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Research paper

MiR-3613-3p impairs IFN-induced immune response by targeting CMPK1 in chronic hepatitis B

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ABSTRACT

Background: This study aims to investigate the effects of miR-3613-3p and its underlying mechanisms on chronic hepatitis B.

Methods: Expressions of miR-3613-3p were determined in clinical samples from chronic hepatitis B patients and healthy volunteers. HBV-transfected hepatoma cell lines were constructed for *in vitro* study. HBV-infected animal model was established *in vivo* study. Quantitative real-time reverse transcription PCR (qRT-PCR) was used to determine mRNA expressions. Western blotting and ELISA were used to determine protein expressions. Luciferase reporter and biotin pull-down assays were used to analyze RNA-RNA interactions. siRNA silencing was used to knockdown miR-3613-3p and CMPK1.

Results: MiR-3613-3p was upregulated in the chronic hepatitis B patients, as compared with healthy volunteers. Inhibition of miR-3613-3p decreased relative expressions of IFN-α and IFN-β, HBV DNA copies, and increased the hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) levels, whereas miR-3613-3p overexpression reversed these changes *in vitro* and *in vivo*. MiR-3613-3p directly targeted CMPK1 and interactions between CMPK1 and miR-3613-3p regulated the anti-HBV efficiency of IFN.

Conclusion: MiR-3613-3p impaired IFN-induced immune response by targeting CMPK1 in chronic hepatitis B.

1. Introduction

Hepatitis B is a serious liver disease caused by hepatitis B virus (HBV) infection. There are approximately 350 million patients infected with hepatitis B virus worldwide (Alexander, 1990; Kane, 1995; Ott et al., 2012). Hepatitis B is transmitted by the exchange of body fluids and the most common routes of transmission are prenatal and sexual contacts. Patients who carried hepatitis B have a high risk to develop into chronic hepatitis, cirrhosis, and even hepatocellular carcinoma, which are accounting for 887,000 deaths in 2015 (Ott et al., 2012). Antiviral medications are highly recommended therapeutic methods for chronic HBV infection by World Health Organization (Milazzo and Antinori, 2009; Rehermann and Nascimbeni, 2005). However, it can't eliminate HBV but is able to stop further development of the HBV infection into cirrhosis or hepatocellular carcinoma (Rehermann and Nascimbeni, 2005). Additionally, antiviral medications are not effective for all chronic hepatitis B patients. Thus, it is crucial to understand the underlying molecular mechanisms of chronic hepatitis B.

MicroRNAs (miRNAs), are 20- to 22-nucleotide noncoding RNAs, have garnered significant interest in recent years. Their important roles have been identified in the occurrence and development of many diseases including cancer, cardiovascular diseases, and chronic hepatitis B (Bhayani et al., 2012; Guo et al., 2018; Reddy, 2015; Wang et al., 2013). Studies have revealed that miRNAs are involved in a series of cellular events including proliferation, differentiation, cell cycle, and apoptosis by regulating the expressions of their cognate target genes by regulating messenger RNAs (Guo et al., 2018; Reddy, 2015). Interestingly, several miRNAs have been identified with abnormal expressions in the process of HBV infection, thereby leading to viral replication and pathogenesis (Ji et al., 2011a; Ji et al., 2011b). For instance, Ji and colleagues have demonstrated that plasma miR-122 and miR-194 are negatively associated with chronic hepatitis B virus infection (Ji et al., 2011b). Additionally, Zhang and colleagues has reported that up-regulation of miRNAs including miR-210 and miR-199a-3p is an effective strategy to regulate HBV replication and thereby suppressing virus production (Zhang et al., 2010). All of these imply that miRNA is closely associated with HBV biology. Identification of novel miRNA with regulatory effects for HBV biology might provide therapeutic strategies for chronic hepatitis B.

MiR-3613-3p is one of the newly identified miRNAs. In 2017, it has

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been identified to be associated with hepatoma by the regulation of cell proliferation and cell cycle (Zhang et al., 2017). In 2018, another study has further revealed that liver miR-3613-3p is upregulated in hepatitis B patients, indicating that expressions of miR-3613-3p are correlated with the occurrence of hepatitis B (Singh et al., 2018). However, its underlying molecular mechanisms remain unclear. Therefore, this study aims to investigate the effects of miR-3613-3p on chronic hepatitis B. Furthermore, we also identified the underlying molecular mechanism of miR-3613-3p in the chronic hepatitis B.

2. Materials and methods

2.1. Clinical samples and cell lines

The procedure for collection of clinical samples was reviewed and approved by the ethic committee of First Affiliated Hospital of Zhejiang University of Traditional Chinese Medicine. Liver sample and plasma were collected from chronic hepatitis B patients (CHB) and healthy donors (HD) ($n = 20$) from May 2017 to September 2018. Hepatoma cancer cell lines including Huh7 and HepG2 were purchased from the Cell Bank of the Chinese Academic of Sciences (Shanghai, China).

2.2. Cell culture and transfections

The cells including Huh7 and HepG2 were cultured at 37 °C at 5% CO_2 in the Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin. pHBV-transfected cells including HepG2.2.15 and Huh7 were constructed according to a previously reported method (Tian and He, 2018). The cells were transfected with plasmids containing HBV sequences using Lipofectamine 2000.

2.3. Animals and protocols

BALB/c mice (weighing 22 ± 2 g) were purchased from SLAC (Shanghai, China). The animals were housed in a 12-h light-dark cycle and fed under experimental conditions with a temperature of 22–24 °C and humidity of 50 ± 5%. The animal experimental procedure used in this study was reviewed and approved by Animal Care and Use Committee of the First Affiliated Hospital of Zhejiang University of Traditional Chinese Medicine.

