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HDAC11 restricts HBV replication through epigenetic repression of cccDNA transcription

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ABSTRACT

Hepatitis B virus (HBV) infection remains an important public health problem worldwide. Covalently closed circular DNA (cccDNA) exhibits as an individual minichromosome and is the molecular basis of HBV infection persistence and antiviral treatment failure. In the current study, we demonstrated that histone deacetylase 11 (HDAC11) inhibits HBV transcription and replication in HBV-transfected Huh7 cells. By using an HBV *in vitro* infection system, HDAC11 was found to affect the transcriptional activity of cccDNA but did not affect cccDNA production. Chromatin immunoprecipitation (ChIP) assays were utilized to analyze the epigenetic modifications of cccDNA. The results show that HDAC11 specifically reduced the acetylation level of cccDNA-bound histone H3 but did not affect that of histone H4. Furthermore, HDAC11 overexpression decreased the levels of cccDNA-bound acetylated H3K9 (H3K9ac) and H3K27 (H3K27ac). In conclusion, HDAC11 restricts HBV replication through epigenetic repression of cccDNA transcription. These findings reveal the novel role of HDAC11 in HBV infection, further broadening our knowledge regarding the functions of HDAC11 and the roles of HDACs in the epigenetic regulation of HBV cccDNA.

1. Introduction

Hepatitis B virus (HBV) infection remains an important public health problem worldwide. According to the Global hepatitis report from the World Health Organization (WHO), an estimated 257 million people are living with chronic HBV infection and are at risk of developing liver cirrhosis or hepatocellular carcinoma (WHO, 2017). HBV is a hepatotropic, noncytopathic, small, enveloped DNA virus with a 3.2 kb partially double-stranded relaxed circular genome (rcDNA) (Mao et al., 2013). Upon entry into a hepatocyte, the rcDNA in the HBV nucleocapsid will be translocated into the nucleus and converted into a covalently closed circular DNA (cccDNA) molecule, which serves as the template for the transcription of all viral mRNAs. HBV cccDNA exhibits as an individual minichromosome with a long half-life, which is responsible for the persistence of the infection during the natural course of chronic infection and prolonged antiviral treatment (Hong et al., 2017). Therefore, targeting cccDNA represents the key approach to cure HBV infection.

Histone acetylation is one of the most studied epigenetic modifications. As a result of the balance of histone acetyltransferases (HATs) and histone deacetylases (HDACs), histone acetylation plays a crucial role in chromatin remodeling and the regulation of gene transcription (Ropero and Esteller, 2007; Barneda-Zahonero and Parra, 2012; Ellmeier and Seiser, 2018). Eighteen human HDACs, namely HDAC1-11 and SIRT1-7, have been identified thus far and are grouped into four classes based on their homology with yeast proteins. Several HDACs have been reported to regulate HBV replication with different mechanisms. For example, HDAC1, belonging to class I HDAC, can be recruited to the cccDNA minichromosome and suppresses HBV transcription via the deacetylation of cccDNA-bound histone H3 and histone H4 (Pollicino et al.,

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2006). HDAC4, a member of the class II HDACs, is involved in the modulation of HBV replication and hepatocyte differentiation by microRNA-1 (Zhang et al., 2011). SIRT1, the most widely studied member of the class III HDACs, facilitates HBV replication and transcription by targeting the transcription factor activator protein-1 (Ren et al., 2014). In contrast, SIRT3, another member of the class III HDACs, can epigenetically restrict HBV cccDNA transcription by acting cooperatively with histone methyltransferases (Ren et al., 2018). However, the role of HDAC11, the newly identified and sole member of class IV HDAC (Gao et al., 2002), in HBV life cycle remains unexplored.

In this study, we investigated the potential role of HDAC11 in the HBV life cycle. The results show that HDAC11 inhibited HBV transcription and replication in HBV-transfected Huh7 cells. By using an HBV *in vitro* infection system, HDAC11 was found to affect the transcriptional activity of cccDNA but did not affect cccDNA production. Further mechanistic analysis revealed that HDAC11 specifically reduced the acetylation level of cccDNA-bound H3 but not that of H4. Moreover, HDAC11 overexpression decreased the levels of cccDNA-bound acetylated H3K9 (H3K9ac) and H3K27 (H3K27ac). Our data suggest that HDAC11 is an important host factor involved in the epigenetic modification of cccDNA, which will provide a reference for the use of HDAC11 or its agonists in the prevention or treatment against HBV infection.

2. Materials and methods

2.1. Cell culture

Huh7 and Huh7-NTCP cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Life Technologies) containing 10% fetal bovine serum (FBS; Life Technologies). HepAD38 cells, which replicate HBV under conditions that can be regulated with tetracycline (Tet-off, Ladner et al., 1997), were maintained in DMEM supplemented with 10% FBS and 400 μg/ml G418 (Life Technologies). All cells were maintained in a humidified incubator at 37 °C with 5% CO2.

