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RESEARCH ARTICLE

Suppression of human hepatoma (HepG2) cell growth by nuclear factor-kappaB/p65 specific siRNA

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Abstract Nuclear factor-kappaB (NF-κB) is a transcription factor and antagonist of apoptosis during liver regeneration and closely related to the formation and development of hepatocellular carcinoma. In the present study, we investigated the effect of small interference RNA (siRNA)-mediated inhibition of NF-κB on growth of human hepatoma (HepG2) cells. Our data indicated that the expression of NF-κB/p65 mRNA was significantly higher in the HepG2 cells than that in the normal liver (LO2) cells before transfection, and the expression of NF-κB/p65 in the HepG2 cells with NF-κB/p65 siRNA (100 nMol/L) transfection at 72 h was reduced at the levels of mRNA (93%) and protein (62%) using real-time reverse transcription-polymerase chain reaction, enzyme-linked immunosorbent assay, and Western blotting. Interestingly, the apoptosis index of the HepG2 cells increased up to 85%, detected by Annexin V-fluorescein isothiocyanate, suggesting that NF-κB is overexpressed in hepatoma cells and can be inhibited by NF-κB/p65 siRNA through the apoptotic mechanism. Thus, we conclude that NF-κB is a potential molecular target for HCC gene therapy.

Keywords Hepatocellular carcinoma · Nuclear factor-kappaB · Apoptosis · Gene therapy · Real-time PCR · Small interference RNA

Abbreviations

ELISA	enzyme-linked immunosorbent assay
GAPDH	glyceraldehydephosphate dehydrogenase
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
NF-κB	nuclear factor-kappaB
PBS	phosphate buffered saline
SD	standard deviation
SDS	sodium dodecyl sulfate
siRNA	small interference RNA
V-FITC	V-fluorescein isothiocyanate

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers and causes of mortality in China [1, 2]. A great deal of progress in understanding the mechanism(s) of hepatocarcinogenesis has been achieved in recent years

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[3–5]. Many genes, such as protooncogenes, tumor suppressor genes, apoptosis genes, and growth factor genes, have been implicated in hepatocarcinogenesis [6–8]. The chronic infection of hepatitis B virus (HBV) or hepatitis C virus (HCV) is involved in HCC development and progression. Many reports have suggested that tumorigenic protein (HBx or core protein of HCV) activates a variety of signaling pathways, including nuclear factor-kappaB (NF-κB) activation [9, 10]. Abnormal activation of NF-κB modulated the transcription and expression of many genes in development of HCC [11–14].

NF-κB is an essential antagonist of apoptosis during liver regeneration and embryonal development of hepatocytes. It may also inhibit the programmed cell death induced by cytokines or cytotoxic drugs in some cancer cell lines, cell proliferation, and immunity against viral infections [15–17]. It is extensively located in the cytoplasm of quiescent cells, with a heterodimeric complex composed of two subunits of the Rel/NF-κB family; these factors include NF-κB1 (p50), NF-κB2 (p52), c-Rel, RelA/p65, and RelB. Activated NF-κB plays important roles in signal transduction pathways of cell differentiation, proliferation, and apoptosis in response to a variety of physiological and pathological stimuli [18–20]. However, the mechanisms of NF-κB to apoptosis inhibition during HCC are still not fully elucidated [5, 21]. In this present study, we used small interference RNA (siRNA) to inhibit the NF-κB expression of human hepatoma cell lines (HepG2 cells) for exploring the effect of siRNA-mediated inhibition of NF-κB expression on the growth of hepatoma cells.

Materials and methods

Cell strain and group

Human hepatoma cell lines (HepG2) and normal liver (LO2) cells were purchased from KeyGen Biotech Co., Ltd., Nanjing, China. The HepG2 cells were divided into blank control (Con), liposome (Lip), negative siRNA control (Neg), and NF-κB/p65 siRNA transfection (Tra). A serial dose of NF-κB/p65 siRNA, including 100, 50, 10, 1.0, and 0.1 nMol/L, were used for this study.