An animal model of hepatitis B infection was established according to a previously reported method(Han et al., 2011). In brief, C57BL/6 mouse was co-injected with pAAV/HBV1.2 vector and miR-3613-3p mimics or negative control mimics (miR-NC) through tail-vein injection. After four weeks, the animals were sacrificed and HBV DNA, hepatitis B surface antigen including Hepatitis B surface antigen (HBsAg), Hepatitis B e antigen (HBeAg), and cytokines including interferon (IFN)-α and IFN-β were determined.

2.4. Quantitative Diagnostic Kit for Hepatitis B Virus DNA

HBV DNA in serum and cell supernatant were determined using qRT-PCR, according to the document of Quantitative Diagnostic Kit for Hepatitis B Virus DNA (Kaijie Biology, Shenzhen, China).

2.5. Isolation of total RNA and quantitative polymerase chain reaction (qPCR)

Isolation of total RNA was performed using a trizol reagent, according to the document of manufacturer (Takara, Japan). RNase-free DNase I was used to avoiding DNA contamination. Primers for miR-3613-3p, IFN-α, IFN-β, Cytidine/uridine monophosphate kinase 1 (CMPK1), and internal control including U6 and GADPH were used for amplification of these genes. The sequences are designed according to a previous literature (Fricke et al., 2018). The sequences are as follows. miR-3613-3p forward: 5′-TGA ACA AAA AAA AAA GCC CAA −3′, and reverse: 5′-GGA ACG CTT CAC GAA TTT G-3′; IFN-α forward: 5′- GAA CTC TAC CAG CAG CT-3′, and reverse: 5′-CAG ATA GAG AGT GAT TC-3′; IFN-β forward: 5′-AAG GCC AAG GAG TAC AGT C-3′, and reverse: 5′-AGT TTC GGA GGT AAC CTG-3′; CMPK1 forward: 5′-GGA AGG CAG ATG TAT CTT TCG TT-3′, and reverse: 5′-TGT TGA CTG AAG GTA GGT CTG A-3′; U6 forward: 5′-ATT GGA ACG ATA CAG AGA AGA TT −3′, and reverse: 5′-GGA ACG CTT CAC GAA TTT G-3′; GADPH forward: 5′-GAA GGT GAA GGT CGG AGT C-3′, and reverse: 5′-GAA GAT GGT GAT GGG ATT TC-3′.

To analyze the accuracy of the PCR reaction, the Melt curves were used. To evaluate the expressions of genes, $2^{-\triangle\triangle}$Ct values were calculated. The mRNA expression values of IFN-α, IFN-β, CMPK1 were normalized to that of GADPH.

2.6. Enzyme-linked Immunosorbent Assay (ELISA)

ELISA was used to detect levels of hepatitis B surface antigen HBsAg, HBeAg, and cytokines including IFN-α and IFN-β in cell supernatant and serum according to the instruction of manufacturer (Cell Biolabs, Inc., San Diego, CA, USA).

2.7. Luciferase reporter assay

The possible target sites between miR-3613-3p with CMPK1 were analyzed using Targetsan website. Luciferase reporter assay was performed according to the instruction of manufacturer (Promega, Madison, MA, United States). The generation of CMPK1 wild-type (CMPK1 WT) was according to a previously reported method (Li et al., 2019). The primers used for amplification of CMPK1 WT are as follows: CMPK1 WT forward: 5′-CTA CTC GAG TTC TAA ACC TGA AGG CA-3′, and reverse: 5′- CTA GCG GCC GCT AGC AAA GGC CAC AGC ACA AAA TT-3′; The CMPK1 WT was then being sequenced to ensure the sequence is correct. After that, dual luciferase vector was incorporated with CMPK1 wild-type (CMPK1 WT) and the miR-3613-3p-binding-site mutated-type (CMPK1 MT). Next, the pHBV-transfected cell line was then co-transfected with dual luciferase vector and miR-3613-3p or miR-NC mimics.

2.8. Biotin pull-down assay

Biotin pull-down assay was performed under RNase free conditions according to a previously reported method(Yamamoto et al., 2015). Biotin-labeled double-stranded RNA of miR-3613-3p (Biotin-miR-3613-3p) or control random RNA (Biotin-miR-NC) was incubated with cell lysates. Next, agarose beads conjugated with streptavidin were added to collect the streptavidin/biotinylated-miRNA/target mRNA complex. Biotinylated miRNA/target mRNA complex was then eluted and mRNA expressions of CMPK1 were determined using qRT-PCR.

2.9. Western Blotting

Protein was extracted according to a previously reported method (Liu et al., 2016). In brief, cells were lysed in Radioimmunoprecipitation assay buffer. After that, cell lysates were centrifuged at 13000*g* to remove insoluble material. Next, a bicinchoninic acid protein assay kit was used for quantification of protein concentrations.

Equal amounts of each cell lysates were separated by 10% SDS-PAGE. Next, the gel was transferred onto the PVDF membrane and blocked with 5% non-fat milk. After binding with primary antibodies against CMPK1 (Abcam, 1:1000) overnight and actin HRP conjugated secondary antibody (Abcam, 1:2000) was added and incubated at room temperature for 2 h. Chemiluminescence was determined using the ChemiDoc MP imaging system (Bio-Rad, Hercules, CA, USA). The expressions of CMPK1 were normalized to the internal control actin.

