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A mutual regulatory loop between transcription factor Yin Yang 1 and hepatitis B virus replication influences chronic hepatitis B

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ABSTRACT

Hepatitis B virus (HBV) infections pose a major threat to human health. HBV can upregulate the expression of the transcription factor Yin Yang 1 (YY1) in *in vitro* cytological experiments, suggesting an association between YY1 and HBV infection. However, data on YY1 expression in chronic hepatitis B (CHB) patients are lacking. In this study, we aimed to assess the correlation between YY1 expression and HBV infection. We detected serum YY1 levels in 420 patients with chronic HBV infection, 30 patients with chronic hepatitis C virus infection, and 32 healthy controls using an enzyme-linked immunosorbent assay. The correlation between YY1 levels and clinical parameters was analyzed. Meanwhile, the changes of YY1 before and after interferon or entecavir treatment were analyzed. YY1 levels in the liver tissues were detected using immunofluorescence staining. The expression of YY1 in HBV-expressing cells was detected through western blotting. Meanwhile, we explored the effects of YY1 on HBV replication and gene expression. We found that YY1 was highly expressed in the serum and liver tissues of CHB patients. Serum YY1 levels positively correlated with HBV DNA and hepatitis B surface antigen (HBsAg). Additionally, HBV DNA levels increased but HBsAg levels decreased after HBV-expressing cells overexpress YY1. In conclusion, our study demonstrates that YY1 plays an important role in HBV replication and gene expression, providing a potential target for the treatment of CHB.

1. Introduction

Hepatitis B virus (HBV) infection poses a significant global public health challenge, affecting an estimated 296 million individuals worldwide (Jeng et al., 2023). Chronic hepatitis B (CHB) patients face elevated risks of developing cirrhosis and hepatocellular carcinoma (HCC). Fortunately, therapies targeting HBV replication, such as nucleos(t)ide analogues (NAs), mitigate complications and halt disease progression, thereby enhancing patient outcomes (Liaw et al., 2004; Marcellin et al., 2013). Presently, the mainstay of anti-HBV treatment comprises NAs and interferons, though these interventions typically do not achieve complete eradication. The treatment goal for CHB now emphasizes achieving a functional cure, characterized by sustained suppression and loss of HBsAg post-treatment cessation (Nguyen et al., 2020). However, despite the efficacy of nucleos(t)ide analogue therapy, HBsAg clearance remains infrequent (Alexopoulou et al., 2020; Gish et al., 2015), necessitating prolonged or indefinite treatment to avert viral reactivation after cessation (Seto et al., 2015). Ongoing research endeavors concentrate on novel agents capable of curing CHB by exploring new mechanisms and therapeutic targets. Given HBV's reliance on host transcription factors for replication, investigations into host protein–HBV genome interactions hold promise for identifying new agent targets against HBV (Turton et al., 2020; Oropeza et al., 2020). Notably, the role of transcription factor Yin Yang 1 (YY1) in HBV regulation has been extensively explored (Shen et al., 2020), with YY1 shown to bind to an integrated sequence adjacent to the direct repeat region 1 in HBV DNA, underscoring its significance in HBV pathogenesis (Nakanishi-Matsui et al., 2000). Thus, further exploration of YY1's impact on HBV warrants attention in therapeutic development efforts.

The transcription factor YY1, a member of the GLI-Kruppel family,

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stands out as a highly conserved and functionally versatile zinc-finger protein (Galvin and Shi, 1997; Meliala et al., 2020; Zhang et al., 2011b). Also referred to as δ, NF-E1, UCRBP, and CF1 (Gordon et al., 2006), YY1 exhibits ubiquitous expression across all vertebrates (Kim et al., 2009). The human YY1 cDNAs and mouse Yy1 cDNAs showcase a 95% identity in both coding and untranslated regions, with a 98.6% identity in the coding region between humans and mice (Hariharan et al., 1991). YY1 possesses various functional domains, including an N-terminal activation domain for transcriptional activation and a C-terminal repression domain for transcriptional repression (Galvin and Shi, 1997; Lee et al., 1995). Thus, YY1 exhibits dual regulatory capabilities, acting as either an activator or a repressor of gene expression, contingent upon cellular stimuli and binding contexts (Gordon et al., 2006; Sarvagalla et al., 2019; Zhang et al., 2017).

YY1 emerges as closely intertwined with various diseases, including HCC (Wang et al., 2020; Xu et al., 2020; Xuan et al., 2020; Yang et al., 2019; Zhang et al., 2011a, 2012), non-alcoholic fatty liver disease (Lai et al., 2018; Yuan et al., 2018), and pancreatic cancers (Chen et al., 2021). Notably, YY1's involvement extends to HBV infection. *In vitro* investigations reveal HBV's ability to induce YY1 expression (Shan et al., 2015; Shang et al., 2015), with Zhang et al. (2011a) demonstrating HBV-mediated upregulation of YY1 in a concentration-dependent manner. However, data supporting upregulated YY1 expression in patients with HBV infection are still lacking. In this study, we analyzed the effect of HBV on YY1 in the CHB patients.

YY1 affects HBV transcription and replication. Teng et al. (2011) reported that YY1 binds to nucleotides 2812–2816 of the pre-S1 promoter and suppresses HBsAg synthesis at the transcriptional level. However, another study (Shen et al., 2020) found that YY1 and HBx proteins could activate HBV transcription by mediating the spatial interaction of the cccDNA minichromosome with cellular chromosome 19p13.11. Furthermore, YY1 binds to the sex-determining region Y box 4 (SOX4) promoter to activate Sox4 transcriptional activity, resulting in the upregulation of Sox4, facilitating HBV replication (Shang et al., 2015). Inconsistent with findings from previous studies, Shi et al. (2020) reported that SOX4 suppressed HBV replication by inhibiting hepatocyte nuclear factor 4α expression.

In this study, we observed a significant upregulation of YY1 in the serum and liver tissues of CHB patients. Furthermore, YY1 plays an important role in HBV replication and gene expression, providing new perspectives for CHB treatment.

2. Materials and methods

An expanded version of the Materials and Methods is provided in supplemental methods.

