LncRNA SNHG7 promotes the proliferation and inhibits apoptosis of gastric cancer cells by repressing the P15 and P16 expression

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Abstract. — OBJECTIVE: To investigate the relative expression of long non-coding small nucleolar RNA host gene 7 (lncRNA SNHG7) in gastric cancer tissues and cells, the effect of lncRNA SNHG7 on proliferation and apoptosis of gastric cancer cells in vivo and in vitro experiments, and the relevant mechanism.

PATIENTS AND METHODS: Real-time quantitative polymerase chain reaction (qRT-PCR) experiment was performed to detect the relative expressions of SNHG7 in the gastric cancer tissues and cells. In presence of Lip2000, SNHG7 interference sequence was transiently transfected into the gastric cancer cells followed by transfection efficiency detection by qRT-PCR. Cell count kit 8 (CCK-8) and clone formation assay were also carried out to detect the effect of SNHG7 on the proliferation of gastric cancer cells, flow cytometry (FCM) to detect the effect of SNHG7 on the cycle and apoptotic rate of gastric cancer cells, in vivo experiment to detect the effect of SNHG7 on biological behaviors of gastric cancer cells, and Western blotting assay to detect the variations in expression of downstream proteins after SNHG7 expression was interfered.

RESULTS: The qRT-PCR experiment showed that in a total of 68 cases of cancer tissues and tumor-adjacent tissues, the relative expression of SNHG7 was upregulated in 48 cases of gastric cancer tissues and 5 gastric cancer cell lines. The in vitro experiments showed that after SNHG7 expression was interfered, the proliferation of gastric cancer cells was inhibited with an increase in apoptotic rate and arrest of cell cycle in G1/G0 phase. Experiment on nude-mouse transplanted tumor model confirmed that after SNHG7 expression was interfered, in vivo tumor growth was inhibited, and the Western blotting assay revealed that regulation of p15 and p16 expressions constituted a part of the potential molecular mechanism.

CONCLUSIONS: Relative expression of SNHG7 is upregulated in gastric cancer tissues and cells, and partially contributes to the development and progression of gastric cancer through regulation of p15 and p16 expressions. Thus, interference on expression of SNHG7 can provide critical theoretical basis for inverting the malignant phenotype of gastric cancer in clinical practice.

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

Introduction

Gastric cancer has been regarded as a major issue of public health in the world, and the most common malignant tumor in digestive system; globally, the incidence rate of gastric cancer has ranked 4th in all cancers, and the mortality rate ranked as high as 2nd. It has been recognized that the pathogenesis of gastric cancer is quite complicated, and according to a study, the development and progression of the majority of gastric cancer cases are affected by the interaction between gene and environment, and also the results caused by the common effects of environment and predisposing factors of tumors. In recent years, treatment of gastric cancer has been improved due to the wide promotion of multidisciplinary synthetic therapy, but the 5-year survival rate of resection for gastric cancer in advance stage has been sustained within 30% to 50% for a period. Hence, through the in-depth research on the specific molecular mechanism of development and progression of gastric cancer, we can identify the high-risk individuals and molecular marker for early diagnosis, and establishment new therapeu-
tic target, which is of great clinical significance for improving the efficacy on gastric cancer. Long non-coding RNAs, also known as lncRNAs, are a kind of non-coding RNAs in length of over 200 nt, and transcribed generally in eukaryotes; lncRNAs, in form of RNA, can regulate the gene expressions in various levels, including pre-transcription, transcription and post-transcription, but do not encode the protein. According to the locus of lncRNAs in genes, they can be further divided into 5 kinds: positive-sense lncRNA, anti-sense lncRNA, double-oriented lncRNA, intragenic lncRNA and intergenic lncRNA. Initially, it was believed that lncRNAs were the by-product in transcription of RNA polymerase II without any biological functions, but recent studies showed that lncRNAs are involved in various regulatory processes, including silencing of the X chromosome, genomic imprinting, chromatin modification, transcriptional activation and interference, intranuclear transport. The abnormal expression of lncRNAs is closely correlated with a variety of tumors. According to the literature, the relative expression of lncRNA HOXD-AS1 is upregulated in hepatocellular carcinoma (HCC) with the effect to promote the metastasis and inhibit the apoptosis of HCC cells. Wu et al. found that in esophageal cancer, lncRNA XIST can facilitate the development and progression of tumor through regulating the activity of miR-101/ enhancer of zeste homolog-2 (EZH2). In gastric cancer, transcription specificity protein 1 (SP1), a transcriptional factor, can promote the transcription of lncRNA urothelial carcinoma-associated 1 (UCA1), and activate the AKT signaling pathway, thereby accelerating the proliferation of gastric cancer cells.

