miR-212-3p targets nuclear factor I A (NFIA) to suppress hepatitis B virus replication and tumor progression in hepatocellular carcinoma via repressing enhancer I activity

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Abstract

Introduction: Emerging evidence identifies that microRNAs (miRNAs) are associated with hepatitis B virus (HBV) infection. In the current study, we mainly focus on the functions and underlying mechanisms of miR-212-3p in HBV replication in hepatocellular carcinoma (HCC).
Material and methods: The levels of miR-212-3p, nuclear factor I A (NFIA) and HBV DNA copies were measured by qRT-PCR. The level of core particle-associated HBV DNA, the production of hepatitis B surface antigen (HBsAg) and hepatitis B e-antigen (HBeAg), and the expression of NFIA were detected via southern blot assay, ELISA and western blot assay, respectively. The putative target of miR-212-3p was predicted by TargetScan and Pictar, followed by the dual luciferase reporter assay and RNA immunoprecipitation (RIP) assay to validate the interaction. The interaction between miR-212-3p and enhancer I/X promoter (EnI/Xp) reporter was also verified by dual luciferase reporter assay. In addition, the cell viability and apoptotic rate were detected by MTT and flow cytometry, respectively.
Results: miR-212-3p mimics or NFIA knockdown inhibited HBV expression and replication in HepG2.2.15 cells, while miR-212-3p inhibitor or NFIA overexpression showed the opposite trend. NFIA was confirmed as a direct target of miR-212-3p. Furthermore, miR-212-3p impeded HBV expression and replication by suppressing NFIA. Also, miR-212-3p lowered EnI/Xp activity by regulating NFIA. In addition, miR-212-3p retarded cell viability and induced apoptosis through targeting NFIA.
Conclusions: miR-212-3p targets NFIA to down-regulate its expression, thereby inhibiting HBV replication and tumorigenesis in HCC. Our findings might provide a promising therapeutic target for HBV infection.

Key words: miR-212-3p, NFIA, HBV, hepatocellular carcinoma.

Introduction

Hepatitis B virus (HBV), a major threat for liver cancer, has infected 350 million individuals worldwide and one-third of them reside in China [1, 2]. HBV, a virus with a partially double stranded DNA genome, is transcribed into four viral transcripts (3.5 kb, 2.4 kb, 2.1 kb, and 0.7 kb) [3]. Due to the small genome of HBV, the four HBV promoter/enhancers (preS1 promoter (S1p), preS1 promoter (S2p), enhancer I/X promoter (EnI/Xp), en-

hancer II/core promoter (EnII/Cp)) are vital for HBV synthesis, and the host factors are indispensably required for HBV expression and replication [4, 5]. Therefore, it is crucial to explore the host-virus interaction in hepatocellular carcinoma (HCC).

MicroRNAs (miRNAs), a type of RNA (~22 nucleotides) with no translation ability, are demonstrated to negatively regulate the target gene expression [6]. MiRNAs have been demonstrated to be involved in kinds of physiological or pathological changes in human diseases [7]. Convincing evidence shows the association between host miRNAs and virus infection [8]. Many miRNAs, such as miR-185-5p [9] and miR-101 [10], have been reported to suppress the amplification of HBV *in vitro*. Tu *et al*. reported that miR-212-3p overexpression decreased cell growth and enhanced apoptosis in HCC via inhibiting FOXA1 expression [11]. However, the underlying molecular mechanism of miR-212-3p in regulating HBV replication is still undefined.

Nuclear factor I (NFI) was initially reported as a cellular factor for adenovirus replication [12]. NFI contains four genes (NFIA, NFIB, NFIC, NFIX) to form homodimers or heterodimers which have the capacity to stabilize and specify DNA [13]. Accumulating evidence indicates that NFIA is related to HBV replication and gene transcription [14, 15]. Nevertheless, the mechanism whereby NFIA regulates HBV replication has rarely been documented. In the present research, we attempted to investigate the interaction between host factors miR-212-3p, NFIA and HBV, providing a new direction for elucidating the mechanism of persistent infection of HBV in HCC.

Material and methods

Cell culture

Human hepatoma HepG2 and HepG2.2.15 cells (containing 2 head-to-tail stably integrated HBV dimers of the D-genotype) (Procell, Wuhan, China) were cultured in Dulbecco's modified Eagle's medium (DMEM; Solarbio, Beijing, China) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific) in a 5% CO_2 incubator at 37°C.

Table I. Oligonucleotide sequences used in the present study

Gene	Sequences
miR-212-3p-F	5′-GGTAACAGTCTCCAGTCA-3'
miR-212-3p-R	5′-GCAATTGCACTGGATACG-3′
NFIA-F	5′-AGGTCTTTACCCAGCACATCCTC-3′
NFIA-R	5′-TCCACTTGACTGACTGCCACTTC-3′
pMov10-F	5′-ACATTCTACATTGCCCGCTTCTTG-3′
pMov10-R	5′-CTCCTTCCTCTATCCGATTGGTCAC-3′
GAPDH-F	5′-TGTTCGTCATGGGTGTGAAC-3′
GAPDH-R	5′-ATGGCATGGACTGTGGTCAT-3′
U6-F	5′-CTCGCTTCGGCAGCACA-3′
U6-R	5′-AACGCTTCACGAATTTGCGT-3′

NFIA – nuclear factor I A, F – forward, R – reverse.