2.1. Study participants and sample collection

Serum samples were collected from 420 patients with chronic HBV infection, 30 with chronic hepatitis C virus (HCV) infection, and 32 healthy individuals at the First Hospital of Jilin University, Jilin, China. The patients with chronic HBV infection were grouped based on hepatitis B e antigen (HBeAg) and alanine aminotransferase (ALT) levels. This resulted in 191 patients classified as HBeAg-negative chronic infection (ENCI, HBeAg-negative, ALT normal), 34 as HBeAg-negative chronic hepatitis (ENCH, HBeAg-negative, ALT elevated), 125 as HBeAg-positive chronic infection (EPCI, HBeAg-positive, ALT normal), and 49 as HBeAg-positive chronic hepatitis (EPCH, HBeAg-positive, ALT elevated). Additionally, 21 patients lacked serum HBeAg testing. The demographic characteristics, laboratory indices, and disease statuses of the patients are detailed in Supplementary Tables S1 and S2. Furthermore, 40 CHB patients were enrolled, with 20 receiving interferon (IFN) treatment and 20 receiving entecavir (ETV). Peripheral serum samples were longitudinally collected at baseline (week 0) and week 48 post-treatment. Demographic characteristics and laboratory indices of CHB patients treated with IFN or ETV are provided in Supplementary Table S3. Liver tissue samples were obtained from three HBeAg positive CHB patients who underwent liver biopsy, with three normal liver tissues adjacent to hemangioma surgical resection serving as controls. Clinical and virological parameters are summarized in Supplementary Table S4. Written informed consent was obtained from all participants, and the study protocol was reviewed and approved by the Ethics Committee of the First Hospital of Jilin University (Ethics approval numbers: #2022-530, #2020-699).

2.2. Immunofluorescence staining

To stain for YY1, paraffin-embedded liver sections from patients with chronic HBV infection and control liver samples were deparaffinized in xylene and rehydrated in a graded ethanol series to phosphate-buffered saline and then incubated with rabbit anti-YY1 primary antibody (1:800, Cell Signalling Technology, USA) overnight at 4 °C. Next, the respective secondary antibodies, cyanine 3(Cy3)-conjugated Goat Anti-Rabbit IgG (1:300, Servicebio, Wuhan, China), were incubated for 1 h after washing the slides. Nuclei were stained with 4,6-diamino-2-phenyl indole (DAPI). Slides were visualized using a fluorescence microscope.

2.3. Cell culture

Human hepatocarcinoma cell lines HepG2, HepG2-sodium taurocholate co-transporting polypeptide (NTCP), HBV-expressing HepG2.2.15 and HBV-expressing HepAD38, and the normal liver cell line L02, were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA). All cells were maintained in a humidified incubator at 37 °C with 5% CO_2.

2.4. HBV infection

HepG2-NTCP were treated with serum from CHB patients (HBV DNA: 8 $\log_{10}$ IU/mL) in the presence of 4% Polyethylene glycol 8000 (PEG8000, Solarbio, Beijing, China) for 24 h. The negative control group was exposed to normal human serum (Solarbio, Beijing, China). After infection of HBV, cells were washed for 5 times with PBS and cultured in DMEM supplemented with 10% FBS. Supernatant and cells were harvested 2 days post infection (dpi).

2.5. Lentiviral transduction

Cells were transduced with the lentiviral vectors encoding YY1 (LV-YY1, GenePharma, Suzhou, China) or control lentiviral vectors (LV-NC, GenePharma, Suzhou, China) in the presence of 5 μg/mL polybrene (GenePharma, Suzhou, China). The following day, the cells were washed twice with PBS and cultured in DMEM supplemented with 10% FBS. The fluorescence expression was observed every day after transduction. Cells and supernatant were harvested at 3 days after transduction.

2.6. Western blot assay

Cells were lysed in radio-immunoprecipitation assay buffer (Beyotime, Shanghai, China) containing phenylmethylsulfonyl fluoride (Solarbio, Beijing, China). Protein concentrations were determined using a bicinchoninic acid protein concentration determination kit (Beyotime, Shanghai, China). Western blot assay was performed using the following primary antibodies: anti-YY1 (1:1000, Cell Signalling Technology, USA). and anti-β actin (1:1000, Cell Signalling Technology, USA).

