Reduced IκBα promotes hepatocellular carcinoma cell proliferation and migration via regulation of NF-κB/Erbin axis

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Abstract. Aberrantly low expression of NF-κB inhibitor α (IκBα) is observed in hepatocellular carcinoma (HCC), yet the underlying mechanism via which IκBα regulates HCC remains largely unknown. Therefore, to determine the potential function of IκBα in hepatocarcinogenesis, the present study used immunohistochemistry (IHC) staining to analyze the associations between IκBα protein expression and clinicopathologic characteristics of 107 patients with HCC. It was found that expression of IκBα was significantly associated with tumor recurrence. Moreover, IκBα protein expression was decreased in 107 HCC tissue samples and was positively associated with overall survival. Mechanistically, it was demonstrated that silencing of IκBα activated NF-κB in both Huh7 and HCCLM3 cells, followed by upregulation of Erbb2 interacting protein (Erbin) at both the mRNA and protein levels, confirmed by reverse transcription-quantitative PCR and western blotting, to promote cell proliferation and migration. Furthermore, knockdown of Erbin significantly attenuated NF-κB-mediated cell proliferation and migration. It was also identified that overexpression of Erbin in HCC tissues promoted both cell proliferation and migration, and was negatively associated with IκBα expression in 107 HCC tissue samples. Thus, these results indicated that downregulation of IκBα promoted HCC tumorigenesis via upregulation of NF-κB-mediated Erbin expression.

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Abbreviations: HCC, hepatocellular carcinoma; IκBα, NF-κB inhibitor α; IKK, IκB kinase

Key words: IκBα, Erbb2 interacting protein, HCC, tumorigenesis

Introduction

Hepatocellular carcinoma (HCC) is one of the most malignant cancer types worldwide, especially in east Asia (1). Although significant improvements in diagnosis and treatment have been accomplished, the mortality rate for patients with HCC remains high, with ~782,000 deaths worldwide annually (1-3). The rapid and unnoticeable development and metastases of HCC are crucial to its progression, resulting in unresectable vascular thrombi, intrahepatic satellite nodules or cancer recurrence (4,5). Hence, it is important to discover effective diagnostic biomarkers to identify HCC in the early stage to improve the prognosis of patients with HCC.

NF-κB inhibitor α (IκBα) is an isoform in the IκB family, members of which have multiple ankyrin repeat domains at the C-terminus (6). IκBα combines with the NF-κB dimer to retain its existence in the cytoplasm and prevent its translocation into the nucleus (6). The IκB kinase (IKK) complex is induced by diverse extracellular signals, such as lipopolysaccharide, tumor necrosis factor (TNF) and growth factors, and the activated IKK complex phosphorylates IκBα. Then, phosphorylated IκBα is specifically recognized by an E3 ubiquitin ligase, leading to its ubiquitination and degradation (7). While previous studies have reported its inhibition of NF-κB, the remaining functions of IκBα in HCC are yet to be fully elucidated.

p65 is a well-known member of the NF-κB family of transcriptional factors participating in numerous pathways in biological responses, such as inflammation, adaptive immunity and stress reactions (8). Previous studies have revealed that NF-κB is involved in cancer oncogenesis and that it specifically promotes proliferation, metastasis and cell survival, particularly in inflammation-related cancer types (9,10). In healthy cells, activation of NF-κB after interaction with cellular stimuli occurs for a short duration and then returns to the inactive form. However, NF-κB is activated aberrantly in cancer cells, and thus continues to upregulate the transcription of its target gene (11). To achieve its transcriptional function, NF-κB/Rel proto-oncogene, NF-κB subunit (Rel) complex should shuttle to nucleus; but, IκBα combines with the dimer to anchor it in cytoplasm and thus inhibit its activity (8). After its phosphorylation is initiated by proinflammation

signals or immune stimulation, IκBα is degraded by the ubiquitin-protease system, which enables the NF-κB/Rel dimer to translocate in nucleus (12). Therefore, reduced expression of IκBα is an important cause of the abnormal elevated expression of NF-κB in cancer cells.

Erbb2 interacting protein (Erbin) is a target gene of NF-κB, encoding Erbin. Containing 16 leucine-rich repeats and one PSD-95/Dlg/ZO-1 (PDZ) domain, Erbin is a member of the leucine-rich repeat and PDZ domain family (13). Furthermore, Erbin is an adaptor of the Erb-b2 receptor tyrosine kinase 2 (ERBB2) protein and, by binding with ERBB2, regulates its function and localization (13). Erbin has also been reported to be involved in several pathways (14). For instance, Erbin suppresses tumorigenesis via inhibiting Akt activity or Ras/Raf signaling (15,16). In addition, Erbin exerts an ontogenetic function by activating estrogen receptor (ER) α or inhibiting STAT3 signaling (17,18). Thus, there is conflicting data regarding the role of Erbin in cancer. A previous study showed that Erbin is upregulated following exposure of macrophages to muramyl dipeptide, TNF α and lipopolysaccharide (19). Although it has been observed that Erbin is upregulated in HCC tissues, the underlying mechanism via which Erbin becomes dysregulated remains unknown.