Plasmid construction and transfection

The cDNA sequence of NFIA was amplified and subcloned into the downstream of the pcDNA3.1 vector (Invitrogen) to construct the NFIA-overexpressing plasmid (pcDNA-NFIA), and a pcDNA3.1 empty vector (pcDNA) was utilized as the negative control. Small interfering RNA (siRNA) antagonistic to NFIA (si-NFIA) and scrambled negative control (si-NC) were obtained from Hanbio (Shanghai, China). miR-212-3p mimics (miR-212-3p), miR-212-3p antagonist (anti-miR-212-3p), and respective scrambled oligomer sequences (miR-NC, anti-miR-NC) were synthesized by Sangon Biotech (Shanghai, China). The wild-type or mutant untranslated region (3'UTR) fragments of human NFIA containing the predictive binding sites with miR-212-3p were amplified and then subcloned into the downstream region of pGL3 (Promega, Madison, WI, USA) to obtain the recombinant luciferase reporter, namely NFIA-WT and NFIA1-MUT. The four HBV promoter reporters were purchased from Sangon Biotech.

qRT-PCR assay

Total RNA extraction was performed using TRIzol reagent (Invitrogen). The cDNA was synthesized by random primers using Moloney murine leukemia virus reverse transcriptase (Promega). SYBR Green PCR Master Mix (Ambion, Carlsbad, CA, USA) was applied to complete the quantitative PCR. MiR-212-3p was normalized by U6 small nuclear RNA, while NFIA was standardized by β-actin. Also, the numbers of HBV DNA copies were determined by qRT-PCR as Ma *et al*. previously described [16]. The sequences of primers are presented in Table I.

HBV replication and southern blot assay

HBV core particle-associated DNA was detected by southern blot as previously described [17, 18].

ELISA assay

ELISA kits (TW-regent co., LTD, Shanghai, China) were applied to examine the production of HBsAg and HBeAg in the culture supernatant in accordance with the manual.

Dual luciferase reporter assay

The luciferase reporter constructs NFIA-WT or NFIA-MUT were transfected into HepG2.2.15 cells

together with miR-212-3p, anti-miR-212-3p or the respective control. At 48 h after transfection, the luciferase activity was measured using the Luciferase Reporter Assay System (Promega), with Renilla luciferase activity as a reference.

RNA binding protein immunoprecipitation (RIP) assay

The binding specificity between NFIA and miR-212-3p was confirmed using a Magna RNA immunoprecipitation kit (Millipore, Billerica, MA, USA). Briefly, cell lysis was performed in RIP lysis buffer comprising protease and ribonuclease inhibitors. Subsequently, 100 µl samples were cultured with magnetic beads coupled with anti-Ago2 (Millipore) or anti-IgG (Millipore). Then the samples were co-cultured with protease K to decompose the protein content and facilitate the isolation of immunoprecipitated RNA. qRT-PCR was used to monitor the levels of NFIA in the precipitates.

Western blot assay

Protein samples from HepG2.2.15 cells were extracted using RIPA buffer (Solarbio, Beijing, China) and then separated by SDS-PAGE. Subsequently, the protein bands were transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore). Following incubation in the blocking buffer (0.1% Tween-20 Tris buffer solution with 5% skim milk; TBST) for 1 h, the membrane was cultivated with moderate concentrations of primary antibodies against NFIA or β-actin (Cell Signaling technology, Boston, Massachusetts, USA) at 4°C overnight. Then the membrane was incubated with an appropriate amount of secondary antibody for 2 h at 37°C. Finally, the chemiluminescence intensity was determined using an enhanced chemiluminescence system (Bio-Rad Laboratories, Shanghai, China). β-actin was considered as an internal reference.

Cell viability detection

The viability of HepG2.2.15 cells was measured by MTT analysis. Briefly, cells (1×10^5) were plated in 96-well plates and maintained in standard conditions. At indicated time points, 10 µl of MTT (Sigma-Aldrich, St. Louis, MO, USA, 12 mM) solution was added into the wells and cultured for 4 h at 37°C. After aspirating the supernatant, 150 µl of dimethyl sulfoxide (DMSO) was supplemented to dissolve the generated formazan. A Multiscan Spectrum (Bio-Rad Laboratories) was utilized to test the absorption value at 490 nm.

Flow cytometry for apoptotic rate analysis

An Annexin V-Alexa Fluor 647/PI apoptosis detection kit (Fcmacs, Jiangsu, China) was used to determine the apoptotic rate in accordance with the manufacturer's manual. Briefly, cells were digested with 0.05% trypsin (Sigma-Aldrich) at 37°C and centrifugated at 200 × g for 3 min. After washing twice with 0.01 M cooling PBS, the re-suspended cells were labelled with Annexin V-fluorescein isothiocyanate (FITC) and propidine iodide (PI) for 20 min. Apoptosis analysis was carried out using a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA, USA).

Statistical analysis

All experimental results were presented as mean ± standard deviation (SD) and analyzed using SPSS19.0 software. Significance of differences was evaluated by Student's *t*-test or one-way analysis of variance (ANOVA). A difference is regarded as significant when $p < 0.05$.

Results

Inhibition of HBV replication and expression by miR-212-3p

Firstly, the expression difference of miR-212-3p in HepG2 and stable HBV-producing hepatocarcinoma cells (HepG2.2.15) was assessed by qRT-PCR. As shown in Figure 1 A, the level of endogenous miR-212-3p was reduced in HepG2.2.15 cells compared to that in HepG2 cells. The transfection efficiency was confirmed by qRT-PCR, indicated by the dramatic up-regulation of miR-212-3p level in HepG2.2.15 cells transfected with miR-212-3p mimics or apparent down-regulation of miR-212-3p level in HepG2.2.15 cells transfected with anti-miR-212-3p (Figure 1 B). As can be seen from Figure 1 C, it was evident that the number of HBV DNA copies in the culture supernatant was decreased by miR-212-3p overexpression, while suppression of miR-212-3p had the opposite effect. As shown by southern blot assay, decreased HBV replicative intermediates were observed in HepG2.2.15 cells with miR-212-3p overexpression; meanwhile, HBV replicative intermediates were markedly augmented in the anti-miR-212-3p group (Figure 1 D). To evaluate the changes of HBV expression mediated by miR-212-3p, the levels of HBsAg and HBeAg were detected in the supernatant. The ELISA results showed that the levels of HBsAg and HBeAg were both strikingly decreased in the miR-212-3p-treated group, while they were remarkably enhanced by the down-regulation of miR-212-3p (Figure 1 E and F). Above all, miR-212-3p was implicated in the suppression of HBV replication and expression.

