European Review for Medical and Pharmacological Sciences 2018; 22: 2653-2661

LncRNA SNHG7 promotes the proliferation of esophageal cancer cells and inhibits its apoptosis

L.-J. XU[1], X.-J. YU[1], B. WEI[1], H.-X. HUI[1], Y. SUN[1], J. DAI[2], X.-F. CHEN[1]

[1]Department of Medical Oncology, The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, Huai'an, China
[2]Department of Orthopedic, The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, Huai'an, China

Lijuan Xu and Xiaojuan Yu contributed equally to this work

Abstract. – OBJECTIVE: Our research studied the expression of long noncoding RNA (lncRNA) SNHG7 in esophageal cancer cells and tissues. The effect of lncRNA SNHG7 on proliferation and apoptosis of esophageal cancer cells has been discussed.

PATIENTS AND METHODS: Si-SNHG7 was transfected into esophageal cancer cells, and qRT-PCR was performed to detect the expression of lncRNA SNHG7 in esophageal cancer cells and tissues. The effect of SNHG7 on the proliferation of esophageal cancer cells was measured by CCK8 assay and plate cloning assay, respectively. Flow cytometry was used to detect the effect of SNHG7 on the cell cycle and apoptosis rate of esophageal cancer cells. Changes in expression of downstream protein p15 and p16 after si-SNHG7 intervention were analyzed by qRT-PCR and Western blot.

RESULTS: QRT-PCR showed that the expression of SNHG7 in esophageal cancer tissues and cells was significantly up-regulated. After the si-SNHG7 intervention, the proliferation of esophageal cancer cells was inhibited, the apoptosis rate increased, and the cell cycle was blocked in G1-G0 phase. QRT-PCR and Western blot showed that, after the si-SNHG7 intervention, the expression of p15 and p16 increased significantly.

CONCLUSIONS: The expression of SNHG7 in the tissues and cells of esophageal cancer is significantly up-regulated. SNHG7 can partly promote the development of esophageal cancer by regulating the expression of p15 and p16.

Key Words:
lncRNA SNHG7, Proliferation, Apoptosis, p15, p16.

Introduction

Esophageal cancer has been regarded as a major public health issue in the world and the most common malignancy in the digestive system. The incidence of esophageal cancer ranks the fourth among all cancers worldwide, and the mortality rate is as high as second[1]. Through in-depth study of the specific molecular mechanisms of esophageal cancer, we can identify high-risk populations and molecular markers for early diagnosis, and establish new therapeutic targets, which is significant for improving the curative effect of esophageal cancer[2]. LncRNA, non-coding RNA with the length of over 200 nt, are usually transcripts in eukaryotes. With no or little protein-coding capacity, lncRNA can regulate different levels of gene expression in RNA form, including pre-transcriptional, transcriptional, and post-transcriptional[3,4]. It was initially thought that lncRNA was merely a by-product of RNA polymerase II transcription, without any biological function. However, recent studies show that lncRNA is involved in activation, interference, and nuclear transport of many regulatory processes such as X chromosome silencing, gene imprinting, chromatin modification, and transcription.

More importantly, numerous research[5-7] have shown that the abnormal expression of lncRNAs is closely related to a variety of tumors. Specifically, lncRNA SNHG7 has been identified to be involved in multiple malignancies. In gastric cancer, the up-regulated expression of SNHG7 is negatively correlated with the prognosis of patients[8]. In lung cancer, SNHG7 can promote the proliferation, invasion, and metastasis of in lung cancer cells[9]. Yet, there have been no reports of the expression and biological effects of SNHG7 in esophageal cancer. Therefore, we intend to study the expression and biological function of SNHG7 in human esophageal cancer tissues *in vitro* and *in vivo*, and to explore its possible molecular mechanism.

Corresponding Author: Jian Dai, MM; e-mail: dj9812@126.com
Xiaofei Chen, MD; e-mail: xiebo83049953@163.com

Patients and Methods

Tissues and Cells

A total of 27 tissue specimens of esophageal cancer in our hospital from January 2012 to December 2015 were included in this study. All the selected patients were in accordance with the following criteria: a) patients who were diagnosed with esophageal cancer by pathological examination for the first time, and were not treated; b) patients with good physical condition and no other major complications. The clinical information of the patients before and after treatment was collected and the statistical analysis was carried out. This study was approved by the Ethics Committee of our Institution. All patients and/or their families received information and signed the written informed consent. Resection around 5 cm of esophageal carcinoma tissues and normal tissues was prepared from each patient. Tissues were made into sections, and some were placed in the 1.5 mL EP tube, preserved in -80°C refrigerator for total RNA extraction. Human esophageal cancer cell lines Eca109, EC9706, TE-10, and TE-11, and human normal esophageal epithelial cells HEEC (Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Beijing, China) were cultured in F12 or 1640 medium containing 10% fetal bovine serum (FBS) (Hyclone, South Logan, UT, USA), penicillin (100 U/mL) and streptomycin (100 μg/mL). Cell culture was maintained in a thermostat incubator (5% CO_2, 37°C) when cell confluency reached 80-90%, then cell passage was routinely conducted.

