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KLF5 silence attenuates proliferation and epithelial-mesenchymal transition induction in Hep-2 cells through NF-κB signaling pathway

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Abstract. – OBJECTIVE: This study aimed at exploring the role and mechanism of Krüppel-like factor 5 (KLF5) in the migration, invasion, epithelial-mesenchymal transition (EMT) induction and proliferation in laryngeal cancer human epithelial type 2 (Hep-2) cells, and to provide a new sight for the treatment of laryngeal carcinoma.

MATERIALS AND METHODS: Hep-2 cells were randomly divided into three groups: control group (Control), KLF5 siRNA group (siKLF5) and control-siRNA group (NC). The effects of KLF5 inhibition on cell proliferation and apoptosis were assessed by 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and flow cytometer, respectively. Wound healing assay and transwell invasion experiments were used to determine cell migration and invasion. Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) and Western blot were used to compare the levels of KLF5, EMT-related genes E-cadherin, N-cadherin, Vimentin and Zinc finger transcription factors (Snail, Slug) expressions. The levels of nuclear transcription factor-κB (NF-κB-p65) and IκBα were also detected by Western blot.

RESULTS: Compared with the Control group, the proliferation rate of Hep-2 cells in the siKLF5 group was significantly decreased while the apoptosis rate was increased ($p<0.05$). Meanwhile, the migration and invasion ability of Hep-2 cells were markedly decreased ($p<0.05$). E-cadherin protein expression was up-regulated while Vimentin, N-cadherin, Snail, and Slug protein expression levels were downregulated in siKLF5 group ($p<0.05$). Silencing KLF5 could inhibit the expression of NF-κB phosphorylation at p65 and the IκBα degradation ($p<0.05$).

CONCLUSIONS: These results revealed that silencing KLF5 expression reduced the proliferation, migration and invasion and EMT abilities by inhibiting the NF-κB pathway in Hep-2 cells. Our results suggest that KLF5 may be a potential therapeutic target in laryngeal carcinoma.

Key Words
KLF5, Laryngeal carcinoma, Cell survival, Nuclear transcription factor-κB, Epithelial-mesenchymal transition.

Introduction

Laryngeal carcinoma, which was classified as the head and neck tumors, is a common cause of death and disability in the world and seriously harms human health[1,2]. The surveys have demonstrated that laryngeal carcinoma has a high incidence and there are about 25% undergoing new patients with nasopharyngeal carcinoma increased every year[3,4]. Treatment for laryngeal carcinoma usually includes a combination of surgery, radiation therapy and chemotherapy. To date, although a great deal of surgical applications and chemotherapeutic drugs has been used in laryngeal carcinoma patients, the survival rate of patients with metastatic laryngeal carcinoma remains dismal[5-7]. Even more, surgery is always associated with the loss of language function, and patients undergoing radiotherapy treatment are prone to relapse[8,9]. In the past years, the pathophysiologic mechanisms of the development of laryngeal carcinoma have been extensively studied. In addition to the above conventional treatments, targeted therapy, also called molecularly targeted therapy, has become one of the major modalities of medical treatment for laryngeal carcinoma[10]. Recent evidence[11,12] demonstrates that the expression levels of several genes such as p16, p53, MGMT, GLUT-1 and HIF-1α are involved in the progress of a variety of cancer cells, but the molecular mechanisms responsible

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or laryngeal carcinoma proliferation and metastasis are still not fully understood[13]. Thus, there is an urgent need to identify new target molecules for improving treatment and overcoming therapy resistance of laryngeal cancer.

A number of studies[14-15] have implicated that Krüppel-like factor (KLF) family, including series of zinc-finger-containing transcription factors, is an essential transcriptional activator which regulates diverse functions, including proliferation, self-renewal, survival, differentiation and development. KLF5 is a new potential target for cancer treatment clinically which belongs to the KLF family and generally acts as a transcriptional activator. In previous studies[16-17], KLF5 is shown overexpression in different types of human malignant cancers and promotes cancer progression, such as intestine, colon, breast, bladder and pancreatic cancer. KLF5 functions as a tumor suppressor in carcinogenesis of various genes controlling different cellular processes including hormonal signaling, epithelial-to-mesenchymal transition, differentiation, protein stability and development.

So far, Ma et al studies[17] have shown that KLF5 may be specifically involved in the promotion of the malignant conversion of the nasopharyngeal epithelium, but there is no clear evidence of its effectiveness in laryngeal carcinoma treatment and the role of KLF5 in laryngeal carcinoma remains unclear. Therefore, in this work, we established the KLF5 siRNA model to determine the role and mechanism of KLF5 in Hep-2 cells, as well as providing a theoretical basis for exploring the molecular mechanisms of laryngeal carcinoma.

Materials and Methods

Cell Culture

Hep-2 cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). The cell culture was performed using the Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, Grand Island, NY, USA) with 10% fetal bovine serum (Hyclone, South Logan, UT, USA), 100 μg/ml streptomycin and 100 IU/ml penicillin. Cells were cultured at 37°C and 5% CO_2 and collected at logarithm phase for further experiments.