2.2. Plasmids and siRNAs

The HBV replication-competent plasmid pSM2 contains a head-to-tail tandem dimer of the HBV genome (genotype D, subtype ayw) and has been described previously (Sommer et al., 2000). The rcccDNA system comprising prcccDNA and pCMV-Cre was a gift from Prof. Qiang Deng (Key Laboratory of Medical Molecular Virology (MOE & MOH), School of Basic Medical Sciences, Fudan University, Shanghai, China). pFlag-HDAC11 was constructed by the in-frame insertion of full-length *HDAC11* into the pXJ40-Flag vector. The coding sequence of *HDAC11* (GenBank accession number NM_024827.4) was PCR-amplified with the following primer pair: forward, 5′-ATGCTACACACAACCCAGCTGT ACC-3' (*Not* I); reverse, 5′-TCAGGGCACTGCAGGGGGAA-3' (*Bgl* II). The small interfering RNA (siRNA) targeting human HDAC11 and the control siRNA (siNC) were purchased from Ribobio, and the siHDAC11 sequences have been reported previously (Toropainen et al., 2010). The plasmids and siRNAs were transfected into cells using the Lipofectamine 2000 Reagent (Invitrogen) or the Lipofectamine RNAiMAX Reagent (Invitrogen) following the manufacturer's instructions.

2.3. HBV infection and enzyme-linked immunosorbent assay (ELISA)

Huh7-NTCP cells were infected as described elsewhere (Zhao et al., 2018; Zhou et al., 2017). The supernatants were collected and centrifuged at 3000 × *g* for 5 min. The levels of HBeAg and HBsAg in the supernatants were examined by ELISA (KHB) according to the manufacturer's instructions.

2.4. HBV DNA and RNA analysis

Intracellular core-associated HBV DNA was extracted and subjected to Southern blot or quantitative real-time PCR (Roche) as described previously (Liu et al., 2017). HBV cccDNA was extracted and then digested by plasmid-safe ATP-dependent DNase (PSD, Epicentre) before it was detected by TaqMan PCR amplification (Toyobo) as reported elsewhere (Qi et al., 2014; Zhang et al., 2017). The total RNA was extracted from cells using TRIzol Reagent (Invitrogen) and was detected by Northern blot (Ambion) or quantitative real-time PCR (Qiagen) according to the manufacturer's protocols. Hybridization signals were quantified with the NIH ImageJ software. The primers used for real-time PCR were as follows: HBV 3.5 kb RNA-F, 5′-CTGGGTGGGTGTTA ATTTGG-3'; HBV 3.5 kb RNA-R, 5′- TAAGCTGGAGGAGTGCGAAT-3'; HBV total RNA-F, 5′- CCGTCTGTGCCTTCTCATCTGC-3'; HBV total RNA-R, 5′- ACCAATTTATGCCTACAGCCTCC-3'.

2.5. HBV capsid detection and western blot

The HBV capsids were extracted and subjected to native agarose gel electrophoresis as described previously (Zhao et al., 2017). The Western blot protocol has also been reported elsewhere (Xu et al., 2012). The used antibodies were as follows: anti-HBc (Dako), anti-Flag (Sigma), anti-β-actin (Proteintech) and anti-HDAC11 (Sigma). The relative band intensities of the proteins were quantified using the NIH ImageJ software.

2.6. Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed as described previously (Qi et al., 2014). Briefly, Huh7 cells were fixed in 1% formaldehyde at 48 h post-transfection and were sheared by sonication in 1% SDS lysis buffer. Cross-linked chromatin was precleaned for 2 h before it was incubated with antibodies for 16 h at 4 °C. Then, it was immunoprecipitated with protein A/G-conjugated agarose beads for 2 h. After washing a few times, the products were digested by RNase A (Omega) and proteinase K (Qiagen). Reverse cross-linking was performed at 65 °C for at least 8 h. Finally, the DNA was purified with a gel extraction kit (Axygen) and was digested by PSD before cccDNA detection. The strategy for quantification and calculation was performed as described elsewhere (Zhang et al., 2017). The used antibodies were as follows: anti-IgG (Proteintech), anti-Flag (Cell Signaling Technology), anti-H3 (Abcam), anti-H4 (Abcam), anti-acH3 (Upstate), anti-acH4 (Upstate), anti-H3K4ac (Diagenode), anti-H3K9ac (Abcam), anti-H3K27ac (Abcam), and anti-H3K36ac (Diagenode). The primers used for real-time PCR were as follows: rcccDNA-F, 5′-CAAGACAGGTTTAAGGAGAC-3'; rcccDNA-R, 5′-GAGAGAAAGGCAAAGTGGAT-3'; GAPDH-ChIP-F, 5′-TCGACAGTCAGCCGCATCT-3'; GAPDH-ChIP-R, 5′-CTAGCCTCCCGG GTTTCTCT-3'.

2.7. Statistical analysis

Data are presented as the mean ± standard error of the mean. Statistics were performed using the Student *t*-test or the nonparametric Mann-Whitney *U* test. $P < 0.05$ was considered statistically significant (NS: not significant, *$p < 0.05$, **$p < 0.01$).

3. Results

3.1. HDAC11 overexpression inhibits HBV transcription and replication in a dose-dependent manner

We screened HDAC1-11 to identify their effects on HBV replication. The results show that HDAC1, HDAC3, HDAC9, HDAC10 and HDAC11 significantly inhibited HBV replication (Fig. S1). Considering that several members of class I-III HDAC family have been reported to regulate