Verification of NFIA as a direct target of miR-212-3p

To elucidate the mechanism of miR-212-3p in suppressing HBV replication, two algorithms, Tar-

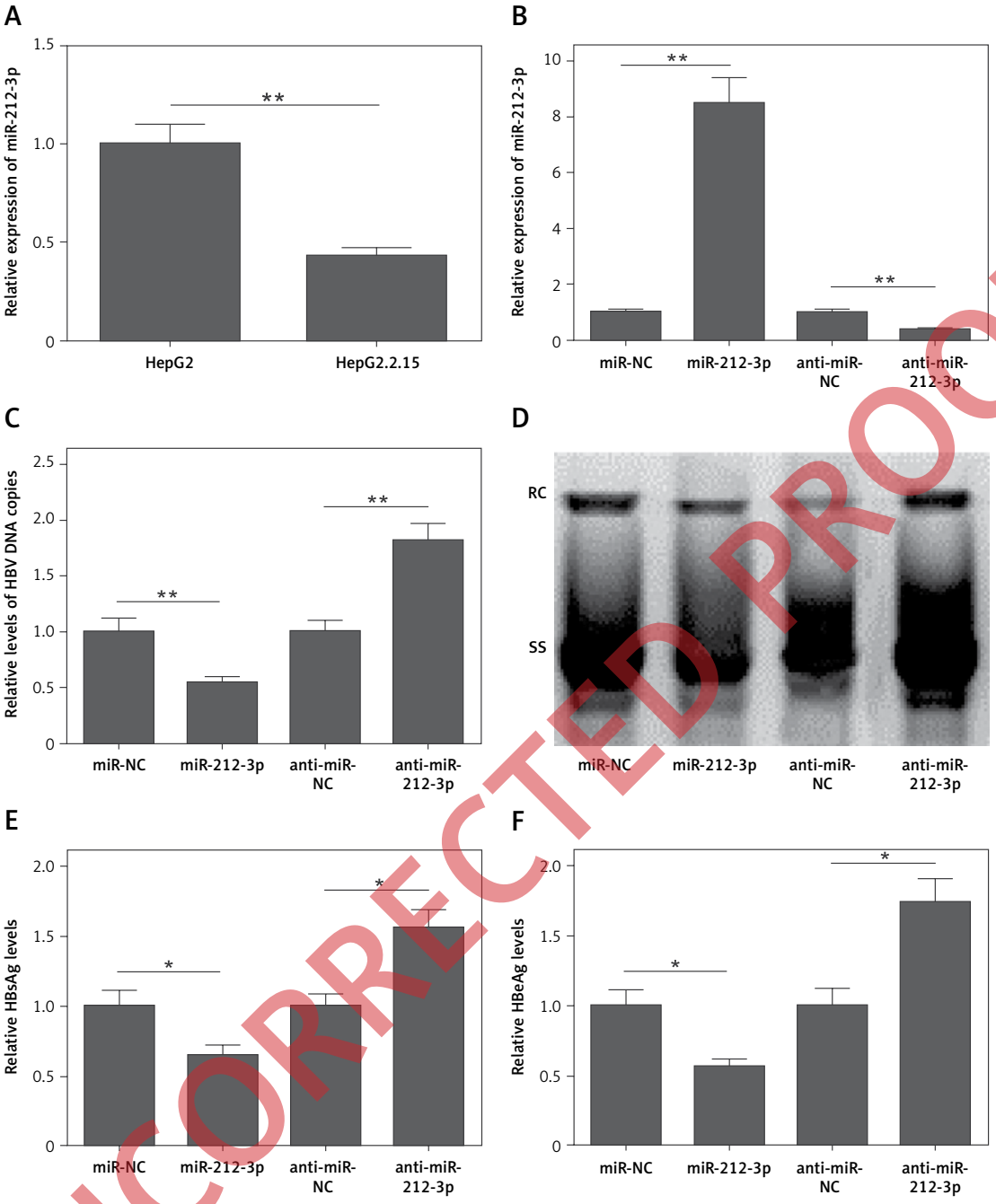

Figure 1. miR-212-3p suppressed hepatitis B virus (HBV) replication and expression. **A** – The level of miR-212-3p in HepG2 or HepG2.2.15 cells was measured by qRT-PCR. HepG2.2.15 cells were transfected with miR-NC, miR-212-3p mimics, anti-miR-NC or anti-miR-212-3p, then the levels of miR-212-3p (**B**) and HBV DNA copies (**C**) were monitored via qRT-PCR. **D** – The core particle-associated HBV DNA in transfected cells was detected by southern blot assay. The positions of relaxed circular (RC) and single-stranded (SS) DNAs were indicated. Levels of HBsAg (**E**) and HBeAg (**F**) in the supernatant of transfected cells were assessed via ELISA. *$P < 0.05$, **$p < 0.01$

getScan (http://www.targetscan.org/) and Pictar (http://pictar.mdc-berlin.de/), were used to search for potential targets of miR-212-3p. Both the results from web-based software found that NFIA, one important regulator of multiple viruses, was the candidate of miR-212-3p. Putative target sites for miR-212-3p had not been detected by the computational programs within the HBV genome (data not shown). The complementary binding sites between the NFIA 3'UTR and miR-212-3p were displayed (Figure 2 A). Transfection of miR-212-3p resulted in a significant reduction of the luciferase activity of WT-NFIA reporter, while the luciferase activity of WT-NFIA reporter was notably elevated by miR-212-3p inhibitor; however, the luciferase activity of MUT-NFIA reporter had no marked fluctuation in any treatment group (Figure 2 B and C). In addition, the specific binding

