European Review for Medical and Pharmacological Sciences 2018; 22: 2671-2679

Long non-coding RNA SNHG15 indicates poor prognosis of non-small cell lung cancer and promotes cell proliferation and invasion

Y.-Z. DONG[1], X.-M. MENG[2], G.-S. LI[3]

[1]Department of Thoracic Surgery, Yantai Yuhuangding Hospital, Yantai, China
[2]Department of Endocrinology, Yantai Yuhuangding Hospital, Yantai, China
[3]Department of Joint Orthopaedics, Yantai Yuhuangding Hospital, Yantai, China

Abstract. – OBJECTIVE: To investigate the expression of human long non-coding ribonucleic acid (RNA) small nucleolar RNA host gene 15 (SNHG15) in non-small cell lung cancer (NSCLC) tissues and its prognostic significance, and to study the influencing mechanism of SNHG15 on biological functions in lung cancer cell lines.

PATIENTS AND METHODS: The expression levels of SNHG15 in 49 pairs of lung cancer tissues and para-carcinoma tissues were detected via quantitative real-time polymerase chain reaction (qRT-PCR). The lung cancer cells were transiently transfected with small-interfering (si)-SNHG15 using RNA interference technique. The effect of si-SNHG15 on the proliferation of lung cancer cells was observed via methyl thiazolyl tetrazolium (MTT) assay, its effect on apoptosis of A549 cells was detected via Hoechst 33342 staining and flow cytometry, and its effects on invasion and migration of A549 cells were studied via wound healing assay and transwell assay.

RESULTS: Results of qRT-PCR showed that the expression of SNHG15 in cancer tissues was increased compared with that in para-carcinoma tissues. Results of cell counting kit-8 (CCK-8) assay showed that knocking down SNHG15 could significantly inhibit the proliferation of lung cancer A549 cells. Hoechst 33342 staining and flow cytometry revealed that knocking down SNHG15 could significantly promote apoptosis of A549 cells. Wound healing assay and transwell assay revealed that knocking down SNHG15 could significantly inhibit the invasion and metastasis capacities of lung cancer A549 cells. Results of Western blotting showed that knocking down SNHG15 could inhibit the invasion and metastasis of A549 cells through inhibiting the expressions of epithelial-mesenchymal transition (EMT), matrix metalloproteinase-2 (MMP-2) and MMP-9 in cells.

CONCLUSIONS: The expression of SNHG15 in lung cancer tissues is significantly higher than that in para-carcinoma tissues, the prognosis of patients accompanied with a high expression of SNHG15 is poor, and knockdown of SNHG15 in A549 cells can inhibit cell proliferation, invasion, and metastasis, and promote apoptosis.

Key Words:
Non-small cell lung cancer, Long non-coding RNA, SNHG15, Cell proliferation, Cell invasion.

Introduction

Lung cancer is a kind of malignant tumor with the highest morbidity and mortality rates in the world, seriously threatening human health and life. According to the conventional pathological classification, lung cancer can be mainly divided into non-small cell lung cancer (NSCLC) and SCLC, the former of which is the most common pathological type in clinic[1]. Although great progress has been made in lung cancer surgery, chemotherapy, radiotherapy, immunotherapy, and other fields in recent years, the prognosis of NSCLC is not satisfactory. According to statistics, the 5-year survival rate of NSCLC is less than 15%, and about 30.55% patients with NSCLC receiving operative treatment will have progression and recurrence[2]. Therefore, clarifying the key molecular biological mechanism of NSCLC cell proliferation, apoptosis, and metastasis will provide a theoretical basis for elucidating the pathogenesis of NSCLC, and also provide references for the development of new therapeutic targets.

Long non-coding ribonucleic acid (lncRNA) is a kind of conserved non-coding RNA with 200 nucleotides in length, which is widely involved in tissue differentiation, cell proliferation, embryonic development, and other processes[3]. LncRNA small nucleolar RNA host gene 15 (SNHG15) is located on chromosome 7p13, which is involved in

Corresponding Author: Guishi Li, MD; e-mail: ytmxm@126.com

the regulation of cell proliferation, apoptosis and tumor occurrence and development in liver cancer, gastric cancer, etc[4,5]. However, the role of lncRNA SNHG15 in NSCLC has not been reported yet.

This study aims to explore the influences of lncRNA SNHG15 on the prognosis of NSCLC and proliferation, apoptosis and invasion capacities of NSCLC cells *in vitro*, as well as its possible mechanism.

Patients and Methods

NSCLC Sample Collection

In this investigation, 49 pairs of lung cancer tissue and para-carcinoma tissue samples were collected from 49 patients with lung cancer receiving operation in our hospital from January 2007 to December 2012. All patients were pathologically diagnosed, and they underwent no neoadjuvant chemotherapy, radiotherapy or any other form of specific treatment for tumors before the operation. The cases were staged according to the lung cancer staging criteria (the 8th edition) of the American Joint Committee on Cancer (AJCC). After surgical resection, samples were immediately cryopreserved in liquid nitrogen and, then, transferred into a refrigerator at -80°C. All patients signed the informed consent. This study was approved by the Ethics Committee of Yantai Yuhuangding Hospital.