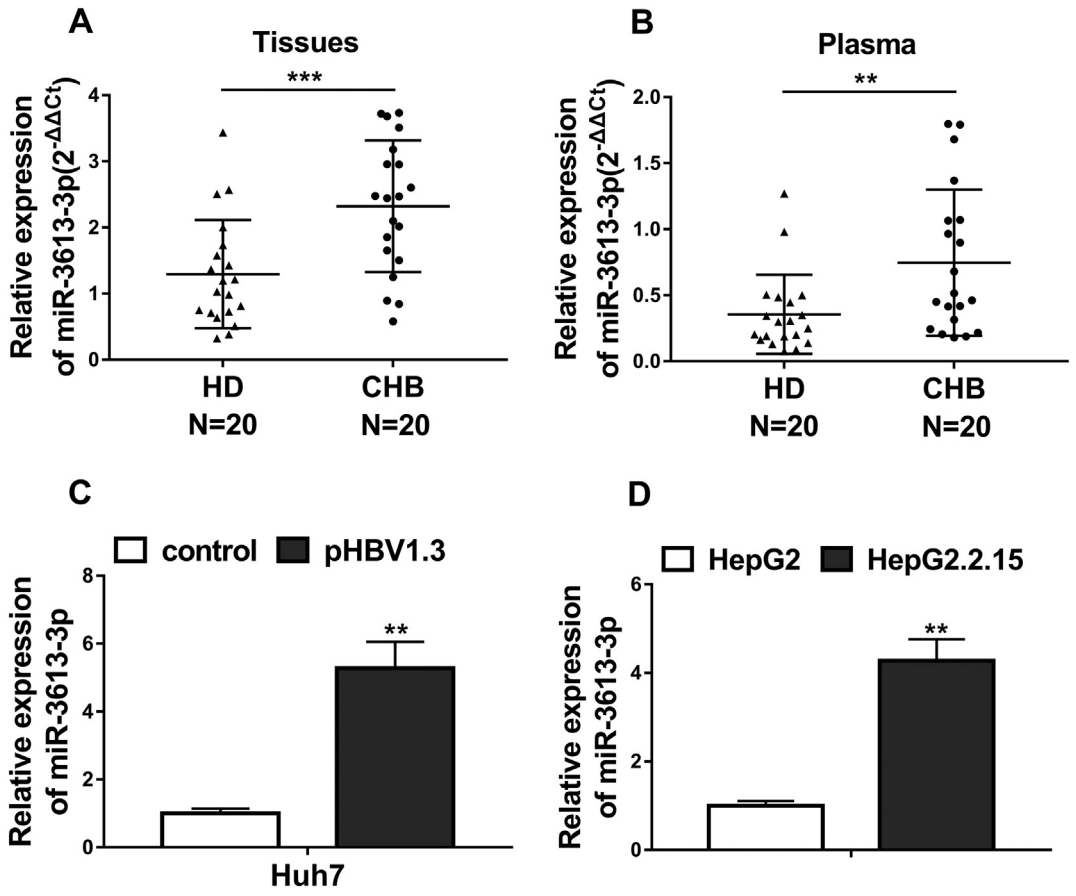

Fig. 1. MiR-3613-3p was upregulated in chronic hepatitis B.
(A-B) qRT-PCR analysis of miR-3613-3p in the liver tissues and plasma from chronic hepatitis B-infected patients (CHB) and healthy donors (HD). (C) The miR-3613-3p expressions in HBV-transfected Huh7 (pHBV1.3) and parental Huh7 (control) cells were detected. (D) The miR-3613-3p expressions in HepG2.2.15 cells and HepG2 cells were detected. *$P < .05$; **$P < .01$.

2.10. Statistical analysis

SPSS (SPSS, Chicago, IL, USA) was used for statistical analysis All Data were expressed as mean ± S.D. One-way ANOVA analysis and Student-Newman-Keuls (SNK) test were performed to calculate the significance among the groups. All P-value < .05 were considered as statistical significance.

3. Results

3.1. MiR-3613-3p was upregulated in chronic hepatitis B

First, we explored miR-3613-3p expression patterns in chronic hepatitis B patients. The miR-3613-3p expressions in serum and liver tissue from CHB and HD were detected. We observed that miR-3613-3p expressions were significantly increased in the liver tissue and plasma in CHB when compared with those in the HD (Fig. 1A and B). These results are supportive with a previous report in which miR-3613-3p is upregulated in the liver biopsy samples from patients with hepatitis B.

Next, HBV-infected stable cell lines including Huh7 and HepG2 were constructed and the miR-3613-3p expressions were determined. Interestingly, we found that miR-3613-3p expressions were significantly enhanced in HBV-infected cells including HepG2.2.15 and Huh 7 + pHBV1.3 (Fig. 1C and D), supporting miR-3613-3p positively correlated with the development of chronic hepatitis B.

3.2. MiR-3613-3p suppressed anti-HBV efficiency of IFN in vitro

We then explored the roles of miR-3613-3p in the anti-HBV efficiency of IFN *in vitro*. First, miR-3613-3p was successfully knocked down in HBV-infected Huh7 cells (Fig. 2A). Interestingly, we found the IFN-α and IFN-β expressions were increased in miR-3613-3p knockdown cells (Fig. 2B and C). Apart from IFNs, HBsAg and HBeAg levels were also significantly decreased in miR-3613-3p knockdown cells (Fig. 2D). HBV DNA copies were significantly decreased in miR-3613-3p knockdown cells (Fig. 2E).

Next, miR-3613-3p overexpressed cells were constructed as shown in Fig. 2F. Interestingly, when we examined the levels of IFNs, the results showed that IFN-α and IFN-β expressions were significantly decreased in miR-3613-3p overexpression cells (Fig. 2G and H). Consistently, HBsAg and HBeAg levels were also significantly increased in miR-3613-3p overexpression cells (Fig. I). HBV DNA copies were significantly increased in miR-3613-3p overexpression cells (Fig. 2J). All of these demonstrated that miR-3613-3p suppressed anti-HBV efficiency of IFN *in vitro*.