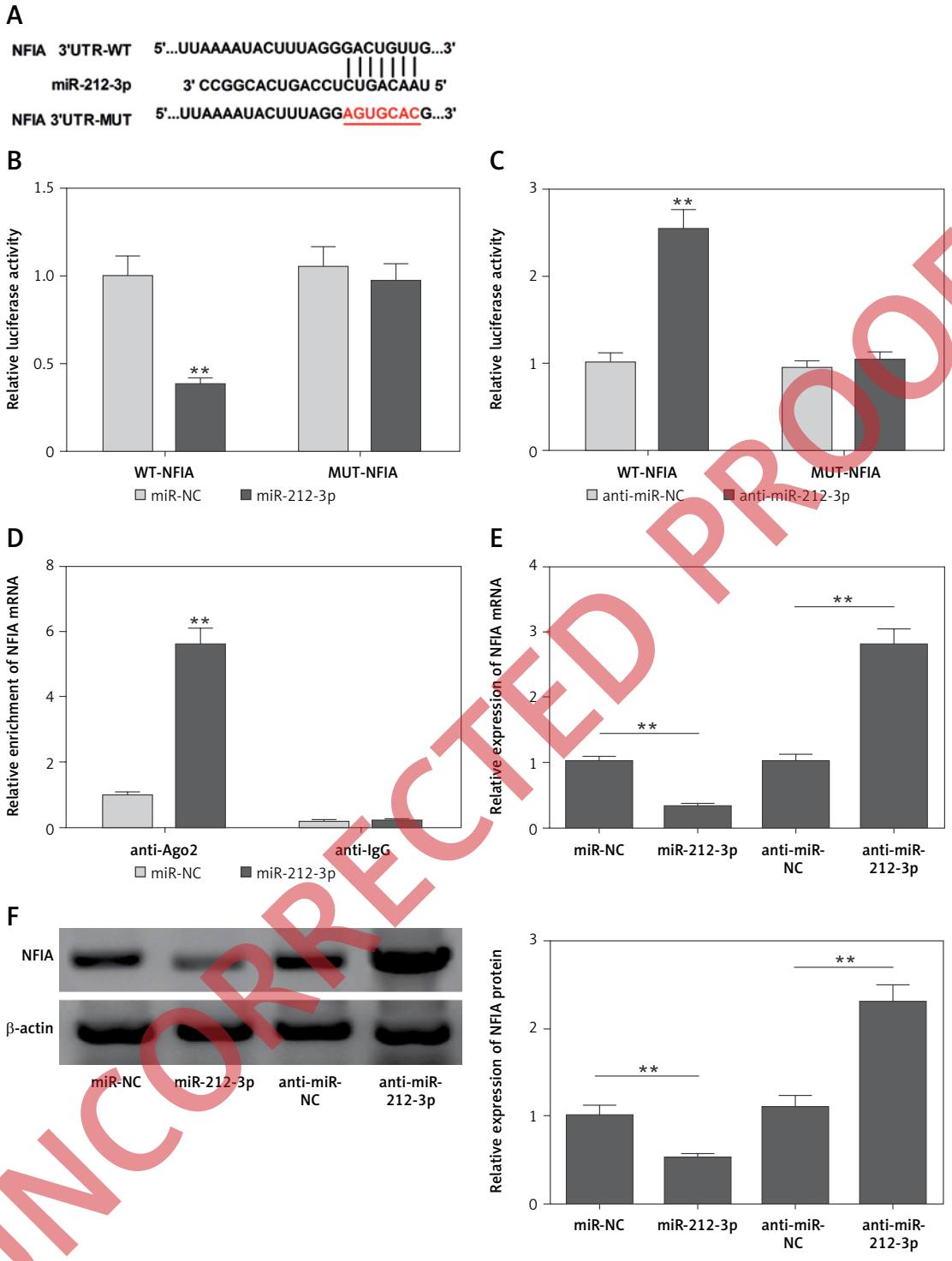

Figure 2. miR-212-3p negatively regulated nuclear factor I A (NFIA) expression. **A** – Complementary sequences between NFIA 3'UTR-WT and miR-212-3p, as well as the mutant sequences of NFIA. **B**, **C** – Luciferase activity of WT-NFIA or MUT-NFIA reporter in HepG2.2.15 cells transfected with miR-212-3p, miR-NC, anti-miR-212-3p, or anti-miR-NC was assessed via dual luciferase reporter assay. **D** – Enrichment degree of NFIA in IgG or Ago2 immunoprecipitation complex of miR-212-3p- or miR-NC-transfected HepG2.2.15 cells was evaluated by RIP assay. **E**, **F** – Effects of miR-212-3p overexpression or knockdown on NFIA mRNA and proteins were measured by qRT-PCR and western blot. $^{**}P < 0.01$

between miR-212-3p and NFIA was further determined depending on RIP analysis. As presented in Figure 2 D, miR-212-3p overexpression led to an obvious enrichment of NFIA mRNA in the Ago2 immunoprecipitation complex compared with that in the IgG control group. Moreover, the mRNA level of NFIA was apparently repressed in the miR-212-3p mimics group but enhanced in the miR-212-3p inhibitor group (Figure 2 E). Also, the western blot assay produced similar results (Figure 2 F). All these results indicated that NFIA was a bona-fide target for miR-212-3p.

Promotion of HBV replication and expression by NFIA

In order to determine whether NFIA was associated with HBV replication and expression, NFIA overexpression or knockdown was performed in HepG2.2.15 cells by transfection with pcDNA, pcDNA-NFIA, si-NC or si-NFIA. qRT-PCR and western blot results manifested that both the mRNA and protein levels of NFIA were elevated in pcDNA-NFIA-transfected cells, but decreased in si-NFIA-transfected cells (Figures 3 A and B). Moreover, NFIA overexpression resulted in increased HBV DNA copies, while NFIA knockdown reduced HBV DNA copies (Figure 3 C). Furthermore, increased HBV replicative intermediates were observed following the transfection of pcDNA-NFIA, whereas the contrary effect was found in the si-NFIA group (Figure 3 D). Also, transfection of pcDNA-NFIA resulted in the promotion of HBsAg and HBeAg levels in the supernatant, while the concentrations of HBsAg and HBeAg were strikingly decreased by depletion of NFIA (Figures 3 E and F). These data suggested that NFIA facilitated HBV replication and expression.