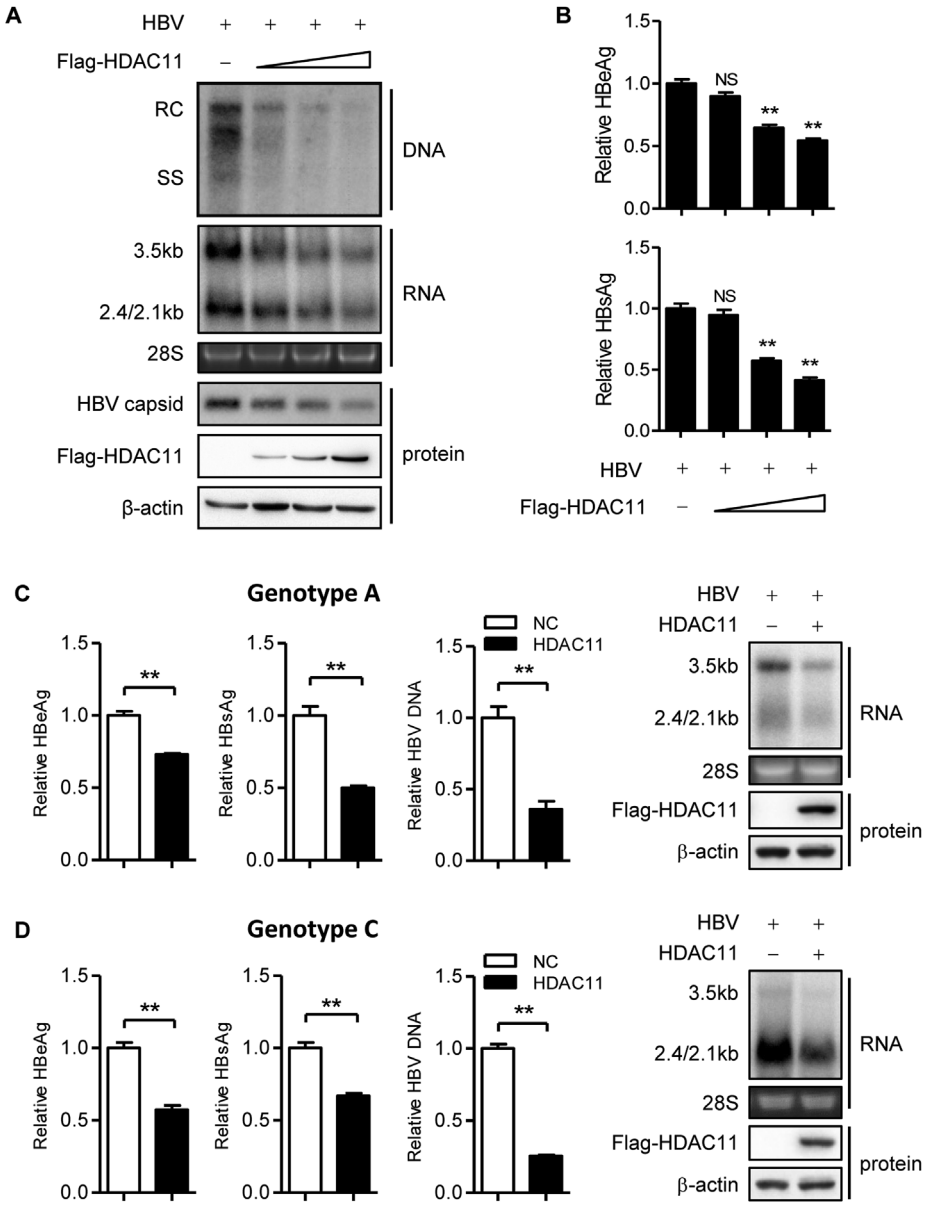

Fig. 1. HDAC11 overexpression inhibits HBV transcription and replication in a dose-dependent manner. Huh7 cells cotransfected with pSM2 and increasing doses of pFlag-HDAC11 were harvested at 72 h post-transfection. (A) Intracellular core-associated HBV DNA and RNA were detected by Southern blot and Northern blot, respectively. The 28S ribosomal RNA served as a loading control. Expression of proteins were detected by Western blot using anti-HBc and anti-Flag antibodies, β-actin served as a loading control. (B) HBeAg and HBsAg in the culture supernatants were detected by ELISA. Abbreviations: RC, relaxed circular DNA; SS, single-stranded DNA. (C, D) Huh7 cells cotransfected with pFlag-HDAC11 and pHBV1.3-A (GenBank accession number AF305422.1) or pHBV1.3-C (GenBank accession number KM999991.1) were harvested at 72 h post-transfection.

HBV lifecycle previously, HDAC11, the newly identified and unique member of class IV HDAC, was selected to be investigated in the following study. To investigate whether HDAC11 plays an important role in HBV life cycle, a plasmid expressing HDAC11 fused with a Flag tag, named pFlag-HDAC11, was constructed. Then, a gradient concentration of pFlag-HDAC11 plasmids was cotransfected with the HBV replication-competent plasmid pSM2 (Yao et al., 2018) into Huh7 cells. Cells were harvested at 72 h post-transfection, and a dose-dependent decrease in the intracellular HBV RNA, core-associated DNA and capsid contents was observed (Fig. 1A), which was also observed for hepatitis B e antigen (HBeAg) and hepatitis B surface antigen (HBsAg) in the cell culture supernatants (Fig. 1B). Consistent results were obtained in the experiments using HBV genotype A or C plasmids (Fig. 1C and D). These results suggest that HDAC11 overexpression can remarkably suppress HBV transcription and replication in a dose-dependent manner.