The present study investigated the clinical importance of IκBα, Erbin and their molecular interactions, which suggested that reduced IκBα expression promoted HCC cell proliferation and migration via the NF-κB/Erbin pathway. Mechanistically, IκBα was downregulated in HCC tissues compared with healthy liver tissues, and decreased expression of IκBα weakened the inhibition of NF-κB. Abnormally activated NF-κB promoted Erbin expression at both the mRNA and protein levels, which served an oncogenic function in HCC. Moreover, IκBα was negatively associated with Erbin, and decreased IκBα and increased Erbin expression levels were associated with poor outcomes in patients with HCC. Therefore, the present results may facilitate the development of a potential strategy to target Erbin for HCC treatment.

Materials and methods

Patients with HCC and sample collection. In all, 107 human HCC tissue specimens were acquired from patients (aged between 19 and 73 years old, and 93 male, 14 female) between January 2011 and December 2013 at the Department of Hepatobiliary Surgery of Southwest Hospital, Army Medical University (Chongqing, China). All patients provided written informed consent and this study obtained approval from the Ethics Committee of Southwest Hospital. All patients used in this study were followed up for 5 years since their cancer diagnosis. Upon acquisition, HCC and paracancerous liver tissues (free of tumor to the eye) were conventionally 10% formalin-fixed for 48 h on a rotator at room temperature, paraffin-embedded and cut into 10-μm sections.

Immunohistochemistry (IHC) staining. To perform IHC staining, sections were first deparaffinized, rehydrated and then boiled for 2.5 min in 100˚C 0.01 mol/l sodium citrate solution for antigen retrieval. Then the IHC detection kit (SP-9001; ZSGB-Bio, Beijing, China) was employed from blockage of peroxidase to incubation of secondary antibodies according to the manufacturer's instructions. Briefly, 3% H_2O_2 was used to block the endogenous peroxidase at room temperature for 15 min. After blocking with 5% goat serum at room temperature for 30 min, tissues were incubated with specific antibodies: Anti-IκBα (sc-945; 1:50 dilution; Santa Cruz Biotechnology, Inc.) and anti-Erbin (22438-1-AP; 1:50 dilution; ProteinTech Group, Inc.) overnight at 4˚C. The following day the sections were incubated with horseradish peroxidase-conjugated secondary antibody, before positive signals were detected using 3,3'-diaminobenzidine and nuclei were stained with hematoxylin at room temperature for 20 sec. After dehydration, representative images were captured with an Olympus BX41 microscope under bright field (magnification, x100). Histological grading was referred to clinical standards (20).

Cell culture and transfection. Huh7 and HCCLM3 cells were purchased from Fudan Cell Bank (Shanghai, China). Cells were maintained in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin solution (Gibco; Thermo Fisher Scientific, Inc.), and cultured at 37˚C with 5% CO_2.

Cell transfection with plasmids (HA-tagged IκBα, Flag-tagged NF-κB p65 and corresponding empty vector as control; all Shanghai GeneChem Co., Ltd.) or small interfering RNAs (siRNAs; Shanghai Genepharma Co., Ltd.) was performed using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. In total, 400,000 cells/well were seeded into 6-well plates 1 day before transfection. Plasmids or siRNAs were diluted in Opti-MEM (Gibco; Thermo Fisher Scientific, Inc.) before mixing with Lipofectamine® 2000. Overexpression vector plasmids were pcDNA3.1 (Shanghai GeneChem Co., Ltd.). Each well of the 6-well plate was added with 2 μg plasmid for transfection. The sequences of siRNA oligos were: siIκBα, 5'-CUC CGAGACUUUCGAGGAATT-3'; siNF-κB p65, 5'-GAUUGA GGAGAAACGUAAAdTdT-3'; and siErbin, 5'-CACACUGUU GUAUGAUCAACCAU-3'. Each well of a 6-well plate was added with 8 μl siRNA (20 μmol/l) for transfection. After 48 h, cells were harvested for successive experiments.

Cell Counting Kit (CCK)-8. Cell proliferation was assessed using CCK-8 according to the instructions of the manufacturer (Bimake). Then, 24 h after transfection, cells were trypsinized and counted manually. Cells (2,000-3,000 cells/well) were seeded into a 96-well plate and after another 24 h, 10 μl CCK-8 solution was added into each well. The cells were incubated at 37˚C for another 2-4 h. A spectrophotometer was then used to measure absorbance at 450 nm.

Clonogenic survival assay. Transfected cells in DMEM with 10% FBS were seeded into 6-well plate, ~500 cells per well. After 10-14 days, cells that formed visualized colonies were stained with 0.1% crystal violet staining solution (Beijing Solarbio Science & Technology Co., Ltd.) at room temperature for 30 min and images were captured. Colonies with >50 cells were counted manually.