RNA Extraction and qRT-PCR Detection of SNHG7 Expression

TRIzol reagent was added to the tissue and cell samples for total RNA extraction. The obtained RNA was kept in the 4°C or -80°C refrigerator for long-term preservation. The spectrometer was used to detect RNA concentration. RNA samples with A260/A280 ratio between 1.8 and 2 were used for future experiments. According to the instructions of the Prime Script RT kit (Perfect Real-time), cDNA was prepared by RNA reverse transcription. The qRT-PCR reaction system was prepared according to the instruction of SYBR Premix Ex Taq TM (TliRNaseH Plus). Reaction procedure was as follows: 95*1 minute, 95* 30 seconds, 60* 40 seconds, for total 40 cycles. In this experiment, each sample was repeated in triplicates, and the entire experiment was independently repeated three times. Relative expression of the target gene was examined by $-\Delta Ct$ and $2^{-\Delta\Delta Ct}$ method.

Synthesis of Interference Sequences and Primers of SNHG7

The interference sequence and primers of SNHG7 were synthesized by Invitrogen (Shanghai, China) using Primer 5 (PRIMER Biosoft) for designation. The primer sequences are as follows: SNHG7 forward primer 5-CCGTGGGCACTGCTGAAT-3, reverse primer 5-CTGCTGGCAAGCGGAAC-3; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward primer 5-AGCCACATCGCTCAGACAC-3, reverse primer 5-GCCCAATACGACCAAATCC-3; SNHG7 interference sequence 5-CCAGAGAACCTGCCTTCCTTCCTAA-3.

Cell Counting Kit-8 (CCK8) Assay and Plate Cloning Assay

Cell proliferation was detected by using Cell Counting Kit-8 assay. 6 hours after SNHG7 interference, the sequence was transiently transfected into obtained esophageal cancer cells and the cells were collected. The density of cells was determined as 4 x 10^4/mL. The cells were evenly seeded on the 96 well-plate (80 μL/wells); 6 repeated wells of each treatment were set for both the experimental group and the control group. CCK-8 mixture containing serum free medium (1:10) was added to each well of 96-well plate (110 μL/well). The plate was incubated for an appropriate length of time. Then, the culture plates were taken out at 5-time points, 0, 24, 48, 72, and 96 hours respectively. The growth curve was performed with a spectrophotometer, by measuring the optical density (OD) of light at 450 nm wavelength.

The cells in the experimental and the control group were placed on the 6-well plate (1000/well) in the recommended medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin, which was maintained at 37°C in an atmosphere of 5% CO_2 and 95% air; the culture medium was replaced after 3 days. 14 days later, the cells were fixed in the formaldehyde and stained with viola crystalline. The colonies in each dish were counted and photographed.

Flow Cytometry

The esophageal cancer cells in the logarithmic growth period were adjusted to 3 x 10^5/mL and seeded on the 6-well plate (2 mL/well). After cell adhesion, cells were transiently transfected into SNHG7 interference sequence. Then, the cells were stained with Annexin-V/PI (Propidium Iodide, PI) and placed in the dark for 15 minutes. The apoptosis rate in the two groups was detected by flow cytometry. The cells were treated and collected in a similar way, and then fixed overnight in the 75% alcohol pre-cooled in the -20°C refrigerator. The content of DNA was detected by PI staining. The cell cycle was divided into G1/G0, S, G2/M phase, and the percentage of G1 was calculated by the software.

Western Blotting

The cells of the experimental and control group were collected, and processed with radioimmunoprecipitation assay (RIPA) for total protein extraction. The standard curve of protein concentration was prepared by bicinchoninic acid (BCA, Beyotime, Shanghai, China) Kit, and the protein concentration in all samples was detected. All the protein samples were loaded to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After that, the separated protein was transferred to the 0.22 μm polyvinylidene difluoride (PVDF) membrane, and the membrane was incubated with the Tris buffered saline-Tween (TBS-T) containing 5% skimmed milk for blocking. Rabbit anti-human p15 antibody (1:1000 dilution, CST, Danvers, MA, USA) and rabbit anti-human p16 antibody (dilution of 1:1000, CST, Danvers, MA, USA) were added for overnight incubation in the 4°C refrigerator, and GAPDH as an internal reference. After washing the membrane three times with TBS-T (5 minutes/time), secondary antibody (1:1000 dilution, CST, Danvers, MA, USA) was added to the membrane. After 2 hours incubation at room temperature, the membrane was washed with TBS-T for three times (10 minutes/time). Pre-prepared ECL solution was then added together with 20 μL chromogenic agent to the membrane. Lastly, the membrane was exposed with chemiluminescence device and the image is preserved.

