European Review for Medical and Pharmacological Sciences 2019; 23: 9277-9285

LncRNA SNHG7 contributes to cell proliferation, invasion and prognosis of cervical cancer

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Abstract. – OBJECTIVE: Long non-coding RNAs (lncRNAs) have been identified as oncogenes or tumor suppressor genes in the development of various cancers. However, the function of lncRNA SNHG7 in cervical cancer remains unclear. Therefore, the aim of this study was to investigate the potential role of lncRNA SNHG7 in cervical cancer and to explore the possible underlying mechanism.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was used to detect the expression of lncRNA SNHG7 in 60 cervical cancer tissues and adjacent normal tissues. Cell Counting Kit-8 (CCK-8) and transwell assays were used to study the effects of lncRNA SNHG7 on the proliferation and invasion of cervical cancer cells, respectively. Furthermore, Western blot was used to detect the expression levels of proteins in the epithelial-mesenchymal transition (EMT) process.

RESULTS: LncRNA SNHG7 expression increased significantly in cervical cancer tissues. The inhibition of lncRNA SNHG7 expression markedly inhibited cervical cell proliferation and invasion. Meanwhile, the inhibition of lncRNA SNHG7 resulted in the increased protein expression level of E-cadherin, and decreased protein expressions of N-cadherin and Vimentin. In addition, the survival time of patients with high expression of lncRNA SNHG7 was remarkably shortened.

CONCLUSIONS: LncRNA SNHG7 contributes to cell proliferation and invasion of cervical cancer. Moreover, SNHG7 has emerged as an independent and significant factor associated with poor survival of cervical cancer patients.

Key Words:
LncRNA SNHG7, Cervical cancer, Cell proliferation, Invasion.

Introduction

Cervical cancer is one of the most common malignant tumors in women, showing a younger trend. There are more than 500,000 new cases every year in the world. It is estimated that cervical cancer is the second most common malignant tumor in women, seriously threatening women's health[1]. Around 85% of cervical cancer patients occur in developing countries. The incidence of cervical cancer in China is as high as 7.5/100,000, and the mortality rate is 3.4/100,000. Meanwhile, the annual incidence rate is increasing in recent years[2]. Previous studies have found that human papillomavirus (HPV) infection can be detected in most patients with cervical cancer. However, HPV infection alone is not enough to result in cervical cancer. Currently, the main mechanism of cervical cancer development is still unclear. Therefore, an in-depth study of the pathogenesis of cervical cancer is of great significance for guiding the prevention and clinical treatment of cervical cancer, as well as improving the survival rate of patients.

More than 98% of transcripts in the human genome do not have the function of coding proteins, namely non-coding RNAs. According to their molecular weight, non-coding RNAs can be divided into three groups: short-chain non-coding RNA (22-23 nt), including miRNA, siRNA, piRNA; medium-chain non-coding RNA (50-200 nt); and long-chain non-coding RNA (>200 nt)[3]. Long non-coding RNAs (lncRNAs) are non-coding RNA molecules with transcripts longer than 200 nucleotides in length. In recent years, the expression and mechanism of lncRNAs in cancer have attracted more and more attention among researchers. Meanwhile, various mechanisms have already been discovered. LncRNAs can regulate protein-encoding genes at three levels, including epigenetic level (genomic imprinting, chromatin modification, chromosome silencing), transcriptional level and post-transcriptional level[4]. Several studies[5,6] have shown that human diseases, including cancer, cardiovascular disease, and diabetes, are associated with the dysregulation of lncRNAs.