3.3. MiR-3613-3p enhanced HBV-induced immune suppression in vivo

We then evaluated the effects of miR-3613-3p on a HBV-infected animal model. Mice infected with HBV were injected with miR-3613-3p or miR-NC. After four weeks, we found that levels of HBV DNA in serum were significantly increased in the miR-3613-3p group (Fig. 3A). Similarly, HBsAg and HBeAg levels were also increased in miR-3613-3p group (Fig. 3B and C). Secretion of IFNs including IFN-α and IFN-β in

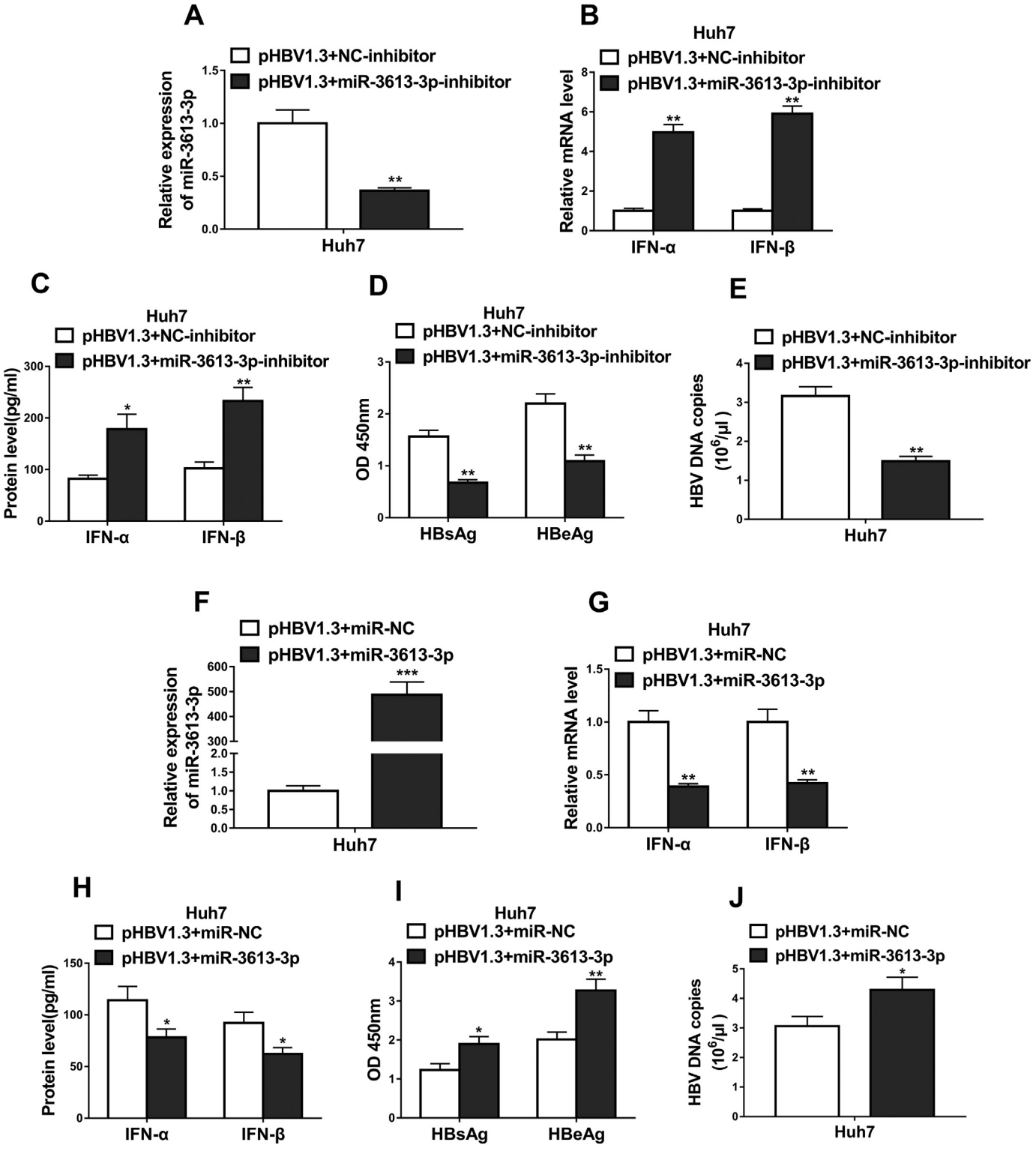

Fig. 2. MiR-3613-3p suppressed anti-HBV efficiency of IFN *in vitro*.

(A) The miR-3613-3p knockdown efficiency in Huh7 cells co-transfected with pHBV1.3 and miR-3613-3p inhibitor or inhibitor negative control was determined. (B) The IFN-α and IFN-β mRNA expressions were determined. (C-D) The secreted levels of IFN-α, IFN-β, HBsAg, and HBeAg in the Huh7 cells were determined. (E) HBV DNA levels were analyzed. (F) The miR-3613-3p overexpression efficiency in Huh7 cells co-transfected with pHBV1.3 and miR-3613-3p mimics or negative control mimics was confirmed. (G) The IFN-α and IFN-β mRNA expressions were determined. (H) Secreted cytokines including IFN-α and IFN-β from the co-transfected Huh7 cells were determined. (I) HBsAg and HBeAg levels in supernatants from the co-transfected Huh7 cells were measured. (J) HBV DNA levels were analyzed. The data were shown as mean ± SD from three independent experiments. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

