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ORIGINAL PAPER

Activation-induced cytidine deaminase (AID) suppresses the activity of HBV EnhII/CP by downregulating FANCE expression

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Abstract

Activation-induced cytidine deaminase (AID) is a host restriction factor known to suppress hepatitis B virus (HBV) replication. However, the precise mechanisms underlying its antiviral activity remain incompletely understood. Combining RNA-Seq and functional assays, our study reveals a novel pathway by which AID inhibits HBV replication via downregulating the expression of Fanconi anemia complementation group E (FANCE). Our findings demonstrate that AID significantly reduces FANCE expression, and that FANCE promotes viral replication by specifically activating the HBV enhancer II/core promoter (EnhII/CP), a key transcriptional regulatory element. Combinatorial regulation experiments further confirm that FANCE is involved in AID-mediated HBV transcription. These results identify a novel downstream gene of AID, broaden the perspective on the host antiviral immune network against HBV, and provides a rationale for potential antiviral strategies targeting the FANCE regulatory axis.

Keywords HBV · Activation-induced cytidine deaminase (AID) · Fanconi anemia complementation group E (FANCE)

Abbreviations

AID	Activation-induced cytidine deaminase
CHB	Chronic hepatitis B
PgRNA	Pregenomic RNA
Pol	Polymerase
EnhII/CP	Enhancer II/core promoter
ORFs	Open reading frames
HBc	Hepatitis B core protein
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
cccDNA	Covalently closed circular DNA
APOBEC	Apolipoprotein B100 mRNA-editing catalytic polypeptide
IFN-α	Interferon-α
ActD	Actinomycin D
DEGs	Differentially expressed genes

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Introduction

According to World Health Organization (WHO), an estimated 296 million people worldwide are chronically infected with hepatitis B virus (HBV), with 1.5 million new infections each year [1–3]. In 2022, 1.1 million people deaths are annually caused by HBV-related diseases [4]. Although the availability of HBV vaccines has reduced the incidence of HBV infection, chronic HBV infection remains a global health problem [5].

HBV is an enveloped DNA virus that is a member of the Hepadnaviridae family. Its 3.2 kb genome contains four partially overlapping open reading frames (ORFs) termed as PreC/C, PreS1/PreS2/S, P and X, and these ORFs encode structural and non-structural viral proteins: hepatitis B e antigen (HBeAg), hepatitis B core protein (HBc), the small, middle and large surface antigens (SHBsAg, MHBsAg, LHBsAg), the viral polymerase (pol) and X protein (HBx) [6, 7]. The covalently closed circular DNA (cccDNA) acts as the sole template for viral transcription and is driven by four major promoters——CP, Sp1, Sp2 and X promoters——as well as two enhancer elements, enhancer I (EnhI) and enhancer II (EnhII), producing 3.5, 2.4, 2.1, and 0.7 kb RNA transcripts, respectively [8]. Following assembly, the mature HBV nucleocapsid undergoes coordinated packaging with viral surface proteins via multivesicular bodies (MVBs), ultimately either generating complete virions that are secreted extracellularly or re-entering the nucleus to produce more cccDNA [9].

Activation-induced cytidine deaminase (AID) belongs to apolipoprotein B100 mRNA-editing catalytic polypeptide (APOBEC) proteins family members, which plays a significant role in host innate immunity [10]. The primary function of APOBEC/AID family is to catalyze the deamination of cytosine (C) to uracil (U) in DNA/RNA, which can lead to nucleotide mismatches and potentially cause DNA/RNA strand breaks [11–13]. AID-mediated somatic hypermutation and class-switch recombination in germinal center B lymphocytes [14, 15]. Beyond its physiological roles, AID potently restricts the replication of various viruses and other elements, including human immunodeficiency virus, retrotransposons and HBV [16–18]. Our previous studies have shown that AID mediates the recruitment of the RNA exosome to HBV ribonucleoprotein (RNP) complexes containing viral transcripts and pol protein, leading to strong inhibition of viral replication [17, 19]. Specifically, the molecular mechanism is initiated by AID-mediated site-specific deamination of HBV cccDNA, a key triggering event that sets off a subsequent cascade of molecular reactions.

Fanconi anemia complementation group E (FANCE) is a key member of the Fanconi anemia (FA) genes [20–22]. As a core component of the FA complex, FANCE is not only involved in maintaining genomic stability, but recent studies have also suggested its role in STAT1-mediated immune activation and the regulation of cytotoxicity [23, 24]. Clinical research has demonstrated that FANCE expression is regulated in various cancer types, with its overexpression strongly associated with tumor advancement and chemoresistance, thus highlighting its potential as both a prognostic biomarker and a therapeutic target in malignancies [25]. Despite the absence of direct and unambiguous mechanistic investigations into the association between FANCE and hepatitis B virus (HBV) replication at present, the maintenance of cellular genomic stability and the regulation of intracellular antiviral immune states mediated by FANCE are probably critical factors influencing the successful establishment of HBV persistent infection and the subsequent progression of HBV-related diseases.

In this study, we identify FANCE as a key downstream molecular of AID that inhibits HBV replication. Specifically, FANCE facilitates HBV replication at the transcriptional level through augmenting the activity of the EnhII/CP promoter. These findings provide a new research direction for exploring the mechanisms of AID-mediated anti-HBV.