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Epithelial-mesenchymal transition (EMT) is a biological process, in which epithelial cells lose cell polarity and adhesion, and obtain mesenchymal cell migration and invasion characteristics. The mechanism of EMT is complex, including the loss of E-cadherin, the induction of transforming growth factor beta (TGF-β), the activation of the Wnt signaling pathway, and the regulation of miRNAs. During the development of EMT, epithelial markers E-cadherin and zonal ZO-1 (zonula occludens-1) are down-regulated. However, the expressions of mesenchymal markers Vimentin and N-cadherin are up-regulated[7]. During the transition, the E-cadherin expression level is an important indicator of EMT. With the continuous understanding of EMT, it has been found that it participates in embryonic development, tissue repair, and organ fibrosis. Furthermore, EMT also plays an important role in tumor development, invasion, and metastasis. This discovery is particularly important for the study of tumors. Therefore, more and more scholars are committed to explore the specific mechanism of EMT to obtain more and greater breakthroughs. At present, some studies have shown that EMT is closely related to primary infiltration and secondary metastasis of various cancers such as breast cancer, colon cancer, and lung cancer. However, its specific mechanism in cancers needs further verification.

In this study, we detected the expression of lncRNA SNHG7 in cervical cancer tissues and adjacent normal tissues. Its relation to the clinical prognosis of cervical patients also was analyzed. We might provide a theoretical basis for lncRNA SNHG7 in the diagnosis, treatment and prognosis evaluation of cervical cancer. In addition, this might be helpful to find new markers for cervical cancer.

Patients and Methods

Collection of Cervical Tissue Samples

A total of 60 patients with cervical cancer who received treatment in our hospital from July 2014 to March 2018 were enrolled in this study. Specimens were surgically resected and diagnosed by histopathological examinations. 60 pairs of cervical cancer tissues and adjacent normal tissues (greater than 5 cm from tumor group) were obtained within 10 min after surgical resection. All collected tissues were stored at −80°C for use. This investigation was approved by the Ethics Committee of Wuhan Children's Hospital Tongji Medical College, Huazhong University of Science and Technology. Signed written informed consents were obtained from all participants before the study.

Cell Culture and Transfection

HeLa and C-33A cervical cancer cell lines were first seeded into 6-well plates. HeLa cells were cultured in Dulbecco's Modified Eagle's Medium-F12 (DMEM-F12) medium (Gibco, Rockville, MD, USA) containing 10% of fetal bovine serum (FBS; Gibco, Rockville, MD, USA). C-33A cells were cultured in modified 1640 medium (HyClone, South Logan, UT, USA). All cells were maintained in an incubator with 5% CO_2 at 37°C. Cell transfection was performed according to the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) at a cell density of 40% of the bottom of the culture flask. 5 μL of Lipofectamine 2000 and lncRNA SNHG7 siRNA dissolved in opti-MEM were added to each well. Meanwhile, a control group was established as well.

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

After transfection of lncRNA SNHG7 siRNA and negative siRNA for 48 h, total RNA was extracted using Pure-Link RNA MiniKit (Thermo Fisher Scientific, Waltham, MA, USA). DNase I was then used to remove genomic contamination. The extracted total RNA was reverse transcribed into complementary deoxyribose nucleic acid (cDNA) by Reverse Transcription Kit. Subsequently, the expression of lncRNA SNHG7 was detected. Specific qPCR conditions were as follows: 95°C for 2 min; 95°C for 10 s, 56°C for 10 s, 68°C for 12 s, for a total of 40 cycles; 95°C for 1 min; 55°C for 1 min; 70°C for 6 s; and 70°C for 6 s. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal reference. Primers used in this study were: lncRNA SNHG7-F: 5′-GTGACTTCGCCTGTGATGGA-3′, and lncRNA SNHG7-R: 5′-GGCCTCTATCTGTACCTTTATTCC-3′; GAPDH-F: 5'-ACCCAGAAGACTGTGGATGG- 3', and GAPDH-R: 5'-TTCTAGACGGCAGGTCAGGT-3'. PCR conditions were the same as above, and 3 duplicate wells were set in each group.

Cell Counting Kit-8 (CCK-8) Assay for Cell Proliferation

After transfection for 12 h, the cells were seeded into 96-well plates at a density of 2×10^3 /100 μL. CCK-8 (Dojindo Molecular Technologies, Kumamoto, Japan) assay was performed after culture for 24, 48, and 72 h, respectively. Brief-

ly, serum-free medium was replaced, and 10 μL of CCK-8 reagent was added to each well. After incubation for 2 h at 37°C and 5% CO_2, optical density (OD) value at 450 nm was measured by a micro-plate reader. 4 replicate wells were set in each group.