3.2. HDAC11 silencing promotes HBV transcription and replication

To further elucidate the impact of endogenous HDAC11 on HBV life cycle, RNA interference was employed. Three siRNAs targeting human HDAC11 were examined in Huh7 cells. The siHDAC11-3 had the best efficiency to downregulate the level of HDAC11 mRNA (Fig. 2A), and was selected to be used in the following experiments. Huh7 cells were cotransfected with pSM2 and siNC or siHDAC11-3. The results show that the knockdown of HDAC11 increased the levels of HBV 3.5 kb RNA and total RNA (Fig. 2B and C). The levels of intracellular core-associated HBV DNA and capsid were also enhanced (Fig. 2C and D). Consistently, HBeAg and HBsAg in the cell culture supernatants were increased with HDAC11 silencing (Fig. 2E and F). As the antibodies could not detect the endogenous HDAC11 protein in Huh7 cells, the silencing effect of siHDAC11-3 on the HDAC11 protein was verified in HDAC11 stably transfected Huh7 cells (Huh7-HDAC11 cells) (Fig. 2G). These results indicate that endogenous HDAC11 silencing promotes HBV transcription and replication.

3.3. HDAC11 inhibits the transcriptional activity of HBV cccDNA in HBV infection system

To analyze how HDAC11 is involved in the HBV natural life cycle, Huh7-NTCP, a stable human NTCP-expressing Huh7 cell line, was subjected to HDAC11 overexpression or silencing and the following HBV virions inoculation as described previously (Zhao et al., 2018;

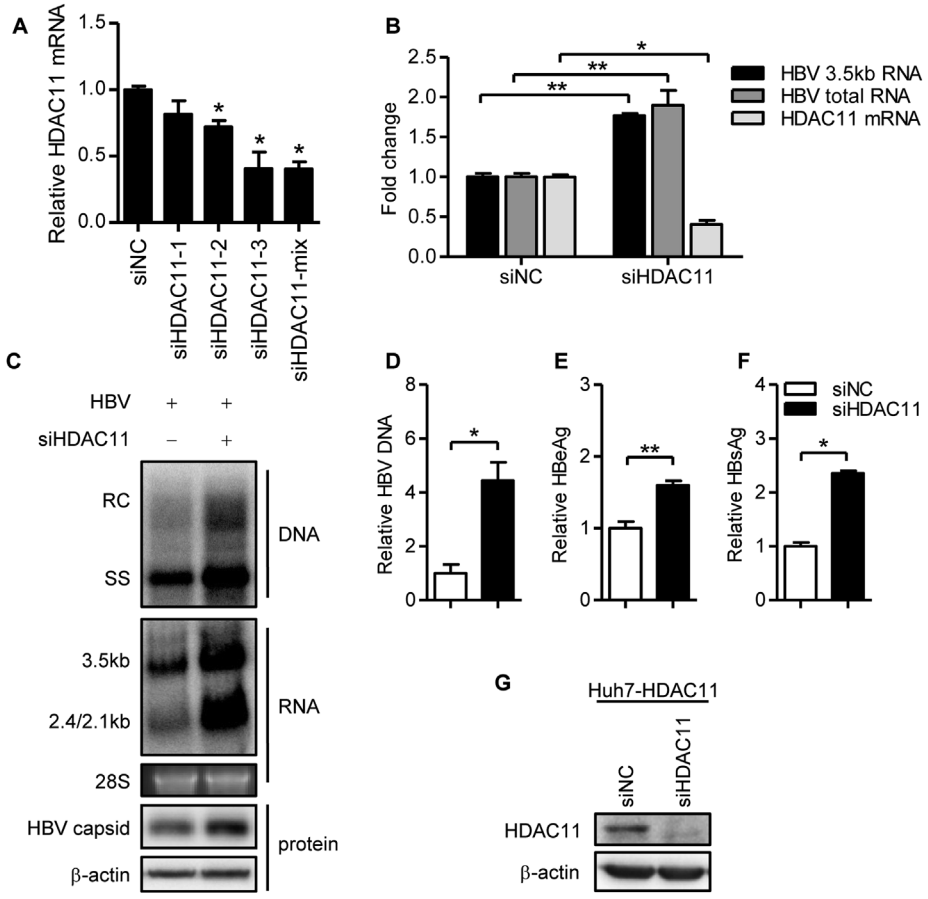

Fig. 2. HDAC11 silencing promotes HBV transcription and replication. (A) Huh7 cells co-transfected with pSM2 and siRNAs were harvested at 72 h post-transfection. The efficiency of three siRNAs targeting human HDAC11 was examined by real-time PCR and siHDAC11-3 was selected to be used in the following experiments. (B–D) HBV replication and transcription intermediates in Huh7 cells were detected by real-time PCR, Southern blot and Northern blot, respectively. Expression of proteins were detected by Western blot. (E, F) HBeAg and HBsAg in the culture supernatants were detected by ELISA. (G) The efficiency of siHDAC11-3 to knockdown HDAC11 protein was examined in HDAC11 stably transfected Huh7 cells (Huh7-HDAC11 cells). HDAC11 protein was detected by Western blot using anti-HDAC11 antibody, β-actin served as a loading control.