Statistical Analysis

Statistical product and service solutions (SPSS) 19.0 software was used for statistical analysis (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY, USA). The normal distribution of measurement data was represented by mean ± standard deviation. The comparison between groups was done using One-way ANOVA test followed by LSD (Least Significant Difference). The skewed distribution was represented by median (four percentile). Wilcoxon matched pair rank test was used to show the statistical difference among treatments. $p<0.05$ was considered as statistically significant.

Results

The Correlation Between the Relative Expression of SNHG7 and the Clinical-Pathological Features

The relative expression of SNHG7 in 27 esophageal carcinoma cases was 1.402, of which the 95% CI was 0.363-0.595. The relative expression level of SNHG7 was not correlated ($p>0.05$) with the general data of patients, such as age, sex, tumor size, location, depth of invasion, tumor stage, differentiation, and distant metastasis. However, the relative expression of SNHG7 with lymphatic involvement was significantly up-regulated ($p<0.05$), while there was no significant difference ($p>0.05$) in relative expression of non-lymphatic involvement tissues with the normal group. This result indicates that the up-regulation of SNHG7 was significantly in relation to the risk of lymph node metastasis (Table I).

High Expression of SNHG7 in Esophageal Cancer Tissues and Cells

QRT-PCR showed that the expression of SNHG7 was significantly increased in 20 of 27 esophageal cancer tissues (Figure 1A). The relative expression of SNHG7 in 4 kinds of esophageal cancer cells (Eca109, EC9706, TE-10, and TE-11) was significantly higher than that in human normal esophageal epithelial cells (HEEC) (Figure 1B). Si-SNHG7 and control sequences were transiently transfected into esophageal cancer cell lines (Eca109, TE-10). After 48 h, the relative expression of SNHG7 decreased significantly (Figure 1C, 1D). The results showed high expression of SNHG7 in esophageal cancer tissues and cells and high efficiency of Si-SNHG7.

Table I. The correlation between the relative expression of SNHG7 and the clinicopathological features.

Clinicopathological features	Number of cases	SNHG7 ($\bar{x}$±s)	*p* value
Sex			
Male	15	1.45±0.18	0.361
Female	12	1.36±0.21	
Age			
>60	16	1.45±0.26	0.825
≤60	11	1.48±0.17	
Lauren Classification			
Enteric adenocarcinoma	14	1.49±0.15	0.892
Diffuse carcinoma	13	1.44±0.26	
Degree of differentiation			
High	3	1.46±0.25	0.934
Moderate	13	1.45±0.25	
Low	11	1.46±0.28	
UICC Classification (Stage)			
I+II	9	1.45±0.15	0.851
III+IV	18	1.46±0.15	
Infiltration depth			
T1+T2	16	1.61±0.18	0.723
T3+T4	11	1.58±0.25	
Lymphnodemetastases			
Not	10	1.35±0.21	0.024
Yes	17	1.87±0.26	
Distant metastasis			
Not	15	1.46±0.24	0.427
Yes	12	1.45±0.17	
Location of the tumor			
Upper esophagus	10	1.48±0.15	0.453
Mid-esophagus	9	1.25±0.14	
Lower esophagus	8	1.46±0.25	
Tumor size			
≤5 cm	12	1.43±0.54	0.262
>5 cm	15	1.48±0.35	

Note: Compared with the control group, $^{*}p<0.05$.

Interference with the Expression of SNHG7 Inhibits the Proliferation of Esophageal Cancer Cells

CCK-8 detection showed that after Eca109, TE-10 esophageal cancer cells were transfected with si-SNHG7, cell proliferation was significantly inhibited (Figure 2A, 2B). The plate cloning assay also confirmed the inhibition of the proliferation of esophageal cancer cells after si-SNHG7 interference (Figure 2C, 2D).

Interference of the SNHG7 Expression Can Inhibit the Apoptosis of Esophageal Cancer Cells and Lead to Cell Cycle Arrest

Flow cytometry results showed that compared with the control group, the cell cycle of si-SNHG7 transfected Eca109 and TE-10 esophageal cancer cells was arrested in G1-G0 phase (Figure 3A, 3B). Meanwhile, compared with the control group, the apoptosis rate of the experimental group was significantly increased (Figure 3C, 3D).

SNHG7 Regulates the Expression of p15 and p16

The results of qRT-PCR and Western blotting showed that si-SNHG7 intervention could promote the expression of p15 and p16 (Figure 4). After the si-SNHG7 intervention in esophageal cancer cells, qRT-PCR detection shows up-regulated expression of p15 and p16 mRNA (Figure 4A). Western blot detection shows that p15 and p16 protein expression is also significantly up-regulated (Figure 4B).