Transwell migration assay. To compare the difference of cell migration after different transfection, $5x10^4$-$1.5x10^5$ transfected

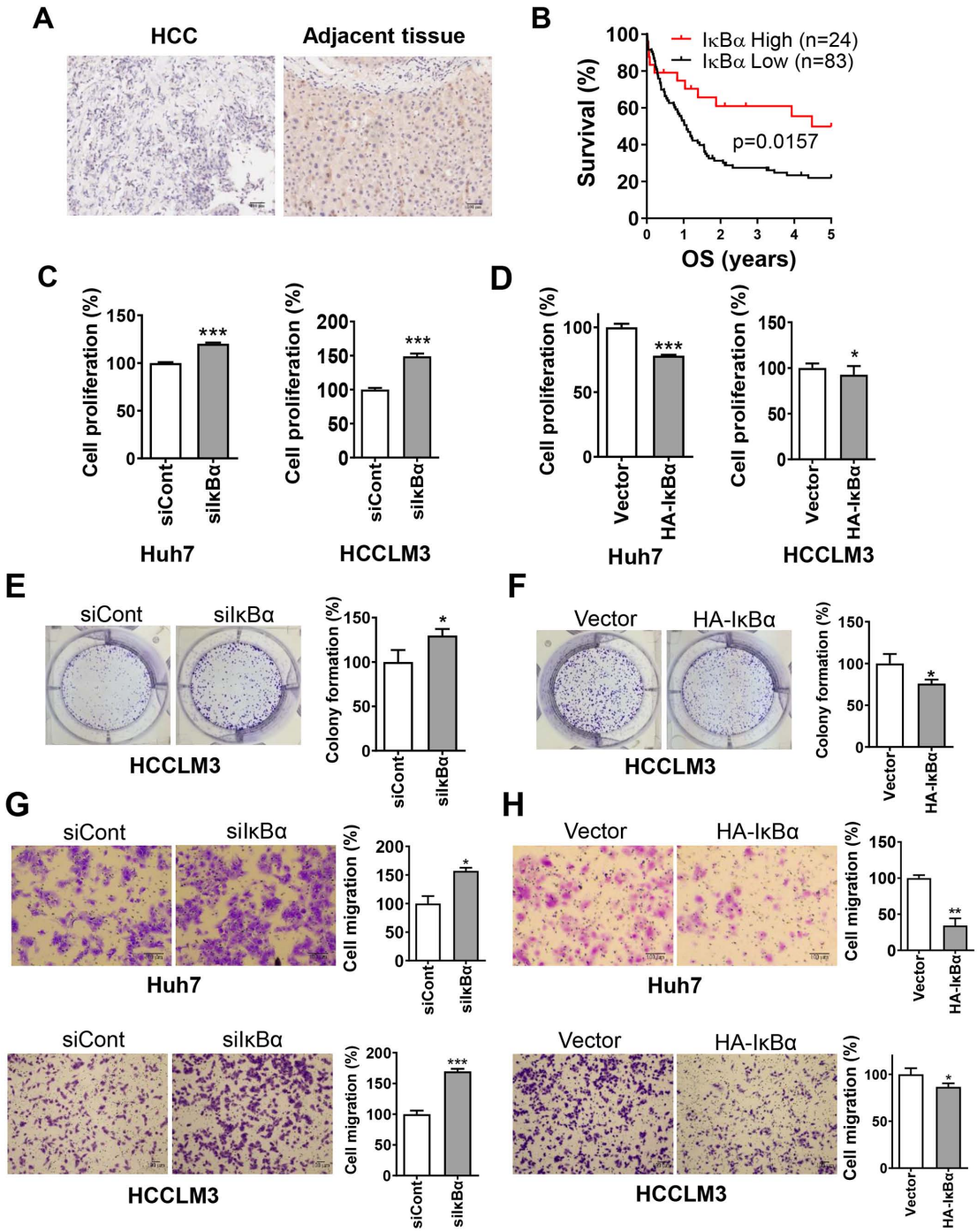

Figure 1. IκBα expression is downregulated in HCC and associated with poor prognosis of patients with HCC. (A) Representative images of HCC and adjacent liver tissues from 107 cases stained with anti-IκBα antibody. (B) Association between OS time of 107 patients with HCC and IκBα expression. Cells were transfected with (C) siIκBα or (D) HA-IκBα and proliferation was assessed after 24 h of transfection using Cell Counting Kit-8 assay. Cells were transfected with (E) siIκBα or (F) HA-IκBα for 48 h and proliferation was assessed with a clonogenic survival assay. Cells were transfected with (G) siIκBα or (H) HA-IκBα for 48 h and then analyzed with a Transwell migration assay. Scale bar, 100 μm. Error bars represent SEM from three independent experiments. *P<0.05, **P<0.01 and ***P<0.001 vs. corresponding control group. siRNA, small interfering RNA; IκBα, NF-κB inhibitor α; Cont, control; HCC, hepatocellular carcinoma; OS, overall survival; HA-IκBα, overexpression plasmid.

cells were plated into an 8 μm-pore Transwell chamber, fit for a 24-well plate, with serum free-DMEM. The lower section of the chamber, a 24-well plate, contained normal medium with 10% FBS. FBS acted as attractant to stimulate cell migrate through the membrane of chambers. After incubating for 24 h, cells were fixed by 4% paraformaldehyde for 15 min at room temperature and stained with 0.1% crystal violet staining solution (Beijing Solarbio Science & Technology Co., Ltd.) at room temperature for 30 min. Three different fields were captured under bright field and cells were counted manually (magnification, x40).