Transient Transfection of Hep-2 Laryngeal Carcinoma Cells

Hep-2 cells were randomly divided into three groups: control group (Control), KLF5 siRNA group (siKLF5), and control-siRNA group (NC). The cell models were established by SiRNAs targeting KLF5 and control siRNAs (scrRNA; Shanghai GenePharma Co., Ltd. Shanghai, China). The sequence of the KLF5 siRNA was 5'-GACCAC-CGACAGATACGTG-3'. Each cell line was established by transfection of 8 μl siRNA/4 μg DNA. Also, Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) were added following the manufacturer's instruction as KLF5 siRNA group (siKLF5). A scrambled siRNA sequence was cloned into the lentivirus vector (Shanghai GeneChem Co., Ltd. Shanghai, China) as control-siRNA group (NC). The expressions of KLF5 mRNA were examined by quantitative Real Time-Polymerase Chain Reaction and Western blot.

Cell Viability Assay

Cell viability was detected by methyl thiazolyl tetrazolium (MTT) assay. Hep-2 cells (2×10^3 per well) were seeded into 96-well plates. Following this, 10 μl of MTT reagent (5 mg/ml) was added and incubated for another 4 h in 37°C, 5% CO_2. After removing the culture medium, 150 μl of dimethyl sulfoxide (DMSO) was added, then the plate was shaken in the dark for 15 min. The optical density (OD) values were measured at the absorbance of 490 nm by a SpectraMax 190 Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). The cell viability was calculated using the following equation: cell viability = $(OD_{treated}—OD_{blank})/(OD_{control}—OD_{blank}) \times 100\%$.

Wound Healing Assay

Cells were digested with 0.25% trypsin-EDTA solution (Sigma-Aldrich, St. Louis, MO, USA), centrifuged (1000 rpm, 5 min), the pellets were collected and resuspended to 3×10^5 cells/ml. 1 ml of cell suspension was added to 6-well plate to get 100% confluence within 24 h. Scratch was performed using a 10 μl pipette tip; the cells were washed twice with 1 × Phosphate-Buffered Saline (PBS; Gibco, Grand Island, NY, USA) to remove suspending cells; the serum-free medium was slowly added and incubated at 37°C in a humidified incubator with 5% CO_2 for 0 h, 24 h; then, photos were taken for analysis.

Transwell Invasion Assay

Cells were collected using a 0.25% trypsin-EDTA solution, the cell density was 2×10^5/ml. 50 μl of Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) was added on the membrane of the upper chamber and solidified at 37°C for 30 min. 600 μl of complete medium containing

10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) was added to a 24-well plate and 100 µl of cell suspension was seeded on the membrane, incubated at 37°C in an atmosphere of humidified air and 5% CO_2 incubator. Transwells were collected 24 h after incubation, fixed in cold methanol for 30 min at -20°C, then stained with 0.5% crystal violet (Solarbio, Beijing, China) at room temperature for 20 min. Invasive cells were observed under an optical microscope (Olympus, Tokyo, Japan). Nine bright fields were randomly selected for photographing to calculate the number of invading cells on the membrane.

Quantitative Real Time-Polymerase Chain Reaction Analysis

A TRIzol kit (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from cells. RNAs were then reverse-transcribed to cDNA using the SuperScript III Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA, USA). Polymerase Chain Reaction (PCR) was performed using Mastercycler ep realplex2 (Eppendorf, Hamburg, Germany) using the following conditions: 95°C for 30 s, 95°C for 5 s, and 60°C for 45 s (40 cycles). Data analysis was carried out by the $2^{-\Delta\Delta Ct}$ method and β-actin mRNA was used as the internal control. Primer sequences used are listed in Table I.

Western Blot

Cells were collected as indicated and lysed in a radioimmunoprecipitation assay (RIPA; Beyotime, Shanghai, China) buffer with proteinase inhibitors. The protein concentration was determined by the bicinchoninic acid (BCA) method following the manufacturer's instructions; then, the protein samples were loaded on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred onto a polyvinylidene difluoride (PVDF) membrane (Merck, Darmstadt, Germany) at 80 V for 30 min, blocked for 1 h with 5% nonfat dry milk in a Tris-Buffered Saline and Tween (TBST; Sigma-Aldrich, St. Louis, MO, USA) solution. Antibodies were diluted with 3% bovine serum albumin in TBST. Antibodies included: E-cadherin (1:2000, SC-7870, Santa Cruz Biotechnology, Santa Cruz, CA, USA), N-cadherin (1:2000, ab18203, Abcam, Cambridge, MA, USA), Vimentin (1:2000, SC-6260, Santa Cruz Biotechnology, Santa Cruz, CA, USA), Snail (1:2000, ab31787, Abcam, Cambridge, MA, USA), Slug (1:2000, ab27568, Abcam, Cambridge, MA, USA), Phospho-NF-κB-p65 (1:2000, AF-3393, Affinity Biosciences, Cincinnati, OH, USA), NF-κB-p65 (1:2000, 06-418, Calbiochem, Darmstadt, Germany), and IκBα (1:1500, Assay-R12-2938, Los Angeles, CA, USA), incubated with horseradish peroxidase (HRP) goat anti-rabbit IgG (1:2000, Proteintech, Chicago, IL, USA) for 1 h. The electrochemiluminescence (ECL; Thermo Fisher Scientific, Waltham, MA, USA) system was used to detect the signal. The protein expression level was standardized with GAPDH, and grayscale scanning and quantification were performed by Image J (NIH) software (Bethesda, MD, USA).