Materials

Lung adenocarcinoma cell lines (PC9, SPC-A1 and A549) and lung squamous cell carcinoma cell lines (H1703 and SK-MES-1) were all purchased from Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Hyclone (South Logan, UT, USA). The primary antibody used in Western blotting was bought from BD (Franklin Lakes, NJ, USA). Horseradish peroxidase (HRP)-labeled secondary antibody was bought from Wuhan Boster Biological Technology Co., Ltd. (Wuhan, China). TRIzol reagent was purchased from Invitrogen (Carlsbad, CA, USA). SNHG15 small-interfering RNA (siRNA) and control siRNA sequences were synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China).

Cell Culture and Transfection

Cell lines were cultured in DMEM containing 10% FBS with 5% CO_2 at 37°C. After A549 cells grew to the logarithmic phase, they were inoculated into a 6-well plate (3×10^4 cells per well) and kept warm at 37°C overnight. Then, A549 cell lines were divided into two groups, namely SNHG15 gene silencing group and control group. SiRNA (concentration: 300 nmol/well) of SNHG15 and negative control siRNA sequences were transfected using Lipofectamine^{TM} 2000, respectively. Methyl thiazolyl tetrazolium (MTT) assay was performed at 24 h after transfection, and Hoechst 33342 staining and flow cytometry were performed at 48 h after transfection. The specific siRNA sequences are as follows: si-SNHG15: 5'-GGGATGAGGCCTGCCTGTGTTAATA-3', si-NC: 5'-UUCUCCGAACGUGUCACGUTT-3'.

Cell Proliferation Assay

Cell proliferation was detected *via* MTT assay according to instructions of the reagent. After digestion and passage of A549 cell lines in a logarithmic growth phase, cells in SNHG15 gene silencing group and control group were inoculated into each well of a 96-well plate (8×10^3 cells per well), and the volume of complete medium was 200 μL. Cell proliferation in each group at 24 h, 48 h, 72 h, and 96 h was detected using the MTT kit. The optical density value was read at a wavelength of 450 nm, and the cell growth curves were drawn.

Hoechst 33342 Staining

A549 cells were transfected with si-NC and si-SNHG15. After 48 h of transfection, cells were collected, inoculated into a 12-well plate, and cultured for 24 h. Then, the supernatant was discarded, and cells were washed twice with phosphate-buffered saline (PBS) and added with Hoechst 33342 fluorescent dye, followed by incubation at 37°C for 15 min. After the fluorescent dye was discarded, cells were washed again with PBS, observed and photographed under an inverted fluorescence microscope.

Flow Cytometry

Cells in SNHG15 gene silencing group and control group were digested into single cell suspension, and the cell concentration was adjusted into 5×10^3 cells/mL. 1 mL cells were taken and centrifuged at 1000 rpm and 4°C for 10 min. After the supernatant was discarded, 1 mL of cold PBS was added and vibrated gently, followed by centrifugation at 1000 rpm and 4°C for 10 min. After the supernatant was discarded, cells were resuspended into 200 binding buffer. 10 μL An-

nexin V-fluorescein isothiocyanate (FITC) and 5 μL propidium iodide (PI) were added, and the mixture was mixed gently, followed by reaction in a dark place at room temperature for 15 min, and detection using flow cytometer within 1 h. The experiment was repeated for 3 times, the apoptotic rate was calculated, and the average was taken.

Western Blotting

Lysis solution was added to extract the total protein from two groups of cells. The total protein was loaded (30 g per well), followed by spacer gel electrophoresis under 80 V for 40 min, and separation gel electrophoresis under 100 V for 2 h. After the protein was transferred onto a membrane using the conventional wet method, it was sealed using 5% skim milk powder for 2 h. B-cell lymphoma 2 (Bcl-2), Bcl-2 associated X protein (Bax), caspase-3, E-cadherin and other primary antibodies (1:200) and goat anti-rabbit secondary antibody (1:1000) were added for incubation for 2 h. Enhanced chemiluminescence (ECL) gel imaging system was used, and Western blotting bands were quantified using Quantity One1-D analysis software. The relative expression level of target protein = measured value $_{\text{target protein}}$ / measured value $_{\text{GAPDH}}$.

Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

The total messenger ribonucleic acid (mRNA) was extracted from cells with TRIzol and reversely transcribed into complementary deoxyribonucleic acid (cDNA). Conditions of reverse transcription reaction are as follows: 25°C for 10 min, 50°C for 30 min, 85°C for 5 min, and detection using the fluorescence quantitative PCR kit. Primer sequences of SNHG15: forward primer: 5'-GCTGAGGTGACGGTCTCAAA-3', reverse primer: 5'-GCCTCCCAGTTTCATGGACA-3'. Primer sequences of the internal reference GAPDH: forward primer: 5'-GGTCTCCTCTGACTTCAACA-3', reverse primer: 5'-AGCCAAATTCGTTGTCATAC-3'. Conditions of qRT-PCR are as follows: 95°C for 5 min, 95°C for 15 s, 60°C for 1 min, a total of 40 cycles. Solubility curve temperature was set to 60-95°C, and 3 repeated wells were set for each sample.