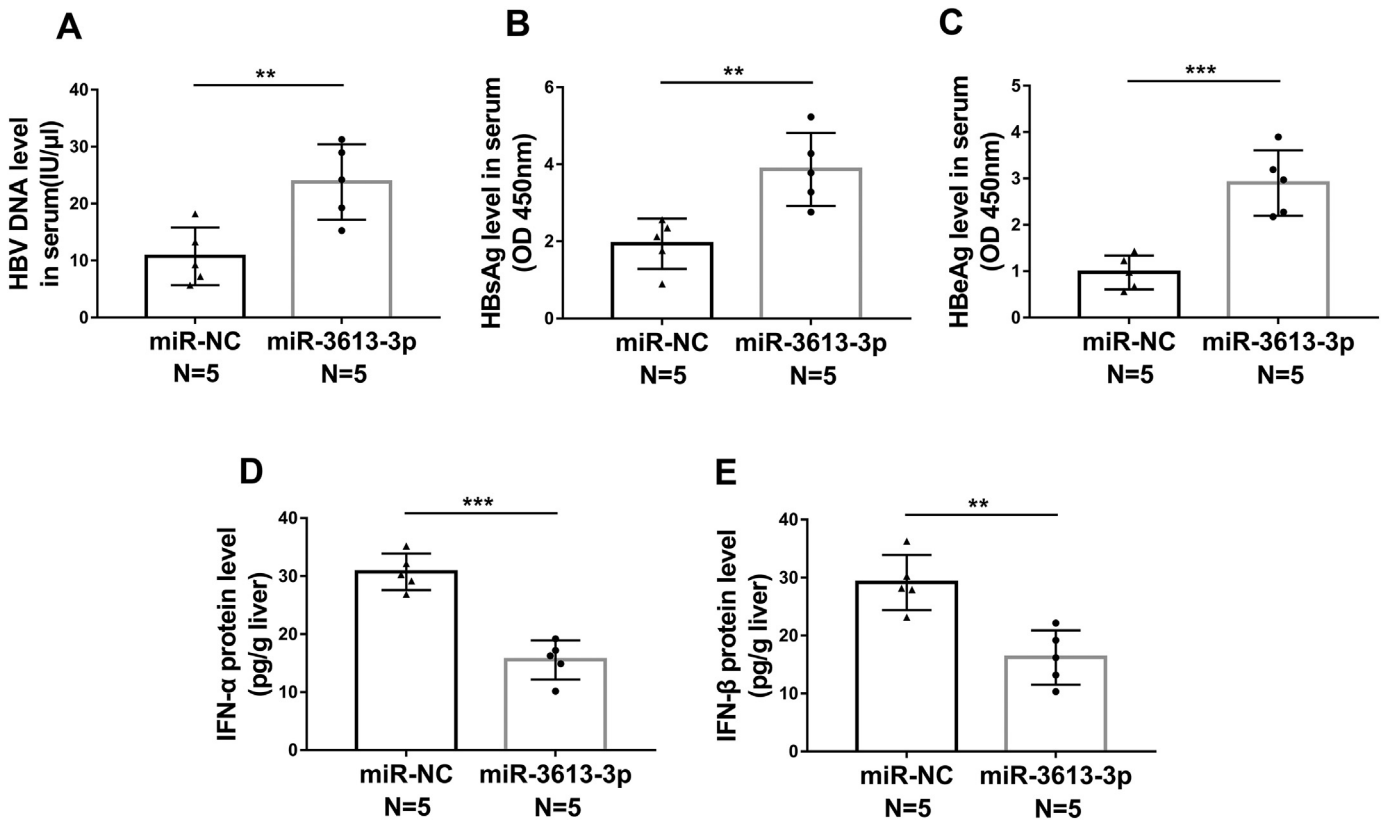

Fig. 3. MiR-3613-3p enhanced HBV-induced immune suppression *in vivo*. HBV-carrying BALB/c mice were injected with miR-3613-3p mimics (miR-3613-3p) or negative control mimics (miR-NC) by tail-vein injection. Four weeks later, (A) serum HBV DNA levels were measured. (B−C) Levels of HBsAg and HBeAg in serum were measured. (D-E) The IFN-α and IFN-β expressions in liver sample were evaluated. *$P < .05$; **$P < .01$; ***$P < .001$.

serum was also reduced in miR-3613-3p group (Fig. 3D and E). Overall, these results supported that miR-3613-3p enhanced HBV-induced immune suppression *in vivo*.

3.4. CMPK1 was recognized to be a target for miR-3613-3p

Furthermore, we analyzed the possible targets of miR-3613-3p for the regulation of chronic hepatitis B. CMPK1 was identified as a direct target of miR-3613-3p using Targetsan. Fig. 4A showed the binding sites between miR-3613-3p and CMPK1 WT and CMPK1 MT.

To confirm the interactions between CMPK1 and miR-3613-3p, a luciferase reporter assay was performed in two HBV-infected cell lines. We found that luciferase activities of CMPK1 WT were significantly decreased in HBV-infected cell lines including Huh 7 + pHBV1.3 (Fig. 4B) and HepG2.2.15 cells (Fig. 4C). Additionally, biotin pull-down assay demonstrated that mRNA levels of CMPK1 were significantly enriched by using bio-miR-3613-3p in both Huh 7 + pHBV1.3 and HepG2.2.15 cells (Fig. 4D), indicating the interactions between miR-3613-3p and CMPK1 WT.

Moreover, the cells were transfected with miR-3613-3p mimics or miR-3613-3p inhibitors and the expressions of CMPK1 were determined. MiR-3613-3p mimics significantly reduced mRNA and protein levels of CMPK1 in HBV-infected cell lines including Huh 7 + pHBV1.3 and HepG2.2.15 cells (Fig. 4E and G). Contrarily, miR-3613-3p inhibitor significantly enhanced mRNA and protein levels of CMPK1 in HBV-infected cell lines including Huh 7 + pHBV1.3 and HepG2.2.15 cells (Fig. 4F and G). Taken together, all of these implied that CMPK1 is a direct target of miR-3613-3p.

3.5. CMPK1 mediated the regulation of miR-3613-3p on anti-HBV efficiency of IFN

To further elucidate CMPK1 mediated regulation of miR-3613-3p on anti-HBV efficiency of IFN, miR-3613-3p inhibitors and CMPK1 siRNA were used in the pHBV-transfected Huh7 cells. First, CMPK1 expressions were successfully enhanced in HBV-infected Huh7 cells co-transfected with miR-3613-3p inhibitors. Additionally, mRNA and protein levels of CMPK1 were successfully decreased in HBV-infected Huh7 cells co-transfected with miR-3613-3p inhibitors and CMPK1 siRNA (Fig. 5A and B). These results demonstrated CMPK1 expressions were regulated.