Cell Apoptosis

Cell apoptosis was analyzed by flow cytometry. Cells were trypsinized, collected and counted. 1×10^6 cells were washed with cold 1×PBS twice and centrifuged at 1000 rpm for 5 min. Then,

Table I. Primers sequence (Shanghai Shenggong Biological Engineering Technology Service Co., Ltd.).

Name	Sequences
KLF5	Forward: 5′-ACACCAGACCGCAGCTCCA-3′ Reverse: 5′-TCCATTGCTGCTGTCTGATTTGTAG-3′
E-cadherin	Forward: 5'-TCC CAT CAGCTG CCC AGA AA-3' Reverse: 5'-TGA CTC CTG TGT TCC TGT TA-3'
Vimentin	Forward: 5'-CCA GTG CGTGAA ATG GAA G-3' Reverse: 5'-TCA AGG TCA TCG TGA TGC TG-3'
N-cadherin	Forward: 5′-GAC CCA GAA GAT GAT GTA AG-3′ Reverse: 5′-CTC AGC GTG GAT AGG C-3′
Snail	Forward: 5'-TCGGAAGCCTAACTACAGCGA-3' Reverse: 5'-AGATGAGCATTGGCAGCGAG-3'
Slug	Forward: 5'-TGTGACAAGGAATATGTGAGCC-3' Reverse: 5'-TGAGCCCTCAGATTTGACCTG-3'
β-actin	Forward: 5′-TTGCCGACAGGATGCAGAA-3′ Reverse: 5′-GCCGATCCACACGGAGTACT-3′

cells were re-suspended in 100 µl of 1×binding buffer, followed by adding 5 µl of Annexin V-fluorescein isothiocyanate (FITC) and 5 µl propidium iodide (PI). After incubation for 15 min, 400 µl 1 × binding buffer was added and the cell suspension was measured by flow cytometry (CyFlow Cube, CyFlow Ploidy Analyser, Partec, Germany).

Statistical Analysis

SPSS 20.0 (SPSS IBM, Armonk, NY USA) was used to analyze the monitoring data. The data analysis results were expressed as Mean ± standard deviation (mean ± SD). The *t*-test was used for the data analysis between the two groups. Multiple comparisons were evaluated by repeated measures analysis of variance (ANOVA). One-way ANOVA was used to compare the mean of multiple samples. The comparison between any two means was performed by the LSD method. The LSD method was applied in the comparison between the two groups. $p < 0.05$ was considered statistically significant.

Results

Expression of KLF5 in Hep-2 Cells

To determine the role of KLF5 in Hep-2 cells, we established stable siKLF5 cell models. Real Time-quantitative Polymerase Chain Reaction and Western blot confirmed the decrease of KLF5 in cells. As shown in Figure 1, it was verified that SiKLF5 resulted in a marked decrease in KLF5 mRNA levels (Figure 1A). Concomitantly, compared with the control group, the siKLF5 group showed a marked reduction of KLF5 protein ($p < 0.05$; Figure 1B). There was no difference between the NC group and the control group ($p > 0.05$; Figure 1).

SiKLF5 Inhibits the Proliferation of Hep-2 Cells

To verify the effects of KLF5 on the proliferation of Hep-2 cells, we first tested whether KLF5 inhibition was able to decrease the viability of Hep-2 cells. Compared with the control group, KLF5 inhibition caused a marked reduction of cell viability ($p < 0.05$; Figure 2). There was no difference between the NC group and the control group ($p > 0.05$; Figure 2).

SiKLF5 Promotes the Apoptosis of Hep-2 Cells

To verify the effects of KLF5 inhibition on suppressing Hep-2 progression, we tested whether KLF5 promoted cell apoptosis. Flow cytometry analysis of cells after Annexin-V-FITC/PI staining (Figure 3A, B, C) suggested a dramatically increased apoptosis rate with KLF5 inhibition (Figure 3D). Compared with the control group, KLF5 inhibition caused a marked promotion of cell apoptosis ($p < 0.05$; Figure 3D). There was no difference between the NC group and the control group ($p > 0.05$; Figure 3D). This evidence indicated that KLF5 inhibition exerted anti-tumor effects in Hep-2 cells.

SiKLF5 Inhibits Migration and Invasion of Hep-2 Cells

To determine the role of KLF5 in cell migration and invasion of Hep-2 cells, the wound scratch migration assay and transwell invasion