Synthesis NF-κB/p65 siRNA and cell transfections

The NF-κB/p65 siRNA expression vector was constructed. The primers were designed according to NF-κB/p65 sequences obtained from GeneBank (NM-021975) and synthesized with a synthesizer (Biomics Biotechnologies Co., Ltd, Nantong, China). The sequences of Neg-siRNA are: 5′-UUCUCCGAACGUGUCACGUTTdTdT-3′ and 3′-TdTdTTAAGAGGCUUGCACAGUGCA-5′. The sequences of siRNA are: 5′-GA UGAGAUCUUCCUACU GUdTdT-3′ and 3′-TdTdCUACUCUAGAAGGAUGACA-5′. The cells were seeded the day before transfection, using RPMI-1640 with 10% FBS, but without antibiotics. Transient transfection of NF-κB/p65 siRNA vectors and the nonsilence vectors were carried out using lipofectamine 2000 and plus reagent in OptiMEM, as suggested by the manufacturer. Cells were incubated with the plasmid vectors, lipofectamine, and plus reagent complexes for 6 h at 37°C, added FBS to the cells to achieve a final concentration of 10% in RPMI-1640, harvested at 24, 48, and 72 h after transfection, and kept at −85°C for subsequent study.

Synthesis of NF-κB cDNA

The cells (5×10^5) added 1.0 ml of TRI_{Zol} reagent (Promega, USA). Total RNA was isolated according to standard procedures and the protocols outlined by the manufacturer. RNA purity was estimated from the ratio of absorbance (A) readings at 260 and 280 nm, with an $A_{260/280}$ ratio between 1.8 and 2.0 indicating sufficient purity. The RNA samples were kept frozen at −85°C until required. For synthesis of cDNA, 2 μg of total RNA was denatured in the presence of random hexamers (100 pMol/L, Promega) and reverse-transcriptase (GIBCO, BRL) at 23°C for 10 min, 42°C for 60 min, 95°C for 10 min, on ice for 5 min, and then stored at −20°C for PCR amplification.

PCR amplification of NF-κB gene

A set of primers, NF-κB-P1 (sense), 5′-AGCACAGATAC CACCAAGAC-3′ (nt 398~417) and NF-κB-P2 (antisense), 5′-TGGTCCCGTGAAATACACCT-3′ (nt 523~542), were designed according to NF-κB/p65 sequences obtained from GeneBank (NM-021975) and synthesized in the Shanghai Institute of Cell Biology, Chinese Academy Sciences, China, and the size of amplified fragment was 145 bp. The PCR amplification consisted of initial denaturation at 94°C for 5 min, followed by 94°C for 25 s, 55°C for 30 s, and 72°C for 90 s for 30 cycles. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genome was used as a control. A set of primer sequences were GAPDH-1 (sense), 5′-AGAAGGCTGGGGCTCATT TG-3′, and GAPDH-2 (antisense), 5′-AGGGGCCATCCACAGTCTTC-3′, and the size of designed fragment was 258 bp. The amplified products were separated by electrophoresis on 2% agarose gels with ethidium bromide staining. The fragment sizes were evaluated using DNA markers (Promega) as molecular weight standards and Molecular Imager Gel Doc™ System.

Quantitative real-time PCR analysis

The human NF-κB/p65 gene expression vector was constructed and confirmed by DNA sequencing as NF-κB/p65 DNA standard. For quantitative real-time PCR, 1 μg of NF-κB cDNA and a serial dilution of standard NF-κB/p65 DNA templates, a set of primers: NF-κB-P1 and NF-κB-P2, and NF-κB/p65 probe: 5′-AGTGTGTGAAGAA GCGGGACCTG-3′ (synthesized in the Shanghai Institute of Cell Biology, Chinese Academy Sciences, China) were performed with Taqman qPCR Kit (Shanghai Shinegene Molecular Biotechnology Co., Ltd, China) as follows: initial denaturation at 94°C for 4 min and then 94°C for 30 s and 60°C for 60 s for 40 cycles using the Bio-Rad iQ5 system (Bio-Rad, Hercules, CA). The C_t values were determined, and the copies of each mRNA were calculated according to the absolute standard curve of NF-kB/p65 DNA. Each run was completed with a melting curve analysis in order to confirm the specificity of amplification and lack of primer dimmers.