Long non-coding small nucleolar RNA host gene 7 (lncRNA SNHG7) is located on chromosome 9q34.3 in length of 984 bp, and, so far, there remain few studies reporting the expression and biological functions of SNHG7 in tumors. In gastric cancer, the upregulation of SNHG7 is negatively correlated with the prognosis of patients; in lung cancer, SNHG7 can promote the proliferation, invasion and metastasis of lung cancer cells. However, no studies have reported the expression and biological effect of SNHG7 in gastric cancer. Hence, we investigated the expression and biological functions of SNHG7 in gastric cancer from the experiments of human gastric cancer tissue specimen, cells and living animals, and preliminarily explored the potential molecular mechanism.

Patients and Methods

Tissues and Cells

We collected a total of 68 pairs of gastric cancer tissue specimens from the patients who were admitted to the general surgery department of Jiangyin Hospital Affiliated to Southeast University Medical College between January 2012 and December 2015, and all enrolled patients conformed to the following criteria: a) patients who were firstly diagnosed as gastric cancer by pathological examination and had not received any treatment; b) patients without any other major complications, especially malignant tumors, and in good physical condition. This study was approved by the Ethic Committee of Jiangyin Hospital Affiliated to Southeast University Medical College, and all patients and their family had been informed consent and signed the written informed consent. Gastric cancer tissues and normal tissues that were 5 cm or so away from the cancer tissues were resected, sliced and placed in 1.5 mL Eppendorf (EP) tubes followed by preservation at -80°C refrigerator for extraction of total RNA.

Human gastric cancer cell lines (BGC823, MGC803, SGC7901, N87 and AGS) and human normal gastric mucosa epithelial cell GES-1 (Institute of Biochemistry and Cell Biology, SIBS, CAS, Beijing, China) were cultured in F12 or 1640 medium (Hyclone, South Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS) (Hyclone, South Logan, UT, USA), where penicillin (100 U/mL) and streptomycin (100 mg/mL) were added to prevent the contamination. Cell suspension was placed in a thermostat incubator (5% CO₂, 37°C), and digestion and subculture of cells were routinely carried out.

RNA Extraction and Detection of SNHG7 Expression Through Real-Time Quantitative Polymerase Chain Reaction (qRT-PCR)

TRIzol reagent was added in the tissue and cell samples to extract the total RNA, and the obtained RNA was placed at 4°C or -80°C refrigerator for long-term preservation. Biological spectrometer was used to detect the concentration of RNA, in which RNA samples with an A260/A280 ratio was within 1.8 to 2.0 was used for later experiment. According to the instruction of Prime Script RT Reagent Kit (Perfect Real-time), cDNA was prepared through reverse transcription of RNA; per the requirement of SYBR Pre-
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mix Ex Taq™ (Tli RNaseH Plus), qRT-PCR reaction system was prepared, and reaction was performed in following conditions: initial denaturation at 95°C for 1 min, 95°C for 30 s, 60°C for 40 s for a total of 40 cycles. In this experiment, 3 replicate wells were set and all samples were detected in triplicate. The relative expression of target gene was expressed in -ΔCt and 2^(-ΔΔCt).