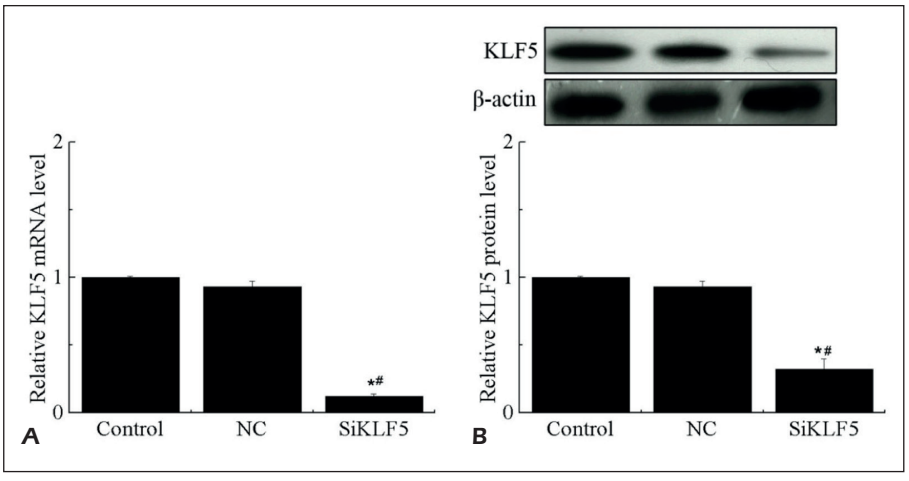

Figure 1. The relative expression of KLF5 in Hep-2 cells. **A**, Real Time-quantitative Polymerase Chain Reaction showed that the expression of KLF5 was down-regulated by siRNA. **B**, Western blot was performed to determine the KLF5 protein level in each group *$p<0.05$ *vs.* control group, $^{\#}p<0.05$ *vs.* NC group.

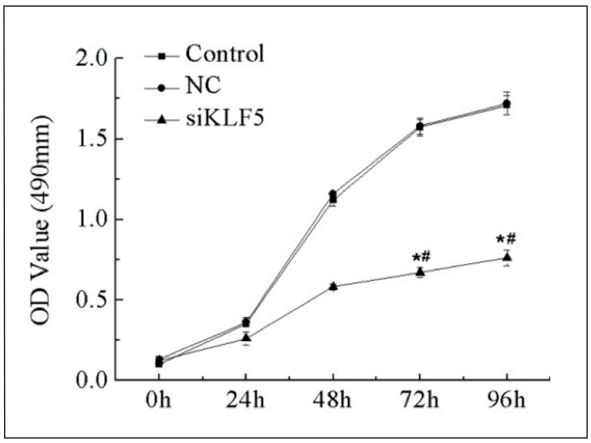

Figure 2. KLF5 inhibition attenuated Hep-2 cells viability. The cell viability of Hep-2 cells was analyzed by MTT assay. *p<0.05 *vs.* control group, #p<0.05 *vs.* NC group.

assay demonstrated that cells with KLF5-siRNA showed decreased cell migration and invasion capability (p < 0.05; Figure 4). There was no difference between the NC group and the control group (p > 0.05; Figure 4). These results suggested that SiKLF5 inhibits migration and invasion of Hep-2 cells.

KLF5 Regulates Expression of mRNAs and Proteins Related in EMT

To elucidate the suppressive effects of KLF5 inhibition on the cell epithelial-mesenchymal transition (EMT), we next examined how E-cadherin, N-cadherin, Snail, Slug and Vimentin expression changed after KLF5 inhibition. N-cadherin, Snail, Slug and Vimentin proteins demonstrated a significant decrease after KLF5 inhibition, while E-cadherin exhibited a significant upregulation (p < 0.05, Figure 5A). The decreased mRNA levels of N-cadherin, Vimentin, Snail, Slug (Figure 5B) and increased mRNA level of E-cadherin (Figure 5B) further verified this observation.

KLF5 Regulates Protein Levels of NF-κB-p65 and IκBα

To understand the mechanism by which SiKLF5 could inhibit cell proliferation and EMT, we examined the expression of NF-κB-p65, phosphor-NF-κB-p65 and its inhibitor, IκBα (Figure 6). The proteins levels were compared using the ratio of Phospho-NF-κB-p65, NF-κB-p65 and IκBα to β-actin to evaluate the activation of NF-κB in the Hep-2 cells. The results showed that SiKLF5 led to inhibition of the activation of phosphor-NF-κB-p65, NF-κB-p65 as well as the degradation of IκBα (p < 0.05; Figure 6).