Besides, we found that IFN-α and IFN-β expressions were significantly increased when miR-3613-3p inhibitors were applied (Fig. 5C and D). Interestingly, when CMPK1 was silenced, IFN-α and IFN-β expressions were significantly decreased. In addition to IFNs, the results demonstrated that levels of HBsAg, HBeAg, and HBV DNA copies were significantly reduced in miR-3613-3p inhibitors group, whereas CMPK1 silencing reversed these changes (Fig. 5E and F). Overall, these results supported that CMPK1 regulated the effects of miR-3613-3p on anti-HBV efficiency of IFN.

4. Discussion

This study explored the roles of miR-3613-3p in the development of chronic hepatitis B. We found upregulated miR-3613-3p in chronic hepatitis B. In addition to its inhibitory effects on anti-HBV efficiency of IFN *in vitro*, miR-3613-3p also enhanced HBV-induced immune suppression *in vivo*. Furthermore, for the first time, we identified that CMPK1 is a direct target of miR-3613-3p and revealed the underlying molecular mechanisms of miR-3613-3p, which is to impair IFN-induced immune response by targeting CMPK1 in chronic hepatitis B. These

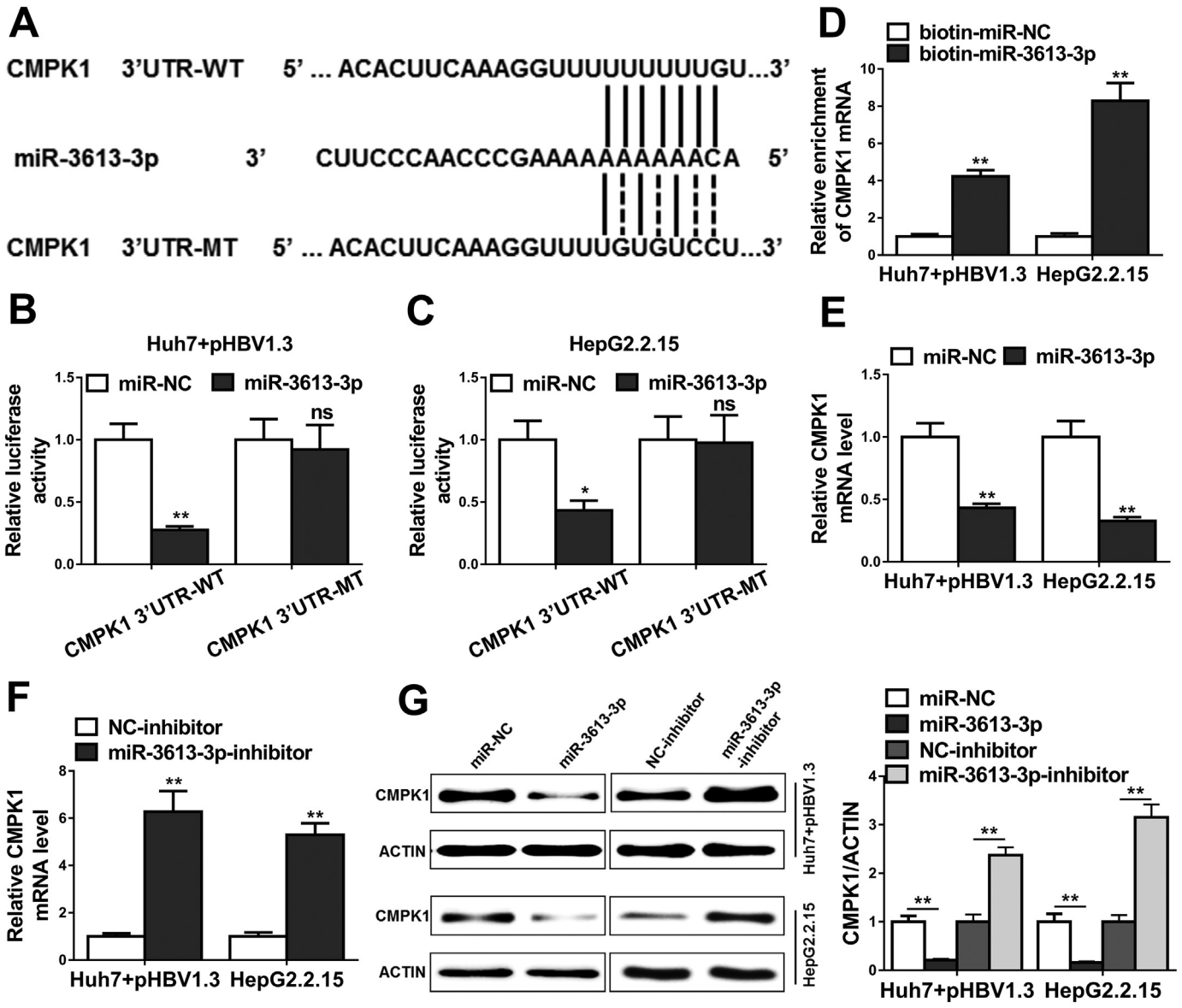

Fig. 4. CMPK1 was identified as a direct target of miR-3613-3p.
(A) The binding sites between miR-3613-3p and wide type CMPK1 mRNA 3′UTR (CMPK1 3′UTR-WT) and mutation sites in CMPK1 3′UTR (CMPK1 3′UTR-MT) reporter. (B—C) Luciferase activity was evaluated in pHBV-transfected cell line co-transfected with CMPK1 3′UTR-WT or CMPK1 3′UTR-MT together with miR-3613-3p or miR-NC. (D) CMPK1 mRNA expressions in biotinylated miRNA/target mRNA complex were determined. (*E*-G) The CMPK1 expressions in pHBV-transfected cells co-transfected with miR-3613-3p mimics and miR-3613-3p inhibitors or the negative controls were detected. $^{*}P < .05$; $^{**}P < .01$; ns indicates not significant.

results confirmed that miR-3613-3p has regulatory effects for HBV biology and thereby providing therapeutic strategies for chronic hepatitis B.