Matrigel Cell Invasion Assay

300 μL of serum-free medium was added to the chamber, followed by incubation for 30 min. The medium was then removed, and matrix gel and serum-free medium were diluted at 1:5. 100 μL of the dilution was added to each chamber and incubated at 37°C. After 30 min, the gel was coagulated. 12 h after transfection, the cells were digested and centrifuged at 1000 × g for 1 min. After re-suspension in the serum-free medium, cells were added to the upper chamber at a density of 3×10^5 cells per well. Meanwhile, 600 μL of 10% FBS medium was added to the lower chamber and cultured at 37°C under 5% CO_2 for 48 h. After that, the chamber was removed, and the culture medium was discarded. After washing twice with phosphate-buffered saline (PBS), the cells were fixed with 90% of ethanol for 30 min to remove 90% of ethanol. Then, the cells were washed twice with PBS and stained with 0.1% of crystal violet for 30 min. The upper un-migrated cells were gently wiped with a cotton swab. Finally, migrating cells were observed under a microscope at 400 times, and the number of migrating cells was counted. 5 fields of view were randomly selected for each sample.

Western Blot

Total protein of each group was extracted, and the extracted protein was denatured. Subsequently, the extracted proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes. After blocking with 5% of skim milk powder solution at room temperature for 2 h, the membranes were incubated with primary antibodies [dilution concentration: E-cadherin (Cell Signaling Technology, Danvers, MA, USA), N-cadherin (Abcam, Cambridge, MA, USA) and Vimentin (Cell Signaling Technology, Danvers, MA, USA), 1:1000; GAPDH 1:5000] overnight at 4°C. On the next day, the membranes were washed with 0.05% in Tris-Buffered Saline and Tween-20 (TBST) and incubated with the corresponding secondary antibody (dilution concentration: 1:5000, Southern Biotech, Birmingham, AL, USA) at room temperature for 2 h. The membranes were washed again with 0.05% TBST solution for 3 times. The gray value of the strip was determined by an image analyzer.

Statistical Analysis

Statistical Product and Service Solutions (SPSS; IBM, Armonk, NY, USA) was used for all statistical analysis. The Student's *t*-test was used to compare the difference between the two groups. Overall survival was determined using the Kaplan-Meier method. Prognostic factors were assessed by multivariate analysis using the Cox proportional-hazards regression model. $p<0.05$ was considered statistically significant.

Results

Expression of LncRNA SNHG7 in Cervical Cancer Tissues

The expression of lncRNA SNHG7 in 60 paired cervical cancer tissues and adjacent normal tissues was detected by RT-PCR. The results showed that lncRNA SNHG7 expression in cervical cancer tissues was significantly higher than in adjacent normal tissues ($p<0.01$, Figure 1A). The relation between lncRNA SNHG7 expression and clinic-pathological features (including age, tumor size, pathological type, tumor differentiation, TNM stage, lymph node metastasis, and depth of tumor invasion) was shown in Table I. The expression of lncRNA SNHG7 was correlated with TNM stage, lymph node metastasis and depth of tumor invasion of cervical cancer patients, and the difference was statistically significant ($p<0.05$). However, lncRNA SNHG7 expression was not related to age, tumor size, pathological type, and tumor differentiation of patients.

Transfection of siRNA Interferes with LncRNA SNHG7 Expression in HeLa and C-33A Cells

Two cervical cell lines were transfected with lncRNA SNHG7 siRNA and negative siRNA in parallel. Total RNA in cells was extracted 48 h later, and the expression of lncRNA SNHG7 in transfected cells was detected by RT-PCR. Results showed that the expression level of lncRNA SNHG7 decreased significantly in the two groups (Figure 1B and 1C). This indicated that lncRNA SNHG7 siRNA could effectively interfere with the expression of lncRNA SNHG7 and significantly reduce lncRNA SNHG7 expression in cells.