RNA extraction and reverse transcription-quantitative (RT-q) PCR. RNA extraction, reverse transcription (RT) and real-time PCR were performed using RNAiso Plus, PrimeScript™ RT reagent kit with gDNA Eraser (Perfect Real Time) and TB

Green Premix Ex Taq (Tli RNaseH Plus) (all Takara Bio, Inc.), following the manufacturer's instructions. Temperature and duration for reverse transcription were 37˚C for 15 min and 85˚C for 5 min. The thermocycling conditions were as follows: Activation of enzyme at 95˚C for 30 sec; 30 cycles of denaturation and annealing at 95˚C for 5 sec and 60˚C for 30 sec. GAPDH was used as the reference gene. The quantitative method to analyze relative gene expression was $2^{-\Delta\Delta Cq}$ (21). The 5'-3'sequences of primers used in RT-qPCR were as follows: GAPDH forward, TGGCACCGTCAAGGCTGAGAA and reverse, TGGTGAAGACGCCAGTGGACTC; NF-κB p65 forward, CGCTGCATCCACAGTTTCCA and reverse, AGG GGTTGTTGTTGGTCTGG; Erbin forward, TGTGGGTGT GAAGACCTCAG and reverse, GTCGCATCTCCGCCA TTTTC; c-Rel forward GGTTGGTCCTGCCTCCTTAC, and reverse, GCTGGAGTCCCAATGACGAA; and enhancer of zeste 2 polycomb repressive complex 2 subunit (Ezh2) forward, GGACCACAGTGTTACCAGCAT and reverse, GTG GGGTCTTTATCCGCTCAG.

Western blot analysis. Proteins were extracted using RIPA lysis buffer (Beyotime Institute of Biotechnology) with complete protease inhibitor cocktail tablet (Bimake). Protein concentration was calculated by a BCA protein concentration kit (Beyotime Institute of Biotechnology) and 5X SDS loading buffer (Shanghai Shenggong Biology Engineering Technology Service, Ltd.) was added for extraction. The samples were gently vortexed and boiled at 100˚C for 10 min before 30 μg was loaded onto 10% polyacrylamide gel (EpiZyme, Inc.). Following electrophoresis, proteins were transferred to nitrocellulose membranes. The membranes were blocked in 5% non-fat milk solution at room temperature for at least 1 h, incubated with primary antibodies at 4˚C overnight and then HRP-conjunct secondary antibodies at room temperature for 1 h. Protein blots were detected using the super ECL kit (Nanjing KeyGen Biotech Co., Ltd.). Antibodies used in this study were: Anti-IκBα (sc-945, 1:600 dilution, Santa Cruz Biotechnology, Inc.); anti-NF-κB p65 (#8242, 1:1,000 dilution, Cell Signaling Technology, Inc.); anti-GAPDH (10494-1-AP, 1:5,000 dilution, ProteinTech group, Inc.) and anti-Erbin (22438-1-AP, 1:500 dilution, ProteinTech group, Inc.).

Statistics analysis. Data are presented as the mean ± SEM, which represented ≥3 independent trials. Comparison between two different groups was performed using unpaired Student's t-test. Comparison among multiple groups was performed using one-way ANOVA and least significance difference was used as the post hoc test. χ^2 test was used to detect associations between protein expression and clinical characteristics. Survival analysis was performed using Kaplan-Meier method and comparisons with the log-rank test. Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, Inc.) and SPSS software (22.0 version; IBM Corp.). P<0.05 was considered to indicate a statistically significant difference.

Results

IκBα expression is downregulated in HCC and positively associated with poor prognosis of patients with HCC. The results indicated that IκBα protein expression was markedly decreased in HCC tissues compared with adjacent tissues (Fig. 1A). In total, 77.6% (83/107) of HCC cases exhibited low expression compared with the matched peri-tumor tissues. Kaplan-Meier survival analysis demonstrated that patients with HCC with low expression of IκBα had a significantly shorter survival time compared with patients with high expression (Fig. 1B). In addition, the association between the expression of IκBα and cancer recurrence was statistically significant (Table I).

Table I. Association between IκBα expression and clinicopathological variables of 107 patients with HCC.