NFIA overexpression reversed the suppressive effect of miR-212-3p on HBV replication and expression

To further confirm whether miR-212-3p suppressed HBV replication and expression through mediating NFIA expression, miR-NC + pcDNA, miR-NC + pcDNA-NFIA, miR-NC + pcDNA-NFIA-MUT, miR-212-3p + pcDNA, miR-212-3p + pcDNA-NFIA, or miR-212-3p + pcDNA-NFIA-MUT were transfected into HepG2.2.15 cells. The results showed that pcDNA-NFIA-mediated increase of HBV progeny, HBV replicative intermediates, as well as HBsAg and HBeAg concentrations was abrogated due to the miR-212-3p transfection, while restoration of NFIA expression reversed the suppressive effect of miR-212-3p on HBV transcripts, HBV replication, and HBsAg and HBeAg levels (Figures 4 A–D). Moreover, pcDNA-NFIA-MUT transfection displayed similar stimulatory effects in regulating HBV replication and expression as pcDNA-NFIA transfection; however, the changes induced by pcDNA-NFIA-MUT transfection were not affected by miR-212-3p overexpression (Figures 4 A–D). Therefore, miR-212-3p inhibited HBV replication and expression through targeting NFIA.

miR-212-3p inhibited HBV enhancer I activity via repressing NFIA

From the above results, it was obvious that miR-212-3p repressed HBV replication and expression partly via suppressing NFIA. To illustrate whether miR-212-3p exerted inhibitory effects on HBV replication and expression by HBV enhancers or promoters, the correlation between miR-212-3p and HBV promoter activities was firstly investigated. As a result, transfection of miR-212-3p remarkably decreased the enhancer I/X promoter (EnI/Xp)-regulated luciferase activities in comparison with the miR-NC group, but there was no significant change in the luciferase activities of other reporters between miR-212-3p and miR-NC groups (Figure 5 A). In contrast, miR-212-3p inhibitor drastically improved EnI/Xp activities (Figure 5 B). Subsequently, the potential effects of NFIA on EnI/Xp activity were investigated in HepG2.2.15 cells. As indicated in Figure 5 C, overexpression of NFIA significantly elevated EnI/Xp activities compared with the control group, while knockdown of NFIA repressed EnI/Xp activities. Furthermore, co-transfection of miR-212-3p + pcDNA or miR-212-3p + pcDNA-NFIA was performed to identify whether the effects of miR-212-3p on the suppression of EnI/Xp activities were mediated by NFIA. NFIA restoration partially counteracted the suppressive effect of miR-212-3p on the luciferase activity of EnI/Xp (Figure 5 D). Consequently, the above results indicated that miR-212-3p suppressed HBV EnI/Xp activities by directly targeting NFIA.

miR-212-3p repressed cell growth via negatively regulating NFIA

Then, we further explored whether the effect of miR-212-3p on HBV pathogenesis was associated with cell proliferation. MTT assay revealed that cell viability was greatly reduced by miR-212-3p overexpression, while this effect was partly weakened with the introduction of pcDNA-NFIA (Figure 6 A). Flow cytometry assay showed that NFIA up-regulation mitigated miR-212-3p-induced apoptosis (Figure 6 B). Taken together, the results showed that miR-212-3p impeded cell proliferation and promoted apoptosis by inhibiting NFIA expression.

Discussion

HBV infection is considered as a central reason for hepatocarcinogenesis. It is well known that