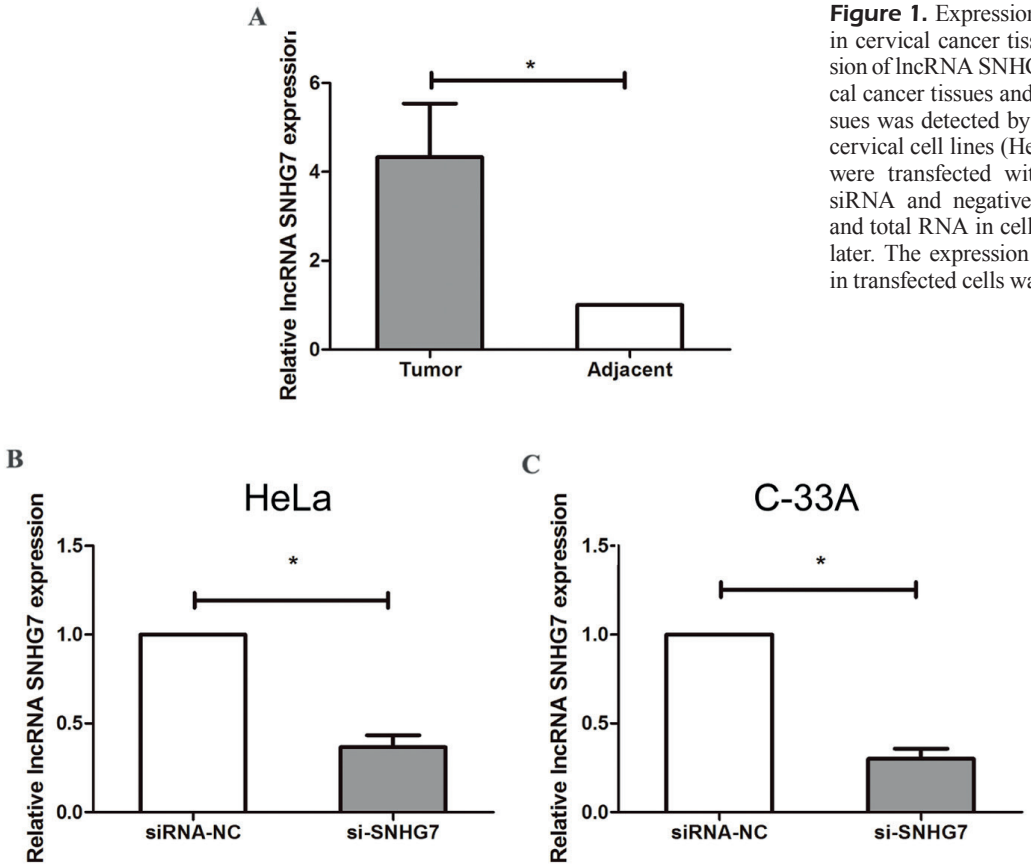

Figure 1. Expression of lncRNA SNHG7 in cervical cancer tissues. **A,** The expression of lncRNA SNHG7 in 60 paired cervical cancer tissues and adjacent normal tissues was detected by RT-PCR. **B-C,** Two cervical cell lines (HeLa and C-33A Cells) were transfected with lncRNA SNHG7 siRNA and negative siRNA in parallel, and total RNA in cells was extracted 48 h later. The expression of lncRNA SNHG7 in transfected cells was detected by qPCR.

Table I. The relationship between lncRNA SNHG7 expression and clinicopathological features.