Discussion

Although many oncogenes, tumor suppressor genes, and tumor-related signaling pathways have

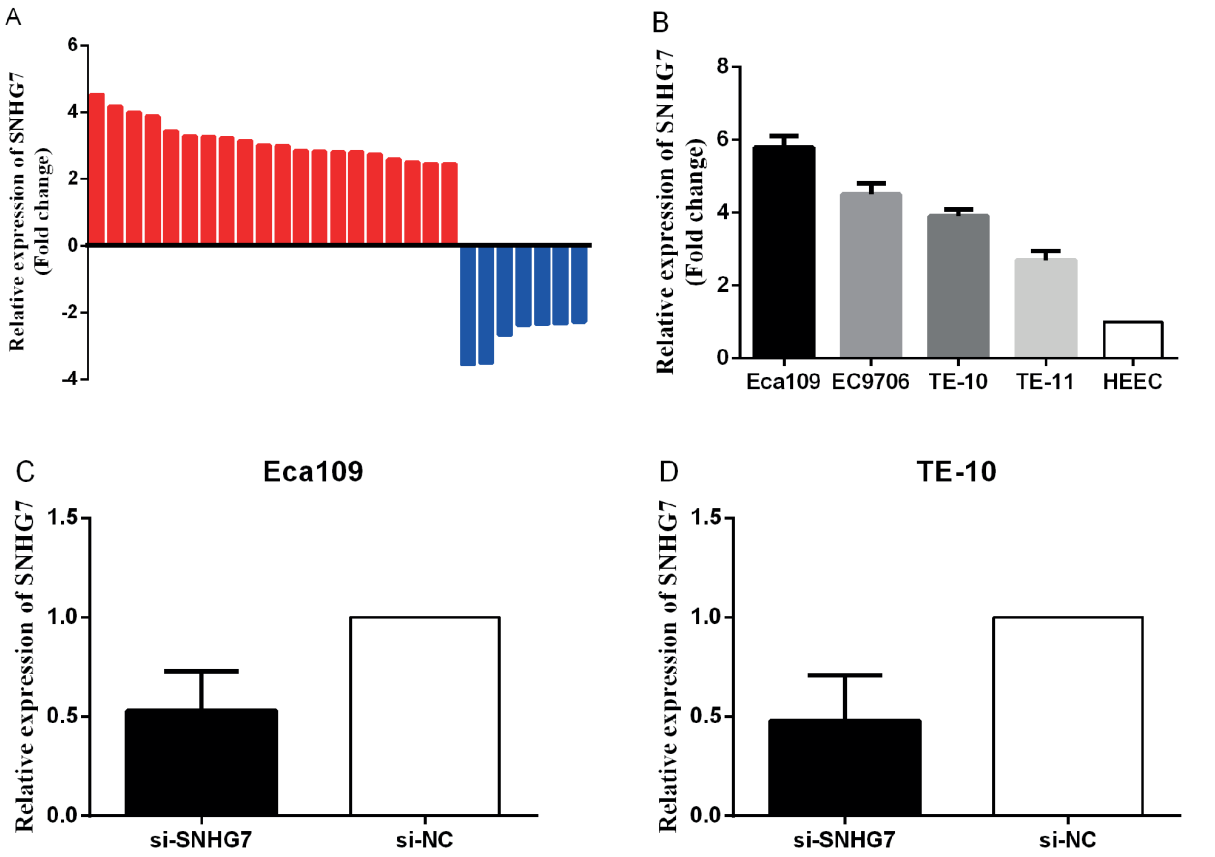

Figure 1. Expression of SNHG7 in esophageal cancer tissues and cells. ***A,*** qRT-PCR is used to detect the relative expression of SNHG7 in 27 samples of esophageal carcinoma, of which 20 samples of SNHG7 are up-regulated. ***B,*** qRT-PCR is used to detect the relative expression of esophageal carcinoma cell lines and human normal esophageal epithelial cells. ***C,*** & ***D,*** After transfection of si-SNHG7 to esophageal cancer cells, the expression of SNHG7 decreases significantly (*p<0.05).

been discovered and confirmed in recent years, the pathogenesis of esophageal cancer is still unclear.

At first, lncRNA was considered to be the "noise" of the human genome transcription, without any biological effects in human[10,11]. However, with the development of science technology, scientists found that although lncRNA doesn't exert protein-encoding function and it has a key role in diverse genetic regulation. The pathogenesis of cancer is complex, which renders the regulation of lncRNA especially important for this process[12,13]. In recent years, new studies have reported the relationship between lncRNAs and cell proliferation, apoptosis, invasion, metastasis, and drug resistance of esophageal cancer. For example, Su et al[14] found that lncRNA SNHG1 can promote the proliferation of gastric cancer cells by regulating the expression of DNA methyltransferase 1 (DNMT1). LncRNA 00152 can promote the invasion and metastasis of gastric cancer cells by regulating epithelial-mesenchymal transition[15]. Our study first finds that SNHG7 was highly expressed in esophageal cancer tissues and cells. After the si-SNHG7 intervention, the proliferation of tumor cells was inhibited, and the apoptosis of tumor cells was promoted, leading to cell cycle arrest.

