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Histone deacetylases and acetylated histone H3 are involved in the process of hepatitis B virus DNA replication

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ABSTRACT

Aims: The aim of this study was to investigate the relationship between anti-HBV treatment and the regulation of HDACs during HBV DNA replication.

Methods: HDAC activities and HBV DNA levels in CHB patients' sera were measured and correlation analysis was made. The changes of HDAC2, HDAC6, AH3 and histone H3 levels in normal control and 4 CHB patient liver tissue samples before and after antiviral treatment were examined. The HDAC inhibitor, TSA, anti-HBV agents, ETV and IFN-α were used to stimulate HepG2.2.15 cells. The levels of HBV DNA, pgRNA in supernatants, and cccDNA in the cells were determined by PCR. The HDAC activity, HDAC6, HDAC2, AH3 and H3 protein levels in cells were tested at days 3, 6, and 9 after treatments.

Key findings: HDAC activity was positively correlated with HBV DNA in the HBV patients' sera. The levels of HDAC2, HDAC6 and AH3 were notably decreased after antiviral treatment. When compared with antiviral treatment group, the normal liver tissue showed obviously decreased HDAC2, HDAC6 and AH3 protein levels. In vitro study, the level of HBV DNA, the HDAC activity, and the HDAC2, HDAC6 and AH3 protein levels decreased in the ETV, IFN-α and TSA groups compared with the control group. The pgRNA level in supernatants was declined in the IFN-α group and increased in the ETV and TSA groups. cccDNA expression was suppressed by IFN-α.

Significance: The changes of HBV replicative products during antiviral treatment are associated with histone deacetylation. Acetylated histone H3 is involved in the process of hepatitis B virus DNA replication.

1. Background

Hepatitis B virus (HBV) is a partially double-stranded DNA virus, and the partially double-stranded DNA genome is shielded by a 27 nm internal capsid (core particle) [1,2]. To infect hepatocytes, HBV enters through Na^+-taurocholate cotransporting polypeptide (NTCP), and after capsid disassembly, circular DNA (rcDNA) is relaxed, transported into the nucleus and then converted into a covalently closed circular DNA (cccDNA) molecule through the actions of the involved enzymes. cccDNA is the template for transcription of the 3.5 kb preC RNA and pgRNA, 2.4 and 2.1 kb preS/S mRNAs, and 0.7 kb HBx mRNA. Pregenomic (pg) RNA is packaged, reverse transcribed and then subsequently used for forming virions or generating cccDNA [1,3].

HBV infection remains a major public health problem worldwide. Current therapies for chronic hepatitis B (CHB) mainly involve interferon-α (IFN-α) and nucleos(t)ide analogs (NAs). Persistent HBV infection relies on the cccDNA pool in the nucleus of infected hepatocytes. Control of the cccDNA level or the transcriptional activity of the cccDNA pool is critical for successful antiviral treatment in CHB patients [4,5].

Regardless, there is still no effective therapeutics to specifically target this mini-chromosome. The transcription of pgRNA from cccDNA is the critical step in HBV replication, and the function of cccDNA can be regulated by the activity of nuclear transcription factors, transcriptional coactivators, corepressors, and chromatin-modifying enzymes [5,6]. These regulators of cccDNA provide various ideas to define new targets and develop new antiviral drugs for CHB control. The modification of HBV DNA, HBV viral proteins (X protein, core protein) and HBV viral protein-binding protein by acetylation is involved in HBV cccDNA anabolism. When one or more of these links are inhibited, the

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synthesis of HBV cccDNA can be affected [5,7]. cccDNA persists as a very stable mini-chromosome that binds histones/non-histones and is the template for the transcription of viral mRNAs. HBV replication is regulated by the acetylation status of cccDNA-bound histones [8,9]. A previous study reported that IFN-α inhibited HBV transcription and replication by targeting the epigenetic regulation of the nuclear cccDNA mini-chromosome [10]. However, there have been no reports on the relationship between antiviral therapy and the regulation of histone deacetylases during the process of HBV DNA replication.

In the present study, we detected HDAC activity and HDAC expression in patients with different HBV DNA levels. The histone deacetylase (HDAC) inhibitor trichostatin A (TSA), anti-HBV agents, entecavir (ETV) and interferon-α (IFN-α) were applied independently to stimulate a HBV-transfected HepG2.2.15 cell line to investigate the effects of anti-HBV treatments on HDAC expression. The relationship between anti-hepatitis B virus therapy and the regulation of histone deacetylases in the process of HBV DNA replication was explored.

2. Materials and methods

2.1. Patient enrollment

A total of 40 CHB patients were recruited from the Department of Infectious Diseases, Renmin Hospital of Wuhan University (Wuhan, China) in 2016. The study was approved by The Clinical Research Ethics Committee of Renmin Hospital of Wuhan University. Informed consent was obtained from each participant in the study. The CHB patients were diagnosed using the EASL guidelines (2017) for CHB [11]. Patients with hepatitis A, C, D and E virus infections, alcoholic liver disease, autoimmune liver disease, drug-induced hepatitis, and fatty liver disease were excluded from this study.