Materials and methods

Cell culture, plasmids, and transfection

Cells were cultured as previously described [26, 27]. Plasmids and siRNAs transfection were performed according to the manufacturer's guidelines with Lipofectamine 2000 (Invitrogen, USA). Recombinant human interferon-α (IFN-α) was obtained from MedChemExpress (USA). Actinomycin D (ActD) was purchased from Sigma-Aldrich (USA). The HBV replicon plasmid (pPB) contains 1.04 copies of HBV genomic DNA (subtype adw R9) and expresses pgRNA under the control of the CMV promoter. Nano-luciferase (NL), a highly sensitive reporter gene is inserted into the core region of the HBV genome. A 1.2-fold HBV genome construct was generated by cloning into the HindIII/EcoRI sites of pUC19, yielding pUC1.2 × HBV/NL. Expression vectors used in this study are listed in Supplementary Table 1.

RT-qPCR and qPCR

Total RNA from hepatoma cell lines was isolated with RNAex Pro Reagent according to the manufacturer's instructions (Accurate Biology, China). RNA was transcribed to cDNA with a reverse transcription kit (Vazyme, China). For HBV DNA, the procedures are just as previous description [27]. ChamQ Universal SYBR qPCR Master Mix kit (Vazyme, China) was used to quantitate HBV RNA and DNA. The primers are listed in Supplementary Table 2.

RNA-Seq

Huh7 cells were co-transfected with HBV replication plasmid (pPB) and AID expression vector (or empty vector). Total RNA isolated from the cells was analyzed by RNA-Seq as previously described (Nanjing Personal Gene Technology Co. Ltd., China) [26]. The data presented in the study are deposited in the BioProject database of the National Center for Biotechnology Information (Accession number PRJNA1100181).

Western blot analysis

Cells were lysed with lysis buffer supplemented with proteinase inhibitor for protein extraction (Solarbio, China). Western blot was as described before [27]. Primary antibodies were as following: anti-GFP antibody (bs-0639R) and anti-FANCE antibody (bs-13142R) were purchased from Bioss (China). Anti-Flag antibody (PM020) was purchased from MBL (Japan). Anti-GAPDH antibody (YN5585) was purchased from Immunoway (China). The proteins were detected with ECL by Intelligent Image Workstation (BLT, China).

Native agarose gel electrophoresis (NAGE) and southern blot

NAGE analysis was performed as described previously [28]. In brief, crude extracts of HBV-expressing cells were loaded into agarose gel for electrophoresis to separate intact nucleocapsid particles. Nucleocapsid DNA was detected using Southern blot with a double-stranded HBV DNA probe spanning the whole viral genome [19].

Dual-luciferase reporter assay and luciferase assay

To measure pgRNA expression, Nano-luciferase (NL) activity was determined in HBV-NL transfectants using the Nano-Glo Luciferase Assay System (Promega, USA) on a PowerScan HT reader (BioTek, USA) as described. The NL gene in this construct replaces the HBV core region, making its activity directly report pgRNA levels [26, 27].

The HBV promoter fragments were cloned into the pGL3-basic luciferase reporter vector. Each construct was co-transfected into Huh7 cells. After a 72-h incubation, cells were lysed with lysis buffer from the Bright-Glo Luciferase Reporter Assay kit (Promega, USA), and luciferase activity was detected.

Enzyme-linked immunosorbent assay (ELISA)

The expression levels of HBeAg and HBsAg in supernatants were detected by ELISA kit (Lvye Biotechnology) and TECAN infinite M200PRO (Switzerland), according to the manufacturer's instructions.