**Synthesis of Interference Sequence and Primers of SNHG7**

Interference sequence and primers of SNHG7 were designed and synthesized by Invitrogen (Shanghai, China) with Primer 5.0: SNHG7 upstream primer 5'-CCGTGGGCACTGCTGAAT-3', downstream primer 5'-CTGGCTGGCAAGCGGAAC-3'; glyceraldehyde-phosphate dehydrogenase (GAPDH), upstream primer 5'-AGCCACATCGCTCAACTACCA AATCC-3', downstream primer 5'-GCCAATACGACCA AATCC-3'; interference sequence of SNHG7 #3, 5'-CCAGAGAACCTGCCTTCTC TTCCTAA-3'.

**Cell Count kit 8 (CCK8) and Clone Formation Assay**

Transient transfection with interference sequence and control sequence was performed for gastric cancer cells, and after 6 hours, we collected the cell suspension, in which cell density was adjusted to 4×10^4/mL. Cells were then evenly seeded on the 96-well plate (80 μL/well), and 6 replicate wells were set in both experiment group and control group. Plates were taken out at five time points with the medium being discarded, namely 0 h, 24 h, 48 h, 72 h and 96 h, and CCK-8 mixture with serum-free medium (1:10) was added into the 96-well plate (110 mL/well). Optical density (OD) at wavelength of 450 nm was detected with a microplate reader for preparation of growth curve. Cells in experiment group and the control group were seeded on a 6-well plate (1000/well), and, 3 days later, medium was exchanged. After 14 days, cells were fixated in formaldehyde and stained with crystal violet followed by cell count and photographing.

**Flow Cytometry (FCM)**

Gastric cancer cells in logarithmic phase were adjusted to 3×10^5/mL, and seeded on a 6-well plate (2 mL/well). After cell adherence, cells were transfected with SNHG7 interference sequence and control sequence transiently. Following 48 h of culture, cells in both groups were collected and washed with phosphate buffer saline (PBS) for preparation of cell resuspension. Cells were then stained with Annexin-V/PI and placed in a dark place for 15 min followed by detecting the apoptotic rate of cells in both groups with flow cytometer; similar methods were applied for treatment and collection of cells, and thereafter, cells were fixed in pre-cooled 75% ethanol in -20°C refrigerator overnight. DNA content was detected *via* propidium iodide (PI) staining, and cell cycles were divided into G1/G0, S, G2/M stages, and the percentage of previous stages was calculated with software.

**Western Blotting Assay**

Cells in the experiment group and control group were collected and treated with radio-immunoprecipitation assay (RIPA) kit for extraction of total protein. Bicinchoninic acid (BCA) kit (Beyotime, Beijing, China) was used to prepare the standard curve of protein concentration and detect the protein concentrations in all samples, in which all of the protein samples were adjusted to the same concentration for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Thereafter, proteins that were separated were transferred on a 0.22 μm polyvinylidene fluoride (PVDF) membrane, and the PVDF membrane was incubated with tris-buffer saline tween 20 (TBST) containing 5% skimmed milk. With GAPDH as internal reference, rabbit anti-human antibody of p15 (dilution at 1:1000, CST, MA, USA), and rabbit anti-human antibody of p16 (dilution at 1:1000, CST, MA, USA) were added to the membrane for incubation overnight at a 4°C refrigerator. After the membrane was washed three times with TBST (5 min/time), secondary antibody (dilution at 1:1000, CST, Danvers, MA, USA) was added to the membrane for incubation at room temperature for 2 h followed by washing membrane with TBST three times (10 min/time). Enhanced chemiluminescence (ECL) solution that was prepared in advance, together with 20 μL color development reagent, was added to the membrane followed by exposure in chemiluminescence apparatus and saving the images.