2.2. Immunohistochemistry examination

Four CHB patients who underwent antiviral therapy and were recruited from the Department of Infectious Diseases, Renmin Hospital of Wuhan University were included in the study. The normal human liver tissue was provided by the liver transplantation center of the Zhongnan Hospital of Wuhan University. All the patients signed informed consent forms. The patients received entecavir (ETV) (Bristol-Myers Squibb, Shanghai, China, 0.5 mg/tablet, once daily) for a total of 24 weeks. Blood and liver biopsy samples at baseline and 24 weeks were collected for subsequent experiments. The study protocol was approved by The Clinical Research Ethics Committee of Renmin Hospital of Wuhan University. The liver biopsy specimens were fixed with 4% paraformaldehyde, and paraffin-embedded sections were incubated with primary antibodies (anti-HDAC2, 1:100; anti-HDAC6, 1:100; anti-acetylated histone H3 (AH3), 1:200; and anti-histone H3 (H3), 1:200) and enzyme-conjugated secondary antibodies for immunohistochemistry (IHC) experiments. The primary antibodies against HDAC2, AH3 and H3 were purchased from Cell Signaling Technology (Danvers, MA, USA). The HDAC6 antibody was obtained from Proteintech (Wuhan, China).

2.3. Cell cultures and drug intervention

HBV-transfected HepG2.2.15 cells were obtained from the China Center for Type Culture Collection (Wuhan University). The cells were cultured in modified eagle's medium (MEM) with 10% fetal bovine serum (FBS) (Gibco, Australia) and 1% penicillin-streptomycin solution (Gibco, Thermo Fisher Scientific, China). The cells were maintained in a humidified incubator at 37 °C with 5% CO_2. HepG2.2.15 cells were plated into 6-well plates at a density of 10^5 cells/mL. Trichostatin A (TSA, Selleck Chemicals, Shanghai, China), IFN-α (Recombinant Human Interferon α1b for injection, Shenzhen Kexing Biotech Co., Ltd., China), and ETV (Bristol-Myers Squibb, Shanghai, China) were added directly into the culture medium at final concentrations of 10 mM, 1000 IU/mL, and 1 mM, respectively. The supernatants and cells were harvested at days 3, 6, and 9. The control groups did not have any drugs intervention. The entire process is shown in Fig. 4A.

2.4. Examination of HDAC activity and protein expression

The HDAC activity in the sera from the CHB patients and the supernatants of the HepG2.2.15 cell cultures were measured using an HDAC assay kit (BioVision, Inc., Milpitas, CA, USA) according to the manufacturer's protocol. HDAC2, HDAC6, H3 and AH3 protein expression levels in the HepG2.2.15 cells were determined by a western blot assay.

2.5. Determination of HBV DNA by PCR assay

The quantification of HBV DNA from sera and supernatants was performed by using an HBV DNA quantitative fluorescence diagnostic kit (PCR-fluorescence probing) (Sansure Biotech, Hunan, China) according to the manufacturer's protocol. The levels of HBV DNA in the specimens were calculated using the $2^{-\Delta\Delta Ct}$ method. Amplification was performed as follows: 50 °C for 2 min, 94 °C for 5 min, 45 cycles of 95 °C for 15 s and 57 °C for 10 s, and then 25 °C for 10 s. 40 CHB patients were selected and divided into three groups: the HBV DNA > 10^5 copies/mL group (20 patients), the HBV DNA between 10^5 copies/mL and 10^2 copies/mL group (10 patients) and the HBV DNA < 10^2 copies/mL (10 patients) group.

2.6. HBV pgRNA levels in supernatants by RT-PCR assay

Total RNA from the supernatants was extracted by using RNAiso Plus (TAKARA Bio, Dalian, China) and reverse transcribed using a PrimeScript™ RT reagent Kit with gDNA Eraser (TAKARA Bio, Dalian, China) to produce cDNA; RT-PCR was performed using Premix Ex Taq™ (Probe qPCR) (TAKARA Bio, Dalian, China) and an Applied Biosystems™ 7500 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, Inc. USA). The forward primer was 5′-CACCTCTGCC TAATCATC-3′ (Position: 1826-1843) and the reverse primer was 5′-GGAAAGAAGTCAGAAGGCAA-3′ (Position: 1974-1955). Amplification was performed as follows: 95 °C for 30 s, 40 cycles of 95 °C for 5 s and 60 °C for 34 s.

2.7. Quantification of cccDNA levels in cells by PCR assay

The cccDNA levels in cells were quantified by quantitative polymerase chain reaction (qPCR) as described in our previous study [12]. Briefly, DNA was extracted from the biopsy specimens using a TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). ATP-dependent DNase (TAKARA Bio, Dalian, China) was used to degrade rcDNA and single-stranded DNA (ssDNA) to enhance the specificity of the cccDNA detection method. Two forward primers, [CCC1, 5′-GCGGWCTCCCCGT-CTGTGCC-3′; diaphanous-related formin 1 (DRF1), 5′-GTCTGTGCCTT CTCATCTGC-3′], and one reverse primer, (CCC2, 5′-GTCATGCCCCAA AGCCACC-3′), were used for cccDNA amplification using SYBR Premix Ex Taq (TAKARA Bio, Dalian, China). Serial dilutions of the pHBV1.3 plasmid were used as a quantification standard. The plasmid was purified, and the concentration was measured. The copy number of the standard plasmid was calculated according to the formula Copies/μL = (C ng/μL × 6.02 × 10^{14})/(660 × 7056 bp). Then, the above plasmid equivalence ratio was diluted to different concentrations (10^{12}, 10^{11}, 10^{11}, 10^{10}, 10^{9}, 10^{8}, and 10^{7}). Amplification was performed as follows: 95 °C for 30 s, 40 cycles of 95 °C for 5 s and 60 °C for 34 s, 95 °C for 15 s and 60 °C for 1 min.