Clinicopathological features	n	lncRNA SNHG7		χ^2	*p*
		Low (n)	High(n)		
Age (years)					
<40	31	15	16		
≥40	29	13	16	0.076	0.782
Tumor size (cm)					
<3	27	12	15		
≥3	33	16	17	0.097	0.755
Pathological type					
Squamous carcinoma	47	22	25		
Adenocarcinoma	13	6	7	0.002	0.967
Tumor differentiation					
High/moderate	32	16	16		
Low	28	12	16	0.306	0.580
TNM					
I-II	31	22	9		
III-IV	29	6	23	15.218	0.000**
Lymph node metastasis					
No	31	25	6		
Yes	29	3	26	29.752	0.000**
Depth of tumor invasion					
<2/3	31	24	7		
≥2/3	29	4	25	24.371	0.000**

Interference With LncRNA SNHG7 On the Proliferation and Invasion of Cervical Cancer Cells

Cell proliferation results of CCK8 assay were plotted with time as horizontal axis and OD450 as vertical axis. Results showed that compared with negative control cells, OD450 values of HeLa and C-33A cells transfected with lncRNA SNHG7 siRNA decreased significantly, indicating that the cell proliferation was inhibited (Figure 2A and 2B).

The Matrix gel chamber invasion assay results showed that the number of HeLa and C-33A cells entering the lower compartment of the chamber was remarkably reduced after interfering with ln-