Parameter	IκBα expression		χ^2	P-value
	High (n=24)	Low (n=83)		
Sex			0.873	0.35
Male	19	74		
Female	5	9		
Age, years			0.97	0.325
≤50	17	49		
>50	7	33		
TNM stage			0.410	0.522
I	11	32		
II-IV	13	51		
Tumor thrombi			1.706	0.192
Yes	4	25		
No	20	58		
Histological grade			0.001	0.98
I	3	8		
II-III	21	75		
Intrahepatic metastasis			0.363	0.547
Yes	6	14		
No	18	69		
Tumor recurrence			7.035	0.008[a]
Yes	10	59		
No	14	24		

Statistical analyses were performed using Pearson χ^2 test. [a]P<0.05. IκBα, NF-κB inhibitor α.

To determine the tumor suppressive function of IκBα, CCK-8, clonogenic survival and Transwell migration assays were used to detect proliferation and migration of HCC cells. Knockdown of IκBα significantly promoted cell proliferation and migration, whereas overexpression of IκBα significantly inhibited cell proliferation and migration (Fig. 1C-H). These findings suggested that IκBα was downregulated in HCC tissues and that knockdown of IκBα was positively associated with poor prognosis of patients with HCC.

IκBα negatively regulates Erbin expression via NF-κB. Although it has been reported that elevated Erbin promotes HCC tumorigenesis (18), the underlying mechanism via which