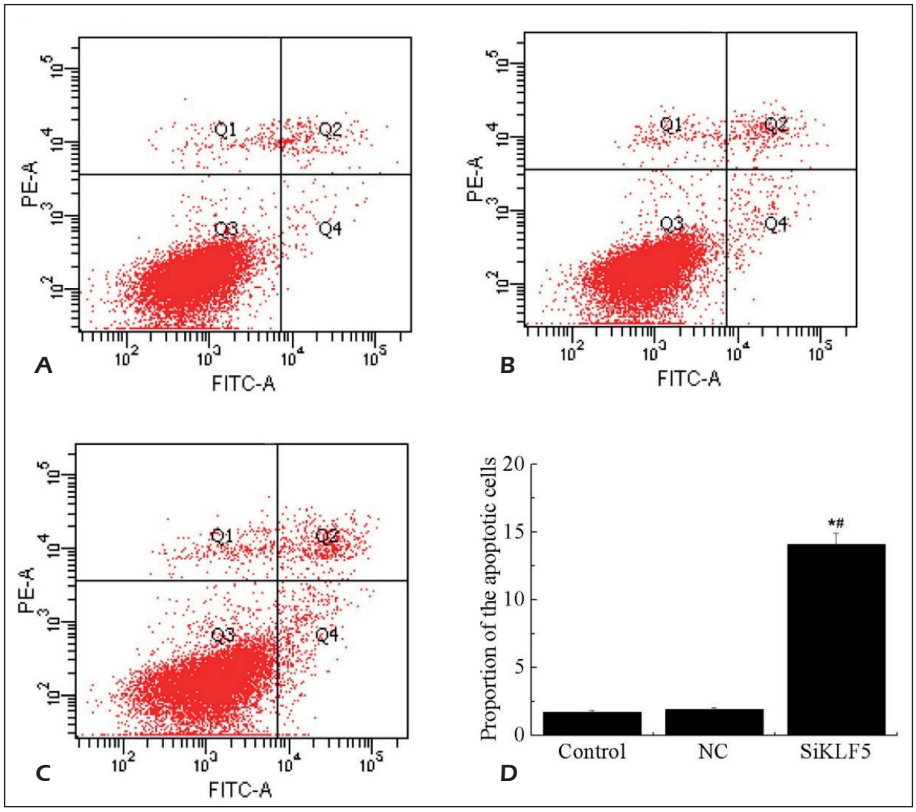

Figure 3. KLF5 inhibition promotes cell apoptosis. Representative flow cytometry graphs of Hep-2 cells, analyzed after Annexin V-FITC/PI staining. **A**, Control group. **B**, NC group. **C**, siKLF5 group. **D**, Quantitative comparison of apoptosis. *p<0.05 *vs.* control group, #p<0.05 *vs.* NC group.

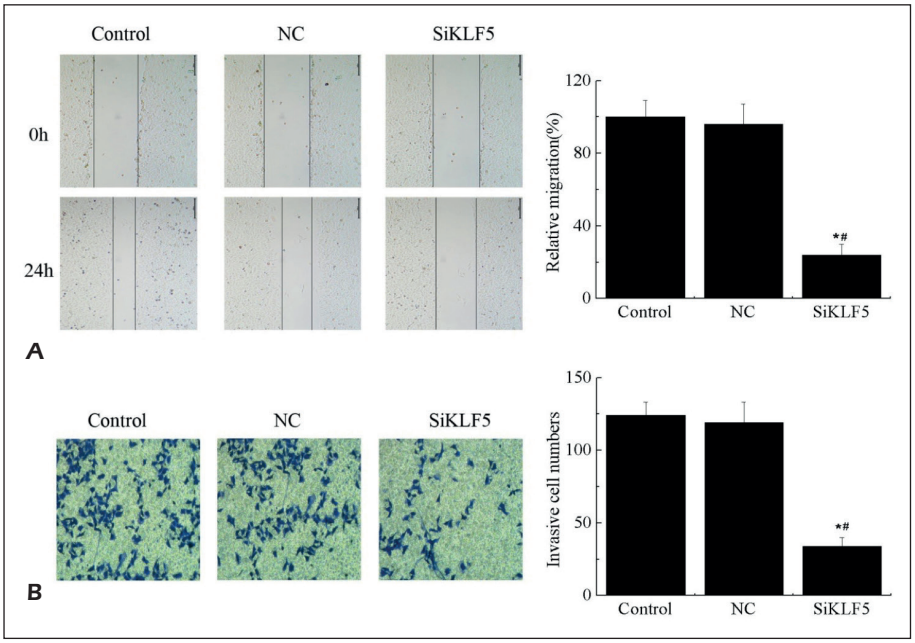

Figure 4. KLF5 inhibition attenuated migration and invasion of Hep-2 cells. A, Scratch migration assay showed KLF5 depletion decreases migration of Hep-2 cells. B, The transwell invasion assay showed KLF5 depletion decreases invasion of Hep-2 cells. (Magnification: 200×). *p<0.05 *vs.* control group, $^{\#}p$<0.05 *vs.* NC group.