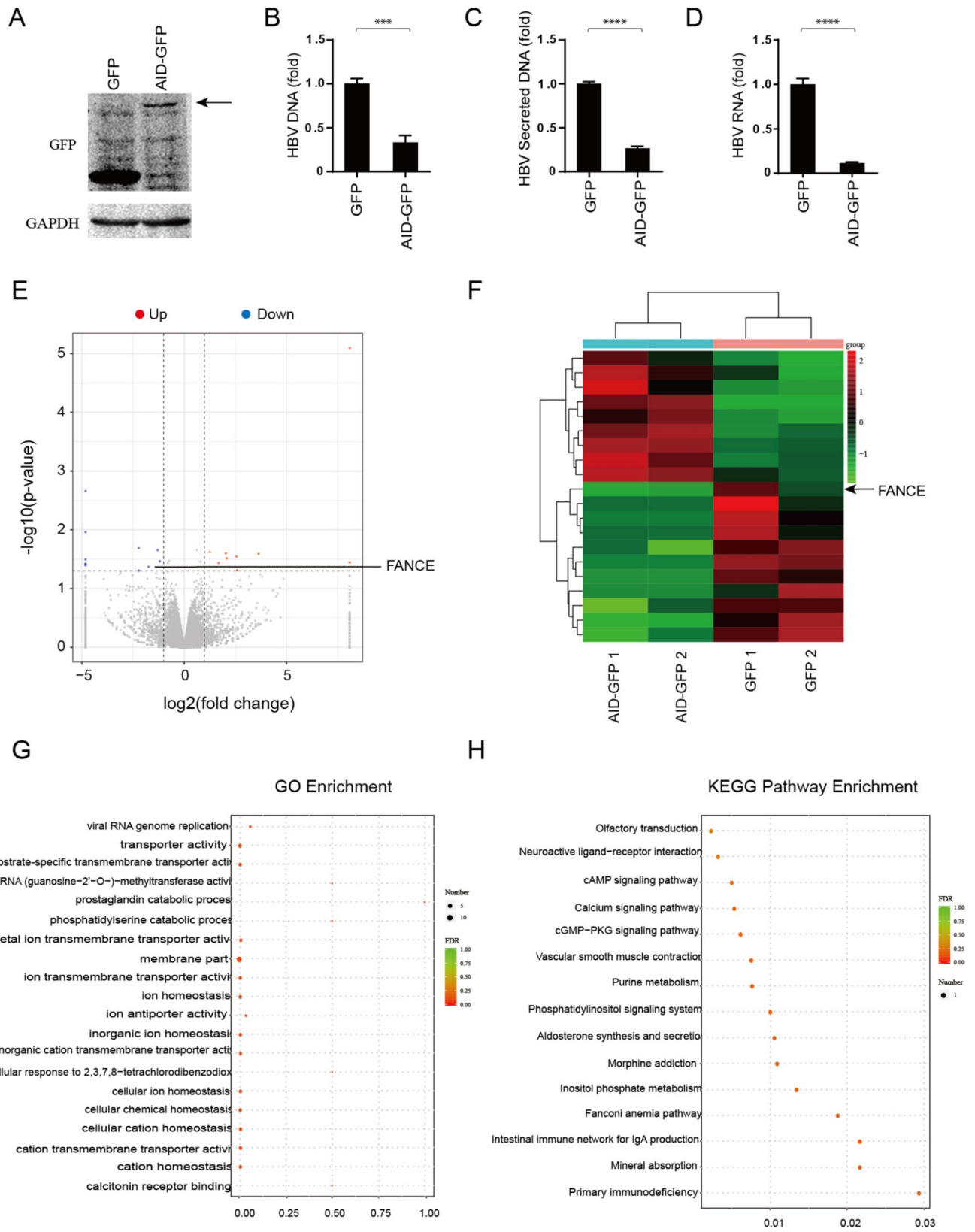
Statistical analysis

Statistical analysis was performed using GraphPad Prism software. For comparisons of the mean between two groups, two-tail Student's *t* test and one-way analysis of variance test were performed for data analysis. All data were presented as the mean ± SD. P values less than 0.05 were considered statistically significant.

Results

The expression of transcripts during AID inhibition of HBV replication through RNA-Seq

AID is a crucial component of the host intrinsic defense system and exerts protective effects against a variety of viruses, including HBV. Consistent with previous reports, our findings showed a marked inhibitory effect of AID on HBV replication (Fig. 1A–D) [17, 19, 29, 30]. To investigate the involvements of downstream genes in the AID-mediated anti-HBV process, Huh7 cells were co-transfected with HBV replicon plasmid (pPB) and AID-GFP or GFP plasmids, followed by RNA sequencing (RNA-Seq) analysis. Data analysis from the RNA-Seq identified significantly differentially expressed genes (DEGs) in the AID-expressing group, comprising 9 upregulated genes (fold change ≥ 2, $P < 0.05$) and 11 down-regulated genes (fold change ≤ -2, $P < 0.05$, Fig. 1E). These differentially expressed genes were subsequently clustered, and a heatmap depicting their expression levels was constructed (Fig. 1F). Gene Ontology (GO) functional enrichment analysis indicated predominant


A
GFP
AID-GFP
GFP
GAPDH
B
HBV DNA (fold)
***
GFP
AID-GFP
C
HBV Secreted DNA (fold)
****
GFP
AID-GFP
D
HBV RNA (fold)
****
GFP
AID-GFP
E
Up
Down
-log10(p-value)
log2(fold change)
FANCE
F
group
FANCE
AID-GFP 1
AID-GFP 2
GFP 1
GFP 2
G
GO Enrichment
viral RNA genome replication
transporter activity
substrate-specific transmembrane transporter acti
rRNA (guanosine-2'-O-)-methyltransferase activi
prostaglandin catabolic proces
phosphatidylserine catabolic proces
metal ion transmembrane transporter activ
membrane part
ion transmembrane transporter activi
ion homeostasis
ion antiporter activity
inorganic ion homeostasi
inorganic cation transmembrane transporter acti
cellular response to 2,3,7,8-tetrachlorodibenzodiox
cellular ion homeostasis
cellular chemical homeostasis
cellular cation homeostasis
cation transmembrane transporter activi
cation homeostasis
calcitonin receptor binding
Number
FDR
H
KEGG Pathway Enrichment
Olfactory transduction
Neuroactive ligand-receptor interaction
cAMP signaling pathway
Calcium signaling pathway
cGMP-PKG signaling pathway
Vascular smooth muscle contractio
Purine metabolism
Phosphatidylinositol signaling system
Aldosterone synthesis and secretio
Morphine addiction
Inositol phosphate metabolism
Fanconi anemia pathway
Intestinal immune network for IgA production
Mineral absorption
Primary immunodeficiency
FDR
Number

◂**Fig. 1** RNA-Seq of AID-expressing Huh7 cells. Huh7 cells were transfected with pPB and AID-GFP (or GFP) plasmids; 72 h later, the cells and supernatants were collected. **A** The levels of proteins were detected by Western blot. **B**, **C** The cellular and secreted HBV DNA levels were quantified by qPCR analysis. **D** HBV RNA was quantified by RT-qPCR analysis. **E**–**H** The total RNA from above-mentioned samples was identified by RNA-Seq. **E** Volcano plot and **F** heatmap. The DEGs were analyzed by Gene Ontology (GO, **G**) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment (**H**). The HBV DNA or RNA level of the GFP sample was defined as value 1. $N=3$, $^{****}P<0.0001$, $^{***}P<0.001$

enrichment of DEGs in biological processes associated with viral genome replication, metabolism, and homeostasis functions (Fig. 1G). Meanwhile, KEGG pathway analysis highlighted significant associations with signal transduction cascades, the FA pathway, and immune-related pathway (Fig. 1H).