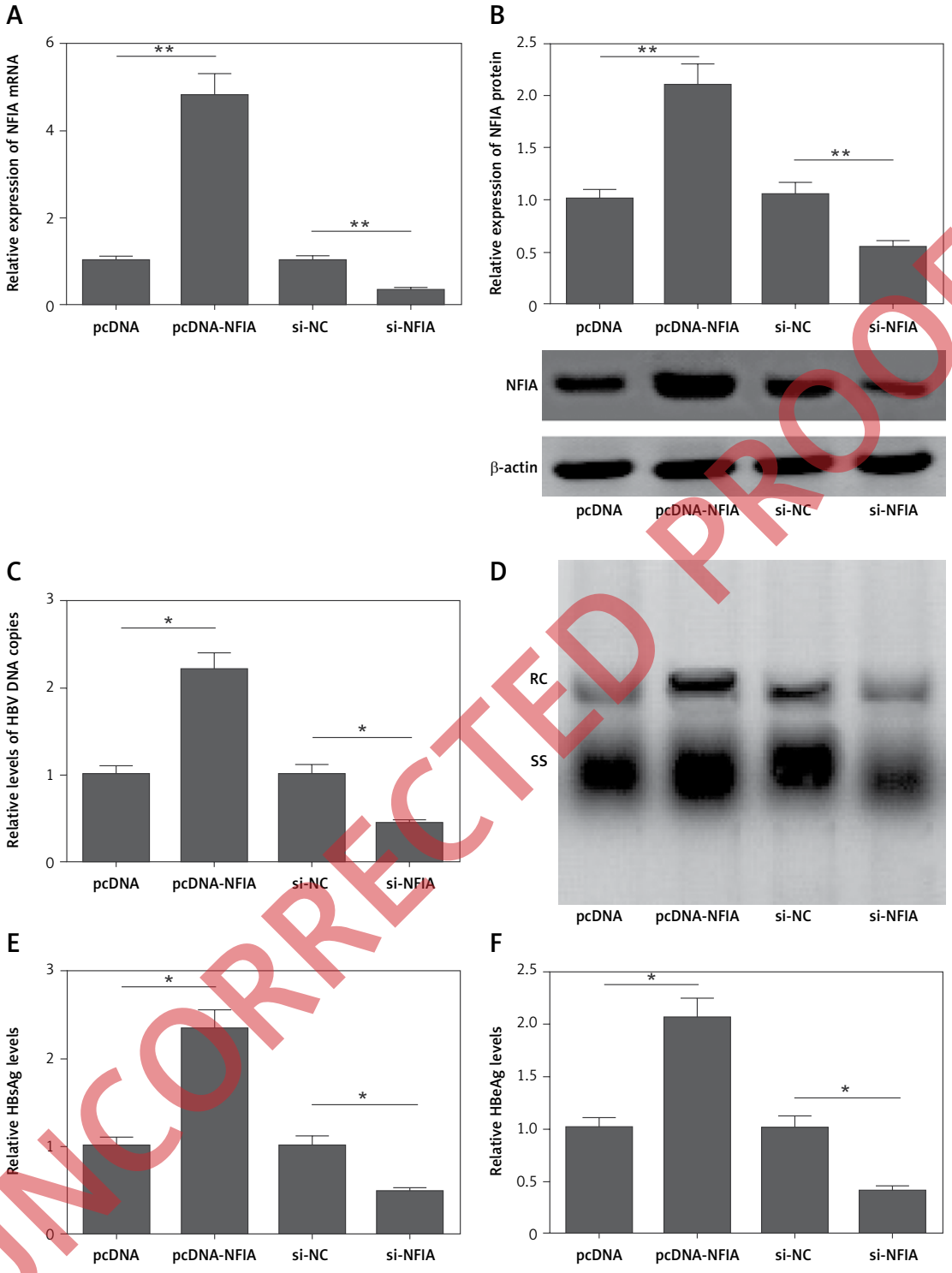

Figure 3. Nuclear factor I A (NFIA) promoted hepatitis B virus (HBV) replication and expression. HepG2.2.15 cells were transfected with pcDNA, pcDNA-NFIA, si-NC or si-NFIA. **A**, **B** – mRNA and protein levels of NFIA in transfected cells were detected by qRT-PCR and western blot. **C** – The level of HBV DNA copies in transfected cells was measured by qRT-PCR. **D** – The level of core particle-associated HBV DNA in transfected cells was detected by southern blot assay. Positions of relaxed circular (RC) and single-stranded (SS) DNAs were indicated. **E**, **F** – Levels of HBsAg (**E**) and HBeAg (**F**) in the supernatant of transfected cells were evaluated via ELISA. *$P < 0.05$, **$p < 0.01$

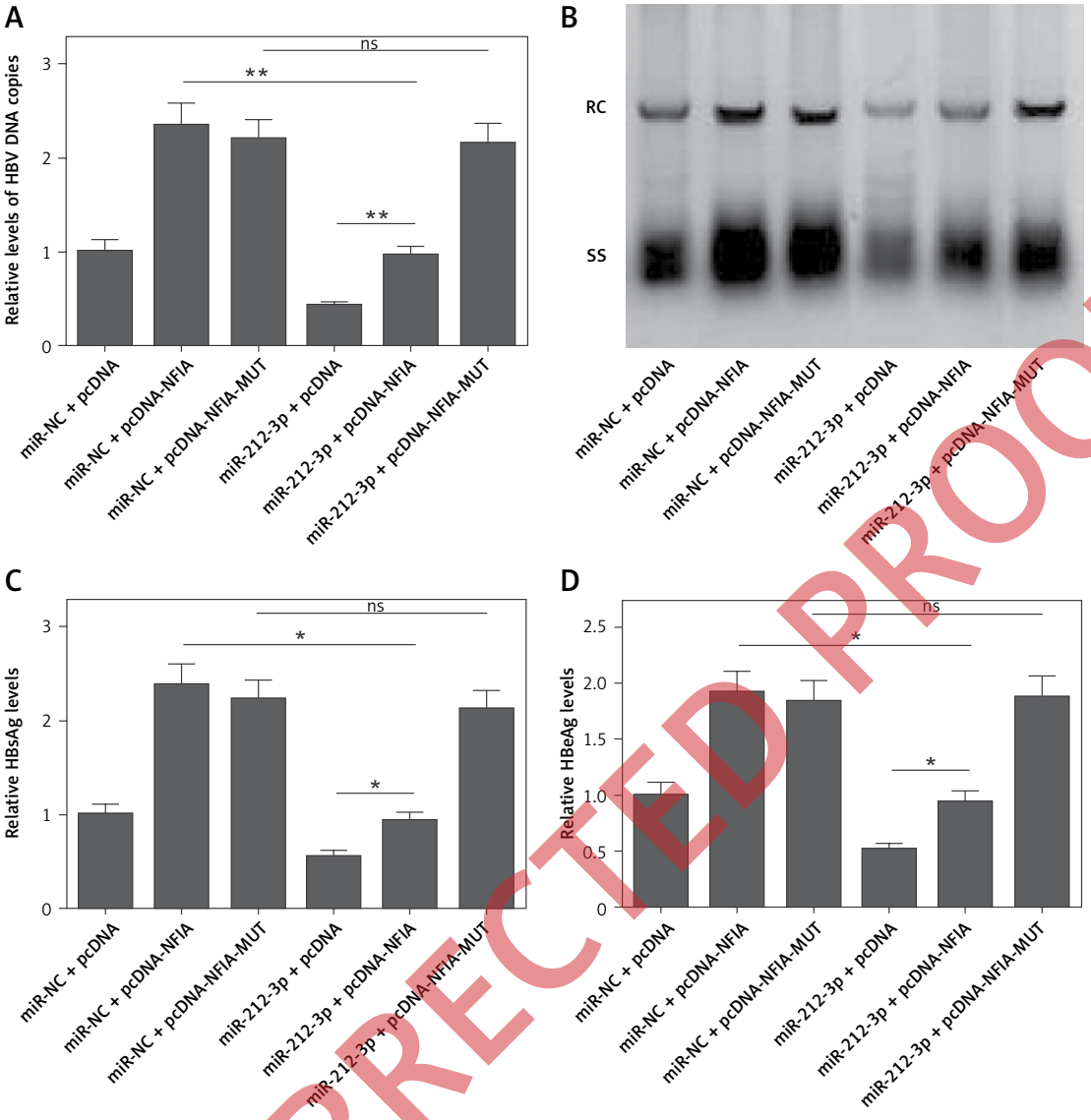

Figure 4. miR-212-3p suppressed hepatitis B virus (HBV) replication and expression by targeting nuclear factor I A (NFIA). The HepG2.2.15 cells were transfected with miR-NC + pcDNA, miR-NC + pcDNA-NFIA, miR-NC + pcDNA-NFIA-MUT, miR-212-3p + pcDNA, miR-212-3p + pcDNA-NFIA, or miR-212-3p + pcDNA-NFIA-MUT. **A** – The level of HBV DNA copies in transfected cells was measured by qRT-PCR. **B** – The level of core particle-associated HBV DNA in transfected cells was detected by southern blot assay. Positions of relaxed circular (RC) and single-stranded (SS) DNAs were indicated. **C**, **D** – Levels of HBsAg and HBeAg in the supernatant of transfected cells were evaluated via ELISA. *$P < 0.05$, **$p < 0.01$

host miRNAs are implicated in virus infection and replication [7, 19]. In this project, we concentrated on understanding the molecular mechanism of miR-212-3p in modulating HBV replication and HCC progression. Our data demonstrated that miR-212-3p inhibited HBV enhancer I activity to regulate HBV expression, replication and HCC progression by targeting NFIA.