In advanced tumors[16,17], p15 and p16, as tumor suppressors, exerted suppressed expression. Importantly, lncRNA was found to participate in the regulation of p15 and p16 expression. Adams et al[18] reported that lncRNA BLACAT1 can silence the expression of p15 epigenetically in rectal carcinoma, thereby promoting the proliferation of colorectal cancer cells. In breast cancer, lncRNA PANDAR inhibits the expression of p16 and further regulates the tumor cell cycle[19]. In this study, the transfection of si-SNHG7 can increase the ex-

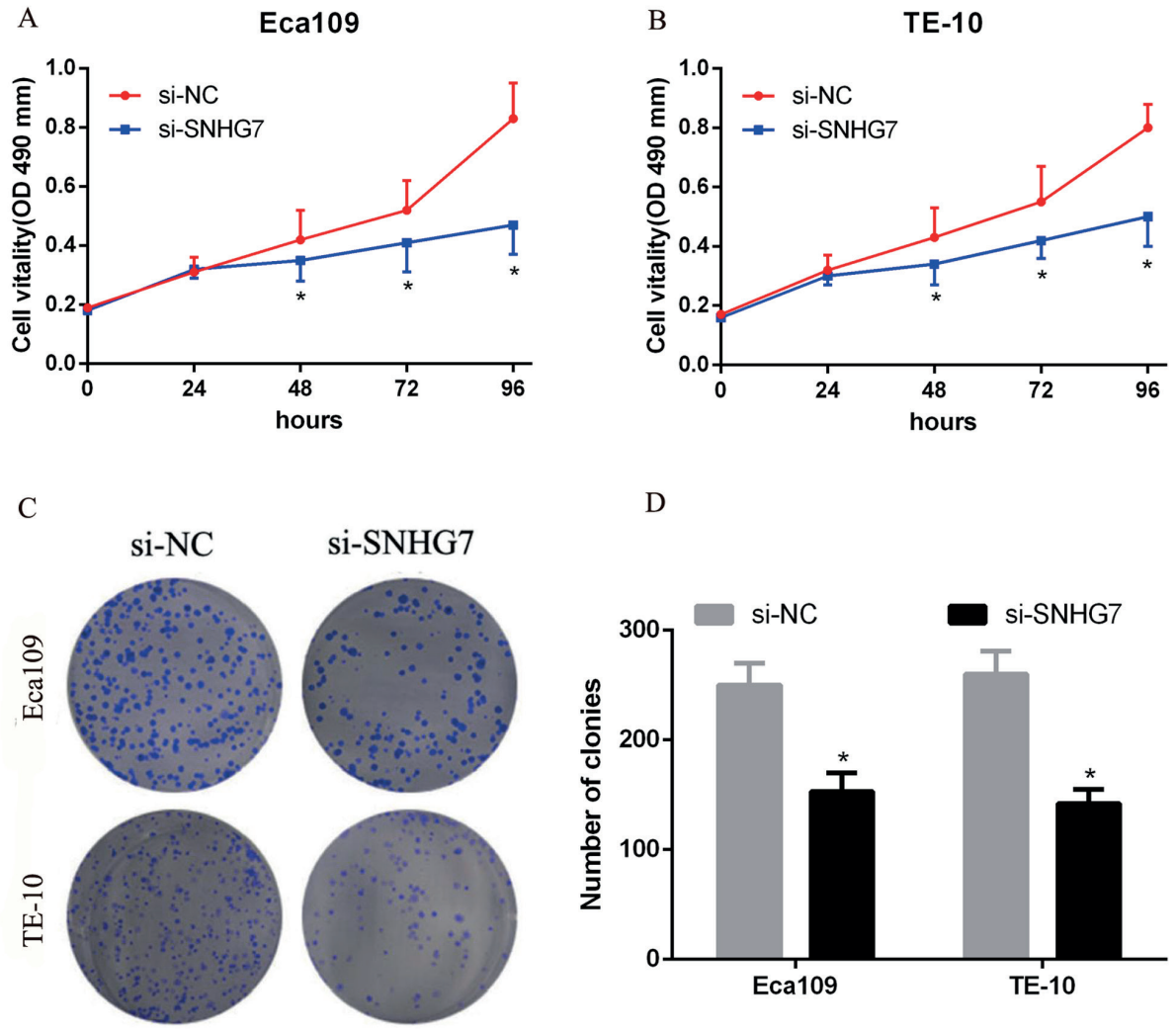

Figure 2. The effect of SNHG7 on the proliferation of esophageal cancer cells. ***A,*** & ***B,*** CCK8 assay detects that after the intervention of si-SNHG7, the proliferation of esophageal cancer cells is significantly inhibited. ***C,*** & ***D,*** Plate cloning assay show that in esophageal cancer cells, the proliferation of esophageal cancer cells is significantly reduced by the intervention of si-SNHG7 (*p<0.05).