Table 1
The clinical data in HBC level.

HBV DNA (IU/mL)	Gender (F/M)	Age (y)	ALT (IU/L)	AST (IU/L)	TBiL (μmol/L)	DBiL (μmol/L)
> 10^5	20(2/18)	43.95 ± 12.54	291.75 ± 296.93	207.25 ± 218.23	120.78 ± 150.10	69.27 ± 91.60
10^2–10^5	10(1/9)	50.80 ± 6.36	315.80 ± 139.30	157.4 ± 19.80	70.18 ± 4.32	41.43 ± 2.05
< 100	10(3/7)	63.40 ± 10.22	36.60 ± 47.64	40.30 ± 23.33	56.13 ± 100.54	31.24 ± 69.12

ALT, alanine transaminase. AST, alanine aminotransferase. TBiL, total bilirubin. DBiL, direct bilirubin. F, female. M, male. Y, years. IU, international unit. OD, optical density.

2.8. Statistical analysis

Statistical analysis was performed using statistical product and service solutions (SPSS) software version 17.0. Data were presented as the mean ± standard deviation. Determining statistical significance between groups was performed by Student's *t*-test. Logistic regression analysis was used for correlation analysis. $P < 0.05$ was considered to be a statistically significant difference.

3. Results

3.1. HDAC activity was positively correlated with HBV DNA in the sera of CHB patients

The basic information of the patients whose samples were measured for HDAC activity is shown in Table 1. As shown in Fig. 1, the HDAC activity was positively correlated with HDAC activity in the sera of CHB patients ($R^2 = 0.7153$, $P < 0.01$).

3.2. HDAC2, HDAC6, H3 and AH3 protein expression in CHB patients before and after antiviral treatment

The HDAC2, HDAC6, H3 and AH3 protein expression in CHB patients was examined with IHC before and after 24 weeks of antiviral treatment. The biochemical and pathological indexes of four patients before and after treatment are shown in Table 2. The biochemical indexes and pathological changes studied in the 4 selected patients were obviously improved after treatment. HDAC2, HDAC6 and AH3 expression in the nucleus was decreased after 24 weeks of antiviral treatment ($P < 0.05$, Fig. 2A–B and Fig. 3A). When compared with the patients' liver tissue after 24 weeks of antiviral treatment, the normal control liver tissue showed obviously decreased HDAC2, HDAC6 and AH3 protein levels ($P < 0.05$, Fig. 2A–B and Fig. 3A). However, there was no difference in H3 protein expression (Fig. 3B).

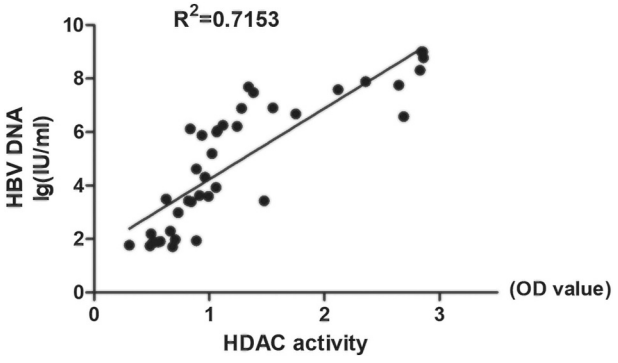

Fig. 1. HDAC activity was positively correlated with HBV DNA in the serum of CHB patients. $R^2 = 0.7153$, $P < 0.01$.

3.3. HDAC activity and HBV DNA, pgRNA and cccDNA expression in vitro experiments

PCR analysis showed that the expression of HBV DNA in supernatants was suppressed in the IFN-α and ETV groups at days 3, 6 and 9 ($P < 0.05$) and suppressed in the TSA group at day 9 compared with the control group at the same time points ($P < 0.05$, Fig. 4B). The HDAC activity in the supernatants of the HepG2.2.15 cell cultures was suppressed in the TSA group at days 3, 6 and 9 ($P < 0.05$), in the IFN-α group at days 3, 6, 9 ($P < 0.05$), and in the ETV group at day 6, 9 ($P < 0.05$, Fig. 4C). The expression of pgRNA was enhanced in the TSA group at days 3, 6, 9 and in the ETV group at day 9, but it was suppressed in the IFN-α group at days 3, 6, 9 ($P < 0.05$, Fig. 4D). cccDNA expression was suppressed by only IFN-α at days 3, 6, 9 ($P < 0.05$, Fig. 4E). In contrast, TSA and ETV had no direct inhibitory effect on the cccDNA levels.

3.4. HDAC2, HDAC6, H3 and AH3 expression in HepG2.2.15 cells

The protein levels of HDAC2, HDAC6 and AH3 were suppressed in the TSA and IFN-α groups compared with the control group at days 3, 6 and 9 ($P < 0.05$, Fig. 5A–F). ETV also had effects on the HDAC2, HDAC6 and AH3 protein levels in HepG2.2.15 cells at day 6 and day 9 compared with the control treatment ($P < 0.05$, Fig. 5C–D).