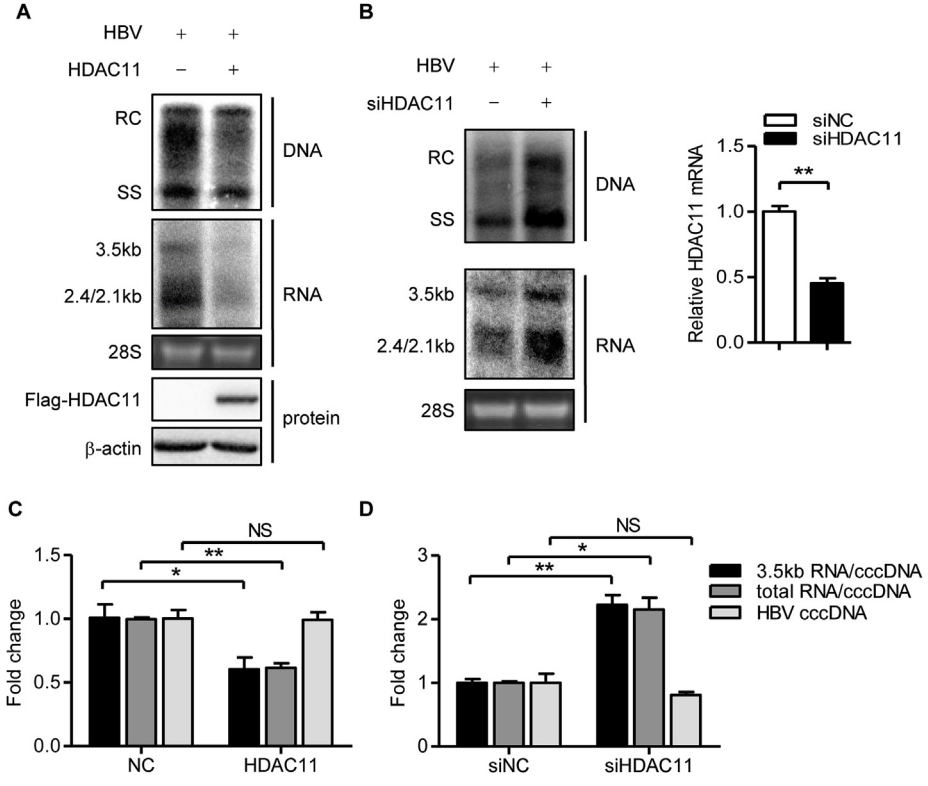

Fig. 3. HDAC11 inhibits the transcriptional activity of HBV cccDNA in HBV infection system. Huh7-NTCP cells were transfected with indicated plasmids or siRNAs and then inoculated with 1000 genome equivalents/cell of HBV for 16 h. Cells were harvested at day 3 post-infection. (A, B) HBV replication and transcription intermediates were detected by Southern blot and Northern blot. HDAC11 expression was detected by Western blot or real-time PCR. (C, D) HBV cccDNA was extracted and then digested by the plasmid-safe DNase before it was detected by real-time PCR using the cccDNA-selective primers.