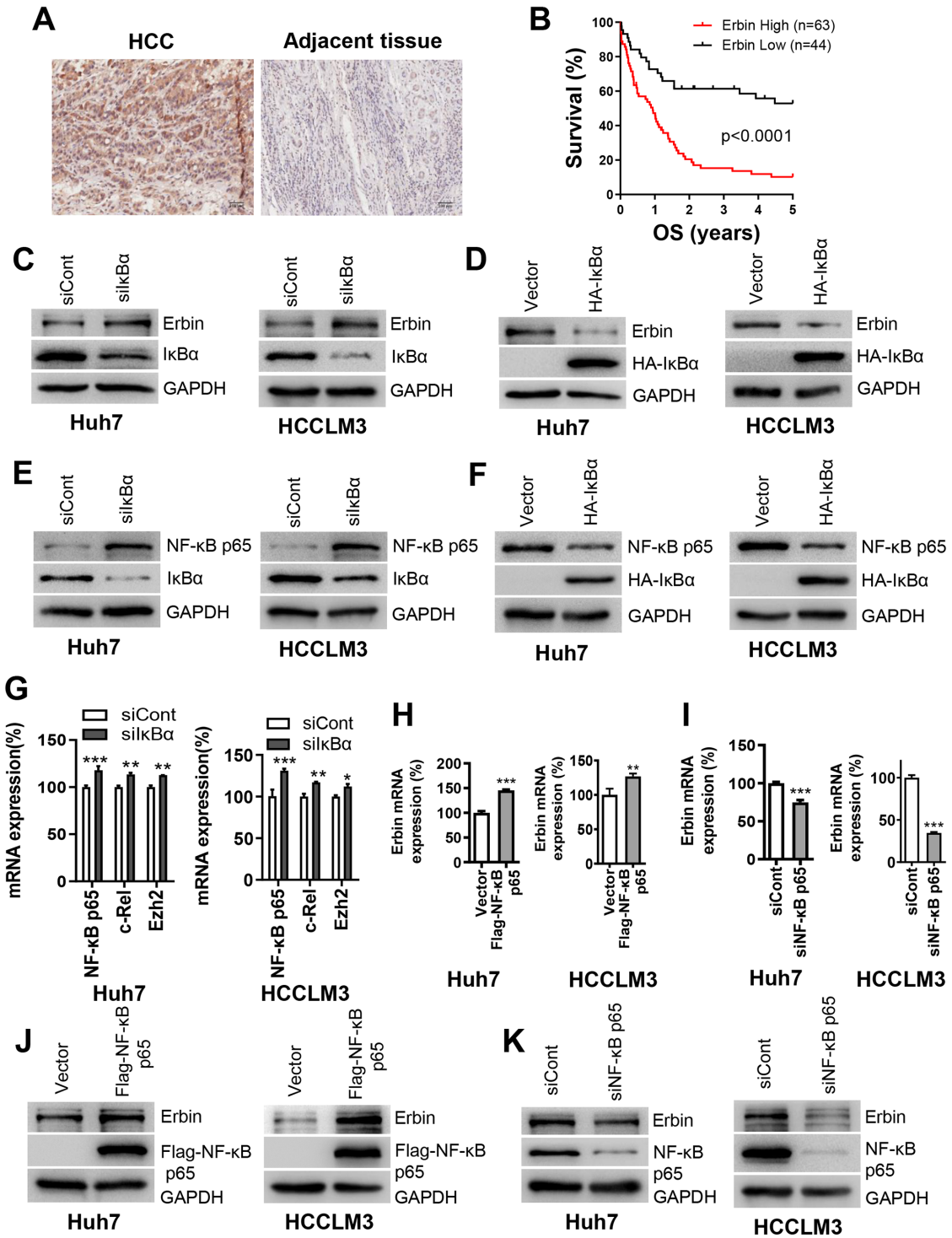

Figure 2. IκBα negatively regulates Erbin expression via NF-κB. (A) Representative images of HCC and adjacent liver tissues from 107 cases stained with anti-Erbin antibody. (B) Association between OS time of 107 patients with HCC and Erbin expression. Cells were transfected with (C) siIκBα or (D) HA-IκBα for 48 h and Erbin expression was determined using western blot analysis. Cells were transfected with (E) siIκBα or (F) HA-IκBα for 48 h and NF-κB p65 expression was determined using western blot analysis. (G) Cells were transfected with siIκBα for 48 h and mRNA expression levels of c-Rel and Ezh2 were assessed using RT-qPCR. Cells were transfected with (H) Flag-NF-κB p65 or (I) siNF-κB p65 for 48 h and then the mRNA expression of Erbin was measured using RT-qPCR. Cells were transfected with (J) Flag-NF-κB p65 or (K) siNF-κB p65 for 48 h, then Erbin expression was determined using western blot analysis. Scale bar, 100 μm. The error bars represent SEM from three independent experiments. *P<0.05, **P<0.01 and ***P<0.001 vs. corresponding control group. siRNA, small interfering RNA; IκBα, NF-κB inhibitor α; Cont, control; HCC, hepatocellular carcinoma; OS, overall survival; Erbin, Erbb2 interacting protein; RT-qPCR, reverse transcription-quantitative PCR; Rel, Rel proto-oncogene, NF-kB subunit; Ezh2, enhancer of zeste 2 polycomb repressive complex 2 subunit.

Erbin is upregulated in HCC remains unknown. The results indicated that Erbin expression was upregulated in the HCC tissues from 107 patients in southwest of China (Fig. 2A). Kaplan-Meier survival analysis demonstrated that high expression of Erbin was positively associated with short survival time of patients with HCC (Fig. 2B). Moreover, expression of Erbin

was associated with malignant characteristics of HCC, such as TNM stage, existence of vascular tumor thrombi and cancer recurrence (Table II). As both IκBα and Erbin are associated with poor survival of patients with HCC, it was determined whether there was an association between IκBα and Erbin. It was found that knockdown of IκBα promoted Erbin protein expression, while overexpression of IκBα reduced this expression (Fig. 2C and D).

NF-κB/Rel transcription factors present in the cytosol in an inactive state form a complex with inhibitory IκB proteins (7). It has been suggested that NF-κB p65 is released from combination with IκBα and nuclear translocation occurs when IκBα is downregulated in HCC (6). The results indicated that knockdown of IκBα increased NF-κB p65 expression (Fig. 2E), while overexpression of IκBα inhibited NF-κB p65 expression (Fig. 2F). Subsequently, NF-κB activation upon IκB knockdown was examined, as indicated by activation of canonical and non-canonical transcriptional targets of NF-κB, c-Rel and Ezh2 after transfection of siIκBα (Fig. 2G).

To assess whether the transcription factor NF-κB regulates Erbin, the effect of knockdown or overexpression of NF-κB on Erbin at the mRNA and protein levels was measured. In contrast to IκBα, overexpression of NF-κB enhanced both mRNA and protein expression levels of Erbin, whereas knockdown of NF-κB inhibited these expression levels (Fig. 2H-K). Collectively, the results indicated that reduced IκBα promoted NF-κB-mediated Erbin expression at both mRNA and protein levels.

IκBα is negatively associated with Erbin in patients with HCC. To further evaluate the association between IκBα and Erbin, the expression levels of IκBα and Erbin were analyzed in 107 HCC tissues using IHC staining. It was determined that 77.6% of HCC samples were IκBα-negative or low expression, demonstrating active NF-κB signaling in the majority of cases (Fig. 3A and B). In line with this finding, Erbin protein expression in 58.9% (63/107) of cases was upregulated compared with the adjacent tissues. Thus, there was a negative association between IκBα and Erbin expression levels in HCC tissues (Fig. 3).

Erbin is responsible for NF-κB-mediated HCC cell proliferation and migration. Based on the oncogenic function of NF-κB (22) and its positive regulation over Erbin, it was evaluated whether Erbin was responsible for NF-κB-mediated cell proliferation and migration. Overexpression of NF-κB upregulated Erbin expression, and overexpression of Erbin exerted oncogenic effects on the proliferation and migration of HCC cells. However, knockdown of Erbin attenuated the aforementioned cellular abilities (Fig. 4A-F). In addition, increased proliferation and migration of HCC cells by NF-κB overexpression was reversed by Erbin knockdown (Fig. 4G-I), indicating that NF-κB promoted its activities via Erbin signaling. In healthy liver tissues, expression of IκBα limited the activity of NF-κB (6) and, in turn, its downstream target Erbin according to the present results. However, in HCC, NF-κB was abnormally activated due to the absence of IκBα, and transcriptionally upregulated expression of Erbin stimulated the proliferation and migration of HCC cells.

Table II. Association between Erbin expression and clinicopathological variables of 107 patients with HCC.