FANCE promotes HBV replication in human hepatoma cells

To further validate the biological function of DEGs on HBV replication, the top 2 upregulated genes (MRM1 and TINCR) and the top 7 down-regulated genes (P2RX6, FAM155A, SLC4A8, PDE2A, SLC9A5, TMEM251 and FANCE) identified from the expression profile were chosen for gene knockdown assays. Our results showed that knockdown of FANCE (siFANCE) significantly reduced HBV RNA expression compared with the siRNA negative control group (siNC, (Fig. 2A, B). These findings suggest that FANCE may act as an effector molecule downstream of the AID signaling pathway, participating in the positive regulation of HBV replication.

To systematically elucidate the regulatory role of FANCE in HBV replication, hepatomacytes were co-transfected with pPB and siFANCE (or siNC). Results showed that siFANCE markedly decrease HBV expression levels (Fig. 2C–J; Fig. S1A–C). Otherwise, overexpressed FANCE markedly elevated these (Fig. 2K–R; Fig. S1D–F), indicating a positive regulatory effect of FANCE on HBV replication. Similarly, we obtained consistent results in the HBV-replicating human HCC cell line HepG2.2.15 (Fig. S2). These results collectively demonstrate that FANCE, acting as a downstream molecular of AID, promotes HBV replication in human hepatoma cells.

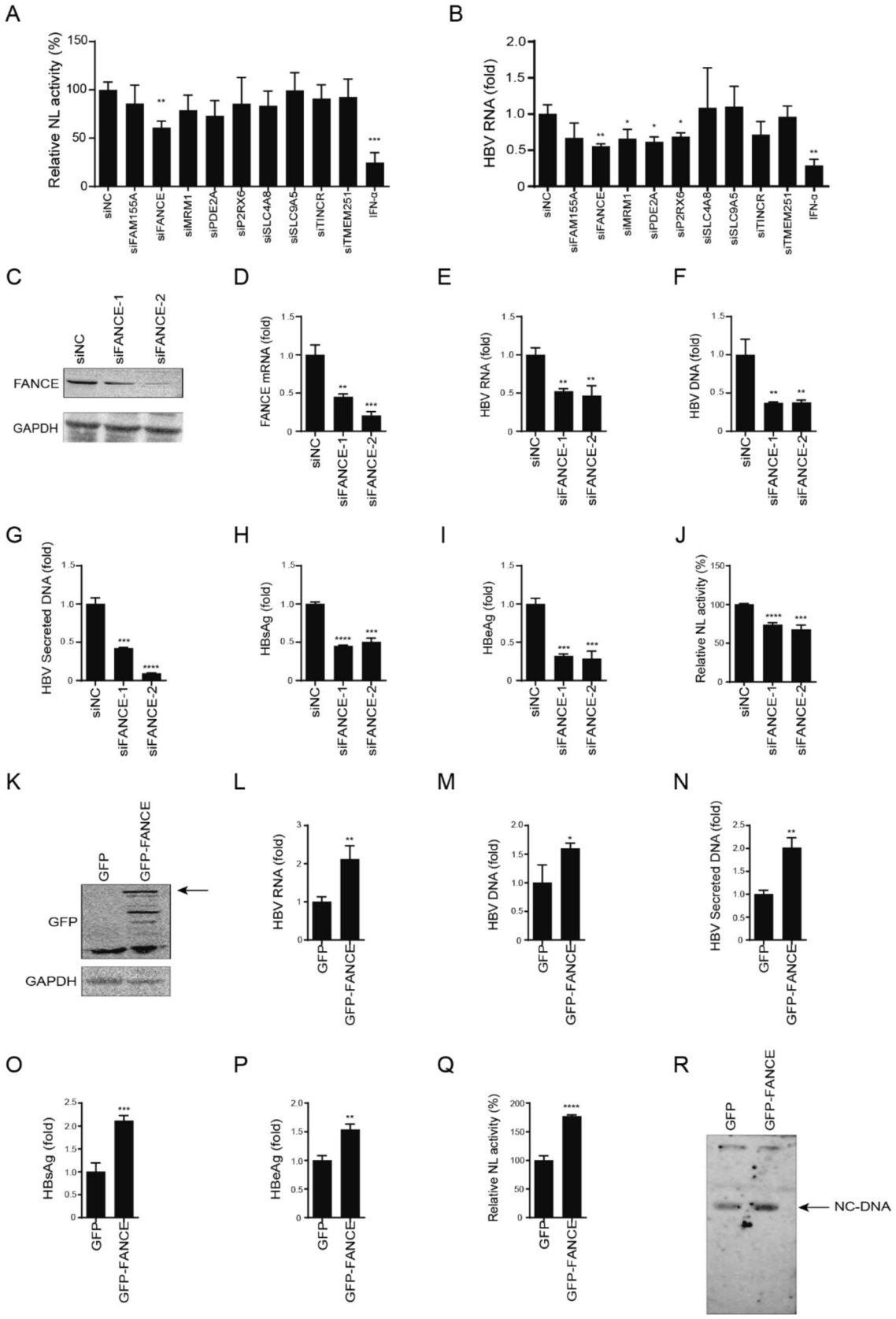
AID reduces HBV RNA by downregulating the expression of FANCE

To further investigate the relationship between AID and FANCE, pPB and AID-GFP (or GFP) plasmids were co-transfected into Huh7 cells. The results showed that AID overexpression significantly reduced both protein and mRNA levels of FANCE (Fig. 3A, B), which was consistent with the sequencing results (Fig. 1F). Conversely, knockdown of AID upregulated FANCE levels (Fig. 3D–F). To investigate the role of FANCE in AID-mediated HBV suppression, we observed that overexpression of FANCE alone increased viral RNA levels by 2.8-fold. This effect was further enhanced to 6.7-fold when FANCE overexpression was combined with AID knockdown (Fig. 3H, lanes 3 and 4). In a complementary manner, AID overexpression reduced viral RNA by 60%, while a more pronounced reduction of 88% was observed upon AID overexpression in FANCE-knockdown cells (Fig. 3I, lanes 2 and 4). Additionally, FANCE significantly attenuates the AID's inhibitory effect on HBV replication (Fig. 3J, K). Consistent results were obtained using a transfected pgRNA reporter plasmid (Fig. 3L). Taken together, these data indicate that AID suppresses HBV replication, at least in part, by downregulating FANCE expression.