Wound Healing Assay

Cells were inoculated into the 6-well plate, and the medium was replaced with serum-free DMEM after cells adhered to the wall. When 90%-100% cells were fused, a 10 μL spearhead was used to uniformly scratch the bottom of the 6-well plate perpendicularly, and cells were washed with PBS for 3 times to wash away the floating cells, followed by incubation. At 0 h and 72 h after the scratch and culture, the migration distance of cells in the scratch area was observed under a microscope, and several different fields of view were randomly selected and photographed.

Transwell Migration and Invasion Assays

Cells in two groups after different treatment were collected, counted and resuspended in serum-free DMEM. 100 μL cell suspension was added to the upper chamber, while 600 μL complete medium containing 10% FBS was added to the lower chamber. After incubation for 24 h, fixation and staining, five fields of view were randomly selected and photographed under the inverted microscope. The number of cells going through the membrane was counted, and the average was taken. The different steps in invasion assay were that a layer of Matrigel was paved onto the upper transwell chamber, and cells were inoculated until Matrigel was air dried in a sterile environment. The remaining operations were the same as those in the migration assay.

Statistical Analysis

Statistical Product and Service Solutions 17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All quantitative data were expressed as mean ± standard deviation. The *t*-test was used for enumeration data. Kaplan-Meier method was used for the calculation of survival rate, the survival curve was drawn, and Log-rank test was used for the comparison of survival rate. Cox regression analysis was used for the analysis of influencing factors of prognosis. Significance level α=0.05.

Results

LncRNA SNHG15 was Highly Expressed in NSCLC Tissues and Correlated with Poor Prognosis

Results of qRT-PCR showed that the expression of SNHG15 in NSCLC tissues was significantly higher than that in paracarcinoma tissue (p<0.05) (Figure 1A), suggesting that SNHG15 exerts a certain promoting effect on the occurrence and development of NSCLC, in other words, it plays a role as an oncogene in NSCLC. NSCLC patients were divided into low SNHG1 expression

Table I. Correlation between SNHG15 expression and clinicopathological characteristics of NSCLC patients.

	SNHG15		*p*
Characteristics	**High No. cases (23)**	**Low No. cases (26)**	**Chi-squared test p-value**
Age (years)			0.469
≤65	10	14	
>65	13	12	
Gender			0.509
Male	12	16	
Female	11	10	
Smoking history			0.357
Smokers	13	18	
Never smokers	10	8	
TNM stage			0.017 *
I + II	9	19	
III	14	7	
Lymph node metastasis			0.032 *
Negative	8	17	
Positive	15	9	
Tumor size			
≤5 cm	9	20	0.007 **
>5 cm	14	6	
Histological subtype			0.390
Squamous cell carcinoma	7	11	
Adenocarcinoma	16	15	

group (n=23) and high SNHG15 expression group (n=26) according to the average expression level of SNHG15 (2.62 relative to GAPDH) in 49 NSCLC patients. The correlations of SNHG15 expression level in NSCLC tissues with clinicopathological data were further analyzed, and it was found that the SNHG15 expression level was related to the tumor size, lymph node status, and tumor-node-metastasis (TNM) staging. Analyses of results revealed that the SNHG15 expression level in patients with advanced lung cancer was higher than that in patients with early lung cancer (p=0.017) (Table I). Also, the SNHG15 expression level in patients with positive lymph node metastasis was higher than that in patients with negative lymph node metastasis (p=0.032) (Table I). There were no significant correlations of SNHG15 expression with patient's age, smoking, and pathological grading (Table I, p>0.05). Next, it was further found *via* Kaplan-Meier survival analysis that the prognosis of patients in low SNHG15 expression group was significantly better than that in high SNHG15 expression group, and both disease-free survival (DFS) and overall survival (OS) were longer (Figure 1B and C). Results showed that the median DFS was 20 months in high SNHG15