As reported, many miRNAs in the host are involved in HBV infection or pathogenesis. For example, Fan *et al.* documented that HBV genome amplification in liver cancer cells was impeded by miR-185-5p [8]. Also, miR-101 [9], miR-370 [15], and miR-539 [10] were identified to suppress HBV genome amplification. However, Wang *et al.* demonstrated that depletion of miR-146a decreased HBV replication and expression in HCC [20]. The different miRNAs caused contrary results in HBV replication, which may imply that miRNAs play different roles in the complex regulatory networks of HBV replication and transfection. In this study, overexpression of miR-212-3p restrained the levels of HBV DNA copies, core particle-associated DNA, HBsAg and HBeAg levels in HepG2.2.15 cells, whereas miR-212-3p inhibitor exhibited the contrary effect. The data illustrated that miR-212-3p blocked HBV expression, replication and tumor progression in HCC.

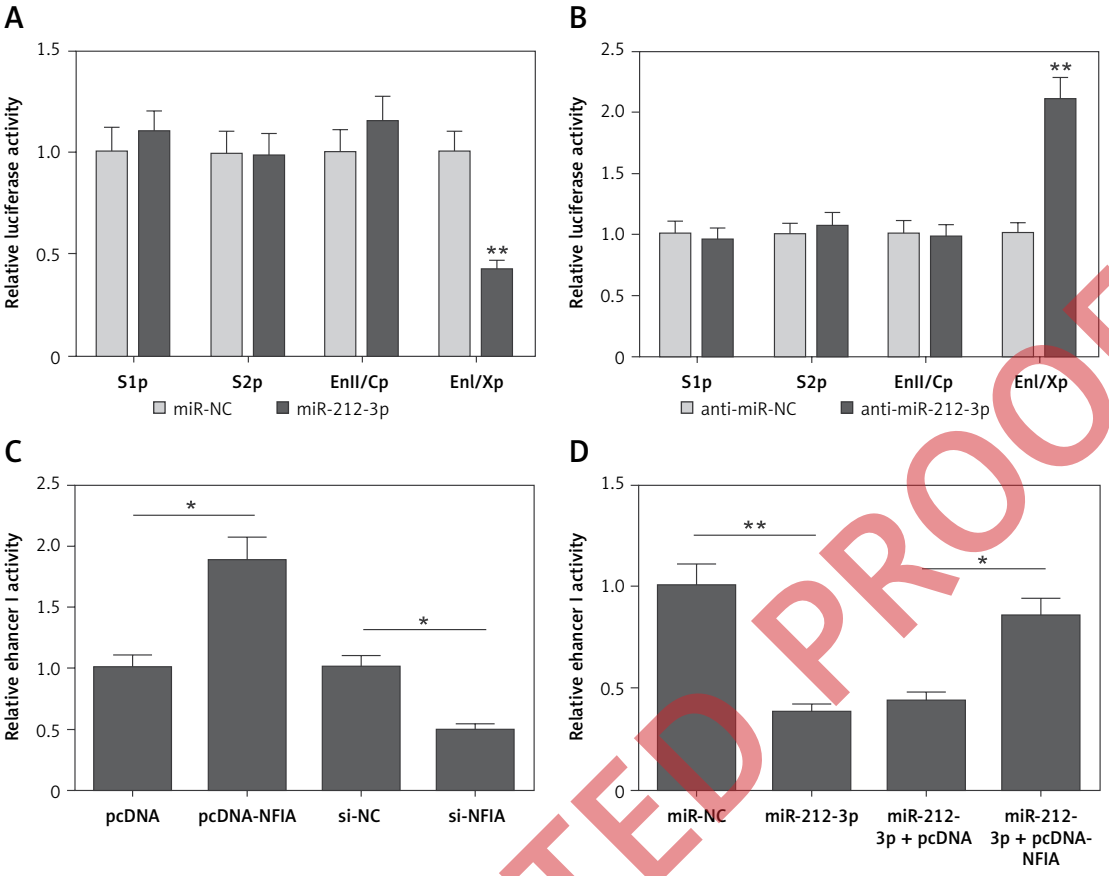

Figure 5. miR-212-3p inhibited hepatitis B virus (HBV) enhancer I activity by targeting nuclear factor I A (NFIA). **A**, **B** – Luciferase activity of preS1 promoter (S1p), preS2 promoter (S2p), enhancer II/core promoter (EnII/Cp) or enhancer I/X promoter (EnI/Xp) reporter in HepG2.2.15 cells transfected with miR-NC, miR-212-3p, anti-miR-NC or anti-miR-212-3p was examined via dual luciferase reporter assay. **C** – The effect of NFIA overexpression or knockdown on enhancer I activity in HepG2.2.15 cells was evaluated by dual luciferase reporter assay. **D** – Enhancer I activity in HepG2.2.15 cells transfected with miR-NC, miR-212-3p, miR-212-3p + pcDNA or miR-212-3p + pcDNA-NFIA was tested via dual luciferase reporter assay. *$P < 0.05$, **$p < 0.01$

Nuclear factor I (NFI) was previously found to be associated with virus replication and viral genes' transcription [12]. For example, Tian *et al.* reported that NFIA modulated by miR-220c promoted HBV replication, demonstrated by the decrease of HBsAg and HBeAg, as well as the decline of DNA and RNA (pgRNA and total RNA) of HBV [14]. Fan *et al.* suggested that the miR-370/NFIA axis regulates HBV gene replication and expression [15]. In this study, NFIA was verified as a direct target of miR-212-3p. Also, NFIA silencing inhibited the levels of HBV DNA copies, core particle-associated DNA, HBsAg and HBeAg in HepG2.2.15 cells. Meanwhile, NFIA overexpression presented the opposite tendency. Further restoration experiments demonstrated that NFIA overexpression regained the levels of HBV DNA copies, core particle-associated DNA, HBsAg and HBeAg in HepG2.2.15 cells blocked by miR-212-3p. To sum up, miR-212-3p inhibited HBV replication by targeting NFIA.