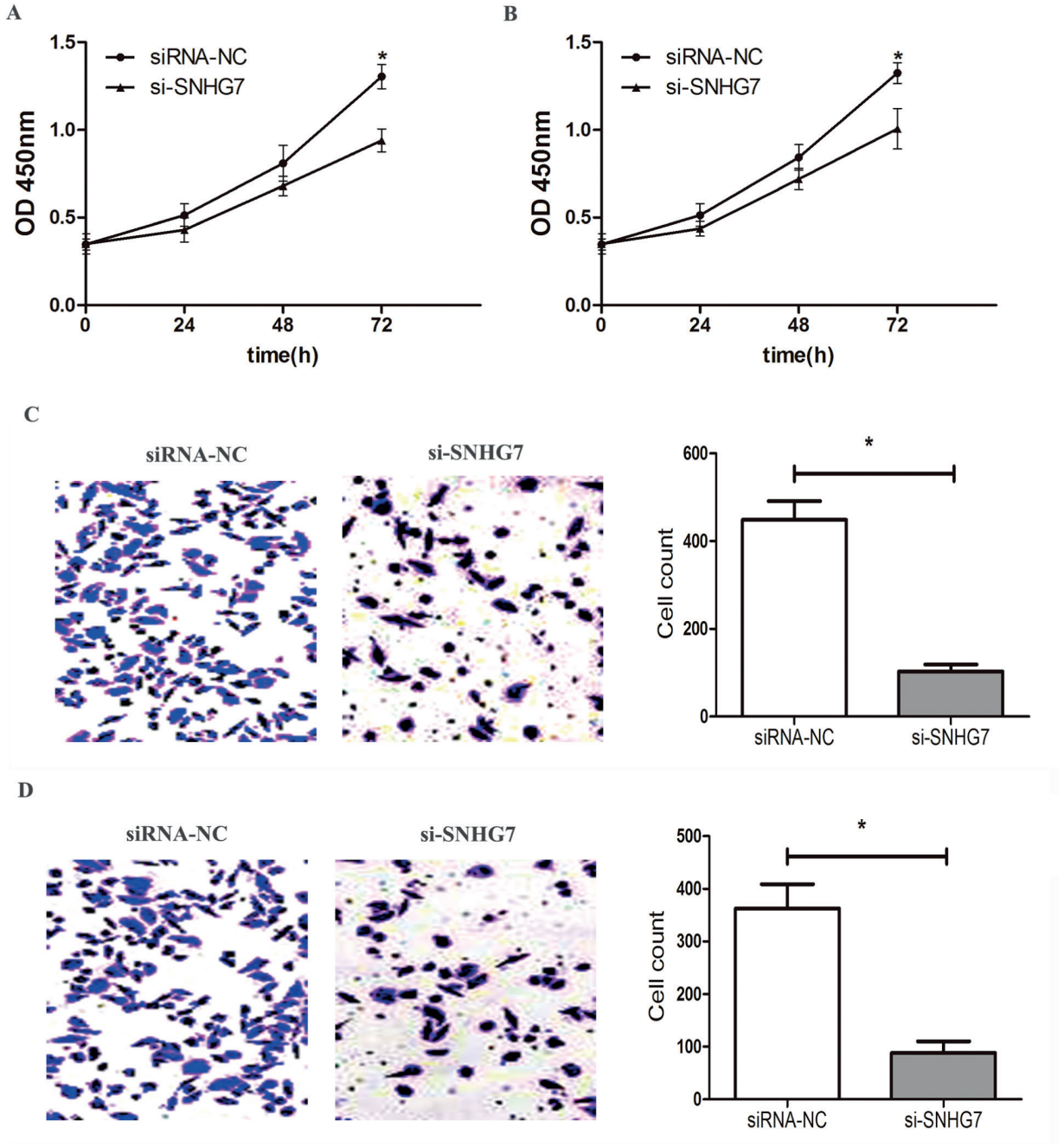

Figure 2. Interference with lncRNA SNHG7 on the proliferation and invasion of cervical cancer cells. **A-B,** Cell proliferation results of CCK8 were plotted with time as horizontal axis and OD450 as vertical axis. **C-D,** Matrix gel chamber invasion assay results showed that the number of HeLa and C-33A cells entering the lower compartment of the chamber was significantly reduced after interfering with lncRNA SNHG7 when compared with negative control cells (magnification: 40×).

cRNA SNHG7 when compared to negative control cells (Figure 2C and 2D). The results indicated that lncRNA SNHG7 could promote cell invasion.

Effect of LncRNA SNHG7 On the Progression of EMT in Cervical Cancer Cells

To evaluate whether lncRNA SNHG7 could influence the progression of EMT in cervical cancer cells, the Western blot assay was conducted. Notably, results showed that, compared with si-NC group, the protein expression of E-cadherin in si-lncRNA SNHG7 group increased significantly. However, the protein expression levels of N-cadherin and Vimentin in si-lncRNA SNHG7 group decreased significantly (Figure 3A and 3B). These findings demonstrated that lncRNA SNHG7 enhanced the EMT process in cervical cancer cells, thereby promoting cell migration and invasion.