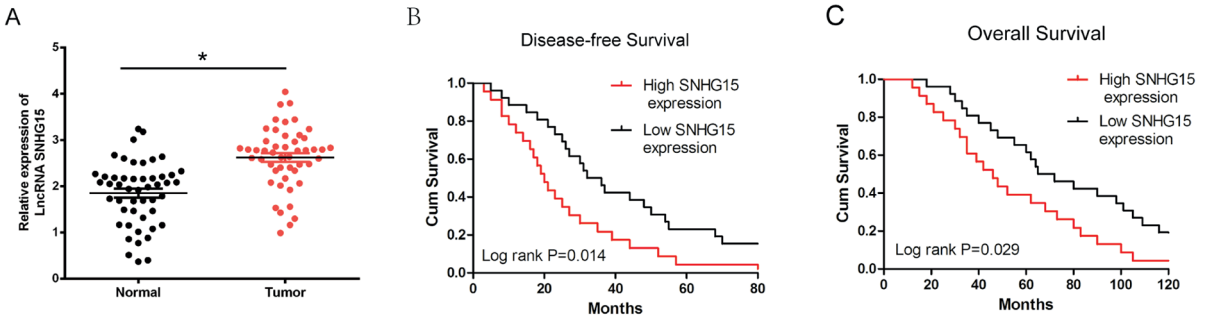

Figure 1. Relative SNHG15 expression in NSCLC tissues and its clinical significance. ***A***, Relative expressions of SNHG15 in NSCLC tissues (n=49) and adjacent non-cancerous tissues (n=49) were examined by qPCR and normalized to GAPDH expression. ***B-C***, Kaplan–Meier disease-free survival curves and overall survival according to SNHG15 expression level. (*p<0.05).

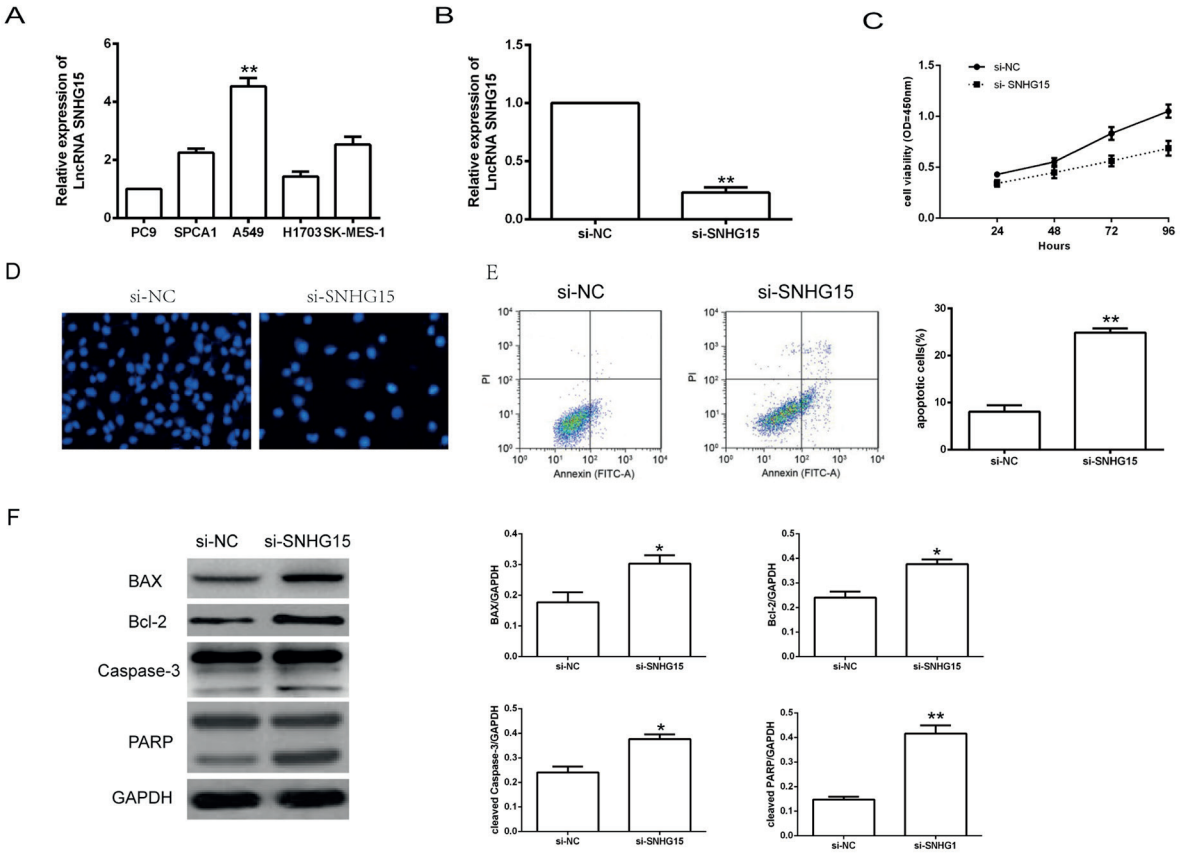

Figure 2. Effects of SNHG15 on A549 cells proliferation and apoptosis *in vitro*. ***A,*** SNHG15 expression was assessed by qRT-PCR analysis in Lung adenocarcinoma cell lines (PC9, SPC-A1, and A549) and lung squamous cell carcinoma cell lines (H1703 and SK-MES-1). ***B,*** Effective knockdown of SNHG15 in A549 cells 48 h after siRNA treatment. ***C,*** MTT assays were performed to determine the proliferation of si-SNHG15 and si-NC transfected A549 cells. D, MTT assays were performed to determine the proliferation of A549 cells following treatment with si-SNHG15 and the negative control. ***D, E,*** Hoechst 33342 staining and Flow cytometry were conducted to determine the apoptosis of si-SNHG15-transfected A549cells. ***F,*** The proteins levels of BAX, Bcl-2, cleaved caspase-3 and PARP were determined by Western blotting in si-SNHG15 transfected A549 cells. The data represent the mean ± SD from three independent experiments. (*p<0.05, **p<0.01).

expression group, and 34 months in low SNHG15 expression group (p=0.014). The median survival time was about 46 months in high SNHG15 expression group, and about 70 months in low SNHG15 expression group (p=0.029).