2.7. Statistical analysis

All statistical analyses were performed using SPSS version 20 for

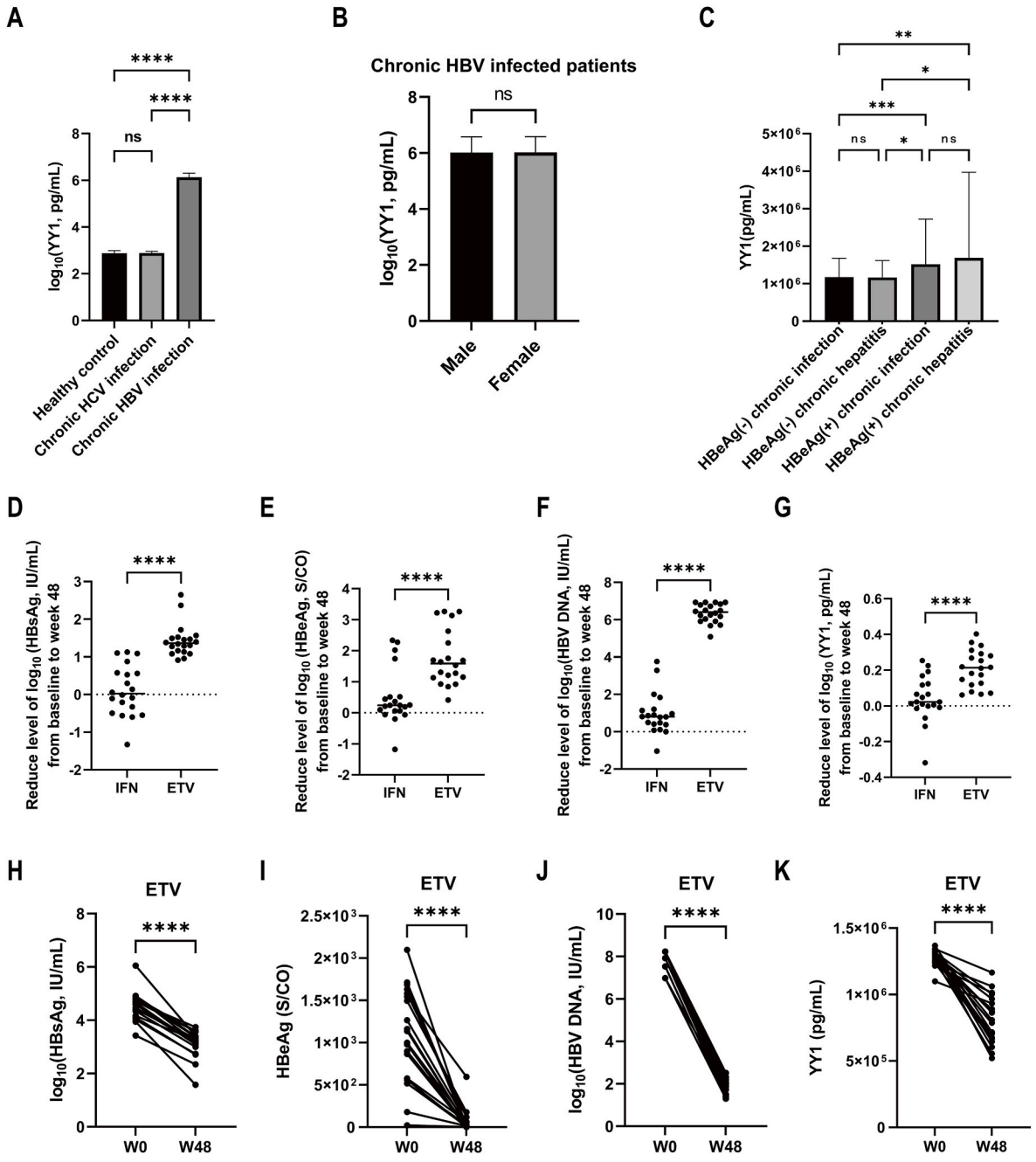

Fig. 1. Serum YY1 levels among the various groups. A: Serum YY1 levels in healthy controls, patients with chronic HCV infection, and patients with chronic HBV infection. B: Serum YY1 levels in male patients with chronic HBV infection and female patients with chronic HBV infection. C: Comparison of serum YY1 levels among ENCI, ENCH, EPCI, and EPCH groups. D–G: The reduced levels of serum HBsAg (D), HBeAg (E), HBV DNA (F), and YY1(G) in CHB patients treated with IFN or ETV from baseline (week 0) to week 48. H–K: The levels of serum HBsAg (H), HBeAg (I), HBV DNA (J), and YY1(K) in CHB patients treated with ETV at baseline (week 0) and week 48. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; ns: not significant, $P > 0.05$.

Windows PC (SPSS, Chicago, IL, USA). Continuous variables were expressed as means ± standard deviation (SD) or median (interquartile range). The two groups were compared using two independent sample *t*-tests, Mann-Whitney test or Welch's T-test. Comparisons between the values before and after treatment were performed using Wilcoxon test. Continuous data were compared among three or four groups using Analysis of Variance or the Kruskal–Wallis test. Categorical variables were analyzed using the chi-squared test. Correlations were calculated using Spearman's correlation analysis.

3. Results

3.1. YY1 levels in serum

We performed enzyme-linked immunosorbent assay to measure serum YY1 levels. The median serum YY1 level was 2.885 $\log_{10}$ (pg/mL) in the healthy control group, 2.888 $\log_{10}$ (pg/mL) in the chronic HCV infection group, and 6.128 $\log_{10}$ (pg/mL) in the chronic HBV infection group. Serum YY1 levels in the chronic HBV infection group were significantly higher than those in the chronic HCV infection and healthy groups. However, no significant differences were found in YY1 levels between the chronic HCV infection and the healthy groups ($P > 0.05$)

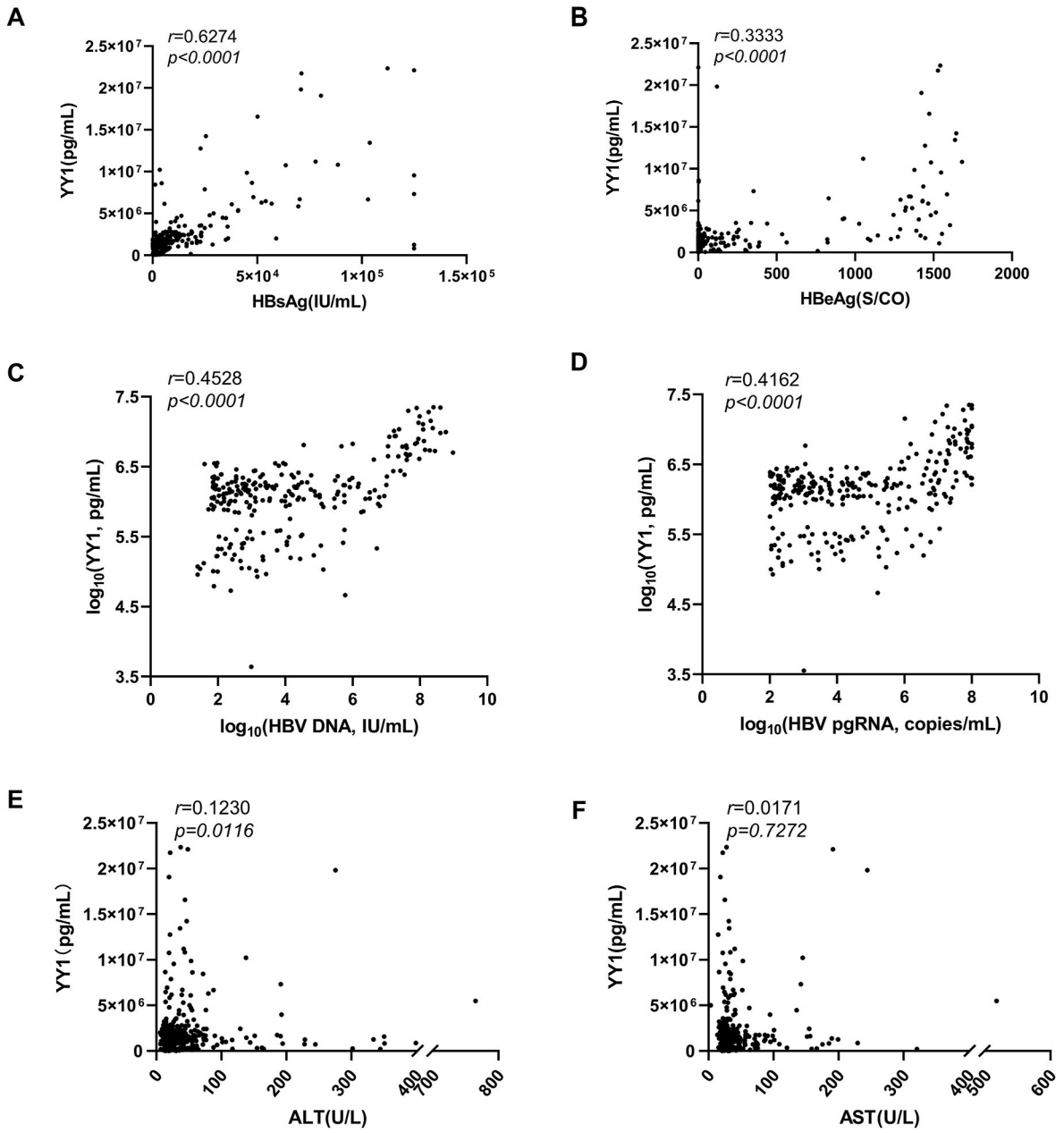

Fig. 2. Correlation between serum YY1 levels and clinical parameters in patients with chronic HBV infection. A–F: Correlation between serum YY1 and serum HBsAg (A), HBeAg (B), HBV DNA (C), HBV pgRNA (D), ALT (E), and AST (F). Notes:*r* is the correlation coefficient, with $r > 0$ indicating a positive correlation. $P < 0.05$ was considered statistically significant. $P > 0.05$ was considered not statistically significant.