AID-mediated the inhibition of HBV EnhII/BCP activity through downregulating FANCE expression

While previous reports demonstrated that AID inhibits HBV RNA expression via a transcription-coupled manner [19], our findings further identify the downregulation of FANCE by AID as a crucial pathway for inhibiting HBV replication (Fig. 3). Therefore, our study aims to investigate whether FANCE similarly modulates HBV RNA at the transcriptional level. To this end, the classic transcriptional inhibitor actinomycin D (ActD) was employed to interfere with nascent transcription [31]. Data suggested that in the presence of ActD, the enhancing effect of FANCE on viral RNA was abolished (Fig. 4A, lanes 7 and 8), providing direct evidence that the upregulation of viral RNA by FANCE depends on the transcriptional process.

Given that both AID and FANCE can affect HBV RNA through transcriptional coupling, we analyzed the impact of AID and FANCE on various HBV promoters and enhancers. Results showed that AID down-regulated the activities of EnhII/CP, Sp1, Sp2 and EnhI/Xp (Fig. 4B, C), while


A
Relative NL activity (%)
150
100
50
0
siNC
siFAM155A
siFANCE
siMRM1
siPDE2A
siP2RX6
siSLC4A8
siSLC9A5
siTINCR
siTMEM251
IFN-α
B
HBV RNA (fold)
2.0
1.5
1.0
0.5
0
C
siNC
siFANCE-1
siFANCE-2
FANCE
GAPDH
D
FANCE mRNA (fold)
E
HBV RNA (fold)
F
HBV DNA (fold)
G
HBV Secreted DNA (fold)
H
HBsAg (fold)
I
HBeAg (fold)
J
Relative NL activity (%)
K
GFP
GFP-FANCE
GFP
GAPDH
L
HBV RNA (fold)
M
HBV DNA (fold)
N
HBV Secreted DNA (fold)
O
HBsAg (fold)
P
HBeAg (fold)
Q
Relative NL activity (%)
R
GFP
GFP-FANCE
NC-DNA

◂**Fig. 2** FANCE promotes HBV replication. **A**, **J** Huh7 cells were transfected with pUC1.2×HBV/NL, pcDNA-CP and indicated siRNA (or siNC); 72 h later, the NL activities were detected. **B** Huh7 cells were transfected pPB and indicated siRNA, 72 h later, HBV RNA was detected by RT-qPCR. **C**–**I** Huh7 cells were transfected with pPB plasmids and siNC or siFANCE, 72 h later, the cells and supernatants were collected. **C** The expressions of proteins were detected by Western blot. **D**, **E** The levels of FANCE mRNA and HBV RNA were detected by RT-qPCR, respectively. **F**, **G** HBV DNA levels of intracellular and extracellular were detected by qPCR, respectively. **H**, **I** ELISA assay was applied to detect HBsAg and HBeAg expression levels in the supernatant. **J** Huh7 cells were transfected with pUC1.2×HBV/NL and pcDNA-CP plasmids and siNC or siFANCE, 72 h later, the NL activities were detected. **K**–**P**, **R** Huh7 cells were transfected with pPB and GFP-FANCE (or GFP) plasmids, 72 h later, the cells and supernatants were collected. **K** The expressions of proteins were detected by Western blot. **L** HBV RNA levels were detected by RT-qPCR. **M**, **N** HBV DNA levels of intracellular and extracellular were detected by qPCR. **O**, **P** HBsAg and HBeAg expression levels in the supernatant were detected by ELISA assay. **Q** Huh7 cells were transfected with pUC1.2×HBV/NL and pcDNA-CP and GFP-FANCE (or GFP) plasmids, 72 h later, the NL activities were detected. The DNA or RNA level of the siNC or GFP sample was defined as value 1. The NL activity of the siNC or GFP sample was defined as value 100. $N=3$, $^{****}P<0.0001$, $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$. **R** Cells extracts were subjected to measure the HBV NC-DNA by Southern blot