4. Discussion

Persistent HBV infection is due to the existence of a cellular reservoir of cccDNA, which is the transcriptional template for all viral mRNAs. cccDNA accumulates as a stable episome formed by mini-chromosomes, which are created by histones/nonhistones, and can be regulated by the activity of nuclear transcription factors, transcriptional coactivators, corepressors, and chromatin-modifying enzymes. Therefore, epigenetic factors play pivotal roles in determining the outcome of HBV infection, viral replication and virion production and the development of HCC. HBV epigenetic regulation involves two major processes: the post-translational modification of histones associated with the cccDNA mini-chromosome and the DNA methylation of the viral or host genome [13–16]. Histone modifications, including acetylation, methylation, phosphorylation, sumoylation, ubiquitination, ADP-ribosylation and deamination, are all reversible. Several studies have indicated that cccDNA transcription is controlled by the epigenetic modifications of cccDNA-bound histones [17,18]. cccDNA transcription and HBV replication are modulated by substances that affect the activity of chromatin-modifying enzymes [5,9].

Histone acetylation is associated with transcriptional activation via the relaxation of the chromatin structure, whereas deacetylation induces a condensed or inactive chromatin state. Histone acetylation has been demonstrated to be related to the activation of transcription, whereas deacetylation has been shown to be associated with gene repression [19,20]. The acetylation of histones might promote inflammatory responses by enhancing the expression levels of proinflammatory genes [21]. Previous studies have controversially shown that HDAC inhibitors activate the transcription of rcDNA and HBV

Table 2
Clinical indexes of four patients before and after treatment.

ID	Gender	Treat time (w)	Age (y)	HBsAg (IU/mL)	HBsAb (mIU/mL)	HBeAg (S/CO)	HBeAb (S/CO)	HBV-DNA (IU/mL)	ALT (IU/L)	TBiL (μmol/L)	HAI	Pathology
1	M	0	33	> 250	1.13	51.25	0.47	1.17E+07	188	14.6	9	Chronic hepatitis, mild. G2/S1
		24	33	47.17	0.03	0	0.03	< 100	10	24.3	1	Chronic hepatitis, mild. G1−/S1−
2	F	0	39	> 250	1.96	410.36	1.96	1.76E+06	126	16.5	18	Chronic hepatitis, severe. G4−/S3
		24	39	> 250	0	1.66	1.87	< 100	16	15.3	4	Hepatitis, mild. G1+/S1
3	M	0	28	> 250	0	85.51	3.95	1.20E+07	238	108	4	Hepatitis, mild. G1+/S1
		24	28	> 250	0	1.08	0.29	< 100	13	22	1	Hepatitis, mild. G1−/S1−
4	F	0	27	> 250	0.23	1208.73	57.88	1.29E+07	125	90	2	Hepatitis, mild. G1−/S1−
		24	27	> 250	0	83.78	5.19	< 100	11	8.8	1	Hepatitis, mild. G1−/S1−

ALT, alanine transaminase. TBiL, total bilirubin. M, male. F, female. Y, years. IU, international unit. S/CO, sample OD/cut off. HAI, histological activity index. HE, hematoxylin eosin.