(Fig. 1A).

In the chronic HBV infection group, no differences in the serum YY1 levels were observed between male and female (Fig. 1B). YY1 levels in EPCI group were higher than those in ENCI ($P < 0.001$) and ENCH groups ($P < 0.05$). Additionally, YY1 levels in EPCH group were higher than those in ENCI ($P < 0.01$) and ENCH groups ($P < 0.05$). However, no significant differences were observed in YY1 levels between EPCH and EPCI groups ($P > 0.05$) or between ENCH and ENCI groups ($P > 0.05$) (Fig. 1C).

As shown in Fig. 1D–K, serum HBsAg, HBeAg, and HBV DNA decreased significantly in CHB patients after 48 weeks of ETV treatment compared to the pre-treatment baseline, followed by a significant decrease in serum YY1 levels. Moreover, the decrease of serum HBsAg, HBeAg and HBV DNA in CHB patients treated with ETV was significantly greater than that in the IFN group, and correspondingly, the decrease of YY1 after ETV treatment was also significantly greater than that in the IFN group. These data suggest that YY1 level in serum of CHB patients is closely related to HBV replication and gene expression.

3.2. Correlation between serum YY1 levels and clinical parameters in patients with chronic HBV infection

To explore the clinical significance of YY1 in patients with chronic HBV infection, correlations between serum YY1 levels and clinical parameters were analyzed. The results showed that serum YY1 levels in patients with chronic HBV infection positively correlated with serum HBsAg ($r = 0.6274$, $P < 0.0001$) and HBeAg levels ($r = 0.3333$, $P < 0.0001$) (Fig. 2A and B). Simultaneously, a correlation analysis on the $\log_{10}$-transformed values of YY1, HBV DNA, and HBV pregenomic RNA (pgRNA) revealed that $\log_{10}$ (YY1) positively correlated with $\log_{10}$ (HBV DNA) and $\log_{10}$ (HBV pgRNA) ($r = 0.4528$ and $r = 0.4162$, respectively) (Fig. 2C and D). However, YY1 was weakly correlated with ALT ($r =$

Table 1
Relationship between virological factors and YY1 expression using generalized linear model analysis.

	B	SD	OR (95% CI)	*P*
$\log_{10}$ (HBsAg)	0.375	0.045	1.455 (1.331, 1.590)	<0.001
$\log_{10}$ (HBeAg)	−0.007	0.032	0.993 (0.932, 1.057)	0.817
$\log_{10}$ (HBV DNA)	0.071	0.017	1.073 (1.038, 1.110)	<0.001
$\log_{10}$ (HBV pgRNA)	0.023	0.026	1.023 (0.973, 1.076)	0.376

0.1230) (Fig. 2E). No significant correlations were observed with aspartate aminotransferase (AST, $P > 0.05$) (Fig. 2F). In the generalized linear model analysis, YY1 was positively correlated with HBsAg ($P < 0.001$) and HBV DNA ($P < 0.001$), but not statistically correlated with HBeAg and HBV pgRNA ($P > 0.05$) (Table 1).

Among the 420 chronic HBV-infected patients, 21 patients with chronic HBV infection did not undergo HBeAg testing. HBV DNA levels (<20 or <50 IU/mL) in 167 patients and HBV pgRNA levels (<100 copies/mL) in 145 patients were below the lowest limit of detection. Therefore, these patients were excluded from correlational analyses.

3.3. YY1 levels in liver tissues

We used immunofluorescence staining to detect YY1 levels in the liver tissues of three CHB patients and three patients who underwent surgery for hepatic hemangioma (normal liver tissues adjacent to the hepatic hemangioma). As shown in Fig. 3, YY1 was dispersed throughout the liver tissues, mainly in the nucleus. YY1 protein levels in the liver tissues of CHB patients was significantly higher than that in the control liver tissues ($P < 0.05$) (Fig. 3). These results indicate an abnormal overexpression of YY1 in the liver tissues of patients with chronic HBV infection, suggesting its key role in chronic HBV infection.

3.4. Regulatory effects of HBV on YY1 expression

To elucidate the exact role of HBV in YY1 expression, we measured YY1 levels in HBV-expressing and non-expressing cells via western blotting *in vitro*.

HepG2 cells were transfected with pAAV-HBV1.3 plasmids or control plasmids. The supernatant of HepG2 cells transfected with pAAV-HBV1.3 plasmids exhibited an elevated HBsAg level (Fig. 4A). A comparison of YY1 protein expression levels between the two groups revealed an upregulation in HepG2 cells transfected with pAAV-HBV1.3 plasmids (Fig. 4B). As shown in Fig. 4C and D, similar experimental results were obtained by transfecting pAAV-HBV1.3 plasmids into L02 cells. To mimic natural HBV infection in liver cells, HepG2-NTCP cells were infected with HBV from serum of CHB patients (Fig. 4E). The levels of HBsAg in the supernatant were measured. After washing away any remaining virus particles, HBsAg was undetectable. However, when the cells were cultured for an additional 24 h, HBsAg levels increased, indicating successful HBV infection (Fig. 4F). HepG2-NTCP cells infected with HBV also showed higher levels of YY1 compared to uninfected cells (Fig. 4G). These findings suggest that HBV infection increases the production of YY1.

ETV treatment reduced the levels of HBsAg in HepG2.2.15 cells (Fig. 4H). These cells also showed lower levels of the YY1 protein compared to untreated control cells (Fig. 4I). Interestingly, when the mRNA level of the hepatitis B core protein (HBc) was reduced in HepG2.2.15 cells using siRNA-HBc (Fig. 4J), the level of the YY1 protein also decreased (Fig. 4K). This suggests that HBc may play a role in increasing YY1 expression.