Knockdown of lncRNA SNHG15 Inhibited the In-Vitro Proliferation of Lung Cancer A549 Cells

The expressions of lncRNA SNHG15 in lung adenocarcinoma cell lines (PC9, SPC-A1 and A549) and lung squamous cell carcinoma cell lines (H1703 and SK-MES-1) were detected *via* qRT-PCR. Results showed that the expression level of SNHG15 in A549 cells was the highest (Figure 2A). In this study, A549 cells with the highest expression level of SNHG15 were selected for subsequent transfection and research. The inhibitory efficiency after transfection with si-SNHG15 for 48 h was verified *via* qRT-PCR. As shown in Figure 2B, si-SNHG15 was effective in inhibiting the expression of lncRNA SNHG15 in MCF-7 cells, and there was a statistically significant difference compared with that in the negative control group (p<0.01), so si-SNHG15 was selected for transfection in subsequent experiments. MTT assay was used to verify the effect of inhibiting SNHG15 on the *in-vitro* proliferation of A549 cells. After A549 cells interfered with si-SNHG15 and si-NC were cultured for 24 h, 48 h, 72 h, and 96 h, respectively, MTT assay was performed. Results showed that the growth rate of A549 cells was significantly inhibited after SNHG15 knockdown (Figure 2C).

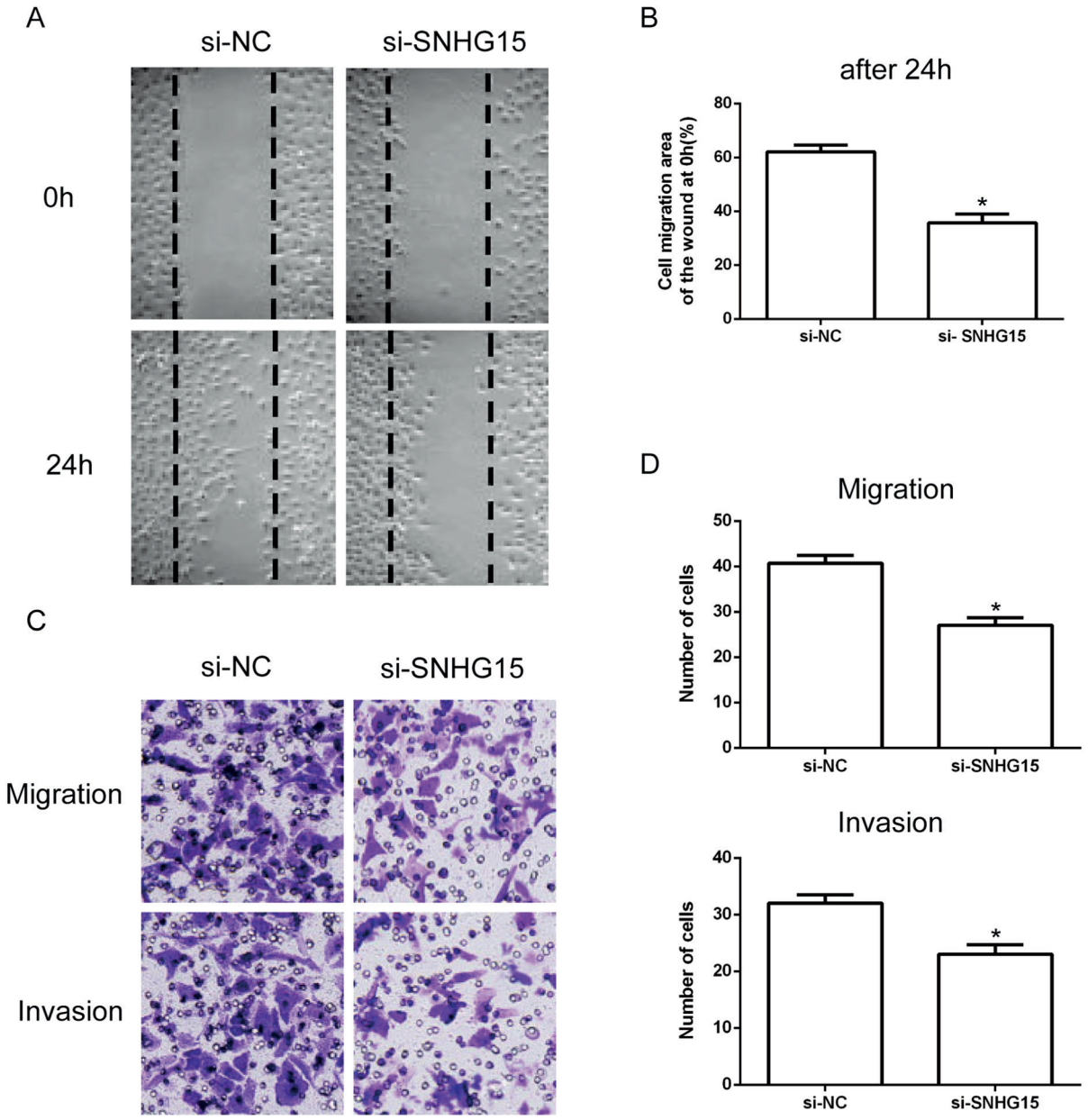

Figure 3. Effects of SNHG15 on A549 cells migration and invasion *in vitro*. ***A-B,*** A549 cells were treated with si-SNHG15 and si-NC, and the effects on cell migration were determined with cell scratch test. ***C-D,*** The effects on cell migration and invasion were determined with cell transwell test. The data represent the mean ± SD from three independent experiments. (*p<0.05).

Knockdown of lncRNA SNHG15 Induced Apoptosis of Lung Cancer A549 Cells

Results of Hoechst 33342 staining showed that the proportion of apoptotic cells in lncRNA SNHG15 knockdown group was significantly increased compared with that in control group (Figure 2D). Results of flow cytometry revealed that the total apoptosis rate in the knockdown group was significantly increased compared with that in the control group (Figure 2E, p<0.05). Results of Western blotting, consistent with the above results, revealed that the expressions of BAX, cleaved caspase-3 and cleaved poly-ADP-ribose polymerase (PARP) were significantly up-regulated after knockdown of lncRNA SNHG15, but the expression level of Bcl-2 was down-regulated (Figure 2F). The above results suggest that inhibiting lncRNA SNHG15 expression can significantly increase the Bax/Bcl-2 ratio, thereby increasing cleaved caspase-3 and cleaved PARP expressions,

initiating mitochondrial apoptotic pathway, and promoting apoptosis of NSCLC cells.