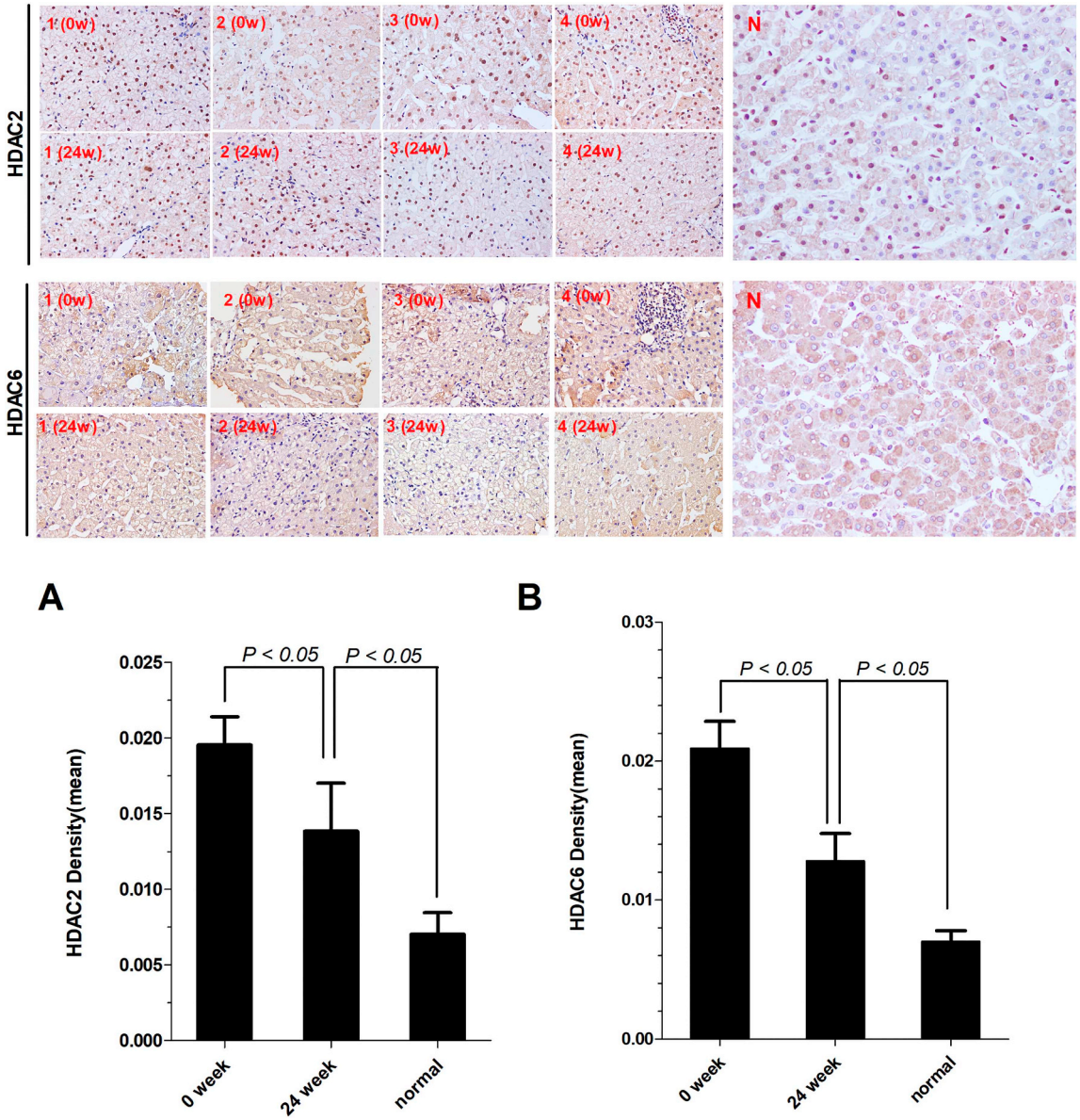

Fig. 2. (A) HDAC2 and (B) HDAC6 protein expression in normal control liver tissues and 4 CHB patients liver tissue before and after 24 weeks anti-virus treatment. The protein was detected by immunohistochemistry.