Parameter	Erbin expression		χ^2	P-value
	High (n=63)	Low (n=44)		
Sex			0.195	0.659
Male	54	39		
Female	9	5		
Age, years			0.009	0.926
≤50	39	27		
>50	24	16		
TNM stage			4.541	0.033[a]
I	20	23		
II-IV	43	21		
Tumor thrombi			4.739	0.029[a]
Yes	22	7		
No	41	37		
Histological grade			0.000	0.988
I	7	4		
II-III	56	40		
Intrahepatic metastasis			2.640	0.014
Yes	15	5		
No	48	39		
Tumor recurrence			9.165	0.002[a]
Yes	48	21		
No	15	23		

Statistical analyses were performed using Pearson χ^2 test. [a]P<0.05. Erbin, Erbb2 interacting protein.

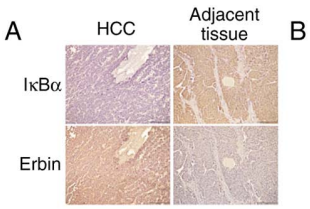

Figure 3. IκBα is negatively associated with Erbin in patients with HCC. (A) Representative images of HCC and adjacent liver tissues from 107 cases stained with anti-IκBα and anti-Erbin antibodies. (B) There was a negative association between IκBα and Erbin expression levels. Scale bar, 100 μm. Erbin, Erbb2 interacting protein; IκBα, NF-κB inhibitor α; HCC, hepatocellular carcinoma.

Discussion

As HCC is a serious and lethal disease worldwide (1), and thus it is important to determine a novel therapeutic target. The present study identified that in HCC tissues, there was low expression of IκBα, the inhibitor of NF-κB, and decreased expression of IκBα promoted cancer cell proliferation and migration. Once IκBα undergoes proteasomal degradation, NF-κB activity is initiated (6), and positively affects cell proliferation and