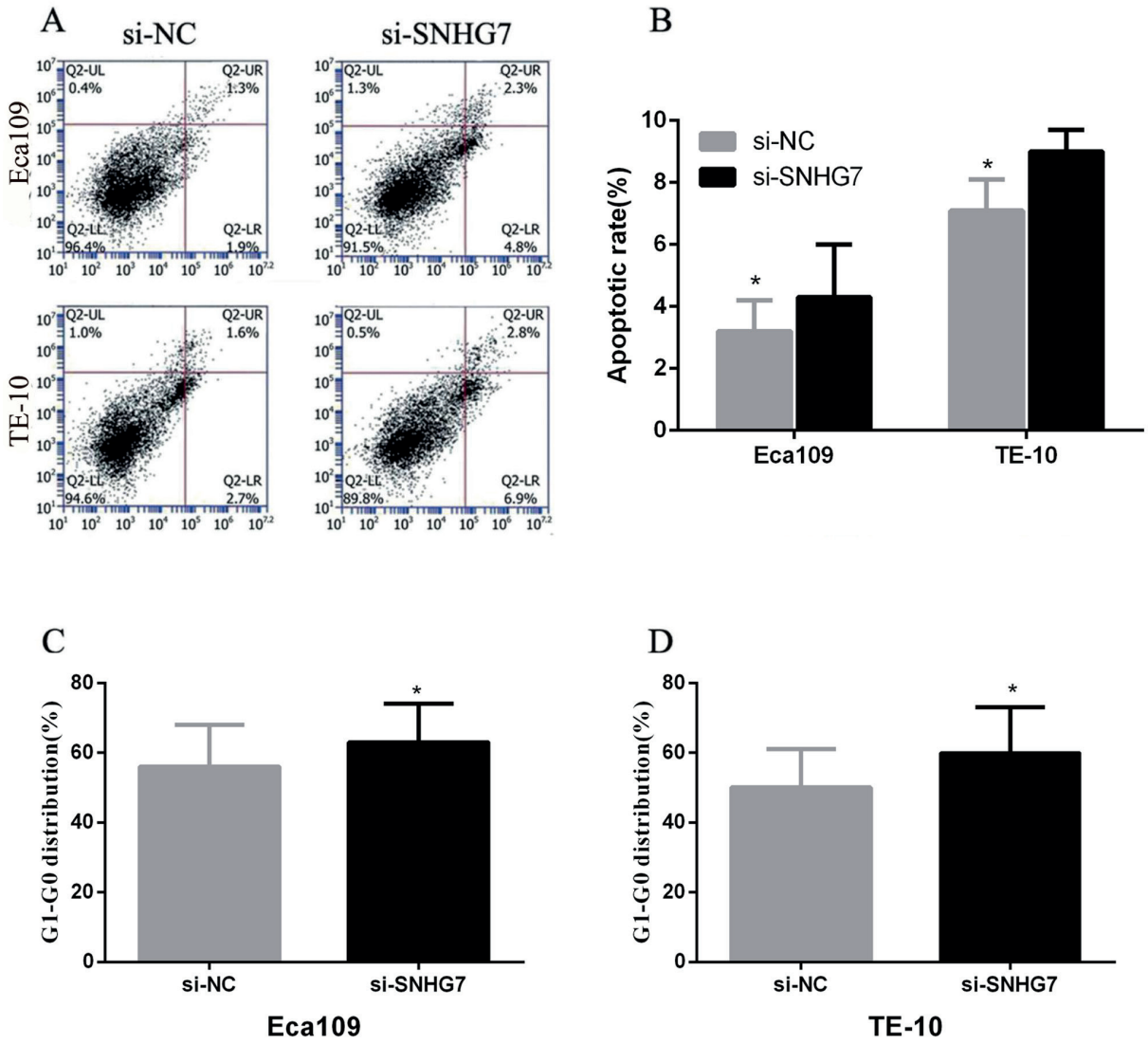

Figure 3. The effect of SNHG7 on the cell cycle and apoptosis of esophageal cancer cells. ***A,*** & ***B,*** Flow cytometry show that si-SNHG7 can promote the apoptosis of esophageal cancer cells. ***C,*** & ***D,*** Flow cytometry show that after the intervention of si-SNHG7 in esophageal cancer cells, the cell cycle is blocked in the G1-G0 phase (*p<0.05).

