p53 knockdown attenuates these increments of pre-miRNAs and mature miRNAs (Suzuki et al., 2009). DNA–protein or protein–protein complexes formed by HBV and p53 caused in inactivation of p53 function and so contributed to hepatocarcinogenesis (Qu et al., 2009; Feitelson, 1998). It is possible that HBV may downregulate miRNA expression by affecting p53. This may be another mechanism underlying the under-expression of miRNAs in the presence of HBV protein.

We also demonstrated that transcription factor SP1 and AP-2α decreased the activity of Drosha promoter. We investigated the effects of HBV on SP1 or AP-2α expression using RT-PCR in HepG2 cells and HepG2.2.15 cells. Unfortunately, we did not find any distinct changes in SP1 or AP-2α expression in HBx-expressing cells (data not shown). Previous studies revealed that HBx can phosphorylate SP1 and up-regulate the DNA-binding activity of SP1. Increasing the DNA binding ability of SP1 phosphorylated by HBx might be an important means of regulating gene expression (Lee et al., 1998; Liu et al., 2010). We supposed that HBx increased the binding of Sp1 to Drosha promoter by phosphorylating SP1, which inhibited activity of Drosha and consequently downregulated Drosha expression. AP-2α was found to act as a tumor suppressor. However, there is little research into how HBV might affect AP-2α expression or its DNA-binding activity. More research in this direction is needed.

In brief, we first demonstrated that HBV could downregulate Drosha expression via HBx-induced inhibition of the activity of Drosha promoter. The transcription factors SP1 and AP-2α might be involved in this process and may be the mechanism underlying HBV-associated miRNA dysregulation. Our reports may provide a new insight into molecular mechanisms underlying HBV-related disease.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.antiviral.2012.04.004.

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