Enzyme-linked immunosorbent assay

The nuclear protein was extracted after cell transfection, according to the instructions for the nuclear and cytoplasmic protein extraction kit, and quantified spectrophotometrically using the BCA assay kit (Beyotime, Haimen, China). The level of NF-κB/p65 was detected according to the human NF-κB/p65 enzyme-linked immunosorbent assay kit (Cusabio Biotech, USA), with 30 μl of complete combining buffer, 10 μl of nuclear protein extraction agent, and 20 μl of complete lysis buffer. A positive control was made by adding 2.5 μg of the provided nuclear extract diluted in 20 μl of complete lysis buffer per well; the blank well contained only 20 μl of complete lysis buffer. Twenty microliters of the appropriate standard diluted in the complete lysis buffer was added to each well. Solutions were incubated with mild agitation for 1 h at room temperature. Each well was washed three times with 200 μl of washing buffer, then 100 μl of diluted NF-κB antibody was added to wells. The plate was then covered and incubated for 1 h at room temperature with mild agitation, the wells were washed four times, and 100 μl of Developing Solution (room temperature) was added to all wells. After incubation for 10 min in the dark, 100 μl of stop solution was added to each well, and within 5 min, the absorption was measured with a spectrophotometer at 450 nm; reference wavelength at 655 nm. The concentration of NF-κB was calculated according to comparison with a standard curve.

Western blotting

The cultured cells were lysed with a sodium dodecyl sulfate (SDS) sample buffer and boiled for 10 min. The protein content was normalized using protein assay kits. Protein (20 μg) was separated by SDS polyacrylamide gel electrophoresis. The separated proteins were transferred to a polyvinylidine difluoride membrane (Millipore, Bedford, MA, USA) using a transfer apparatus at 300 mA for 110 min. The membrane was then blocked with 5% nonfat milk and incubated, with primary antibody against NF-κB/p65 (rabbit anti-human, 1: 500, Beyotime Co., China), overnight at 4°C. After incubation with horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit, 1:500, Beyotime Co., China) for 2 h at room temperature, Western blot analysis was carried out with enhanced chemiluminescence system.

Immunohistochemistry

The cells after NF-κB/p65 siRNA transfection were fixed with 10% formaldehyde, then according to the streptavidin–peroxidase method empirical procedure directions. Phosphate buffered saline was used to substitute for the primary antibody and served as a negative control. The NF-κB/p65 positive material was a brown–yellow fine particle layer localized in the nucleus and cytoplasm. NF-κB/p65 staining was evaluated semiquantitatively on the basis of the percentage of positive cells.

Detection of cell apoptosis

Cell apoptosis was detected after transfection using the Annexin V-fluorescein isothiocyanate Cell Apoptosis Detection Kit with flow cytometry.

Statistical analysis

Data was expressed as the mean±standard deviation. Statistical analyses were done using the SPSS10.0 software package. Differences between groups were assessed using Fisher's exact test or the chi-square test. $P \leq 0.05$ was regarded as statistically significant.

Results

Expression of NF-κB/p65 mRNA before and after siRNA transfection

The amplified fragments (145 bp) of NF-κB/p65 mRNA in human HepG2 or LO2 cells by RT-PCR are shown in Fig. 1. The expression of NF-κB/p65 mRNA was significantly higher in HepG2 cells than that in LO2 cells ($P<0.001$, Fig. 1a). The relative ratio of NF-κB/p65 mRNA to GAPDH was 1.13±0.03 in HepG2 cells and 0.29±0.07 in LO2 cells, respectively. The relative ratio of NF-κB/p65 protein to β-actin was 0.84±0.02 in HepG2 cells and 0.16±0.02 in LO2 cells ($P<0.001$, Fig. 1b). The comparative analysis of NF-