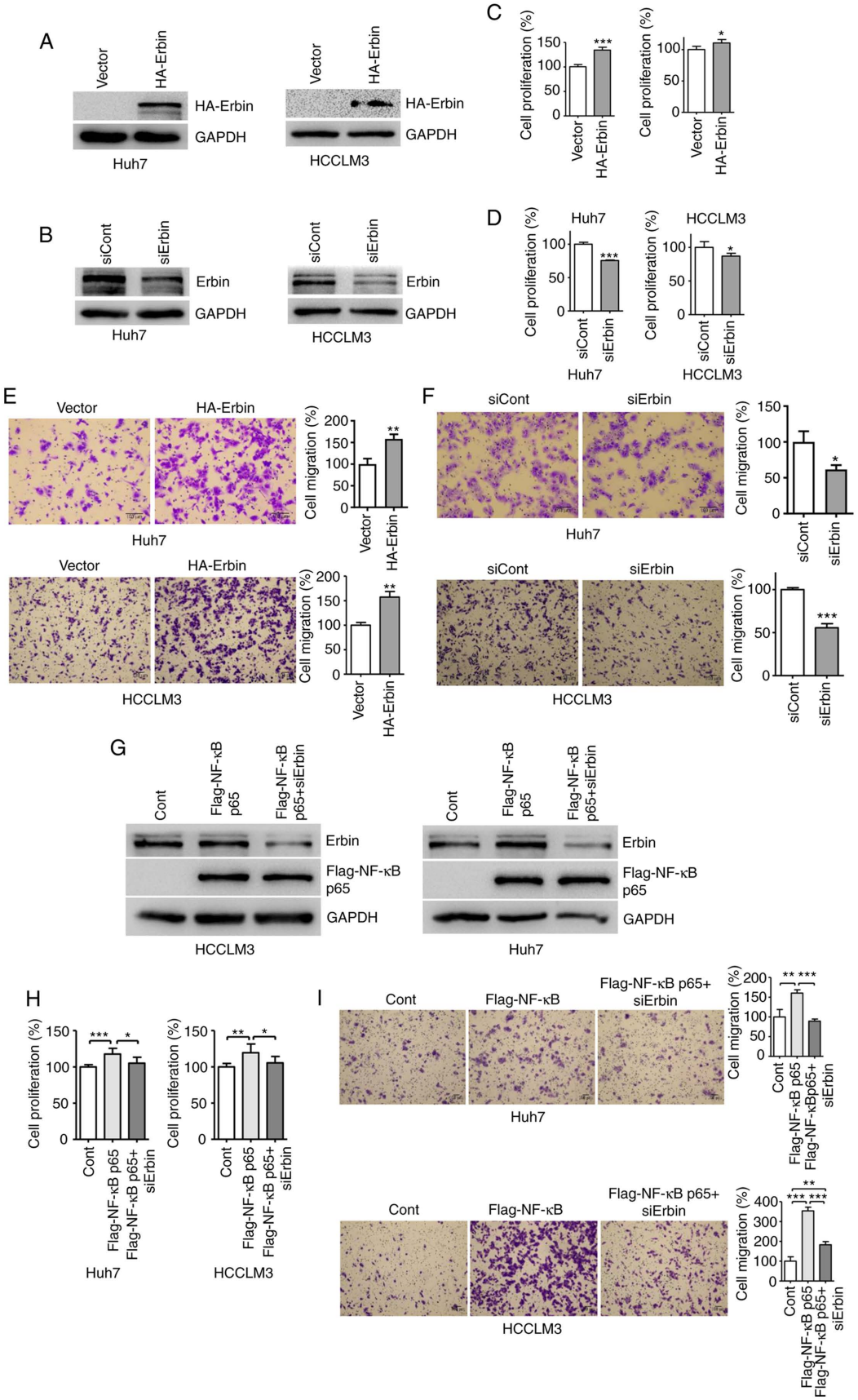

Figure 4. Erbin is responsible for NF-κB-mediated HCC cell proliferation and migration. Cells were transfected with (A) HA-Erbin or (B) siErbin for 48 h and Erbin expression was determined by western blotting. Cells were transfected with (C) HA-Erbin or (D) siErbin and cell proliferation was assessed after 24 h of transfection using CCK-8 assay. After transfection with (E) HA-Erbin or (F) siErbin for 48 h, cells were seeded into Transwell plate for 24 h and stained with 0.1% crystal violet staining solution. Cells were transfected with Flag-NF-κB p65, with or without Erbin siRNA, and analyzed using (G) western blotting, (H) CCK-8 assay or (I) Transwell migration assay. Scale bar, 100 μm. The error bars represent SEM from three independent experiments. *P<0.05, **P<0.01 and ***P<0.001 vs. corresponding control group. siRNA, small interfering RNA; IκBα, NF-κB inhibitor α; Cont, control; Erbin, Erbb2 interacting protein; CCK-8, Cell Counting Kit-8.

migration by promoting the expression of Erbin. In the current study, consistent with previous reports of increased Erbin expression in HCC tissues obtained from Shanghai (18), the results indicated Erbin expression was upregulated in HCC tissues from 107 patients in southwest of China and exerted oncogenic functions. Moreover, downregulation of IκBα and upregulation of Erbin were closely associated with poor prognosis of patients with HCC. Therefore, the present results demonstrated the clinical importance of IκBα and Erbin, identified a novel association between IκBα/NF-κB and indicated the role of the downstream oncoprotein Erbin in the promotion of HCC tumorigenesis.

IκBα is considered to be a tumor-suppressing factor in different types of cancer, such as breast (23,24), ovarian (25), gastric (26) and colorectal (27), mainly due to its inhibition of NF-κB activity; however, the underlying mechanism is yet to be elucidated. Liver tumorigenesis is usually attributed to inflammation (28). Cell proliferation increases during inflammation, in which accelerated DNA replication results in the increased occurrence of oncogenic mutation (29). Moreover, NF-κB, as a hallmark of inflammation, not only participates in the initiation, but also maintains inflammation via blocking apoptosis, together with TNFα to activate anti-apoptosis genes (30). Thus, NF-κB serves a crucial role in promoting inflammation and contributing to malignancy (31).

Erbin has been reported to exert a range of diverse functions. For example, Erbin suppresses the activity of the Ras/Raf pathway to inhibit proliferation and stop epithelial-mesenchymal transition progress in colorectal cancer (32). Deficiency of Erbin significantly promotes ERK phosphorylation in ERBB2-overexpressed breast cancer (33). Furthermore, accumulation of Erbin attenuates Raf activation to halt autophagy and senescence in KRAS proto-oncogene, GTPase-induced skin tumorigenesis (34). Erbin also exerts a pro-tumorigenesis role by negatively regulating ERα (18). Therefore, these findings suggest that the function of Erbin depends closely on cell types or a certain cancer microenvironment. The current study demonstrated a novel signaling via which NF-κB promotes tumorigenesis. It was indicated that in HCC, Erbin is highly expressed and associated with poor prognosis of patients. In HCC cells, it was identified that NF-κB activated the expression of Erbin at both the mRNA and protein levels, and overexpression of Erbin enhanced cell proliferation and migration, while its knockdown reduced these abilities.

In conclusion, the present study identified that: i) IκBα was decreased in HCC and associated with poor survival of patients with HCC; ii) knockdown of IκBα upregulated NF-κB signaling to promote HCC cell proliferation and migration; and iii) NF-κB-mediated Erbin upregulation contributes to HCC cell proliferation and migration and a poor outcome of patients with HCC. Therefore, the present results indicated the important clinical implications of IκBα and Erbin, as well as the novel NF-κB/Erbin oncogenic pathway. Thus, disruption of this signaling pathway may be a novel strategy to slow the progression of HCC.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

CMX designed and supervised the study. YT, XTL, YDL, JZ, LF, YYZ, HQY and LDZ performed the experiments. YT, YYZ, HQY and CMX contributed to immunohistochemistry analysis and pathological analysis. YJ and LS contributed to the clinical sample collection, fixing and embedding, and pathological information processing. YT, PB and CMX analyzed and interpreted the data. YT and CMX drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Southwest Hospital. The informed consent forms were obtained from the patients with HCC.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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