In the previous studies, miR-3613-3p has been identified to be associated with neuroblastoma, dedifferentiated liposarcoma, hepatocellular carcinoma, and chronic hepatitis B (Fricke et al., 2018; Singh et al., 2018; Zhang et al., 2017). MiR-3613-3p is able to regulate cell proliferation and cell cycle in hepatoma cancer tissues as well as cell lines including HepG2 cells (Zhang et al., 2017). Interestingly, miR-3613-3p is associated with several cancer biomarkers including baculoviral IAP repeat containing 5, kinetochore protein Nuf2, and NDC80 kinetochore complex component (Nowak et al., 2018). More recently, a liver based global microRNA expression profiling was performed on hepatitis B patients. Expressions of miR-3613-3p in liver are upregulated and thereby regulating liver cell proliferation in hepatitis B patients (Singh et al., 2018). It is interesting to further explore the roles of miR-3613-3p and its underlying mechanisms in chronic hepatitis B. Thus, the current study is aimed to explore the effects and underlying mechanisms of miR-3613-3p on the regulation of chronic hepatitis B.

First, we determined the expression patterns of miR-3613-3p in plasma and liver tissue in chronic hepatitis B, indicating that liver and plasma miR-3613-3p mRNA expressions were enhanced in CHB, which are in agreement with a previous study in which liver miR-3613-3p is upregulated in hepatitis B patients (Singh et al., 2018). We further investigated the expression patterns of miR-3613-3p in the cells infected with HBV. Again, mRNA expressions of miR-3613-3p were also significantly increased in HBV-infected cells, indicating miR-3613-3p positively correlated with chronic hepatitis B.

After the expression patterns of miR-3613-3p were identified, we then regulated the expressions of miR-3613-3p and verified its effects on anti-HBV efficiency of IFN. In the current study, we first examined expressions of IFNs, which are soluble glycoproteins with potent antiviral activities. IFN-α has been widely used in hepatitis B therapy for many years (Lau et al., 1993; Pramoolsinsup, 2002). IFN-β has also