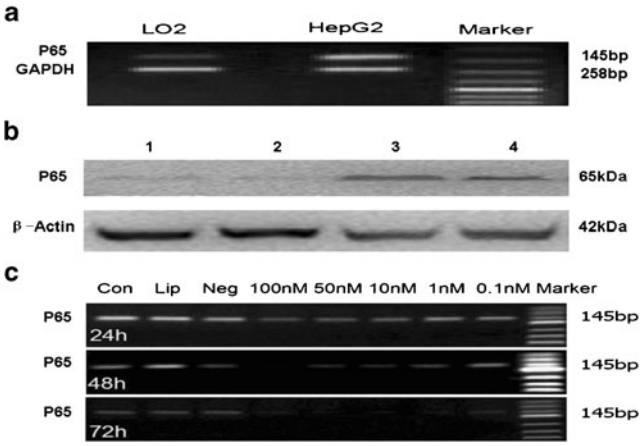

Fig. 1 Comparative analysis of NF-κB/p65 mRNA expression before and after NF-κB/p65 siRNA transfection. The fragments (145 bp) of NF-κB/p65 mRNA in the cells were amplified by RT-PCR assay, separated on 2% agarose gel, and strained with ethidium bromide. **a** The higher expression of cellular NF-κB/p65 mRNA was found in the HepG2 cells not in the LO2 cells before NF-κB/p65 siRNA transfection. **b** Western blot analysis of NF-κB/p65 protein (65 kDa) in HepG2 cells and LO2 cells; β-actin, the molecular weight 42 kDa as the control protein. *Lanes 1–2* LO2 cells, *lanes 3–4* HepG2 cells. **c** The downregulation of NF-κB/p65 mRNA expression in the HepG2 cells depended on the dose of NF-κB/p65 siRNA and the time after transfection. *p65* the amplified fragments of NF-κB/p65 mRNA, *GAPDH* the amplified fragments (258 bp) of glyceraldehyde-3-phosphate dehydrogenase, *HepG2* the human hepatoma HepG2 cell lines, *LO2* human normal liver cell lines, *Marker* DNA molecular marker, *Con* blank control group, *Lip* liposome group, *Neg* negative siRNA control group. The HepG2 cell transfection group with different dose of NF-κB/p65 siRNA: 100 nM, 100 nMol/L; 50 nM, 50 nMol/L; 10 nM, 10 nMol/L; 1 nM, 1.0 nMol/L; and 0.1 nM, 0.1 nMol/L

κB/p65 mRNA expression was performed in different times after the NF-κB/p65 siRNA transfection (Fig. 1c). The expression of NF-κB/p65 mRNA was significantly lower in the cells with NF-κB/p65 siRNA transfection than that in the blank control (Con), liposome (Lip), and negative siRNA control (Neg) groups. The downregulation of NF-κB expression depended on dose of NF-κB/p65 siRNA and time after transfection.

Expression of NF-κB/p65 mRNA depended on dose of siRNA and time

The time course of NF-κB/p65 siRNA transfection and the dynamic alteration of NF-κB/p65 gene copies with siRNA dose-dependent are shown in Fig. 2. The downregulation of NF-κB/p65 expression in HepG2 cells depended on dose of siRNA (Fig. 2a). The copies of NF-κB/p65 gene with siRNA transfection were significantly lower ($P<0.01$) than that in the Con group except the 0.1 nMol/L group. No significant relationship was found between the Con group and the Lip group or the Neg group. The downregulation of NF-κB/p65 gene expression in the HepG2 cells group was time-dependent after siRNA transfection (Fig. 2b), and the copies of NF-κB/p65 gene at 72 h in the 100 nM group were reduced to 93% compared with the Con group.

Alteration of cellular NF-κB/p65 expression after siRNA transfection

The expression and distribution of NF-κB/p65 in HepG2 cells with NF-κB/p65 siRNA transfection are shown in Fig. 3. The NF-κB/p65 positive material was a brown–yellow fine particle layer localized in the cytoplasm and nucleus of HepG2 cells (Fig. 3a). The incidence of NF-κB/p65 expression (Fig. 3b) decreased significantly in cytoplasm of HepG2 cells with siRNA transfection (63% vs.9%, $P<0.001$), and not in nucleus of HepG2 cells (Fig. 3c).

Level of NF-κB/p65 protein after siRNA transfection

The levels of NF-κB/p65 expression after NF-κB/p65 siRNA transfection was significantly lower in the HepG2