3.5. Regulatory effects of YY1 on HBV replication and transcription

After confirming that HBV infection upregulates YY1 expression, we investigated the effect of YY1 on HBV replication and expression. The YY1 overexpression plasmid (pcDNA3.1-YY1) was transfected into cells to regulate YY1 expression. YY1 was confirmed to be overexpressed in the transfected cells using western blotting (Fig. 5A, F, and K). HepG2.2.15 cells transfected with YY1-overexpressing plasmids released more HBV DNA compared with the control group; however, HBsAg levels in the supernatant and cells were downregulated. Overexpression of YY1 in HepG2.2.15 cells resulted in a 3.29-fold increase in HBV DNA in the supernatant ($P < 0.05$) (Fig. 5B), while HBsAg levels in the supernatant and cells decreased by 67.82% and 87.47% ($P < 0.05$, $P < 0.05$) (Fig. 5C and D), respectively, compared with the control group. HepG2 and L02 cells were co-transfected with pAAV-HBV1.3 plasmids and YY1-overexpressing plasmids for further verification. Consistent with the results for HepG2.2.15 cells, co-transfection in HepG2 cells led to a 1.92-fold increase in HBV DNA in the supernatant ($P < 0.05$) (Fig. 5G), while HBsAg levels in the supernatant and cells decreased to

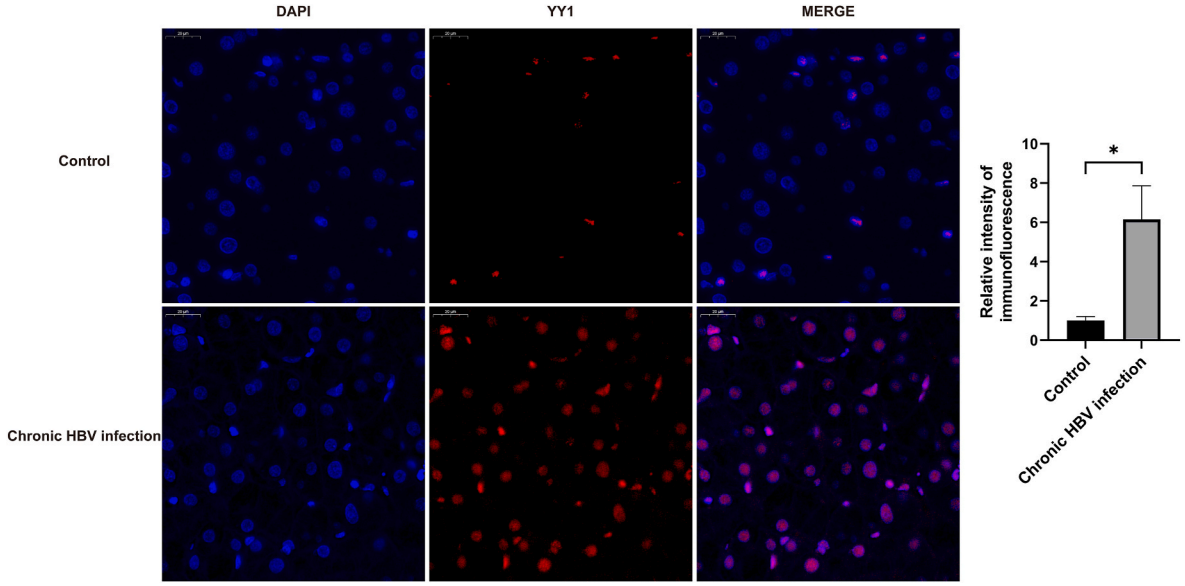

Fig. 3. YY1 levels in liver tissues. The expression of YY1 protein in liver tissues of healthy controls (normal liver tissues adjacent to hepatic hemangioma) and CHB patients. Immunofluorescence staining with an anti-YY1 antibody (red) and 4,6-diamidino-2-phenylindole (DAPI) (blue). Immunofluorescence intensity was measured using ImageJ. *$P < 0.05$.