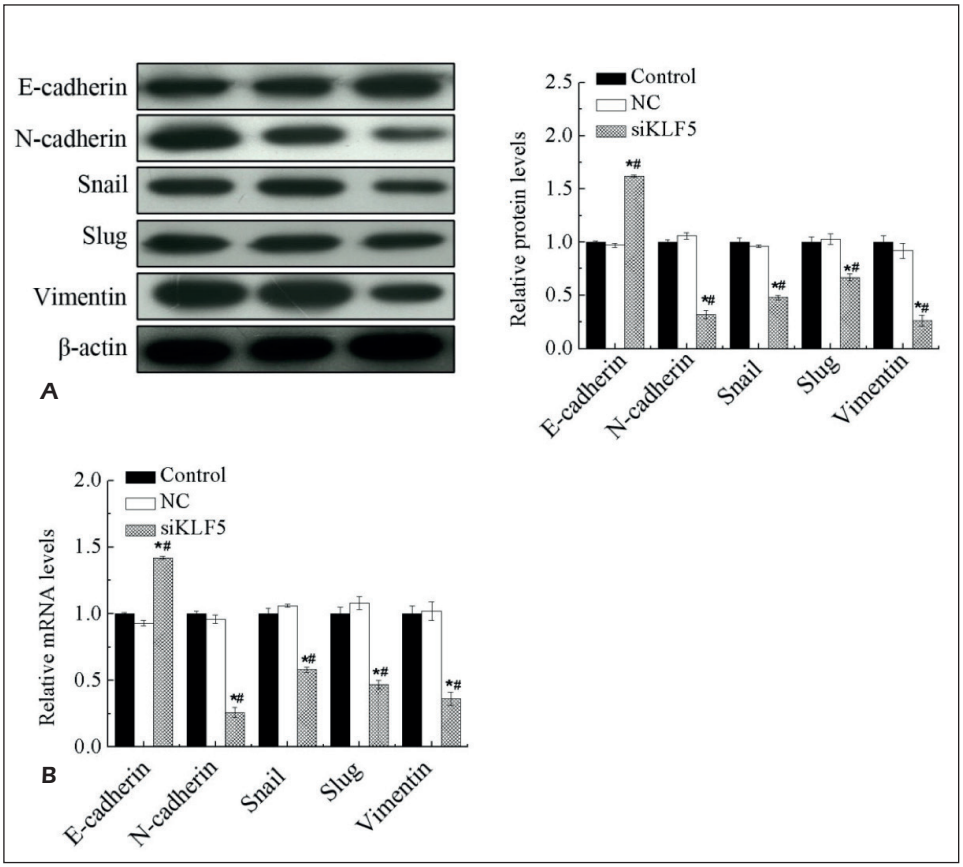

Figure 5. EMT-related expression levels in Hep-2 cells. **A**, Western blot was performed to determine the E-cadherin, N-cadherin, Vimentin, Snail and Slug protein level in each group. **B**, RT-PCR showed the expression of E-cadherin, N-cadherin, Vimentin, Snail and Slug mRNA levels. *p<0.05 *vs.* control group, $^{\#}p$<0.05 *vs.* NC group.

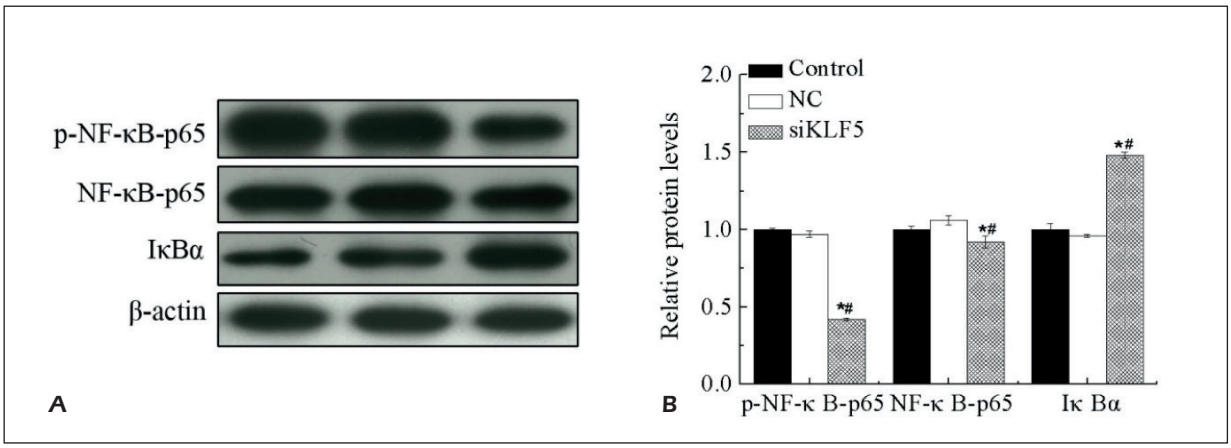

Figure 6. KLF5 regulates the protein levels of NF-κB-p65 and IκBα. **A**, Western blot was performed to determine the NF-κB-p65, NF-κB-p65 and IκBα protein level in each group. **B**, Quantification of the protein expression. *$p<0.05$ *vs.* control group, $^{\#}p<0.05$ *vs.* NC group.

Discussion

Laryngeal carcinoma is featured with high mortality and morbidity and the incidence of this disease is still rising because of the environment, heredity, lifestyle and so on[1,16]. Cancer invasion and metastasis are the most frequent reasons that result in high mortality. The mechanism of the invasion and metastasis is a multistep process that involves numerous biological signals and stimuli. Even though new technologies developed fast in recent years, the mechanisms of these procedures remain unclear[2,18]. In our work, we demonstrated that siRNAs targeting KLF5 could attenuate the proliferation, migration, invasion and EMT of Hep-2 cells. Our results also suggest that siKLF5 may modulate these responses, in part, by suppressing NF-κB p65 activation in Hep-2 cells[19,20]. To our best knowledge, this is the first study that demonstrated the effect of KLF5 on Hep-2 cells and described its underlying molecular mechanism.

We first showed that the proliferation of Hep-2 cells were suppressed by KLF5 inhibition, while the apoptosis was promoted. KLF5 has been recognized as a potential contributor in the regulation of cell proliferation, differentiation and metastasis[21]. Consistent with our result, KLF5 inhibition was shown to result in an attenuated proliferation and promoted apoptosis of Hep-2 cells. This result demonstrated that KLF5 may be a key contributor to the unrestricted growth of Hep-2 cells.

Metastatic cells possess enhanced cell migration ability and enhanced tendency for invasive growth. The previous studies showed that KLF5 could promote cancer cell migration and invasion by regulating the EMT process both *in vitro* and *in vivo*[22]. However, whether KLF5 plays different roles in laryngeal carcinoma hasn't been well studied. In the present work, the scratch migration assay and transwell invasion assay revealed that cells with KLF5-siRNA showed a decrease of migration, as well as invasion capability, confirming previously reported results. These results were in agreement with the previous studies[22]. This finding was further supported by silencing of KLF5 could regulate the EMT process. EMT is a biological process which is considered the first process for cancer invasion and migration[23]. Through EMT, the epithelial cells lose their polarity, the adherence and tight junction function and the cells migratory capacity was enhanced while the apoptosis rate was decreased[24]. Moreover, EMT is often accompanied by the regulations of several epithelial markers, such as the downregulation of E-cadherin and the production of EMT-inducing transcription factors (N-cadherin, Snail, Slug and Vimentin)[25-27]. They play an important role in enhancing cell motility and migration. Consistent with our study, we detected the increase of E-cadherin and the decrease of N-cadherin, Snail, Slug and Vimentin in the KLF5-siRNA group. KLF5 plays an important role in promoting invasion and metastasis of cancer cells by regulating E-cadherin expression and EMT process.