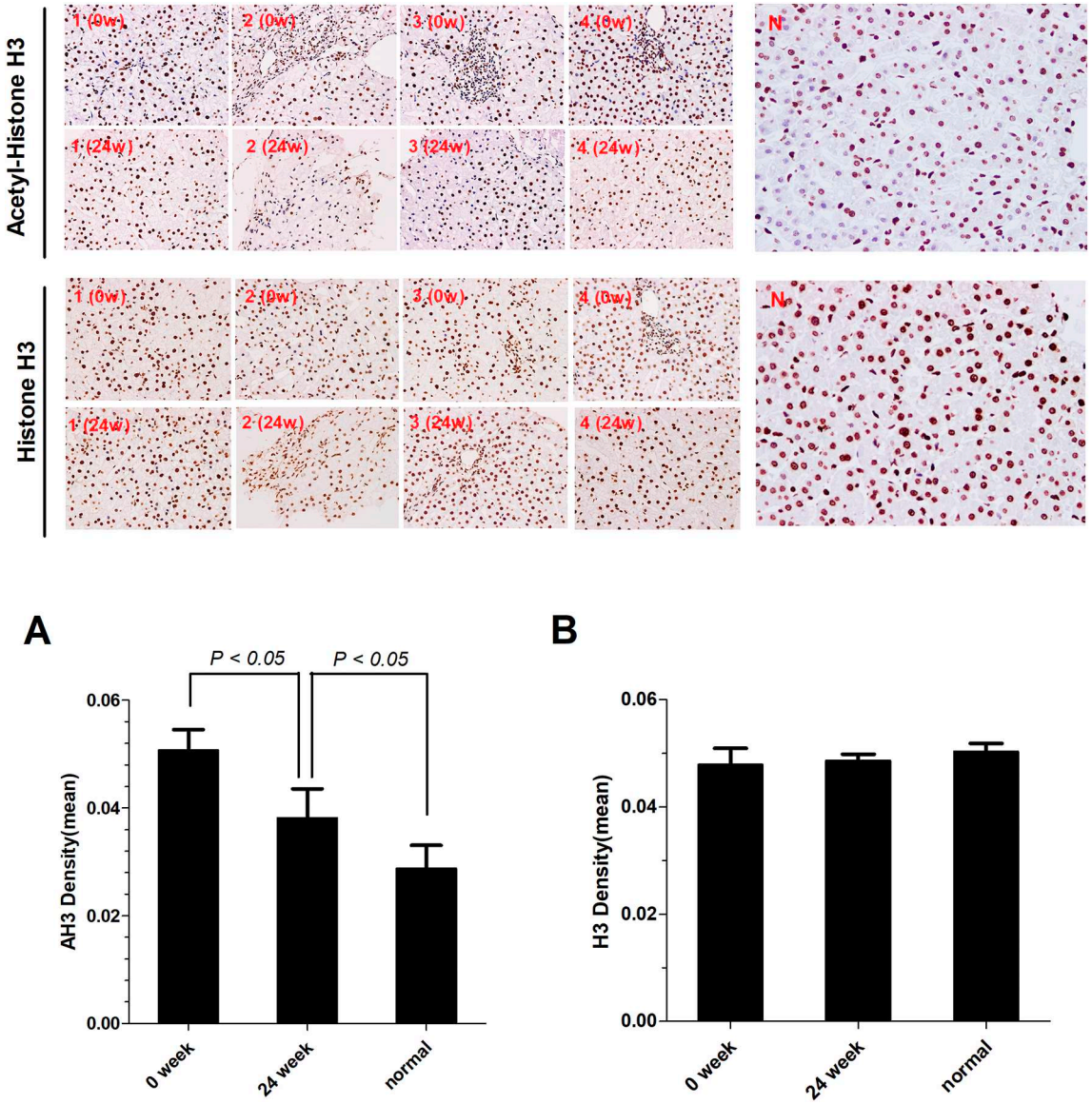

Fig. 3. (A) Acetyl-Histone 3 (AH3) and Histone H3 (H3) protein expression in in normal control liver tissues and 4 CHB patients liver tissues before and after 24 weeks anti-virus treatment. The proteins were detected by immunohistochemistry.

cccDNA in HepG2 cells transfected with unit-length linear HBV genomic DNA [9,22]. Other studies have shown that cccDNA transcriptional activity is 10-fold lower in HBeAg-negative patients than in HBeAg-positive patients [23,24], suggesting that host immunopathological factors can regulate HBV DNA replication in addition to HDAC activity. However, the effects of HDAC activity on the process of HBV DNA replication need further investigation, as which HDACs are predominant during HBV DNA replication remains unknown.

Previous study has been shown that a broad-spectrum HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) prevents the pre-S (2) mutant-induced oncogenic phenotype. SAHA may be a chemoprotective agent for high-risk chronic HBV patients who may develop HCC [25]. Likewise, in our earlier research, HDAC activity was higher in CHB patients without liver failure compared with CHB patients with liver failure. The HDAC1 and HDAC3 inhibitor entinostat (MS275) can suppress inflammatory responses in CHB [26]. In general, HDAC activity has been positively correlated with the HBV loads, and HDACi can inhibit HBV by suppressing the activity of HDAC. HBV replication is regulated by the acetylation status of hepatitis B virus cccDNA-bound H3 and H4 histones [6,9].

In our present study, HDAC activities in the sera from CHB patients with different HBV DNA levels were detected. HDAC activity was positively correlated with HBV DNA levels in the sera of CHB patients. We further verified whether the results obtained from the liver tissue specimens were consistent with the results obtained from the patient blood. Type I HDAC molecule HDAC2, type II HDAC molecule HDAC6, H3 and AH3 protein expression in CHB patients was examined with IHC before and after 24 weeks of antiviral treatment. The biochemical indexes and pathological changes studied in the 4 selected patients were obviously improved after treatment. HDAC2, HDAC6 and AH3 expression in the nucleus was decreased after 24 weeks of antiviral treatment. When compared with the patients' liver tissue after 24 weeks of antiviral treatment, the normal control liver tissue showed obviously decreased HDAC2, HDAC6 and AH3 protein levels. These results are consistent with a previous study, which showed that the histone H3 was involved in antiviral immunity. Histone H3K36 trimethylation (H3K36me3) in the distal promoter region of interferon-inducible genes can directly promote the transcriptional activation and expression of these antiviral genes [27].