Knockdown of SNHG15 Inhibited the Migration and Invasion Capacities of A549 Cells

Invasion and metastasis are important features of the tumor. The effects of SNHG15 on lung cancer cell migration and invasion were further analyzed. First, wound healing assay was used to detect the cell migration capacities in control group and SNHG15 knockdown group. Results showed that the cell migration distance in the SNHG15 knockdown group was significantly shorter than that in the control group after 24 h (p<0.05, Figure 3A and B). Next, transwell migration assay was conducted, and results were consistent with those in wound healing assay. After knockdown of SNHG15, the number of cells passing through the filter membrane in the lower chamber was significantly reduced, and the difference was statistically significant (p<0.05, Figure 3C and D).

Knockdown of SNHG1 Inhibited the Epithelial-Mesenchymal Transition (EMT) Process, Matrix Metalloproteinase-2 (MMP-2) and MMP-9 Expressions in A549 Cells

To further investigate the mechanism of SNHG15 knockdown in inhibiting migration and invasion capacities of A549 cells, expressions of related molecules were further detected *via* Western blotting. After knockdown of SNHG15, the expression of E-cadherin was significantly up-regulated, but N-cadherin and Vimentin expressions were significantly down-regulated (Figure 4). It was further found that knockdown of SNHG15 could also down-regulate the expressions of MMP-2 and MMP-9 in A549 cells. The above results indicate that knockdown of SNHG15 can inhibit the metastasis of A549 cells through inhibiting the EMT process and expressions of MMPs in cells.

Discussion

LncRNA has become a hotspot in tumor research in recent years. LncRNA is the non-coding protein RNA, which can be involved in the regulation of biological processes, such as cell proliferation, apoptosis, and tumorigenesis, through regulating downstream miRNA[3,6-8]. Related studies[9,10] have shown that lncRNA SNHG15 is related to the occurrence and development of a variety of tumors. Zhang et al[11] showed that the expression level of lncRNA SNHG15 in liver cancer tissues is significantly higher than that in para-carcinoma tissues, the expression of SNHG15 is correlated with histological grading, TNM staging and vascular invasion, the high expression of lncRNA SNHG15 is related to the poor prognosis of patients with liver cancer, and SNHG15 is an independent risk factor affecting the prognosis of liver cancer. In gastric cancer, SNHG15 is highly expressed in gastric cancer tissues, the high expression of SNHG15 is correlated with the depth of tumor in-