The activation of NF-κB is believed to play an important role in the development of laryngeal carcinoma[28]. The activation of the NF-κB was reported to trigger at the initiation of laryngeal carcinoma. In resting cells, NF-κB p65 is kept in

the cytoplasm through the interaction with its inhibitor, IκBa. Translocation of NF-κB is triggered by phosphorylation, polyubiquitination and subsequent degradation of IkBα[29,30]. In the present study, siKLF5 had up-regulated the expression of IκBα and reduced phosphorylation of IκBα. We also observed inhibited activities of NF-κB in Hep-2 cells as determined by Western blot and mRNA expression assays. These findings suggest that siKLF5 limited proliferation and EMT by suppressing the NF-κB activation. The exact mechanism underlying the observed interaction between KLF5 and NF-κB needs further elucidations. Regardless, the precise mechanism leading to inhibition KLF5 appears to be promising prophylaxis targeting at the NF-KB activation to reduce the Hep-2 cells proliferation, migration, invasion and EMT.

Conclusions

We revealed in the present study that KLF5 siRNA was potent in inhibiting Hep-2 cells proliferation, migration, invasion and EMT. KLF5 also suppressed the phosphorylation NF-κB p65/IκBα indicating that the inhibitory effect was partly operated by the blocking of NF-κB activation. These observations suggest that KLF5 may be a potential therapeutic target in laryngeal carcinoma.

Conflict of Interests

The authors declared there is no conflict of interest.

References

1) Habib A. Management of advanced hypopharyngeal carcinoma: systematic review of survival following surgical and non-surgical treatments. J Laryngol Otol 2018; 132: 385-400.
2) Okuyama K, Fujita S, Yanamoto S, Naruse T, Sakamoto Y, Kawakita A, Omori K, Tsuchihashi H, Umeda M. Unusual recurrent tongue spindle cell carcinoma with marked anaplasia occurring at the site of glossectomy for a well-differentiated squamous cell carcinoma: a case report. Mol Clin Oncol 2017; 7: 341-346.
3) Aghazadeh K, Dabiri Satri S, Sharifi A, Lotfi M, Maraghehpour B, Hashemiaghdam A. Predictors of thyroid gland invasion in laryngeal squamous cell carcinoma. Iran J Otorhinolaryngol 2018; 30: 153-158.
4) Zhou YH, Huang YY, Ma M. MicroRNA-138 inhibits proliferation and induces apoptosis of laryngeal carcinoma via targeting MAPK6. Eur Rev Med Pharmacol Sci 2018; 22: 5569-5575.
5) Iqbal MS, Chaw C, Kovarik J, Aslam S, Jackson A, Kelly J, Dobrowsky W, Kelly C. Primary concurrent chemoradiation in head and neck cancers with weekly cisplatin chemotherapy: analysis of compliance, toxicity and survival. Int Arch Otorhinolaryngol 2017; 21: 171-177.
6) Tan J, Jing YY, Han L, Zheng HW, Shen JX, Zhang LH, Yu LS. MicroRNA-203 inhibits invasion and induces apoptosis of laryngeal cancer cells via targeting LASP1. Eur Rev Med Pharmacol Sci 2018; 22: 6350-6357.
7) Joshi P, Joshi A, Norohna V, Chaturvedi P, Patil V, Agarwal JP, Juvekar S, Prabhash K. Role of neoadjuvant chemotherapy in advanced carcinoma of the hypopharynx and larynx. South Asian J Cancer 2017; 6: 15-19.
8) Winquist E, Agbassi C, Meyers BM, Yoo J, Chan KKW. Head and neck disease site group. Systemic therapy in the curative treatment of head and neck squamous cell cancer: a systematic review. J Otolaryngol Head Neck Surg 2017; 46: 29.
9) Aghazadeh K, Dabiri Satri S, Sharifi A, Lotfi M, Maraghehpour B, Hashemiaghdam A. Predictors of thyroid gland invasion in laryngeal squamous cell carcinoma. Iran J Otorhinolaryngol 2018; 30: 153-158.
10) Bryant AK, Sojourner EJ, Vitzthum LK, Zakeri K, Shen H, Nguyen C, Murphy JD, Califano JA, Cohen EEW, Mell LK. Prognostic role of p16 in nonoropharyngeal head and neck cancer. J Natl Cancer Inst 2018; 110: 1393-1399.
11) Edwards ZC, Trotter EW, Torres-Ayuso P, Chapman P, Wood HM, Nyswaner K, Brognard J. Survival of head and neck cancer cells relies upon LZK kinase-mediated stabilization of mutant p53. Cancer Res 2017; 77: 4961-4972.
12) Lv Y, Jia C, Jiang A, Zhang H, Wang Y, Liu F, Yang L, Sun Y, Lv R, Song X. Analysis of association between MGMT and p53 gene single nucleotide polymorphisms and laryngeal cancer. Anticancer Res 2017; 37: 4399-4403.
13) Tan J, Jing YY, Han L, Zheng HW, Shen JX, Zhang LH, Yu LS. MicroRNA-203 inhibits invasion and induces apoptosis of laryngeal cancer cells via targeting LASP1. Eur Rev Med Pharmacol Sci 2018; 22: 6350-6357.
14) Mao X, Miao S, He H, Miao S, Pei R, Yang X, Xiu C, Song K, Zhang J, Sun J, Jia S, Guan Q. Krüppel-like factor 5: a novel biomarker for lymph node metastasis and recurrence in supraglottic squamous cell laryngeal carcinoma. Tumour Biol 2014; 35: 623-629.
15) Azami T, Matsumoto K, Jeon H, Waku T, Muratani M, Niwa H, Takahashi S, Ema M. Klf5 suppresses ERK signaling in mouse pluripotent stem cells. PLoS One 2018; 13: e0207321.
16) Ma Y, Wang Q, Liu F, Ma X, Wu L, Guo F, Zhao S, Huang F, Qin G. KLF5 promotes the tumorigenesis and metastatic potential of thyroid cancer cells through the NF-κB signaling pathway. Oncol Rep 2018; 40: 2608-2618.