According to the previous documents, NFI plays vital and complicated roles in regulating HBV replication through different HBV promoters/enhancers. For instance, NFIA promoted HBV enhancer I activity, and was negatively regulated by miR-200c [14]. Guo *et al.* discovered that miR-372/373 negatively regulated NFIB expression to repress enhancer I-Cp activities [21]. In our research, it was found that miR-212-3p strikingly suppressed EnI/Xp activity, but had no effect on S1p, S2p, or EnII/Cp activity. Moreover, silencing of NFIA hindered enhancer I activity. Consistently, NFIA was previously found to induce HBV transcription, gene expression and replication by enhancing HBV enhancer I activities [15]. Furthermore, restoration of NFIA expression recovered the enhancer I activity inhibited by miR-212-3p. To conclude, miR-212-3p repressed HBV enhancer I activity by targeting NFIA.

The tumor-suppressive effects of miR-212-3p have been demonstrated in several malignancies through different mechanisms, such as osteosarcoma [22, 23], glioblastoma multiforme [24], and colorectal cancer [25]. A recent study revealed that miR-212-3p decreased cell invasion in HCC through inhibiting connective tissue growth factor

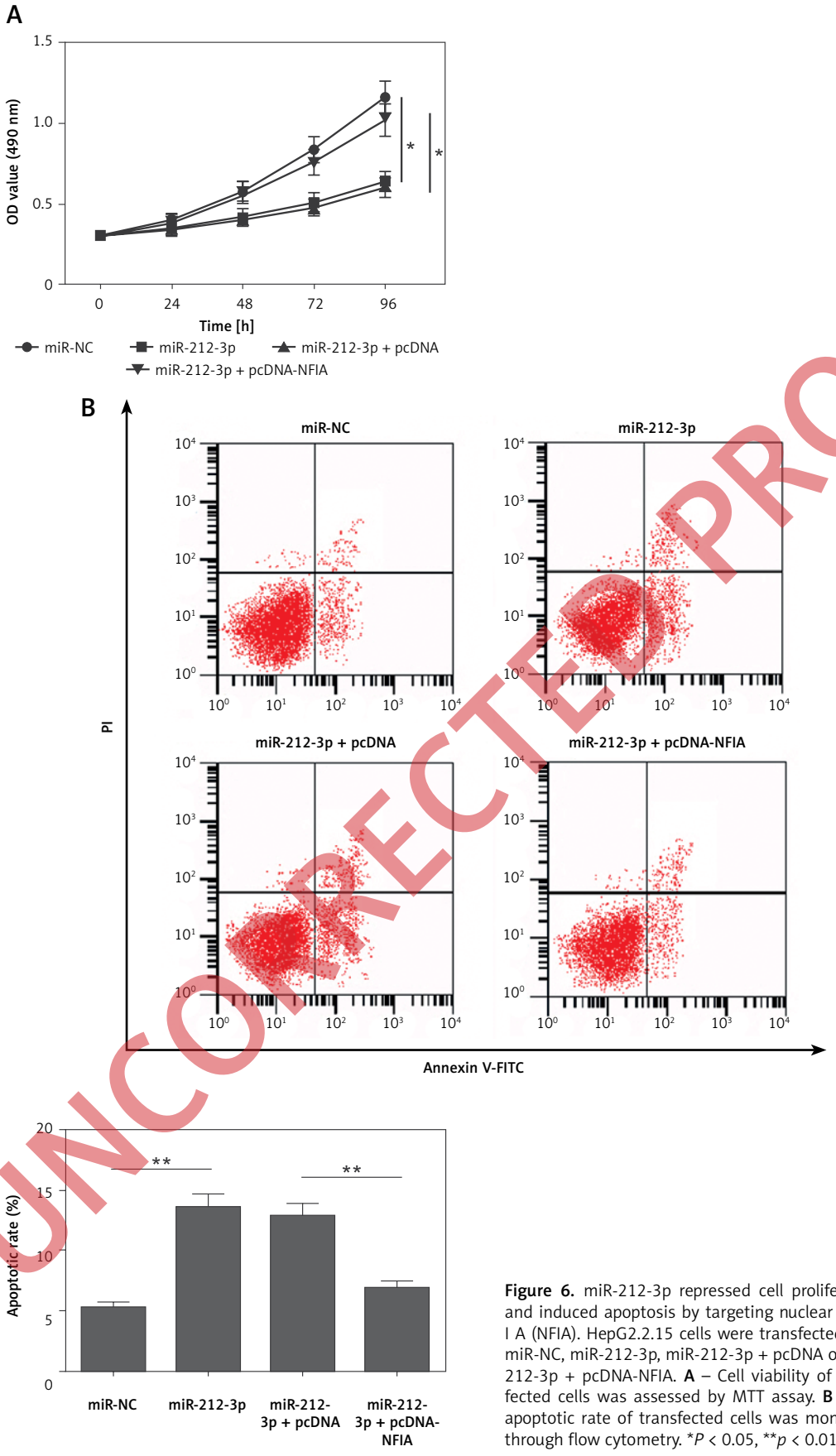

Figure 6. miR-212-3p repressed cell proliferation and induced apoptosis by targeting nuclear factor I A (NFIA). HepG2.2.15 cells were transfected with miR-NC, miR-212-3p, miR-212-3p + pcDNA or miR-212-3p + pcDNA-NFIA. **A** – Cell viability of transfected cells was assessed by MTT assay. **B** – The apoptotic rate of transfected cells was monitored through flow cytometry. *$P < 0.05$, **$p < 0.01$

(CTGF) [26]. Our study confirmed that NFIA overexpression counteracted the inhibitory effect on cell viability and the promotive effect on apoptotic rate in HepG2.2.15 cells caused by miR-212-3p mimics. The above results showed that miR-212-3p impeded cell growth by targeting NFIA in HCC.

In conclusion, miR-212-3p suppressed HBV gene expression, replication and tumor progression in HCC via decreasing HBV enhancer I activity by targeting NFIA. Therefore, the new regulatory pathway may shed light on the mechanism of the host-virus network.

Conflict of interest

The authors declare no conflict of interest.

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