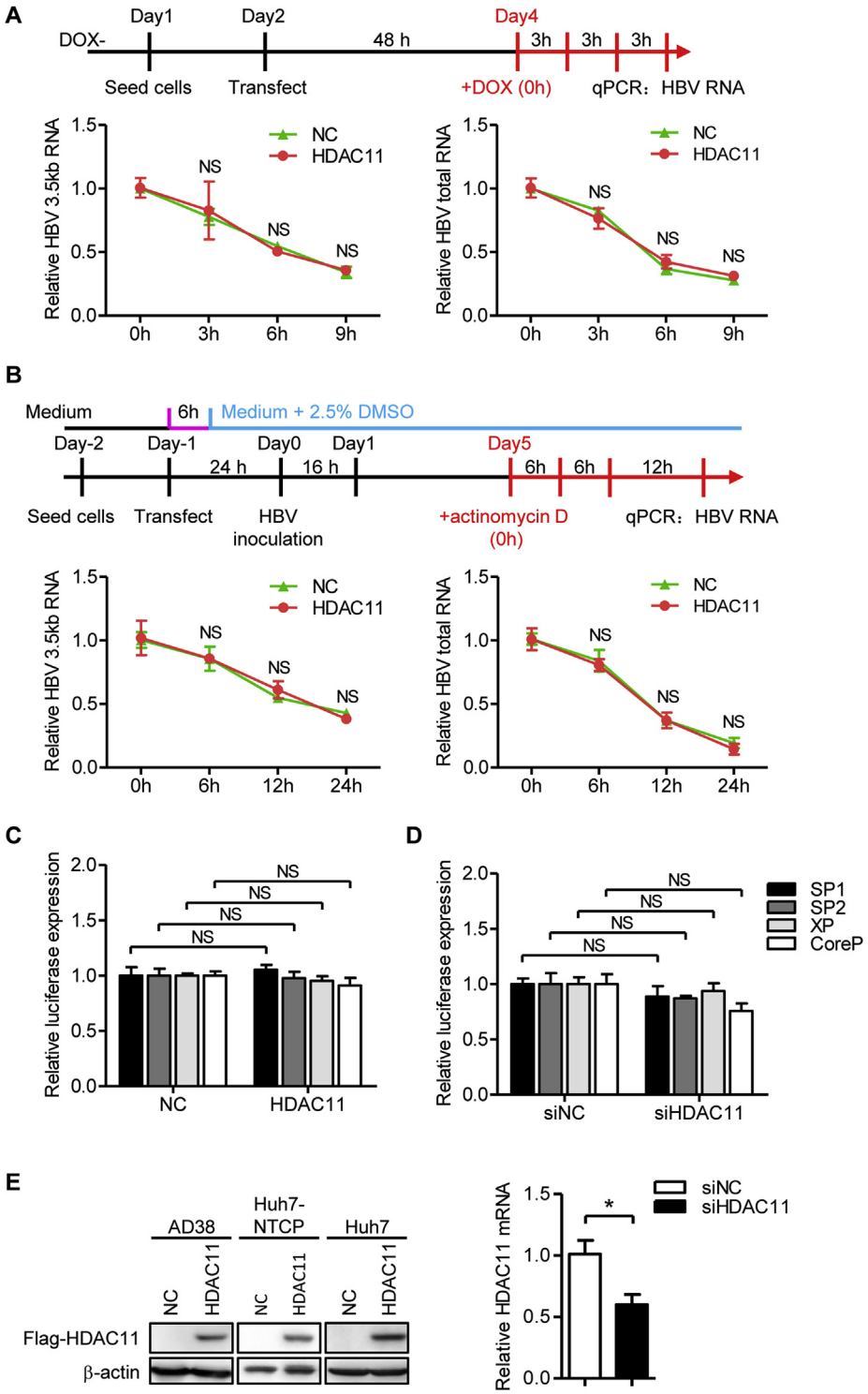

Fig. 4. HDAC11 has no effects on HBV RNA stability and the expression of promoter reporters. (A) Schematic illustration of the experimental design: HepAD38 cells transfected with indicated plasmids were treated with Doxycycline (DOX, 2 μg/ml) at 48 h post-transfection and collected at the indicated times after treatment. HBV RNAs were then quantified by real-time PCR. (B) Schematic illustration of the experimental design: Huh7-NTCP cells transfected with indicated plasmids were treated with actinomycin D (5 μg/ml) at 5th day post-infection and collected at the indicated times after treatment. HBV RNAs were then quantified by real-time PCR. (C, D) Huh7 cells were cotransfected with indicated luciferase reporter plasmids and pFLAG-HDAC11 or siHDAC11. Empty vector and siNC served as negative controls. The luciferase activity was measured at 48 h post-transfection. (E) HDAC11 expression in the above cells was detected by Western blot or real-time PCR.

Zhou et al., 2017). Consistent with the results in Figs. 1 and 2, the levels of intracellular HBV core-associated DNA and RNA were significantly reduced with HDAC11 overexpression (Fig. 3A) and were enhanced with HDAC11 silencing (Fig. 3B). Notably, regardless of whether HDAC11 was overexpressed or silenced, the level of HBV cccDNA remained stable (Fig. 3C and D), which indicated that HDAC11 has no effect on cccDNA synthesis. Furthermore, the relative ratios of both HBV 3.5 kb RNA to cccDNA and total RNA to cccDNA were decreased with the overexpression of HDAC11 (Fig. 3C) but were increased with the knockdown of HDAC11 (Fig. 3D). Altogether, these results indicate that HDAC11 represses HBV cccDNA transcriptional activity without affecting the production of cccDNA.

3.4. HDAC11 inhibits cccDNA transcription through the deacetylation of cccDNA-bound histone H3

To investigate how HDAC11 inhibits HBV transcription, we first checked the possibility that HDAC11 may affect the stability of HBV RNA. HepAD38 cells were cultured in normal medium before and after transfection, then Doxycycline (DOX) was used to block the production of new HBV RNA at 48 h post-transfection and cells were harvested every 3 h. Results show that HDAC11 overexpression did not change the

degradation rate of either HBV 3.5 kb RNA or total RNA (Fig. 4A). Consistent results were obtained in the experiments using actinomycin D, an RNA polymerase II inhibitor which blocks RNA synthesis, in HBV-infected Huh7-NTCP cells (Fig. 4B). Then, a dual-luciferase reporter assay was used to examine the effects of HDAC11 on the activity of the four following HBV promoters: preS1, preS2, Core and X. The overexpression or silencing of HDAC11 did not affect the luciferase expression of the reporters (Fig. 4C and D), which means HDAC11 may not directly regulate the transcriptional activity of HBV by targeting the promoter sequences. Because cccDNA forms minichromosome mainly with histones in cell nuclei and active histone posttranslational modifications (PTMs) are enriched at the transcription start site regions of HBV genome, rather than the promoter regions (Tropberger et al., 2015). We hypothesized that HDAC11, a histone deacetylase, might regulate HBV transcription by epigenetically modifying the cccDNA minichromosome.

To test this hypothesis, cccDNA chromatin immunoprecipitation (ChIP) assays were employed. Considering that the quantity of cccDNA is low in HBV-infected Huh7-NTCP cells, we used a recombinant cccDNA (rcccDNA) system as a surrogate. In this system, rcccDNA can be produced with high efficiency, epigenetically organized as minichromosome similar to the organization of natural cccDNA, and measured more precisely with plasmid-safe DNase (PSD) digesting and using specific primers (Li et al., 2018; Qi et al., 2014). The ChIP results indicated that HDAC11 was recruited to cccDNA (Fig. 5A) and that cccDNA-bound histone H3 was significantly hypoacetylated in Huh7 cells overexpressing HDAC11, whereas the acetylation level of cccDNA-bound histone H4 did not change (Fig. 5B). The levels of total histone H3, total H4, acetylated H3 (acH3) and acetylated H4 (acH4) associated with the GAPDH promoter, which served as a host gene control, remained unchanged (Fig. 5B).

To identify the acetylated lysine residue of H3 that is preferentially targeted by HDAC11, we used ChIP assays to screen four lysine residues of H3, which are the most reported sites to regulate gene transcription. As shown in Fig. 5C, HDAC11 overexpression decreased the levels of cccDNA-bound H3K9ac and H3K27ac without affecting cccDNA-bound H3K4ac and H3K36ac. In the meanwhile, H3K9ac associated with GAPDH promoter was also reduced and the others remained unchanged. Correspondingly, HDAC11 silencing resulted in enhanced levels of cccDNA-bound acH3, H3K9ac and H3K27ac, while H3K9ac associated with GAPDH promoter was also increased (Fig. 5D). The absolute enrichment values and IgG controls were shown in Fig. S2.