In the present study, the relationship between anti-hepatitis B virus

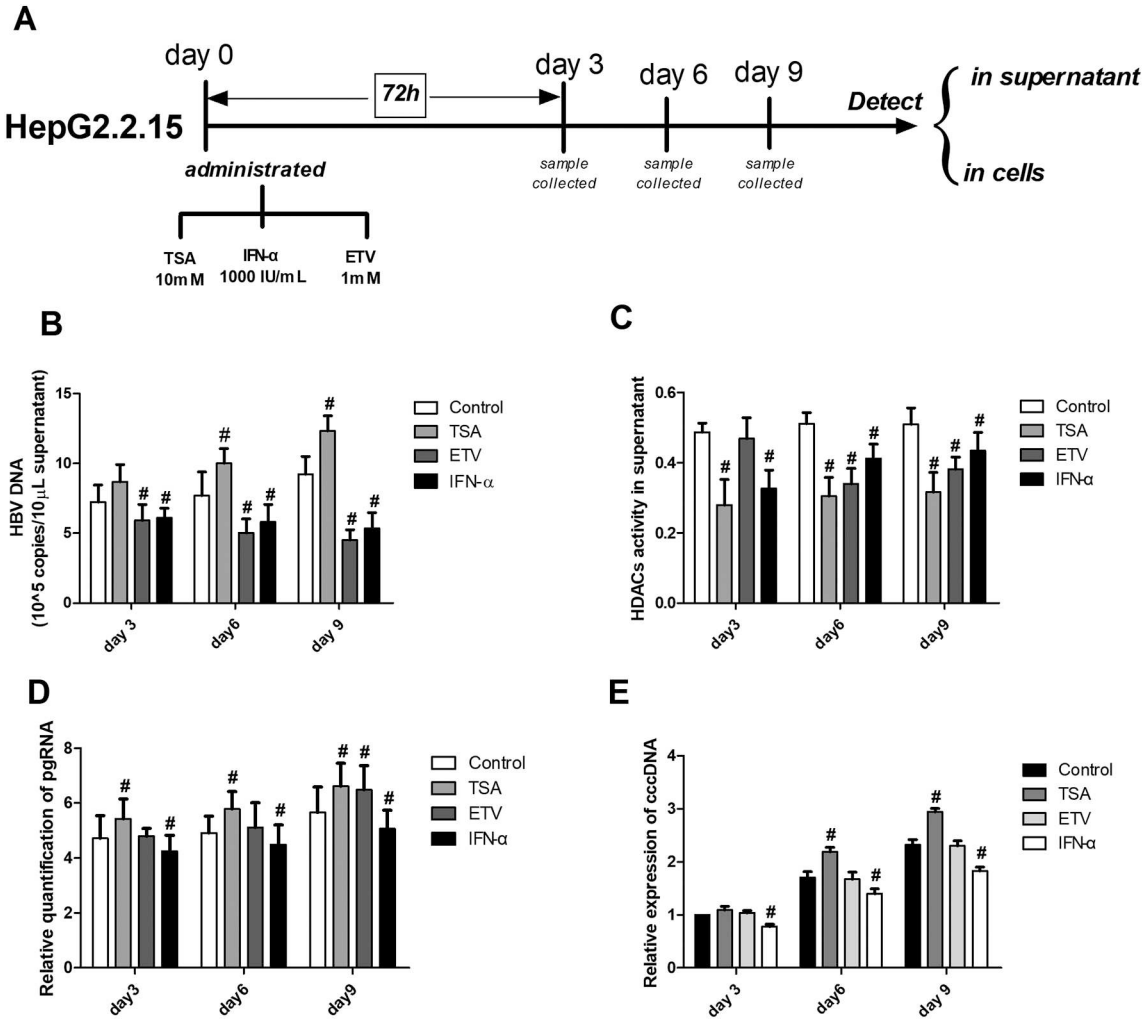

Fig. 4. (A) The administrate time point: Administrated drugs (TSA 10 mM, ETV 1 mM, IFN-α 1000 IU/mL) to the cells in respective groups at day0. At the 3th, 6th and 9th day, the supematant was collected. After interval for 9 days, we detect the HBV DNA levels and pg RNA levels in supernatant, HDAC activity in HepG 2.2.15 cells. (B) The expression of HBV DNA in supernatant of HepG 2.2.15 cells. HBV DNA levels in supernatant of HepG 2.2.15 cells detected by Hepatitis B Viral DNA Quantitative Fluorescence Diagnostic kit (PCR-Fluorescence Probing). (C) HDAC activity in HepG 2.2.15 cells. The HDAC activity performed according to the HDAC assay kit. (D) The expression of pgRNA in supernatant of HepG 2.2.15 cells. pgRNA levels in supernatant of HepG2.2.15 cells detected by RT-PCR using Premix Ex Taq™ (Probe qPCR) and pgRNA specific primers. (E) The relative expression of cccDNA in HepG 2.2.15 cells was assayed by qPCR. # $P < 0.05$, compared to control group.

therapy and the regulation of HDACs and acetylated histone H3 in the process of HBV DNA replication was also explored in vitro. We used TSA, ETV, and IFN-α to stimulate HepG2.2.15 cells and then observed the changes in HDAC activity and the HBV DNA, pgRNA, HDACs, AH3 and H3 levels at different time points. In this study, we found that the supernatant levels of HBV DNA and HDAC activity decreased in the ETV, IFN-α and TSA groups compared with the control group. The level of pgRNA in the supernatant declined in the IFN-α group and increased in the ETV and TSA groups. cccDNA expression was suppressed by IFN-α. The cccDNA levels showed no remarkable changes in the ETV and TSA groups. The protein levels of HDACs and AH3 were inhibited by TSA, IFN-α and ETV compared with the control treatment.

IFN-α has been shown to inhibit cccDNA transcription by targeting the epigenetic control of cccDNA through chromatin remodeling polycomb repressive complex 2 [10], and IFN-α has also been reported to reduce the acetylation of cccDNA-bound H3K9 and H3K27 histone residues [28]. A previous study demonstrated that IFN-α suppressed HBV by reducing active posttranslational modifications (PTMs) on the cccDNA mini-chromosome [29]. These results were consistent with those of previous studies [10,30]. TSA and ETV treatment can reduce HBV DNA levels and HDAC activities but increase pgRNA levels. This may be due to the inhibition of cccDNA transcription by changing the acetylation states of related molecules in viral mini-chromosomes. However, the cccDNA levels of the HepG2.2.15 cells in the TSA and ETV groups did not show significant differences in the short treatment duration. TSA, IFN-α and ETV could inhibit the HDACs and AH3 protein expression in the cells at days 3, 6, 9. The regulation of cccDNA mini-chromatin proteins by acetylation involves in the recruitment of chromatin modification enzymes, including HDACs or HATs, to promoters or transcriptional repressors, in addition to affecting the acetylation status of mini-chromatin histones [31–33]. The TSA treatment decreased HBV DNA replication and increased the pgRNA level, which may change the dynamic acetylation and deacetylation of cccDNA-associated proteins in HepG2.2.15 cells; which cccDNA transcription or pgRNA production-related histones or signaling molecules were modified by TSA need to be further investigated.