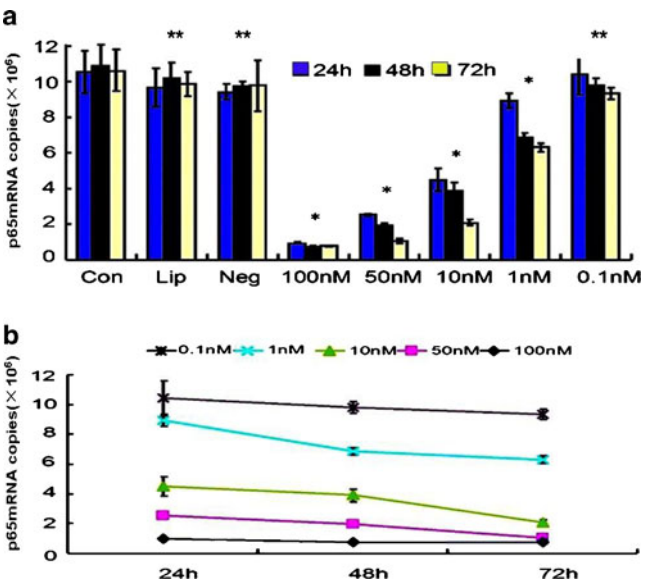

Fig. 2 Quantitative analysis of NF-κB/p65 mRNA after siRNA transfection. The copies of NF-κB/p65 gene in cells were analyzed by real-time quantitative PCR. **a** The downregulation of NF-κB/p65 mRNA in HepG2 cells depended on dose of siRNA. NF-κB/p65 mRNA copies in siRNA transfection cells were significantly lower than in blank control (Con) cells except 0.1 nMol/L group. *Con* blank control group, *Lip* liposome group, *Neg* negative siRNA control group. HepG2 cell transfection group with dose of siRNA: 100 nM, 100 nMol/L; 50 nM, 50 nMol/L; 10 nM, 10 nMol/L; 1 nM, 1.0 nMol/L; and 0.1 nM, 0.1 nMol/L. Statistical analysis. The copies of NF-κB/p65 mRNA were analyzed according to the absolute quantitative standard curve of NF-kB/p65 DNA. The results were expressed as mean±SD from three independent experiments, $*P<0.001$ or $**P>0.05$ vs. blank control group. **b** The time course of siRNA transfection and the dynamic alteration of NF-κB/p65 mRNA copies with different dose of siRNA transfection

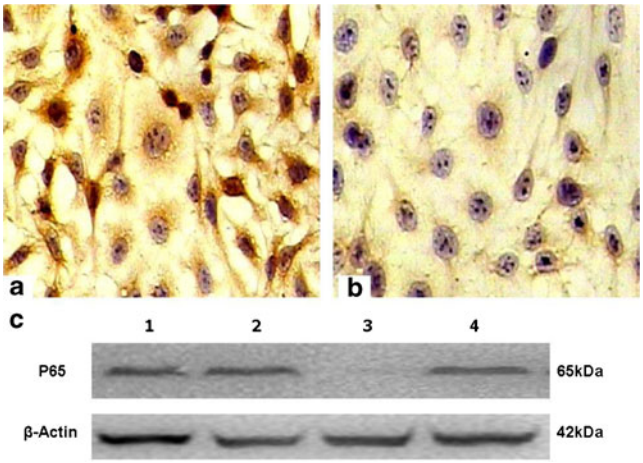

Fig. 3 Alteration of NF-κB/p65 protein expression after siRNA transfection. **a** Immunohistochemical staining with anti-NF-κB/p65 (streptavidin–peroxidase method, original magnification ×40). The NF-κB/p65 positive material was a brown–yellow fine particle layer and localized in cytoplasm and nucleus of HepG2 cells. **b** The expression different of NF-κB/p65 positive material in cytoplasm and nucleus of HepG2 cells with siRNA transfection. **c** Western blot analysis of NF-κB/p65 protein in cytoplasm and nucleus of HepG2 cells with siRNA transfection or without. *Lanes 1* cytoplasm of HepG2 cells, *Lanes 2* nucleus of HepG2 cells, *Lanes 3* cytoplasm of HepG2 cells with siRNA transfection, and *Lanes 4* nucleus of HepG2 cells with siRNA transfection