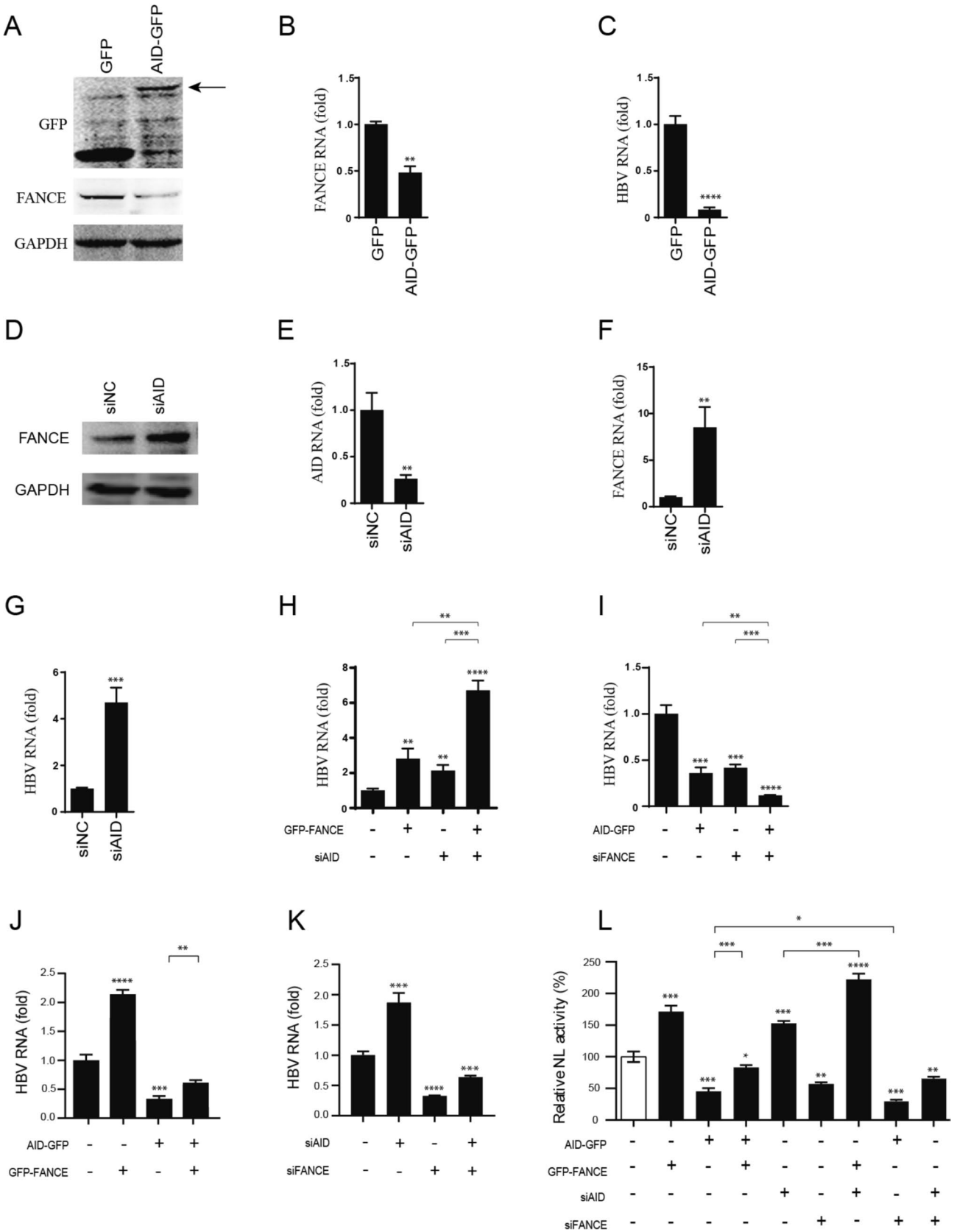
FANCE only specifically enhanced the activity of EnhII/CP (Fig. 4D, E). These effects were specific, as neither manipulation altered activity of a non-HBV control vector (Fig. S3). Additional findings suggested that the reduction of HBV EnhII/CP activity by AID was considerably more obvious when FANCE was silenced, as opposed to merely decreasing the expression levels of AID (Fig. 4F). Conversely, a significant enhancement in EnhII/CP activity was observed (Fig. 4G). Furthermore, our results indicated that the activity of the HBV EnhII/CP was significantly decreased compared with that in the condition of aid overexpression alone; whereas the activity of the HBV EnhII/CP was slightly restored when aid and FANCE were overexpressed simultaneously (Fig. 4H). Nevertheless, concurrent silencing of AID and FANCE resulted in persistently low HBV EnhII/CP activity, in accordance with HBV RNA levels (Fig. 4I). FANCE indeed weakens the AID-mediated enhancement of EnhII/CP activity. These data indicate that AID can specifically suppress HBV EnhII/CP activity by downregulating FANCE expression.

Discussion

By integrating transcriptomic analysis with functional validation, the present study elucidates a novel mechanism by which the host innate immune system suppresses hepatitis B virus (HBV) replication: AID downregulates the expression of FANCE, thereby specifically suppressing the transcriptional activity of HBV EnhII/CP, ultimately impeding viral replication at the transcriptional level. This finding not only adds a new dimension to the antiviral spectrum of AID, but also closely links FANCE, a key regulator of the DNA damage repair pathway, with viral transcriptional regulation, deepening our understanding of the complex host–virus interaction network.

Firstly, our research consolidates and expands the role of AID as a key host restriction factor. Previous studies have fully demonstrated that AID exerts antiviral effects either by directly editing HBV cccDNA through its cytidine deaminase activity or by recruiting the RNA exosome to degrade viral transcripts within ribonucleoprotein complexes [17, 19, 30]. This study reveals that AID possesses a novel function independent of its classic editing activity, that is, as an upstream signal of transcriptional regulation, negatively regulating the expression of the host gene *FANCE* (Figs. 1, 3). These results imply that AID may serve as a multifunctional immune hub, engaging multiple parallel molecular strategies to cooperatively combat viral infection, with its antiviral mechanisms demonstrating notable layering and complementarity.