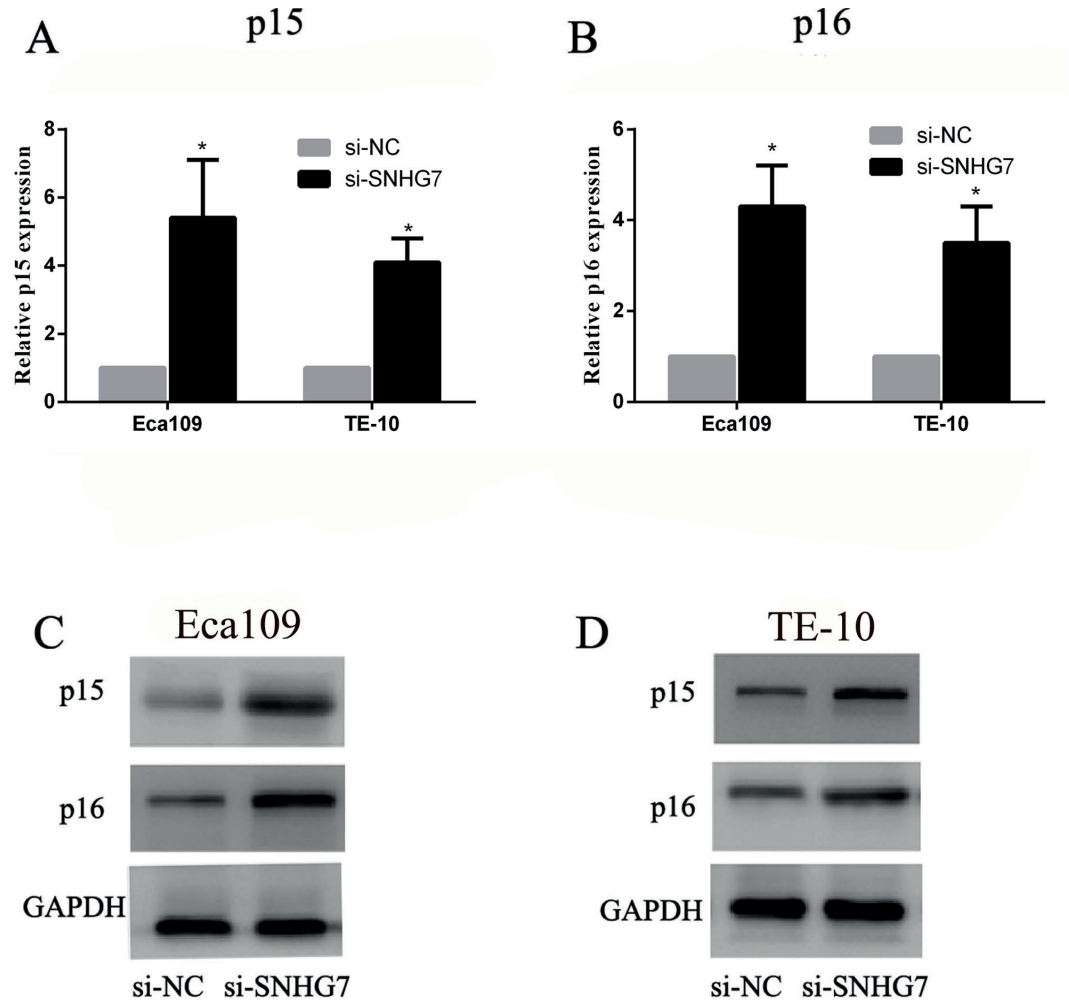

Figure 4. SNHG7 inhibits the expression of p15 and p16. ***A,*** & ***B,*** After si-SNHG7 intervention in esophageal cancer cells, qRT-PCR detection show that the expression of mRNA in p15 and p16 is obviously up-regulated. ***C,*** & ***D,*** Western blotting detection showed that the protein expression of p15 and p16 is also significantly up-regulated.

pression of p15 and p16, suggesting that SNHG7 may partly promote the development of esophageal cancer by regulating p15 and p16. However, the potential molecular mechanisms are not studied in this study. It is reported that the down-regulation of p15 and p16 in the tumor is closely related to the methylation of the gene promoter[20,21]. As a key gene regulator, lncRNA can transfer epigenetic compounds to the promoter of the target gene and inhibit transcription. Zhang et al[22] found that through combining with PRC2, lncRNA 00668 can inhibit the expression of p15 and p16 by epigenetic silencing. In the future, we will study whether SNHG7 can inhibit the transcription of p15 and p16 through PRC2 epigenetic compounds.

In summary, the up-regulation of SNHG7 in esophageal cancer can target p15 and p16 to promote the development and progression of esophageal cancer. Therefore, inhibition of SNHG7 can partly reverse the malignant phenotype of esophageal cancer.