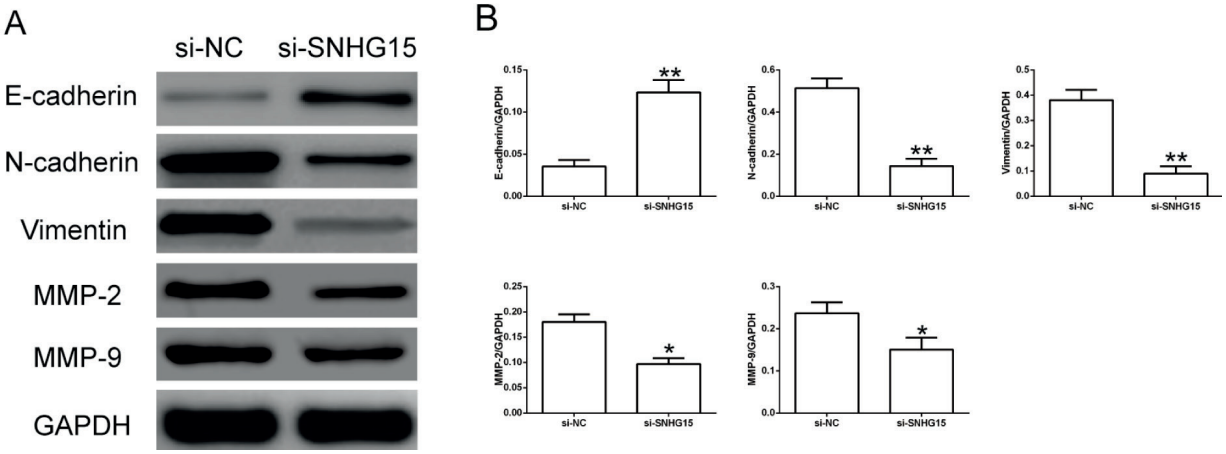

Figure 4. Effects of SNHG15 on the proteins expression related to migration and invasion of A549 cells. ***A,*** The expression levels of target proteins (E-cadherin, N-cadherin, Vimentin, MMP-2, and MMP-9) measured by Western blotting in A549 cells transfected with si-SNHG15 or si-NC. ***B,*** The data represent the mean ± SD from three independent experiments. ($^{*}p$<0.05, $^{**}p$<0.01).

vasion, TNM staging and lymph node metastasis, and SNHG15 is also an independent risk factor affecting the prognosis of gastric cancer. Moreover, *in-vitro* interference and knockdown of SNHG15 expression can inhibit the proliferation and invasion, and induce apoptosis of gastric cancer cells. However, after transfection of SNHG15 into gastric cancer cell lines and up-regulation of its expression, the proliferation and invasion of gastric cancer cells can be promoted *via* regulating MMP-9 and MMP-2 expressions[5]. In this study, it was found that lncRNA SNHG15 expression was significantly up-regulated in NSCLC tissues. Prognostic analysis showed that patients with low lncRNA SNHG15 expression had longer DFS and OS than those with high SNHG15 expression. The above clinical evidence suggests that SNHG15 is an adverse prognostic factor in patients with lung cancer and plays a potential role as an oncogene. To explore the mechanism of lncRNA SNHG15 in lung cancer, A549 cells with the higher expression level of lncRNA SNHG15 were used for *in-vitro* mechanism research. Apoptosis is associated with the occurrence of many malignant tumors. It was found that interference in the expression of lncRNA SNHG15 promoted apoptosis and inhibited cell proliferation. Besides, results of Western blotting showed that knockdown of lncRNA SNHG15 could significantly increase the Bax/Bcl-2 ratio, thereby increasing the expressions of cleaved caspase-3 and cleaved PARP, and ultimately promoting apoptosis. Both Bax and Bcl-2 belong to the Bcl-2 family, and they are important regulatory proteins that regulate the mitochondrial apoptotic pathway, playing important roles in the alteration of mitochondrial outer membrane permeability[12]. The results suggest that knocking down lncRNA SNHG15 can activate the mitochondrial apoptotic pathway, thereby promoting apoptosis and inhibiting proliferation.

Malignant tumors are characterized by invasion against the surrounding tissues and distant metastasis, and patients with advanced lung cancer are often accompanied by distant metastatic lesions, making it difficult to treat lung cancer[13-15]. In this study, it was confirmed *via* invasion and metastasis assays that knockdown of lncRNA SNHG15 in A549 cells could significantly inhibit cell invasion and metastasis capacities. Invasion and metastasis are multi-factor and multi-step dynamic processes. EMT is one of the key steps in invasion and metastasis of epithelial-derived malignant tumors[13], and the EMT process includes the down-regulation of epithelial cell marker (E-cadherin) expression and up-regulation of expressions of interstitial cell markers (Vimentin and N-cadherin). Moreover, Wnt, Hedgehog, Notch and other signaling pathways are involved in the occurrence of EMT[16]. Many researches[17,18] have shown that lncRNA can play corresponding roles in tumor invasion and metastasis *via* regulating EMT in tumors. Results of Western blotting revealed that, after knockdown of lncRNA SNHG15, E-cadherin expression was significantly up-regulated, but Vimentin and N-cadherin expressions were down-regulated, indicating that knockdown of lncRNA SNHG15 can inhibit the EMT of lung cancer A549 cells. Moreover, it was found that knockdown of lncRNA SNHG15 could also down-regulate the expressions of MMP-2 and MMP-9, which was consistent with the research result of SNHG15 in gastric cancer. MMP-2 and MMP-9 are the most important enzymes in MMPs, playing central roles in the development of tumors[19-21]. The results indirectly proved that lncRNA SNHG15 can regulate the expressions of MMPs, thus promoting lung cancer invasion and metastasis capacities.