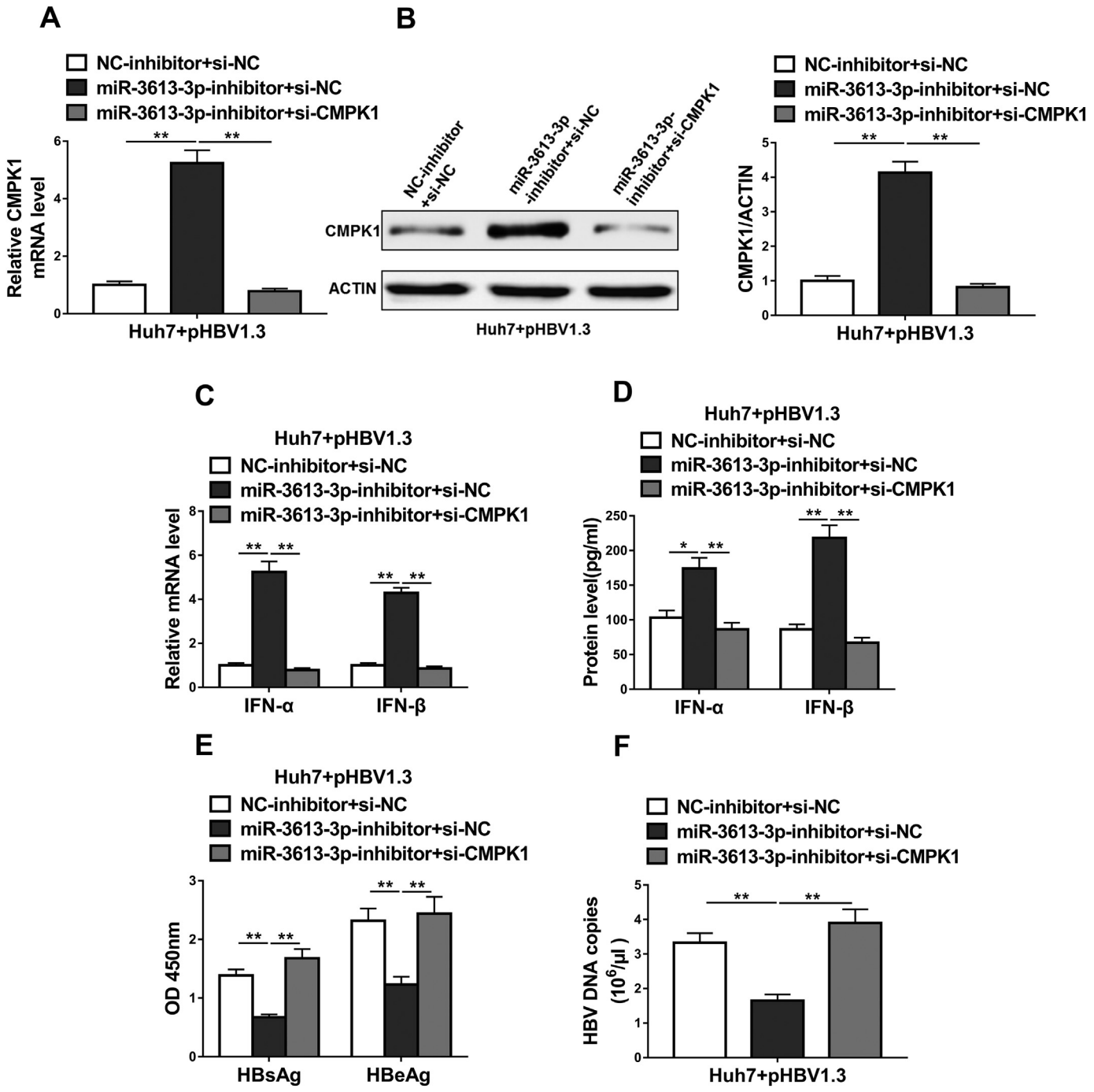

Fig. 5. CMPK1 mediated the regulation of miR-3613-3p on anti-HBV efficiency of IFN.
The pHBV-transfected Huh7 was co-transfected with inhibitor negative control and siRNA negative control (NC-inhibitor + si-NC), miR-3613-3p inhibitor and siRNA negative control (miR-3613-3p-inhibitor + si-NC) or miR-3613-3p inhibitor and CMPK1 siRNA (miR-3613-3p-inhibitor + si-CMPK1). (A-B) The CMPK1 expressions were measured. (C-D) IFN-α and IFN-β expressions were determined in the pHBV1.3-transfected cells co-transfected with 1) negative control inhibitor and negative control siRNA (NC-inhibitor + si-NC), 2) miR-3613-3p inhibitor and negative control siRNA (miR-3613-3p-inhibitor + si-NC), 3) miR-3613-3p inhibitor and CMPK1 siRNA (miR-3613-3p-inhibitor + si-CMPK1). (E-F) HBsAg and HBeAg secretions, and HBV DNA copied were determined. $^*P < .05$; $^{**}P < .01$.

been identified with antiviral properties for chronic hepatitis B therapy (Pramoolsinsup, 2002; Twu et al., 1988). Additionally, IFN-β is able to suppress HBV replication with higher efficiency than IFN-α (Pramoolsinsup, 2002; Seto et al., 2018). In the present study, when miR-3613-3p was knocked down, the expressions of IFN-α and IFN-β were significantly increased. Contrarily, the expressions of IFN-α and IFN-β were significantly decreased with overexpression of miR-3613-3p. These results suggested that anti-HBV efficiency of IFN was suppressed by miR-3613-3p.

Apart from IFNs, HBsAg and HBeAg expressions, HBV DNA copies were also determined. High expressions of HBsAg indicate hepatitis B infection and decreasing the expressions of HBsAg are thought to be an important goal for chronic hepatitis B therapy (Lau et al., 1993; Seto et al., 2018). HBeAg is an antigen located in the outer layer of the hepatitis B virus and its presence in the plasma of patients can be served as an indicator of active viral replication (Lindh et al., 2000; Seto et al., 2018). In the present study, miR-3613-3p knockdown resulted in an increase of levels of HBsAg and HBeAg, whereas miR-3613-3p

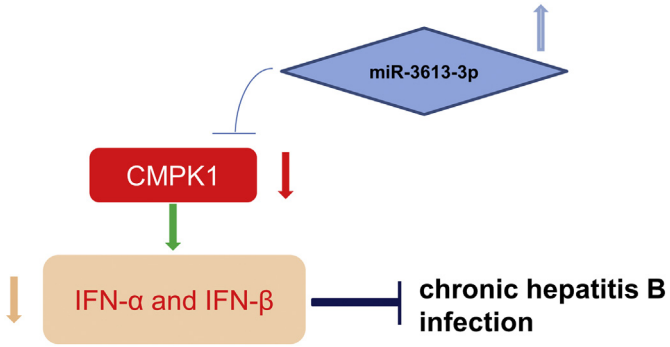

Fig. 6. Schematic figure demonstrating the model of miR-3613-3p/CMPK1 axis regulating chronic hepatitis B infection.

overexpression resulted in a decrease of levels of HBsAg and HBeAg. Furthermore, we also detected HBV DNA copies, a strong predictor for hepatitis B infection independent of HBeAg. The results demonstrated that HBV DNA copies were negatively associated with the expressions of miR-3613-3p *in vitro* and *in vivo*. All of these implied that miR-3613-3p suppressed anti-HBV efficiency of IFN *in vitro* and enhanced HBV-induced immune suppression *in vivo*.

We further predicted the possible targets of miR-3613-3p. CMPK1 was identified as the target of miR-3613-3p. Previous study has been reported that CMPK1 is responsible for nucleic acid biosynthesis (Zhou et al., 2017). CMPK knockdown affects DNA repair in UV-induced cell damage by the local change of deoxycytidine triphosphate at the damage sites (Tsao et al., 2015). In this study, luciferase reporter and biotin pull-down assays demonstrated the interactions between miR-3613-3p and CMPK1 WT. However, apart from CMPK1, some other possible targets should be investigated in further study. This is a limitation for this study. As we know, we can predict other targets for miRNA, but mechanistic insight of the potential targets in a particular cellular context could hardly be satisfied without systematic study.

We then explored whether CMPK1 could regulate the effects of miR-3613-3p on anti-HBV efficiency of IFN. Interestingly, after CMPK1 was silenced, IFN-α and IFN-β expressions were significantly decreased. In addition to IFNs, levels of HBsAg, HBeAg, and HBV DNA copies were significantly increased in CMPK1 knockdown group. Overall, these data supported that CMPK1 regulates anti-HBV efficiency of IFN mediated by miR-3613-3p. However, the underlying molecular mechanisms of CMPK1 on the regulation of chronic hepatitis B should be explored in further studies. Considering the roles of CMPK1 in the nucleic acid biosynthesis, it is interesting to explore its regulatory effects on the process of HBV replication in the development of chronic hepatitis B.

In conclusion, we verified the roles of miR-3613-3p in chronic hepatitis B. First, miR-3613-3p is upregulated in chronic hepatitis B. Second, CMPK1 was identified as a direct target of miR-3613-3p. We then revealed the underlying molecular mechanisms of miR-3613-3p, which is to impair IFN-induced immune response by targeting CMPK1 in chronic hepatitis B (Fig. 6). These results confirmed that miR-3613-3p has regulatory effects for HBV biology and thereby providing therapeutic strategies for chronic hepatitis B.

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Disclosure of potential conflicts of interest

None.

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