Conclusions

We showed that the expression of SNHG7 in the tissues and cells of esophageal cancer was significantly up-regulated. SNHG7 can partly promote the development of esophageal cancer by regulating the expression of p15 and p16.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.
2) Qu LP, Zhong YM, Zheng Z, Zhao RX. CDH17 is a downstream effector of HOXA13 in modulating the Wnt/beta-catenin signaling pathway in gastric cancer. Eur Rev Med Pharmacol Sci 2017; 21: 1234-1241.
3) Peng C, Liu J, Yang G, Li Y. The tumor-stromal ratio as a strong prognosticator for advanced gastric cancer patients: Proposal of a new TSNM staging system. J Gastroenterol 2017:
4) Washietl S, Kellis M, Garber M. Evolutionary dynamics and tissue specificity of human long noncoding RNAs in six mammals. Genome Res 2014; 24: 616-628.
5) Liu JN, Shangguan YM. Long non-coding RNA CARLo-5 upregulation associates with poor prognosis in patients suffering gastric cancer. Eur Rev Med Pharmacol Sci 2017; 21: 530-534.
6) Cui WC, Wu YF, Qu HM. Up-regulation of long non-coding RNA PCAT-1 correlates with tumor progression and poor prognosis in gastric cancer. Eur Rev Med Pharmacol Sci 2017; 21: 3021-3027.
7) Lu S, Zhou J, Sun Y, Li N, Miao M, Jiao B, Chen H. The noncoding RNA HOXD-AS1 is a critical regulator of the metastasis and apoptosis phenotype in human hepatocellular carcinoma. Mol Cancer 2017; 16: 125.
8) Chen DL, Chen LZ, Lu YX, Zhang DS, Zeng ZL, Pan ZZ, Huang P, Wang FH, Li YH, Ju HQ, Xu RH. Long noncoding RNA XIST expedites metastasis and modulates epithelial-mesenchymal transition in colorectal cancer. Cell Death Dis 2017; 8: e3011.
9) Wang ZQ, Cai Q, Hu L, He CY, Li JF, Quan ZW, Liu BY, Li C, Zhu ZG. Long noncoding RNA UCA1 induced by SP1 promotes cell proliferation via recruiting EZH2 and activating AKT pathway in gastric cancer. Cell Death Dis 2017; 8: e2839.
10) He HT, Xu M, Kuang Y, Han XY, Wang MQ, Yang Q. Biomarker and competing endogenous RNA potential of tumor-specific long noncoding RNA in chromophobe renal cell carcinoma. Onco Targets Ther 2016; 9: 6399-6406.
11) She K, Huang J, Zhou H, Huang T, Chen G, He J. LncRNA-SNHG7 promotes the proliferation, migration and invasion and inhibits apoptosis of lung cancer cells by enhancing the FAIM2 expression. Oncol Rep 2016; 36: 2673-2680.
12) Chen D, Zhang Z, Mao C, Zhou Y, Yu L, Yin Y, Wu S, Mou X, Zhu Y. ANRIL inhibits p15(INK4b) through the TGFbeta1 signaling pathway in human esophageal squamous cell carcinoma. Cell Immunol 2014; 289: 91-96.
13) Bodoor K, Haddad Y, Alkhateeb A, Al-Abbadi A, Dowairi M, Magableh A, Bsoul N, Ghabkari A. DNA hypermethylation of cell cycle (p15 and p16) and apoptotic (p14, p53, DAPK and TMS1) genes in peripheral blood of leukemia patients. Asian Pac J Cancer Prev 2014; 15: 75-84.
14) Su J, Zhang E, Han L, Yin D, Liu Z, He X, Zhang Y, Lin F, Lin Q, Mao P, Mao W, Shen D. Long noncoding RNA BLACAT1 indicates a poor prognosis of colorectal cancer and affects cell proliferation by epigenetically silencing of p15. Cell Death Dis 2017; 8: e2665.
15) Sang Y, Tang J, Li S, Li L, Tang X, Cheng C, Luo Y, Qian X, Deng LM, Liu L, Lv XB. LncRNA PANDAR regulates the G1/S transition of breast cancer cells by suppressing p16(INK4A) expression. Sci Rep 2016; 6: 22366.
16) Tang Y, Wang J, Lian Y, Fan C, Zhang P, Wu Y, Li X, Xiong F, Li X, Li G, Xiong W, Zeng Z. Linking long non-coding RNAs and SWI/SNF complexes to chromatin remodeling in cancer. Mol Cancer 2017; 16: 42.
17) Wang PQ, Wu YX, Zhong XD, Liu B, Qiao G. Prognostic significance of overexpressed long non-coding RNA TUG1 in patients with clear cell renal cell carcinoma. Eur Rev Med Pharmacol Sci 2017; 21: 82-86.
18) Adams BD, Parsons C, Walker L, Zhang WC, Slack FJ. Targeting noncoding RNAs in disease. J Clin Invest 2017; 127: 761-771.
19) Hu Y, Ma Z, He Y, Liu W, Su Y, Tang Z. LncRNA-SNHG1 contributes to gastric cancer cell proliferation by regulating DNMT1. Biochem Biophys Res Commun 2017; 491: 926-931.
20) Zhao J, Liu Y, Zhang W, Zhou Z, Wu J, Cui P, Zhang Y, Huang G. Long non-coding RNA Linc00152 is involved in cell cycle arrest, apoptosis, epithelial to mesenchymal transition, cell migration and invasion in gastric cancer. Cell Cycle 2015; 14: 3112-3123.
21) Moselhy SS, Kumosani TA, Kamal IH, Jalal JA, Jabaar HS, Dalol A. Hypermethylation of P15, P16, and E-cadherin genes in ovarian cancer. Toxicol Ind Health 2015; 31: 924-930.
22) Zhang E, Yin D, Han L, He X, Si X, Chen W, Xia R, Xu T, Gu D, De W, Guo R, Xu Z, Chen J. E2F1-induced upregulation of long noncoding RNA LINC00668 predicts a poor prognosis of gastric cancer and promotes cell proliferation through epigenetically silencing of CKIs. Oncotarget 2016; 7: 23212-23226.