Conclusions

We found that lncRNA SNHG15 plays important roles in occurrence and development processes of NSCLC and exerts a function as an oncogene. LncRNA SNHG15 can be a new and effective marker of prognosis or progression of NSCLC, which also provides a potential alternative target for the treatment of NSCLC.

Funding Acknowledgements

This work was supported by Yantai City Science and Technology Development Plan (No. 20150WS39).

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.

2) Lazar V, Suo C, Orear C, van den Oord J, Balogh Z, Guegan J, Job B, Meurice G, Ripoche H, Calza S, Hasmats J, Lundeberg J, Lacroix L, Vielh P, Dufour F, Lehtio J, Napieralski R, Eggermont A, Schmitt M, Cadranel J, Besse B, Girard P, Blackhall F, Validire P,

Soria JC, Dessen P, Hansson J, Pawitan Y. Integrated molecular portrait of non-small cell lung cancers. BMC Med Genomics 2013; 6: 53.

3) Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: Insights into functions. Nat Rev Genet 2009; 10: 155-159.

4) Zhang JH, Wei HW, Yang HG. Long noncoding RNA SNHG15, a potential prognostic biomarker for hepatocellular carcinoma. Eur Rev Med Pharmacol Sci 2016; 20: 1720-1724.

5) Chen SX, Yin JF, Lin BC, Su HF, Zheng Z, Xie CY, Fei ZH. Upregulated expression of long noncoding RNA SNHG15 promotes cell proliferation and invasion through regulates MMP2/MMP9 in patients with GC. Tumour Biol 2016; 37: 6801-6812.

6) Yang G, Lu X, Yuan L. LncRNA: A link between RNA and cancer. Biochim Biophys Acta 2014; 1839: 1097-1109.

7) Isin M, Dalay N. LncRNAs and neoplasia. Clin Chim Acta 2015; 444: 280-288.

8) Huang T, Alvarez A, Hu B, Cheng SY. Noncoding RNAs in cancer and cancer stem cells. Chin J Cancer 2013; 32: 582-593.

9) Chen SX, Yin JF, Lin BC, Su HF, Zheng Z, Xie CY, Fei ZH. Upregulated expression of long noncoding RNA SNHG15 promotes cell proliferation and invasion through regulates MMP2/MMP9 in patients with GC. Tumour Biol 2016; 37: 6801-6812.

10) Ma Z, Huang H, Wang J, Zhou Y, Pu F, Zhao Q, Peng P, Hui B, Ji H, Wang K. Long non-coding RNA SNHG15 inhibits P15 and KLF2 expression to promote pancreatic cancer proliferation through EZH2-mediated H3K27me3. Oncotarget 2017; 8: 84153-84167.

11) Zhang JH, Wei HW, Yang HG. Long noncoding RNA SNHG15, a potential prognostic biomarker for hepatocellular carcinoma. Eur Rev Med Pharmacol Sci 2016; 20: 1720-1724.

12) Boise LH, Gottschalk AR, Quintans J, Thompson CB. Bcl-2 and Bcl-2-related proteins in apoptosis regulation. Curr Top Microbiol Immunol 1995; 200: 107-121.

13) Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell 2011; 144: 646-674.

14) Perlikos F, Harrington KJ, Syrigos KN. Key molecular mechanisms in lung cancer invasion and metastasis: A comprehensive review. Crit Rev Oncol Hematol 2013; 87: 1-11.

15) Zhao Y, Adjei AA. New strategies to develop new medications for lung cancer and metastasis. Cancer Metastasis Rev 2015; 34: 265-275.

16) Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol 2014; 15: 178-196.

17) Liu B, Sun L, Song E. Non-coding RNAs regulate tumor cell plasticity. Sci China Life Sci 2013; 56: 886-890.

18) Xu Q, Deng F, Qin Y, Zhao Z, Wu Z, Xing Z, Ji A, Wang QJ. Long non-coding RNA regulation of epithelial-mesenchymal transition in cancer metastasis. Cell Death Dis 2016; 7: e2254.

19) Bauvois B. New facets of matrix metalloproteinases MMP-2 and MMP-9 as cell surface transducers: Outside-in signaling and relationship to tumor progression. Biochim Biophys Acta 2012; 1825: 29-36.

20) Roeb E, Matern S. [Matrix metalloproteinases and colorectal cancer]. Med Klin (Munich) 2003; 98: 763-770.

21) Gialeli C, Theocharis AD, Karamanos NK. Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. FEBS J 2011; 278: 16-27.