Furthermore, our study characterizes FANCE as a novel host-promoting factor for HBV replication, which specifically enhances the activity of the HBV EnhII/CP in hepatocytes (Fig. 4). Since EnhII/CP serves as a core regulatory switch driving the transcription of viral pgRNA and preC mRNA and thereby determining viral replication efficiency [32, 33], the targeting of this node by FANCE establishes its role as a key host factor supporting viral replication. This finding broadens the functional repertoire of the FA pathway from genome maintenance to the control of viral infection. Mechanistically, we speculate that FANCE may modulate EnhII/CP activity in the following ways: (i) acting as a transcriptional co-factor recruited directly or indirectly to the EnhII/CP region, where it cooperates with liver-enriched transcription factors (e.g., HNF4α, C/EBP) or nuclear receptors (e.g., RXRα/PPARα) to stabilize the transcription initiation machinery [34–36]; (ii) modulating the chromatin state or epigenetic modifications of the cccDNA minichromosome via the FA pathway, thereby creating a transcriptionally favorable microenvironment [25, 37, 38]; or (iii) engaging in the regulation of specific intracellular signaling cascades (e.g., the STAT1 pathway) to indirectly affecting the transcriptional network [23, 24]. These hypotheses warrant further validation in future studies employing advanced techniques such as chromatin immunoprecipitation sequencing


A
GFP
AID-GFP
GFP
FANCE
GAPDH
B
FANCE RNA (fold)
GFP
AID-GFP
C
HBV RNA (fold)
GFP
AID-GFP
D
siNC
siAID
FANCE
GAPDH
E
AID RNA (fold)
siNC
siAID
F
FANCE RNA (fold)
siNC
siAID
G
HBV RNA (fold)
siNC
siAID
H
HBV RNA (fold)
GFP-FANCE
siAID
I
HBV RNA (fold)
AID-GFP
siFANCE
J
HBV RNA (fold)
AID-GFP
GFP-FANCE
K
HBV RNA (fold)
siAID
siFANCE
L
Relative NL activity (%)
AID-GFP
GFP-FANCE
siAID
siFANCE

◂**Fig. 3** AID reduces HBV RNA by downregulating the expression of FANCE. **A**–**C** Huh7 cells were transfected with pPB and AID-GFP (or GFP) plasmids; 72 h later, the cells were harvested. **A** The expression levels of proteins were detected by Western blot. **B** FANCE mRNA was detected by RT-qPCR. **C** HBV RNA was detected by RT-qPCR. The RNA level of the GFP sample was defined as value 1. **D**–**G** Huh7 cells were transfected with pPB and siAID; 72 h later, the cells were harvested. **D** The expression levels of proteins were detected by Western blot. **E**–**G** The RNA levels of AID, FANCE, and HBV were detected by RT-qPCR, respectively. The RNA level of the siNC sample was defined as value 1. **H**, **I** Huh7 cells were transfected with pPB and other plasmids and siRNA as indicated, 72 h later, the cells were harvested and the levels of HBV RNA were detected by RT-qPCR. The HBV RNA level of the GFP + siNC sample was defined as value 1. **J** Huh7 cells were transfected with pPB and AID-GFP and (or) GFP-FANCE plasmids, **K** Huh7 cells were transfected with pPB and siAID and (or) siFANCE; 72 h later, the cells were harvested and the levels of HBV RNA were detected by RT-qPCR. The HBV RNA level of the GFP or siNC sample was defined as value 1. **L** Huh7 cells were transfected with pUC1.2 × HBV/NL, pcDNA-CP and other plasmids, and siRNA as indicated. The cells were then cultivated for 3 days and the NL activities were detected. The NL activity of the GFP + siNC sample was defined as value 100. $N = 3$, $^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$

(ChIP-Seq), proteomic interaction analysis, and epigenetic profiling of cccDNA.

AID acts as an enzyme that associates with single-stranded DNA/RNA and preferentially deaminates cytosines in the AID-preferred WRC motif (W = A/T, R = A/G), rather than other regions of the Ig locus. Notably, FANCE contains a large number of WRC motifs with a high density, which suggests that AID may catalyze cytidine deamination (C to U) on FANCE mRNA and result in its instability [39]. Therefore, these findings corroborate the potential mechanism by which AID downregulates FANCE expression. The chromatin state, structural integrity, and epigenetic modification status of cccDNA directly determine the chromatin accessibility and transcriptional activity of the HBV EnhII/CP region. Emerging evidence indicates that AID mediates cytidine deamination of HBV cccDNA, which compromises cccDNA stability and structural integrity, and consequently modulates the transcriptional activity of the EnhII/CP region [29]. As documented in previous studies, FANCE plays a key role in DNA interstrand crosslink repair and the maintenance of chromatin stability [25]. This function may in turn regulate HBV cccDNA stability and counteract the effects of AID on cccDNA.

Despite some important findings in this study, certain limitations should be acknowledged. First, the mechanistic investigation remains incomplete. Although we have delineated two key events—AID-mediated downregulation of FANCE and FANCE-driven enhancement of EnhII/CP activity—the precise molecular connection between these events and their direct impact on cccDNA remains to be clarified. Additionally, whether FANCE enhanced viral transcription is dependent on an intact FA core complex has not been verified. Second, the relationship between the transcriptional regulatory pathway identified in our study and other established antiviral mechanisms of AID remains undefined. Further studies, potentially utilizing AID deaminase activity-deficient mutants, are needed to clarify whether these mechanisms act independently, synergistically, or in a hierarchical manner during antiviral defense. Third, in vivo evidence is still lacking. Since all experiments were performed in hepatoma cell lines, future investigations should validate the interplay between AID and FANCE and its impact on viral load and hepatic pathology in HBV-infected animal models.