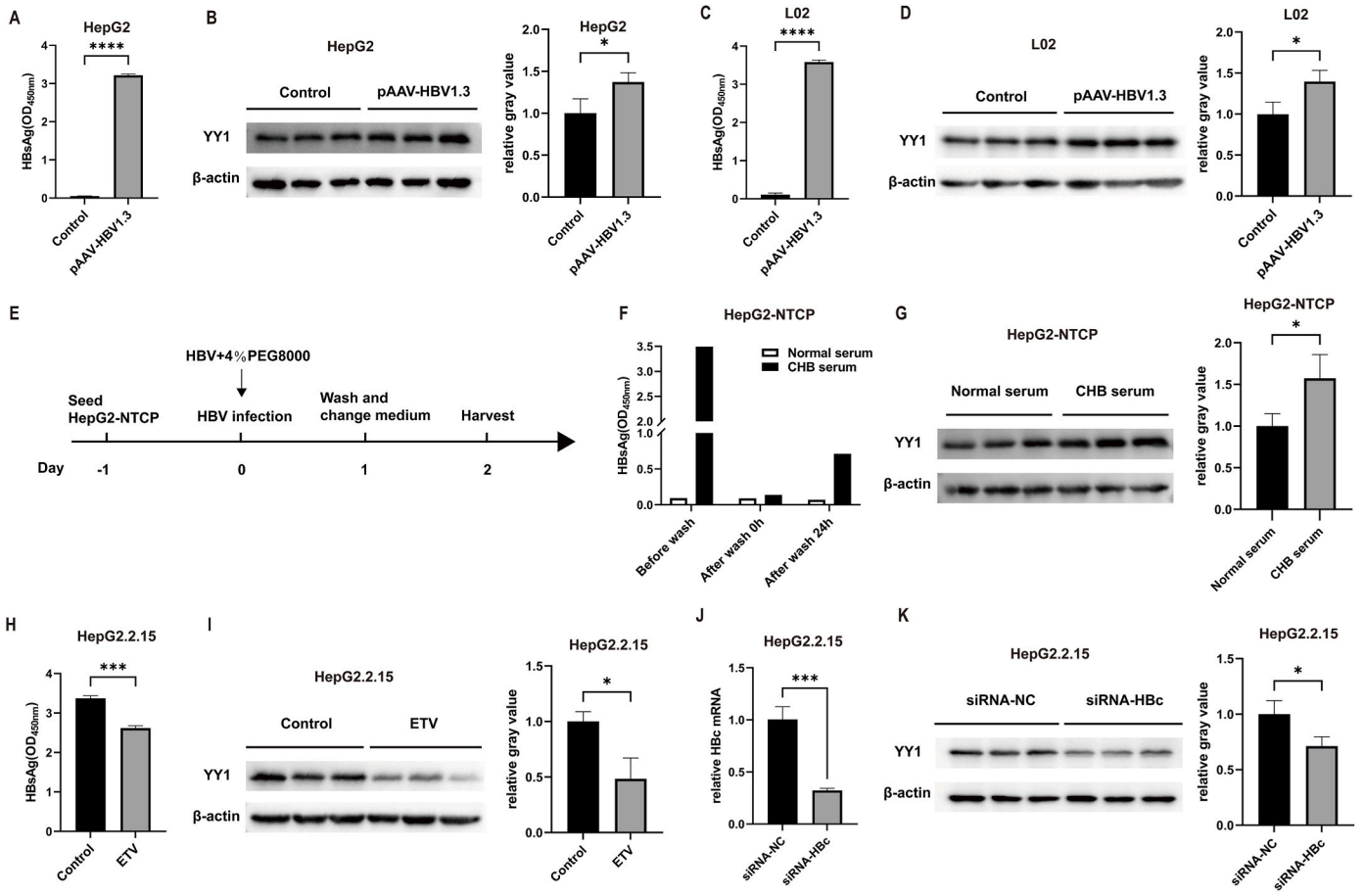

Fig. 4. Regulatory effects of HBV on YY1 expression. A: HBsAg levels in the supernatant of HepG2 cells transfected with pAAV-HBV1.3 plasmids or its control plasmids. B: Expression levels of YY1 protein in HepG2 cells transfected with pAAV-HBV1.3 plasmid or the corresponding negative control plasmid were detected using western blotting. C: HBsAg levels in the supernatant of L02 cells transfected with pAAV-HBV1.3 plasmids or its control plasmids. D: Expression levels of YY1 protein in L02 cells transfected with pAAV-HBV1.3 plasmid or the corresponding negative control plasmid were detected using western blotting. E: Schematic of the experimental design of HBV infecting HepG2-NTCP. F: The changes of HBsAg in the supernatant of HepG2-NTCP cells were exposed to normal human serum or serum of CHB patients. G: Expression levels of YY1 protein in HepG2-NTCP cells infected with HBV and control cells. H: HBsAg levels in the supernatant of HepG2.2.15 cells treated or untreated with ETV. I: Expression levels of YY1 protein in HepG2.2.15 cells treated or untreated with ETV were detected using western blotting. J: HBc mRNA levels in HepG2.2.15 cells transfected with siRNA-HBc or siRNA-NC were detected using quantitative real-time polymerase chain reaction. K: Expression levels of YY1 protein in HepG2.2.15 cells transfected with siRNA-HBc or siRNA-NC. Grey values were measured through ImageJ. *P < 0.05, ***P < 0.001, ****P < 0.0001.

10.39% and 20.21% ($P < 0.05$, $P < 0.05$) (Fig. 5H and I), respectively, compared with the control group. Similar results were observed in L02 cells (Fig. 5L–N).

Finally, the number of cells was assessed at 48 h post-transfection with pcDNA3.1-YY1 or control plasmid pcDNA3.1-NC by cell counting kit-8 (CCK-8) assay. These results revealed no statistically significant differences in cell numbers (Fig. 5E, J, and O). Thus, the differences in HBV DNA and HBsAg levels between the two groups were not due to differences in cell numbers.

To confirm these findings, HepG2.2.15 and HepAD38 cells were engineered to overexpress YY1 using a lentiviral vector (LV-YY1) (Fig. 6A and E). As expected, increased YY1 levels in these cells (Fig. 6B and F) led to higher levels of HBV DNA (Fig. 6C and G) but lower levels of HBsAg (Fig. 6D and H).

4. Discussion

CHB is a complex result of the interaction between HBV and the infected host cells, and its pathogenesis has not been fully elucidated. CHB is a global health concern. The World Health Assembly aimed for hepatitis B elimination by 2030 (Al Awaidy and Ezzikouri, 2020), driving intensified research for novel anti-HBV agents, targeting functional or complete cures. Despite advancements in HBV understanding and medical technology, achieving a definitive cure for CHB remains challenging. Novel, potent treatment approaches are imperative to attain hepatitis B elimination, with multidrug combination therapy potentially leading the way forward.

In this study, we observed a significant upregulation of YY1 in the serum and liver tissues of patients with chronic HBV infection. No significant differences were observed in YY1 serum levels between patients with chronic HCV infection and healthy individuals, indicating a specific association between YY1 levels and HBV infection. Furthermore, YY1 serum levels positively correlated with serum HBsAg and HBV DNA levels, indicating that YY1 levels are closely associated with HBV replication and expression. Moreover, serum HBsAg, HBeAg, and HBV DNA decreased significantly in CHB patients after 48 weeks of entecavir treatment compared to the baseline, followed by a significant decrease in serum YY1 levels. Therefore, YY1 levels are a potential indicator of HBV replication in patients with chronic HBV infection. Furthermore, YY1 protein levels were significantly higher in HBV-expressing cells than in non-expressing cells, confirming that HBV can upregulate YY1 expression.

We also explored the impact of YY1 on HBV replication and expression. *In vitro* cell experiments showed that YY1 promoted HBV DNA replication. However, concurrently, YY1 inhibited HBsAg expression through other mechanisms. Although these results may seem