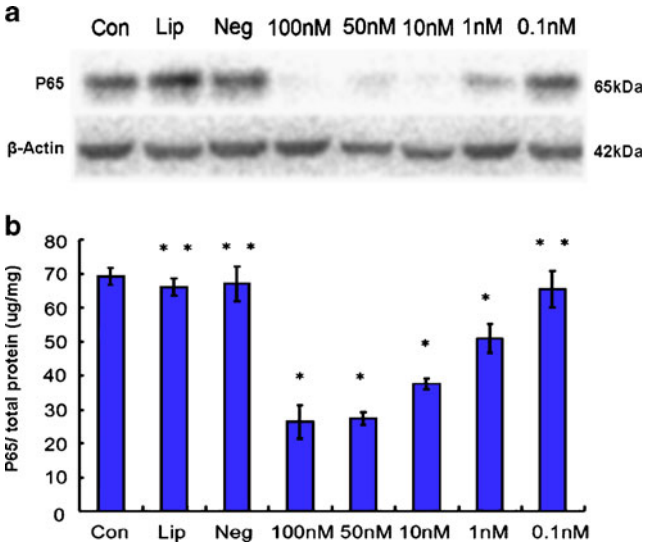

Fig. 4 The inhibition of different dose of siRNA on NF-κB/p65 expression. **a** The NF-κB/p65 expression after siRNA transfection was detected by Western blotting analysis. **b** The expression of NF-κBp65 protein after siRNA transfection was quantitatively analyzed by an enzyme-linked immunosorbent assay. The results were expressed as mean±SD from three independent experiments. Statistical analysis, **P*<0.001 or ***P*>0.05 vs. the Con group. *p65* the molecular weight 65 kDa, the dynamic alteration of NF-κB/p65 protein expression after NF-κB/p65 siRNA transfection; *β-actin* the molecular weight 42 kDa as the control protein

cells group than that in the Con group, and the down-regulation of NF-κB/p65 expression in HepG2 cells was dose-dependent of NF-κB/p65 siRNA. No significant difference of NF-κB/p65 expression was found between the Con group and the Lip group or the Neg groups (Fig. 4a). The level of NF-κB/p65 expression in HepG2 cells (the 100 nM group) was reduced to 62%, compared with the Con group, and confirmed by quantitative analysis of NF-κB/p65 (Fig. 4b).

Apoptosis acceleration of HepG2 cell after siRNA transfection

The summary of HepG2 cell apoptosis rate after a serial concentration of NF-κB/p65 siRNA transfection is shown in Table 1. The increasing of HepG2 cell apoptosis was dose-dependent of NF-κB/p65 siRNA, and the apoptotic index was 85% in the 100 nM group with statistically significant difference in other dose of NF-κB/p65 siRNA groups except of the 0.1 nMol/L group.

Discussion

Hepatocellular carcinoma is one of the most common malignancies worldwide [1, 22, 23]. Despite many therapeutic approaches, the long-term prognosis of HCC is poor because of higher relapse and intrahepatic metastasis [24, 25]. Tumor cells to evade programmed cell death are a major characteristic that enables their uncontrolled growth. NF-κB is a transcription factor that plays a pivotal role in regulating multiple biological functions including inflammation, cell

Table 1 Summary of HepG2 cell apoptosis rate after NF-κB/p65siRNA transfection

Groups	Apoptosis (%)	*P* value
Con	4.25±0.79	
Lip	4.63±0.38	>0.05
Neg	4.48±0.53	>0.05
HepG2 cells + siRNA		
0.1 nM	4.46±0.58	>0.05
1 nM	5.92±0.43	<0.001*
10 nM	6.83±0.47	<0.001*
50 nM	7.58±0.31	<0.001*
100 nM	7.86±0.41	<0.001*

HepG2 cell transfection group with different dose of NF-κB/p65 siRNA: 100 nM, 100 nMol/L; 50 nM, 50 nMol/L; 10 nM, 10 nMol/L; 1 nM, 1.0 nMol/L; and 0.1 nM, 0.1 nMol/L. Apoptosis index was counting 1×10^4 cells, and the results were expressed as mean±SD from three independent experiments

Con blank control group, *Lip* liposome group, *Neg* negative siRNA control group

**P*<0.001 vs. the Con group

growth, and apoptosis [26, 27]. The association of NF-κB pathway activation with associated/driven tumor promotion, progression, and metastasis was demonstrated in several models [5, 28]. However, the mechanism of NF-κB to apoptosis inhibition during HCC has not been fully elucidated [21]. In this present study, we investigated NF-κB/p65 siRNA to inhibit the NF-κB expression of human HepG2 cells for exploring the effect of siRNA-mediated NF-κB expression on tumor cell growth.