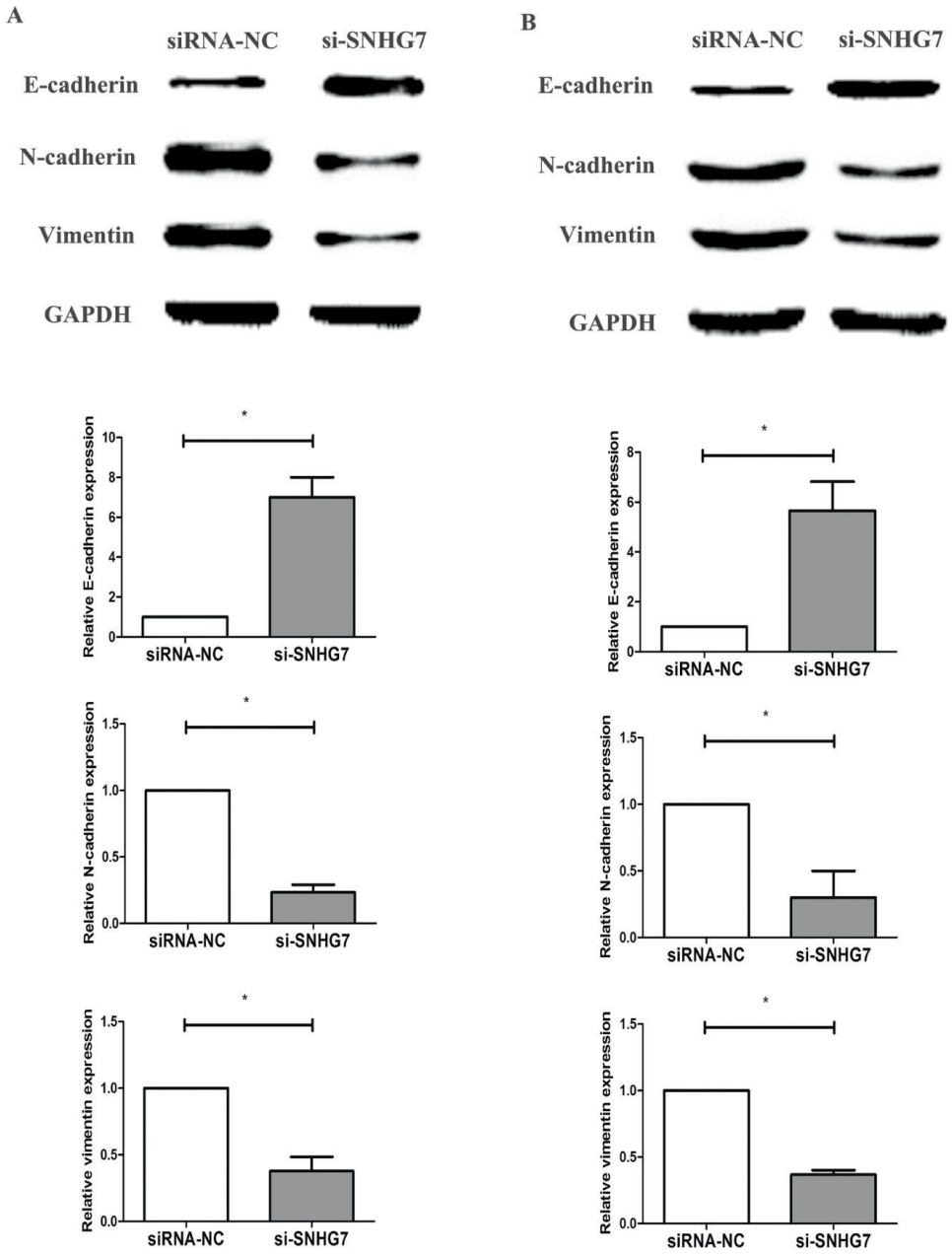

Figure 3. Effect of lncRNA SNHG7 on the progression of EMT in cervical cancer cells. **A-B,** Western blot was used to detect the expression levels of related proteins in EMT process.

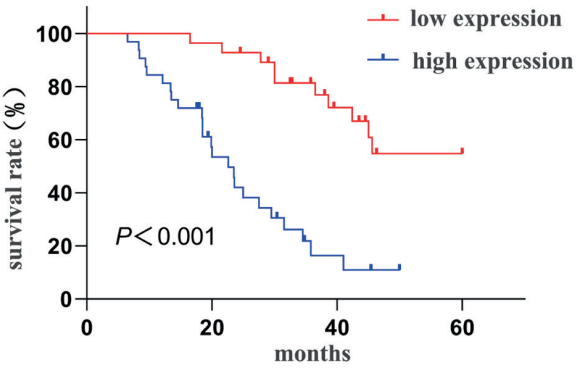

Figure 4. Association of lncRNA SNHG7 expression with clinical prognosis. Kaplan-Meier analysis was performed to investigate the correlation between lncRNA SNHG7 expression and overall survival of cervical cancer patients.

Association of LncRNA SNHG7 Expression With Clinical Prognosis

Kaplan-Meier analysis was performed to investigate the correlation between lncRNA SNHG7 expression and the overall survival of cervical cancer patients. Our results showed that the overall survival time of patients with higher expression of lncRNA SNHG7 was significantly shorter (Figure 4) than those with lower expression of lncRNA SNHG7. Multivariate analysis was conducted using the Cox proportional hazards model. Results confirmed that lncRNA SNHG7 expression emerged as an independent and significant factor associated with a poor survival rate of cervical cancer patients (Table II).