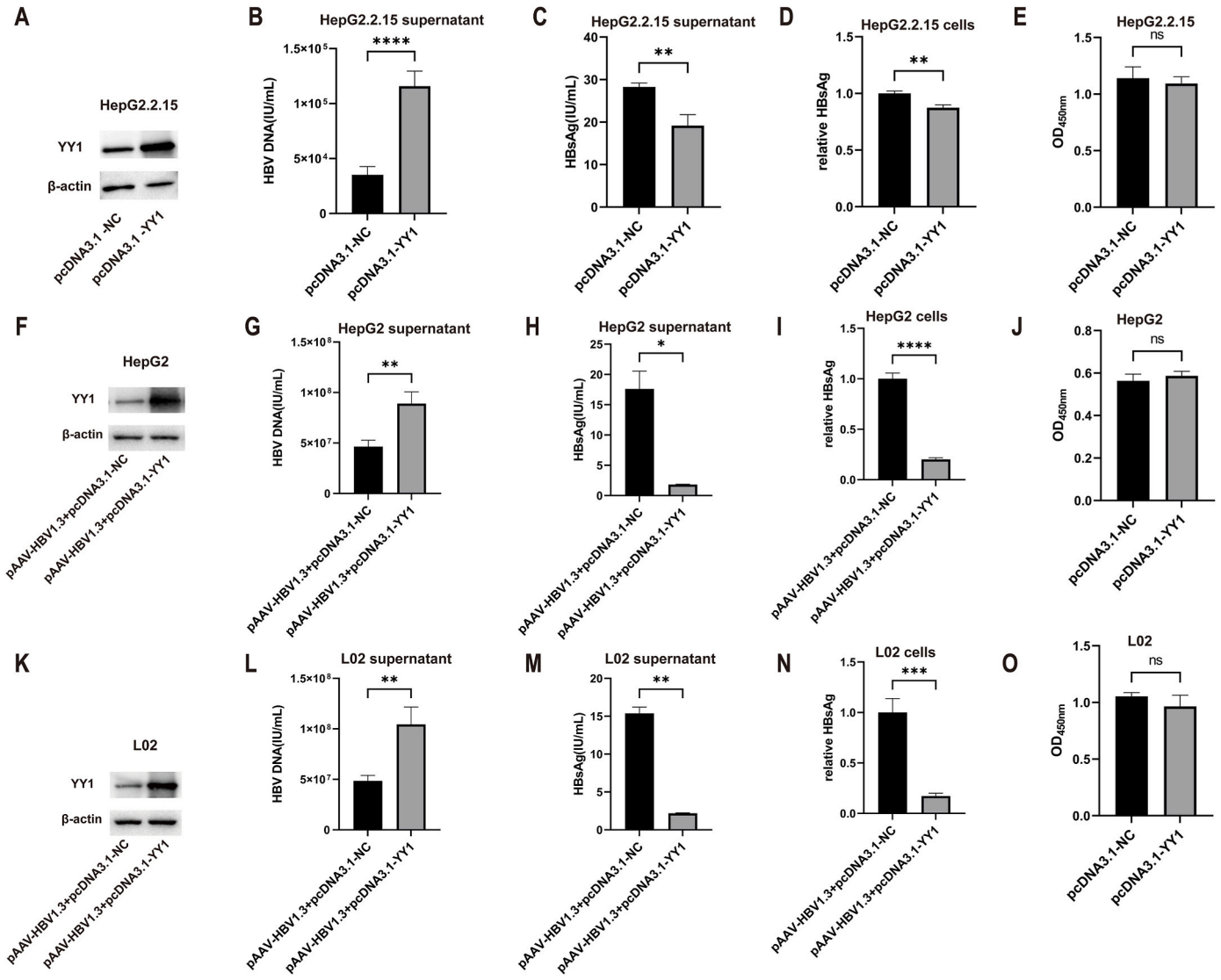

Fig. 5. Regulatory effects of YY1 on HBV were studied by plasmid transfection. A: The protein levels of YY1 in HepG2.2.15 cells transfected with pcDNA3.1-NC plasmid or pcDNA3.1-YY1 plasmid. B: HBV DNA levels in supernatant of HepG2.2.15 cells transfected with pcDNA3.1-NC plasmid or pcDNA3.1-YY1 plasmid. C: HBsAg levels in supernatant of HepG2.2.15 cells transfected with pcDNA3.1-NC plasmid or pcDNA3.1-YY1 plasmid. D: HepG2.2.15 cells transfected with pcDNA3.1-NC plasmid or pcDNA3.1-YY1 plasmid were broken up by ultrasound, and the relative expression of HBsAg intracellular was detected. E: Cells proliferation assay of HepG2.2.15 cells after transfection for 48 h using CCK-8 assay. F: Expression levels of YY1 protein in HepG2 cells co-transfected with corresponding plasmid. G: HBV DNA levels in supernatant of HepG2 cells co-transfected with the corresponding plasmid. H: HBsAg levels in supernatant of HepG2 cells co-transfected with corresponding plasmid. I: HepG2 cells co-transfected with corresponding plasmid were broken up by ultrasound, and the relative expression of HBsAg intracellular was detected. J: Cell proliferation assay of HepG2 cells after transfection for 48 h using CCK-8 assay. K: Expression levels of YY1 protein in L02 cells co-transfected with the corresponding plasmid. L: HBV DNA levels in the supernatant of L02 cells co-transfected with the corresponding plasmid. M: HBsAg levels in the supernatant of L02 cells co-transfected with the corresponding plasmid. N: L02 cells co-transfected with the corresponding plasmid were broken up by ultrasound, and the relative expression of HBsAg intracellular was detected. O: Cell proliferation assay of L02 cells after transfection for 48 h using CCK-8 assay. $^{*}P < 0.05$, $^{**}P < 0.01$,$^{***}P < 0.001$,$^{****}P < 0.0001$.

contradictory, HBsAg synthesis is not completely consistent with the replication of HBV during infection. YY1 is autoregulated through its own DNA-binding sites (Kim et al., 2009). When YY1 levels reach a certain threshold, the transcription factor negatively regulates its own locus (Hays and Bonavida, 2019). Elevated levels of endogenous YY1 induced by HBV in CHB patients may not completely inhibit HBsAg expression. It may be necessary to add exogenous YY1 to keep YY1 level at a high level to effectively inhibit HBsAg. Further studies are required to elucidate whether elevated levels of YY1 induced by HBV can effectively influence HBV replication and expression *in vivo*.

Our findings on YY1 overexpression promoting HBV DNA replication align with previous studies. Ren et al. (2018) demonstrated that YY1 overexpression in transfected HepAD38 cells increased HBV DNA replicative intermediates, total HBV RNAs, and 3.5 kb RNA, indicating the role of YY1 as an activator of HBV replication. Moreover, Teng et al. (2011) reported that YY1, binding to the nucleotide 2812–2816 site of the pre-S1 promoter, suppressed HBsAg synthesis at the transcriptional level, which is consistent with our findings. On the contrary, a study showed that the expression of HBsAg in siRNA-YY1 HepG2 cells was reduced to 78.86% of that in HepG2 cells transfected with siRNA-mock (Shen et al., 2020).

As a multifunctional transcription factor, YY1 represses or activates gene transcription depending on tissue context, downstream targets, and interactions with other regulatory proteins (Zhang et al., 2011b). YY1 possibly upregulates HBV DNA replication while simultaneously inhibiting HBsAg synthesis at the transcriptional level. YY1 may play a