In conclusion, HDAC activity and AH3 are involved in HBV DNA replication. Aberrant HDAC activity is associated with the process of HBV DNA replication. Antiviral treatment of HepG2.2.15 cells with IFN-α and ETV resulted in decreased HDAC activity, especially the

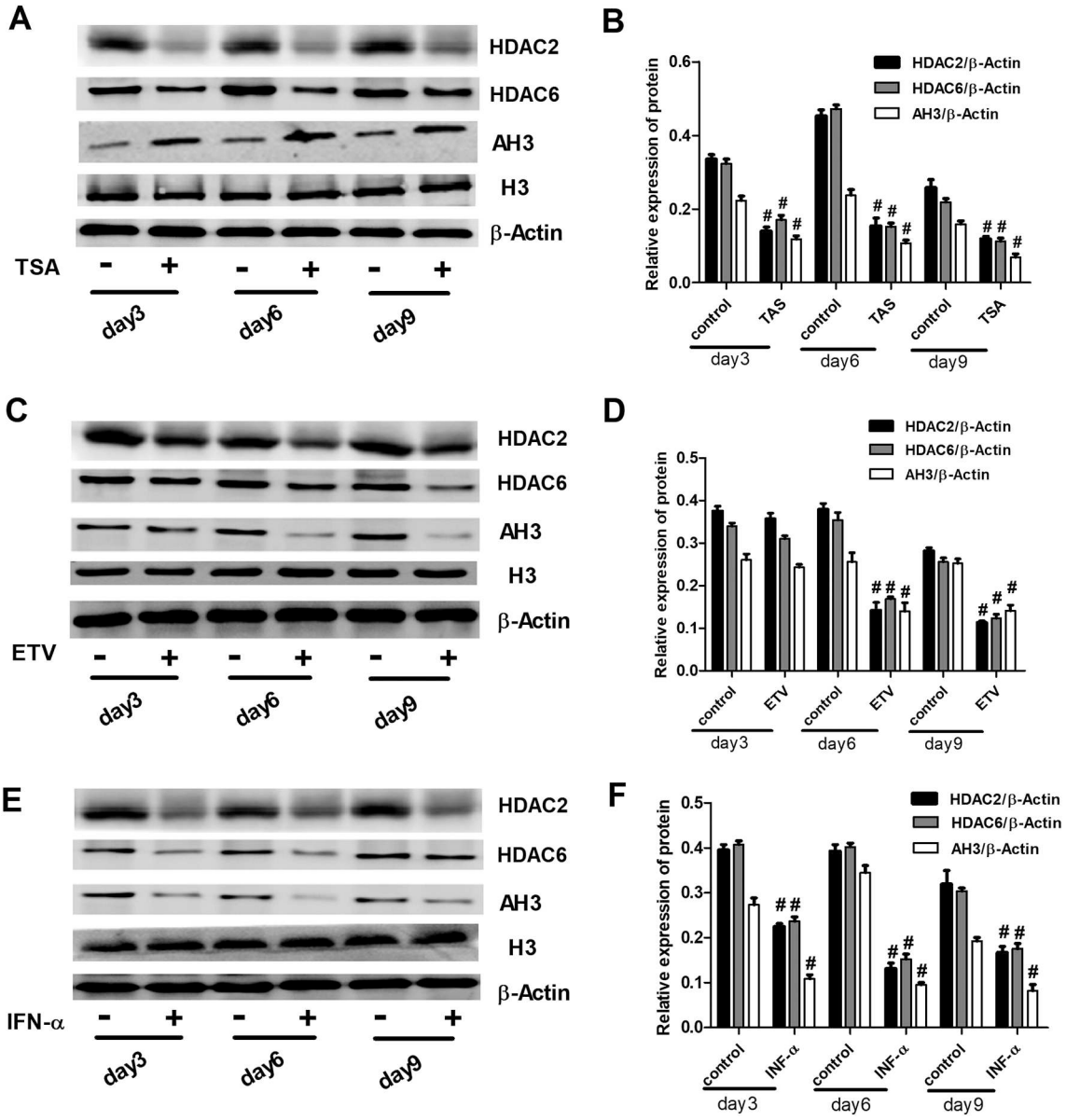

Fig. 5. (A, B) HDAC6, AH3 and H3 protein expression in HepG 2.2.15 cells after treated with TSA. (C, D) HDAC6, AH3 and H3 protein expression in HepG 2.2.15 cells after treated with ETV. (E, F) HDAC6, AH3 and H3 protein expression in HepG 2.2.15 cells after treated with INF-α. The protein expression was detected by western blot. # $P < 0.05$, compared to control group. AH3, acetylated histone H3. H3, Histone H3.

activity of HDAC. TSA strengthened cccDNA transcription and inhibited HBV DNA production in HepG2.2.15 cells. However, the recruitment of HDACs to cccDNA mini-chromosomes and comprehensive acetylation regulation during the process of HBV replication need to be further investigated.

Authors' contributions

Z.J.G. takes responsibility for the integrity of the work as a whole, from inception to published article. D.Z. and Y·W. conceived and designed the experiments. Y.W, H.Y.Z., F.Z.J. and W.B.Z. performed the experiments. D.Z., L.W.W. and H. Zhang analyzed the data. Y.W. and H.Y.Z. contributed reagents/materials/analysis tools. D.Z. wrote and revised the paper. Z.J.G. edited the article. D.Z. and Y.W. contribute equally to this article. All authors approved the final version of the manuscript. Z.J.G. is the guarantor.

Conflict of interest

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

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