A causal relationship between inflammation and cancer has long been suspected. NF-κB is a very important molecule that connects inflammation with the tumor [29] and is involved in similar biological processes of HCC as a critical modulator of genes that promote cell survival, inflammation, angiogenesis, tumor development, progression, and metastasis [30, 31]. Interestingly, NF-κB positive material in our previous rat hepatoma model was a buff-colored layer of fine particles localized in the nucleus, and all incidences during different stages of HCC development were significantly higher than that of controls, with an increasing tendency of hepatic NF-κB expression [21]. The expression of NF-κB/p65 mRNA or protein was significantly higher in HepG2 cells than that in LO2 cells ($P<0.001$) before transfection (Fig. 1a, b), suggesting that the abnormality of NF-κB expression is associated with the malignant transformation of hepatocytes.

A developing siRNA strategy is a powerful technique to inhibit specific gene expression, which has highlighted the potential use of siRNA molecules to study gene function or explore new tumor therapeutic agents [32, 33]. siRNAs are short, double-stranded RNA molecules that can target mRNAs with complementary sequences for degradation via a process termed RNA interference [34, 35]. In this present study, four siRNA sequences (data not shown) targeting at different sites of NF-κB/p65 mRNA were designed by random method. All siRNA sequences were done blast research in GeneBank to confirm that only NF-κB/p65 gene was targeted. However, only one siRNA sequences according to the different suppression effects was selected to be used for the present study. The level of NF-κB/p65 mRNA expression was significantly decreased in the NF-κB/p65 siRNA transfection cells than that in the Con, Lip, or Neg groups (Fig. 1c). The inhibition of NF-κB/p65 mRNA expression was time-dependent and dose-dependent of NF-κB/p65 siRNA (Fig. 2), indicating that NF-κB/p65 siRNA is an effective NF-κB gene silencing method.

NF-κB is a transcription factor with generally biological activity. NF-κB proteins are predominantly cytoplasmic, associating with members of inhibitory IκB family and forming NF-κBIκB complexes without activity. Activated NF-κB plays important roles in signal transduction pathways of cell differentiation, proliferation, and apoptosis in response to a variety of physiological and pathological stimuli [19]. In normal liver cells, NF-κB is present as a latent, inactive complex retained in the cytoplasm through association with IκB. The released NF-κB rapidly enters the nucleus where it can perform its function [12]. NF-κB/p65 positive material was a brown–yellow fine particle layer and localized in the cytoplasm and nucleus of HepG2 cells (Fig. 3a), but mainly in the nucleus of siRNA transfection cells (Fig. 3b), with significantly lower expression at RNA level, confirmed by the downregulation of NF-κB/p65 at protein level (Fig. 4), suggesting that NF-κB/p65 siRNA intervened not only the expression of NF-κB gene but also inhibited the action of transcription.

HBV or HCV infection may activate NF-κB and, thus, may modulate cell apoptosis and may be associated with HCC, and its role has been explored. NF-kB is an essential antagonist of apoptosis during liver regeneration and embryonal development of hepatocytes. It may also inhibit the programmed cell death induced by cytokines or cytotoxic drugs in some cancer cell lines. HCC is one of the most resistant tumors to systemic chemotherapy. NF-κB represents a group of evolutionarily conserved and structurally related proteins and has anti-apoptotic effects which have been implicated in a variety of biological processes [36]. Several studies have recently shown that the tumor is not only a disease of out-of-control cell proliferation, but also a disease of cell apoptotic dysregulation. The anti-apoptotic effect of NF-κB may be a reason for tumor generation. Activation of NF-κB promotes cell survival through different mechanisms [29, 31]. The present data revealed that the apoptosis ratio of hepatoma cells was enhanced with siRNA dose-dependent (Table 1), suggesting that NF-κB as an anti-apoptosis protein can maintain tumor cell viability, and inhibiting its activation can induce tumor cell death through apoptosis mechanism.

In conclusion, siRNA is powerful sequence-specific reagents designed to knockdown the expression of target genes. Although the mechanism underlying siRNA activity has not been completely elucidated, siRNA has already become a powerful reverse genetic method for suppressing the expression of a target gene. Recent studies have demonstrated that siRNA duplexes are long enough to induce gene-specific suppression. NF-κB was associated with the development and progression of HCC. The application of siRNA technology against NF-κB/p65 can significantly inhibit the expression of NF-κB at RNA and protein level, and then promote the apoptosis of hepatoma cells. NF-κB may represent a promising strategy to be used in gene therapy for HCC.

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Disclosure/conflict of interest The authors declare no conflict of interest.

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