17) Ma D, Chang LY, Zhao S, Zhao JJ, Xiong YJ, Cao FY, Yuan L, Zhang Q, Wang XY, Geng ML, Zheng HY, Li O. KLF5 promotes cervical cancer proliferation, migration and invasion in a manner partly dependent on TNFRSF11a expression. Sci Rep 2017; 7: 15683.
18) Hayashi S, Manabe I, Suzuki Y, Relaix F, Oishi Y. Klf5 regulates muscle differentiation by directly targeting muscle-specific genes in cooperation with MyoD in mice. Elife 2016; 5.
19) Jeon H, Waku T, Azami T, Khoa le TP, Yanagisawa J, Takahashi S, Ema M. Comprehensive identification of Krüppel-like factor family members contributing to the self-renewal of mouse embryonic stem cells and cellular reprogramming. PLoS One 2016; 11: e0150715.
20) Farrugia MK, Vanderbilt DB, Salkeni MA, Ruppert JM. Kruppel-like pluripotency factors as modulators of cancer cell therapeutic responses. Cancer Res 2016; 76: 1677-1682.
21) Zinovyeva MV, Nikolaev LG, Kondratyeva LG, Vinogradova TV, Sverdlov ED. Correlation between expression of KLF5 and ZEB1 transcription factor genes in pancreatic cancer. Dokl Biochem Biophys 2018; 481: 219-221.
22) Liu JY, Lu JB, Xu Y. MicroRNA-153 inhibits the proliferation and invasion of human laryngeal squamous cell carcinoma by targeting KLF5. Exp Ther Med 2016; 11: 2503-2508.
23) Drosatos K, Pollak NM, Pol CJ, Ntziachristos P, Willecke F, Valenti MC, Trent CM, Hu Y, Guo S, Aifantis I, Goldberg IJ. Cardiac myocyte KLF5 regulates ppara expression and cardiac function. Circ Res 2016; 118: 241-253.
24) Zhao X, Yu D, Yang J, Xue K, Liu Y, Jin C. Knockdown of Snail inhibits epithelial-mesenchymal transition of human laryngeal squamous cell carcinoma Hep-2 cells through the vitamin D receptor signaling pathway. Biochem Cell Biol 2017; 95: 672-678.
25) von Scotti F, Kapsreiter M, Scherl C, Iro H, Bohr C. A 9-year analysis of transoral laser microsurgery (TLM) of head and neck cancer on their potential suitability for transoral robotic surgery (TORS) for estimation of future TORS-specific caseload. Eur Rev Med Pharmacol Sci 2018; 22: 2949-2953.
26) Zhang L, Sun J, Wang B, Ren JC, Su W, Zhang T. MicroRNA-10b triggers the epithelial-mesenchymal transition (EMT) of laryngeal carcinoma Hep-2 cells by directly targeting the E-cadherin. Appl Biochem Biotechnol 2015; 176: 33-44.
27) Zhao C, Wang XB, Zhang YH, Zhou YM, Yin Q, Yao WC. MicroRNA-424 inhibits cell migration, invasion and epithelial-mesenchymal transition in human glioma by targeting KIF23 and functions as a novel prognostic predictor. Eur Rev Med Pharmacol Sci 2018; 22: 6369-6378.
28) Pradère JP, Hernandez C, Koppe C, Friedman RA, Luedde T, Schwabe RF. Negative regulation of NF-κB p65 activity by serine 536 phosphorylation. Sci Signal 2016; 9: ra85.
29) Zheng JW, Meng B, Li XY, Lu B, Wu GR, Chen JP. NF-κB/P65 signaling pathway: a potential therapeutic target in postoperative cognitive dysfunction after sevoflurane anesthesia. Eur Rev Med Pharmacol Sci 2017; 21: 394-407.
30) Christian F, Smith EL, Carmody RJ. The regulation of NF-κB subunits by phosphorylation. Cells 2016; 5: 10.3390/cells5010012.