Collectively, these results indicate that HDAC11 restricts cccDNA transcription by reducing the acetylation level of cccDNA-bound histone H3.

4. Discussion

HDAC11 was first discovered in 2002. The protein homology determines that HDAC11 is identified as a member of the HDAC family (Gao et al., 2002). Considering that it shares a low sequence homology with the existing HDACs, HDAC11 was classified into class IV HDAC and was the sole member of this class (Gao et al., 2002). HDAC11 has been implied to exert regulatory functions in some important biological processes, such as immunity (Villagra et al., 2009; Yuan et al., 2018), tumorigenesis (Buglio et al., 2011; Deubzer et al., 2013; Sahakian et al., 2015), metabolic homeostasis (Sun et al., 2018), and cell development and differentiation (Byun et al., 2017; Liu et al., 2009). Here, we first demonstrated that HDAC11 negatively regulated HBV transcription and replication in both HBV-transfected and -infected cells, revealing a novel role of HDAC11 in HBV infection. Further mechanistic analysis by using ChIP assays revealed that the HDAC11-mediated inhibition of HBV transcription is linked with the epigenetic modification of the cccDNA minichromosome.

As a negative transcriptional regulator, HDAC11 has been reported to inhibit the transcription of different host genes by decreasing histone acetylation at the promoter regions (Villagra et al., 2009; Byun et al., 2017; Yuan et al., 2018). The cccDNA minichromosome structure allows itself to undergo multiple posttranslational modifications of histones, such as acetylation, methylation, phosphorylation and ubiquitination (Hong et al., 2017). In the current study, we demonstrated that HDAC11 is recruited to cccDNA and inhibits HBV transcription through deacetylating cccDNA-bound histone H3 without affecting histone H4. Furthermore, HDAC11 overexpression decreased the levels of cccDNA-bound H3K9ac and H3K27ac. However, some other reports have shown that HDAC11 overexpression reduces the acetylation of both H3 and H4 at the IL10 and IL13 promoters (Villagra et al., 2009; Yuan et al., 2018). In HDAC11-depleted A549 cells (human alveolar epithelial cells), acH4, rather than acH3, seems to be the main substrate of HDAC11 (Nutsford et al., 2018). These ambivalent results imply that the regulatory methods of HDAC11 are diverse in different biological processes. In addition to deacetylating histones, HDAC11 could also directly modulate the acetylation status of transcription factors, such as E4 binding protein-4 (E4BP4), to regulate interleukin-13 expression in $CD4^+$ T cells (Yuan et al., 2018). Thus, further studies are needed to determine whether HDAC11 could modulate transcription factors to regulate HBV life cycle.

A recent report shows that HDAC11 exhibits anti-influenza A virus (IAV) properties through involving in host innate antiviral response, the IAV-induced phosphorylation of the signal transducer and activator of transcription 1 (STAT1) and the subsequent expression of the interferon stimulated genes (ISGs) were significantly decreased in HDAC11-depleted human lung epithelial cells (Nutsford et al., 2018). Moreover, after LPS stimulation or IFN treatment, HDAC11 mRNA and protein levels increased in macrophages (Lin et al., 2013). In addition, administration of IFN-α resulted in cccDNA-bound histone hypoacetylation and active recruitment of transcriptional corepressors to cccDNA in HepG2 cells (Belloni et al., 2012). Thus, it is possible for HDAC11 to participate in the process of IFN treatment against HBV. Considering that the ISRE (IFN-stimulated response element) in HBV genome is necessary for the recruitment of STATs to cccDNA, and the transcription of the ISRE mutant HBV could not be repressed by IFN-α treatment (Belloni et al., 2012), we constructed a similar ISRE mutant HBV plasmid (ISREm). Unexpectedly, we found that HDAC11 overexpression could still reduce the antigen and RNA levels of ISREm (Fig. S3), which means HDAC11 could inhibit HBV in a STAT-independent manner. Besides, according to another report, HDAC11 antagonizes type I IFN signaling via impeding the recycle of type I IFN receptor, and ISG15 mRNA levels were increased in HDAC11-depleted cells (Cao et al., 2019). These ambivalent results suggest that further substantial studies are needed to answer what is the exact role of HDAC11 in innate antiviral response, and whether this account for the HDAC11-mediated inhibition of HBV.

HDACs are important epigenetic regulatory factors, and some of them have important roles in the regulation of cccDNA transcriptional activity. HDAC1 can inhibit HBV replication via deacetylating both cccDNA-bound H3 and H4 (Pollicino et al., 2006). HDAC4 can inhibit the acetylation of cccDNA-bound H3 (Xing et al., 2019), but it remains unknown whether the acetylation of cccDNA-bound H4 is also affected. SIRT3 was reported to restrict HBV transcription through the deacetylation of cccDNA-bound H3 rather than the deacetylation of cccDNA-bound H4 (Ren et al., 2018), which is similar to HDAC11. However, HDAC11 mainly deacetylates cccDNA-bound H3K9 and H3K27, while SIRT3 deacetylates cccDNA-bound H3K9 (Ren et al., 2018). Unlike the above family members, SIRT1 is recruited to cccDNA (Belloni et al., 2009) but facilitates HBV replication and transcription by targeting the transcription factor activator protein-1 (Ren et al., 2014). Taken together, with the addition of HDAC11, at least one member of each class of the HDAC family can epigenetically regulate cccDNA, and they have preferences for different lysine residues of the histones. Thus, it is attractive to analyze whether HDACs work synergistically and elaborately modify the acetylation of cccDNA-bound histones.