In summary, this study delineates a novel pathway mediated by AID, which inhibits HBV transcription and replication by negatively regulating FANCE. This work advances the comprehension of the complexity inherent of

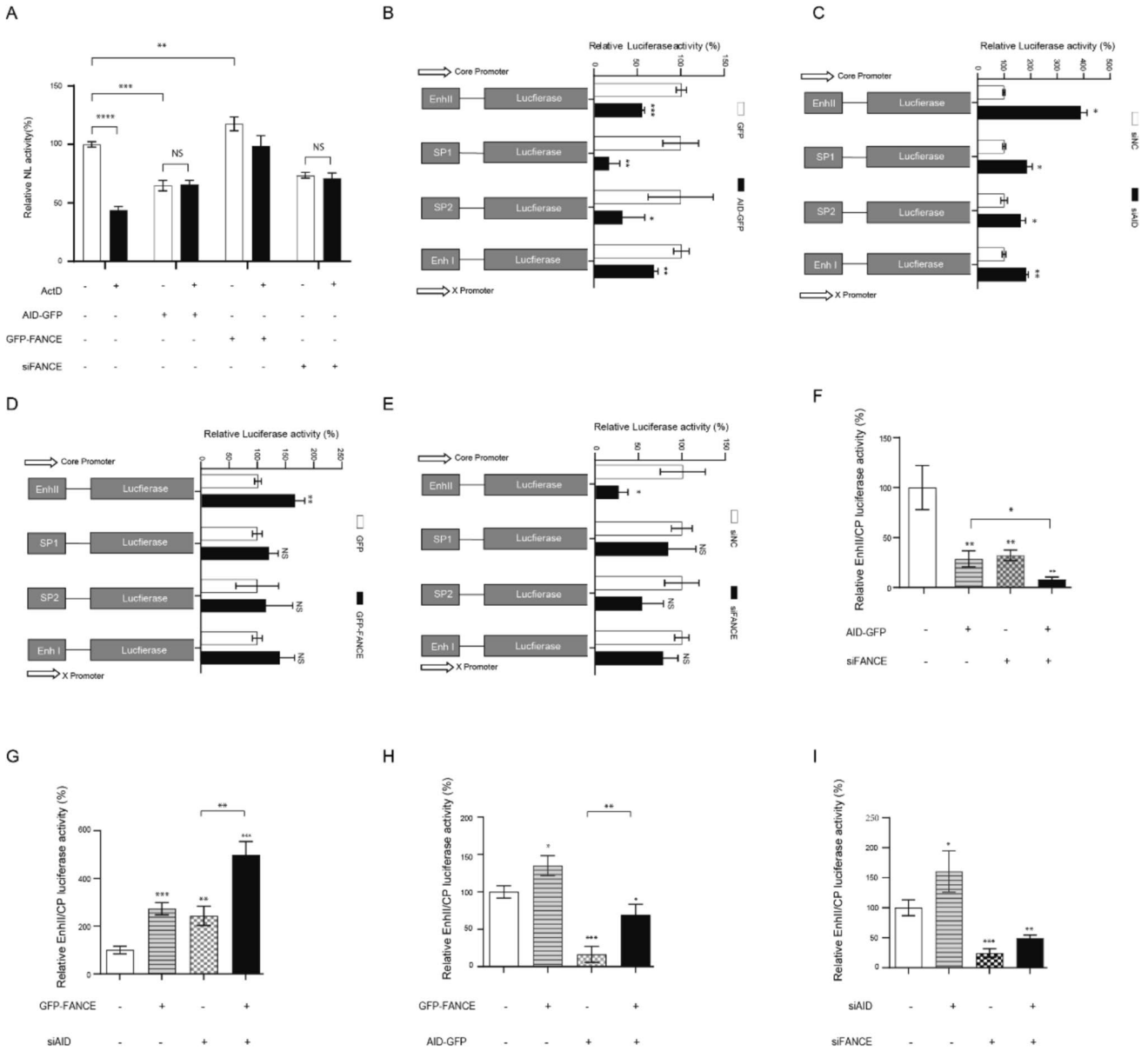


Fig. 4 AID modulates EnhII/CP Activity via FANCE. **A** Huh7 cells were transfected with pUC1.2×HBV/NL, pcDNA-CP expression plasmids and other plasmids and siRNA as indicated and then cells were cultivated for 3 days. ActD was added to inhibit transcription for 54 h of transfection, 18 h later, the NL activities were detected. The NL activity of the GFP + siNC group was defined as value 100. **B**–**E** Huh7 cells were co-transfected with plasmids or siRNA as indicated; 72 h later, the luciferase activities were detected. The luciferase activity of the GFP or siNC group was defined as value 100. **F** Huh7 cells were co-transfected with EnhII/CP-Luc and AID-GFP (or GFP) expression vector and siFANCE (or siNC), **G** Huh7 cells were co-transfected with EnhII/CP-Luc and GFP-FANCE (or GFP) expression vector and siAID (or siNC), **H** Huh7 cells were co-transfected with EnhII/CP-Luc and GFP-FANCE (or GFP) expression vector and AID-GFP (or GFP), and **I** Huh7 cells were co-transfected with EnhII/CP-Luc and siAID (or siNC) and siFANCE (or siNC); then the luciferase activities were detected. The luciferase activity of the GFP + siNC group was defined as value 100. $N = 3$, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, *ns* no significant

host innate antiviral defenses and provides a new potential target for the intervention of HBV infection.

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Data availability The data of the RNA-Seq presented in the study are deposited in the BioProject database of the National Center for Biotechnology Information (Accession number PRJNA1100181).

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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