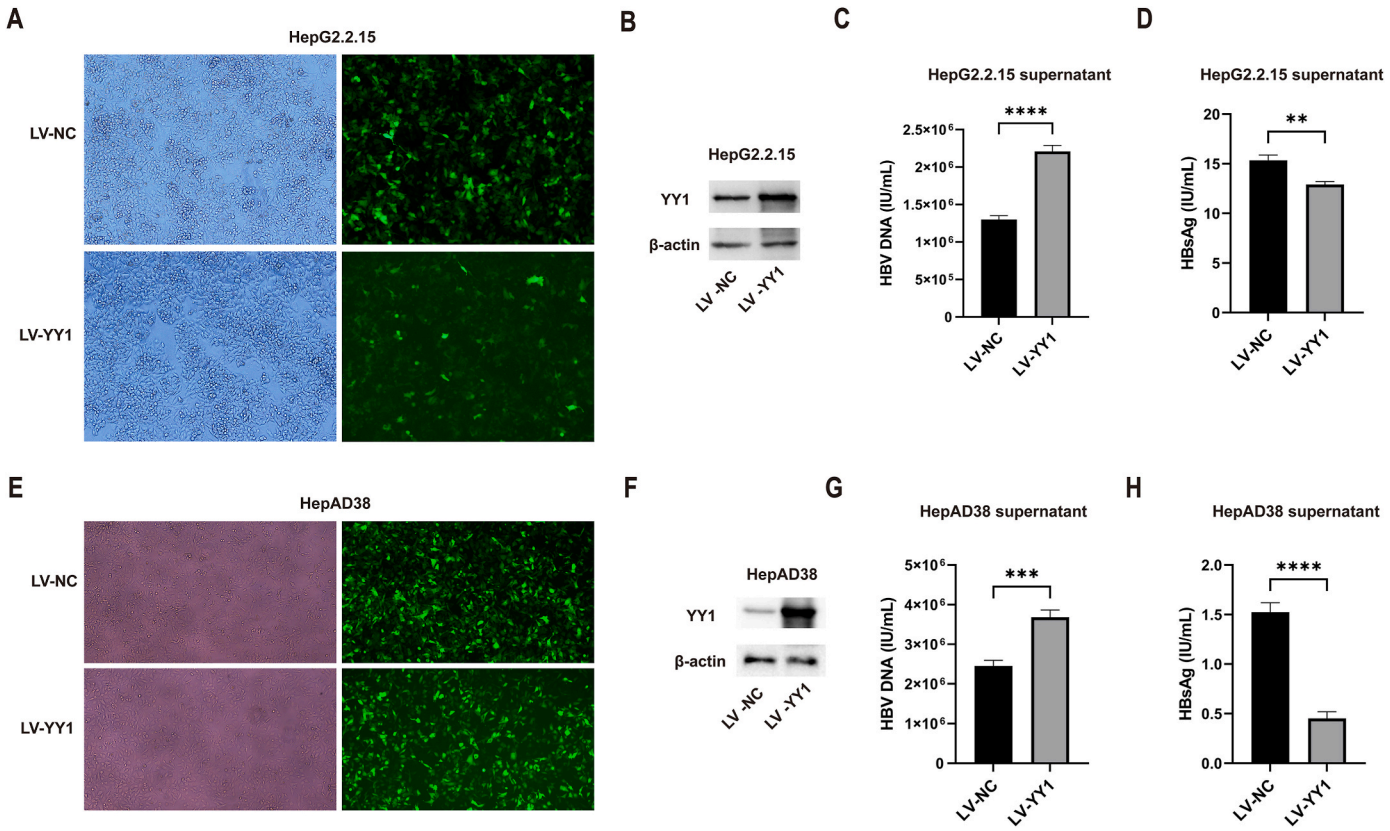

Fig. 6. Regulatory effects of YY1 on HBV were studied by lentivirus transduction. A: Expression of green fluorescent protein (GFP) in HepG2.2.15 cells transduced with LV-NC or LV-YY1. The images were captured by a bright light or fluorescent microscope (× 100) at 3 days post transduction. B: The lever of YY1 in HepG2.2.15 cells transduced with LV-NC or LV-YY1. C: HBV DNA levels in supernatant of HepG2.2.15 cells transduced with LV-NC or LV-YY1. D: HBsAg levels in supernatant of HepG2.2.15 cells transduced with LV-NC or LV-YY1. E: Expression of GFP in HepAD38 cells transduced with LV-NC or LV-YY1. The images were captured by a bright light or fluorescent microscope (× 100) at 3 days post transduction. F: The levels of YY1 in HepAD38 cells transduced with LV-NC or LV-YY1. G: HBV DNA levels in supernatant of HepAD38 cells transduced with LV-NC or LV-YY1. H: HBsAg levels in supernatant of HepAD38 cells transduced with LV-NC or LV-YY1. $^{**}P < 0.01$, $^{***}P < 0.001$,$^{****}P < 0.0001$.

complex role in HBV replication and expression, and its effect on HBV is likely a multipathway process. Different cellular contexts may cause YY1 to interact with diverse cellular factors, thereby exerting varying regulatory functions. The role of YY1 in chronic HBV infection and its regulatory mechanisms remain unclear. Understanding the YY1–HBV interaction and regulatory mechanisms could hold clinical importance and benefit the future development of HBV therapeutics. A deeper understanding of the function of YY1 and its interactions with other proteins could provide fresh insights into chronic HBV infection. Altogether, further investigation into the YY1-related regulation of HBV may provide new perspectives for HBV treatment.

While increasing HBV DNA levels by YY1 may benefit HBV self-replication, the decrease in HBsAg is more worthy of further investigation. The current goal of CHB treatment is a functional cure, achieved by eliminating HBsAg, continuously suppressing HBV DNA, and maintaining undetectable levels after treatment cessation (Nguyen et al., 2020; Yuen et al., 2022). As the first-line therapy for CHB, NAs effectively suppress HBV replication, prevent disease progression, and reducethe risk of HCC (Alexopoulou et al., 2020). However, NAs rarely achieve complete HBV eradication or HBsAg clearance (Kim et al., 2014; Lampertico et al., 2017). Therefore, further studies of other targets are required to improve HBsAg seroconversion.

Our study found that YY1 could effectively inhibit HBsAg synthesis *in vitro*, suggesting a theoretical basis and novel potential target for chronic HBV infection. However, the underlying mechanism remains largely unknown, and whether inhibiting HBsAg synthesis using YY1 based on the inhibition of HBV replication using NAs may improve the functional cure rate of CHB remains to be further studied. Importantly, our conclusion that YY1 affects HBV replication and expression stems from *in vitro* cell models. Therefore, it is necessary to explore the regulatory effects and potential mechanisms of YY1 on HBV in animal models and validate these results in future clinical trials. Such endeavors could unveil potential targets and new treatment perspectives for CHB.

In conclusion, our findings show that HBV increases YY1 production, which in turn elevates HBV DNA levels. However, YY1 reduces the production of HBsAg. This suggests that YY1 regulates HBV through a complex process with both activating and inhibiting effects. The YY1-mediated inhibition of HBsAg synthesis might occur at the transcriptional level.

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CRediT authorship contribution statement

Jie Zhou: Writing – original draft, Methodology, Investigation, Data curation. **Yunhao Hua:** Investigation. **Yuwei Liu:** Investigation. **Tong Wu:** Methodology. **Hongqin Xu:** Software, Data curation. **Zhongfeng Wang:** Investigation. **Xiaomei Wang:** Writing – review & editing, Supervision, Conceptualization. **Junqi Niu:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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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.2024.105889.

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