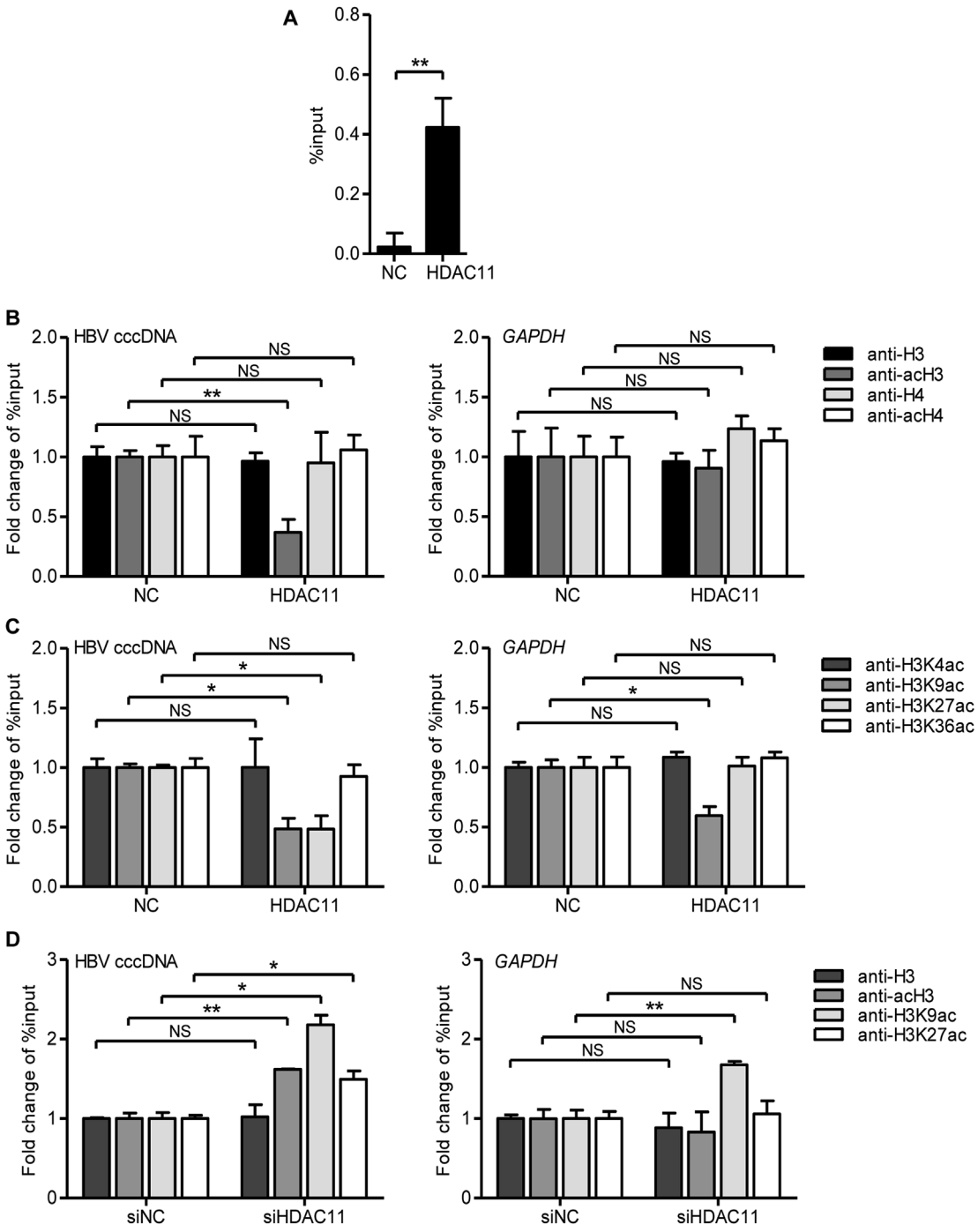

Fig. 5. HDAC11 inhibits cccDNA transcription through the deacetylation of cccDNA-bound H3. (A) Cross-linked chromatins from Huh7 cells cotransfected with rcccDNA system and empty vector or pFlag-HDAC11 were immunoprecipitated with anti-IgG and anti-Flag antibodies, cccDNA was purified and then digested by the plasmid-safe DNase (PSD) before it was detected by real-time PCR. (B–D) Levels of histone H3, acetylated H3, H4, acetylated H4, H3K4ac, H3K9ac, H3K27ac and H3K36ac associated with cccDNA were examined by ChIP assays with indicated antibodies. DNAs were purified and cccDNA was digested by PSD before it was detected by real-time PCR. GAPDH promoter served as a host gene control. The absolute enrichment values and IgG controls were shown in Fig. S2.

In conclusion, our study demonstrates that HDAC11 exhibits an anti-HBV function through the epigenetic regulation of cccDNA, revealing the novel role of HDAC11 in viral infection. These findings further broaden our knowledge regarding the physiological functions of HDAC11 and the roles of HDACs in the epigenetic regulation of HBV cccDNA. Additionally, our results help to provide a reference for the development of anti-HBV agents to functionally cure cccDNA.

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Declaration of competing interest

The authors declare that they have no conflicts of interest to this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2019.104619.

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