Discussion

Cervical cancer is a serious threat to women's health worldwide. Due to its poor prognosis, it is particularly important to study the molecular mechanism of cervical cancer development. More and more studies have shown that non-coding RNAs play important roles in the development of cervical cancer. Some microRNAs (miRNAs) are abnormally expressed in cervical cancer and can affect the proliferation and migration of cervical cancer cells. Meanwhile, some special miRNAs can be used as molecular markers for the diagnosis of cervical cancer[8,9]. The earliest known lncRNA associated with cervical cancer is BC200, which is highly expressed in cervical cancer tissues[10]. It has also been found that knocking down the expression of MALAT1 in cervical cancer cells (Caski) significantly reduces cell migration ability and the tumorigenic ability of nude mice[11]. Qin et al[12] have found that MEG3 is significantly down-regulated in cervical cancer tissues by comparing 18 pairs of cervical cancer tissues and adjacent normal tissues. Overexpression of MEG3 inhibits the proliferation of cervical cancer HeLa and C-33A cells *in vitro*. Liao et al[13] have indicated that XLOC_010588 is down-regulated in cervical cancer tissues. Overexpression of XLOC_010588 inhibits the proliferation of HeLa and SiHa cells, while the knockdown of XLOC_010588 inhibits the growth of HCC94 cells. Studies on the molecular mechanism of XLOC_010588 have shown that XLOC_010588 binds to c-Myc, thereby reducing c-Myc expression. These findings suggest that lncRNA plays an important role in cervical cancer, showing potential applications. Some of these lncRNAs are tumor suppressor genes, while some are cancer-promoting genes. All these lncRNAs play different roles in different tissues[14-16]. These findings indicate that the expressions of lncRNAs are tissue and individual specific. LncRNAs can have multiple targeted functional genes, depending on the function of the downstream target gene being regulated. There is also a difference in the function of the same lncRNA in tumorigenesis. This is also the cause of tissue and individual specificity. In this study, we validated that lncRNA SNHG7 expression increased significantly in cervical cancer tissues. Moreover, inhibition of lncRNA SNHG7 expression significantly inhibited cervical cell proliferation and invasion.

It is an undeniable fact that lncRNA is involved in the development, invasion, and metastasis of various tumors. Previous studies have indicated

Table II. Multivariate analysis was conducted using the Cox proportional hazards model.

Clinicopathological features	HR	95%	p
TNM	2.997	1.038-8.649	0.042*
Lymph node metastasis	4.156	1.177-14.684	0.027*
Depth of tumor invasion	0.249	0.098-0.634	0.004**
lncRNA SNHG7	4.434	1.629-12.069	0.004**

that EMT is also an important factor in tumorigenesis, development, invasion and distant metastasis. The involvement of lncRNA in EMT regulation has attracted much attention among researchers. Richards et al[17] induced a TGF-β-induced EMT model of mammary epithelial cells. They have found that lncRNA-HIT can promote the invasion and migration of tumor cells, and up-regulate the expression of E-cadherin after the down-regulation of lncRNA-HIT. Meanwhile, lncRNA-HIT can down-regulate the expression of Vimentin, and inhibit tumor migration and invasion. Liang et al[18] have found that H19 is highly expressed in mesenchymal tumor cells and tissues. Moreover, H19 competitively antagonizes miR-138 and miR-200A functions, resulting in the rise of Vimentin protein, ZEB1, and ZEB2 expression. Eventually, this may lead to the EMT process. Thus, the expressions of lncRNAs play important regulatory roles in EMT. However, the mechanism of lncRNA in the regulation of EMT is very complicated. In the future, researchers will work to explore more lncRNAs related to tumor EMT and elucidate their specific mechanisms. This will open up wider insights for lncRNAs in the diagnosis and treatment of tumors. In this study, we found that the inhibition of lncRNA SNHG7 expression resulted in the increased expression level of E-cadherin, and decreased expressions of N-cadherin and Vimentin proteins. Furthermore, the survival time of patients with high expression of lncRNA SNHG7 was significantly shortened.

Conclusions

We are first to demonstrate that lncRNA SNHG7 acted as an oncogene in cervical cancer and contributed to cervical cell proliferation and invasion. Moreover, lncRNA SNHG7 might emerge as an independent and significant factor associated with a poor survival rate of cervical cancer patients.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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