**Tumor Formation in Nude Mice**

With male nude mice aged between 4 and 5 weeks as subjects, cells in the experiment group and control group were injected in the axilla of BALB/c mice to establish the experimental xenograft model; during the experiment, nude mice...
should be kept in specific pathogen-free (SPF) animal experiment room. Every three days, mice were observed and weighed. After 18 days of growth, nude mice were executed and the xenograft tumor was taken for hematoxylin and eosin (HE) staining.

**Statistical Analysis**

Data were processed with Statistical Product and Service Solutions 19.0 (SPSS 19.0, Inc., Chicago, IL, USA). Based on the type of data, data in normal distribution were presented as mean ± standard deviation, and in skewed distribution were presented as median or quartile. For measurement data, Kolmogorov-Smirnov test of single sample was firstly performed to testify whether the data were in normal distribution. For data conforming to normal distribution, independent sample t-test and pairwise t-test were performed for comparison; while for those in skewed distribution, Wilcoxon signed-rank test was performed. \( p < 0.05 \) suggested that the difference had statistical significance.

**Results**

**SNHG7 Was Highly Expressed in Gastric Cancer Tissues and Cells**

To detect the expressions of SNHG7 in gastric cancer tissues and tumor-adjacent tissues, we carried out qRT-PCR experiment to detect the relative expression of SNHG7 in 68 pairs of gastric cancer tissues and tumor-adjacent tissues. Results showed that compared with the tumor-adjacent tissues, upregulation in relative expression of SNHG7 was found in 48 cases of gastric cancer tissues (fold ≥ 1.0, Figure 1A). Sequentially, qRT-PCR experiment was again conducted for measuring the relative expressions in 5 gastric cancer cell lines (BGC823, MGC803, SGC7901, N87 and AGS) and the normal gastric cell line (GES-1), and we found that SNHG7 was significantly upregulated in gastric cancer cells (Figure 1B). In order to discover the biological functions of SNHG7, specific interference sequences were designed and synthesized; si-SNHG7 and control sequence were transiently transfected into the gastric cancer cell lines, and after 48 h, we detected the interference efficiency, in which the 3# sequence was used for later experiment (Figure 1C and D).

**Interference on SNHG7 Expression Inhibited Cell Proliferation**

Firstly, we conducted the CCK-8 experiment to investigate the effect of SNHG7 on proliferation of gastric cancer cells, and found that after the interference on SNHG7 expression in AGS and SGC7901, significant inhibition was identified in cell proliferation (Figure 2A and B); besides, clone formation assay also confirmed that interference on SNHG7 expression inhibited the proliferation of tumor cells (Figure 2C and D).

**Interference on SNHG7 Expression Inhibited Cell Apoptosis and Promotes the Arrest of Cell Cycle**

To investigate whether SNHG7 inhibits the cell proliferation through affecting cell cycle, we transfected the AGS and SGC7901 cells with si-SNHG7 transiently, and the FCM results showed that in comparison with the transfection-control group, cell cycles in AGS and SGC7901 cells transfected with si-SNHG7 were arrested in G1-G0 phase (Figure 3A and B). Thereafter, we studied the effect of SNHG7 on apoptosis of gastric cancer cells, and after cells were treated by similar methods, the FCM results showed that in comparison with the control group, apoptotic rate of cells in experiment group was significantly increased (Figure 3C and D).

**Interference on SNHG7 Inhibited the In Vivo Tumorigenesis**

In order to detect the role of SNHG7 in animals, stable transfection of SGC7901 cells with sh-SNHG7 or control sequence was performed followed by the cell injection in the axilla of nude mice to establish the xenograft models. After 18 days, nude mice were executed, and the subcutaneous tumor tissues were taken for photographing and weighing. The results showed that the volume and weight of xenograft tumors formed by injection of sh-SNHG7 transfected cells were significantly lower than those in the control group (Figure 4A and B). Then, we took some tumor tissues for detecting the relative expression of SNHG7 in xenograft tumors via qRT-PCR, and found that the SNHG7 expression in xenograft tumor formed by injection of sh-SNHG7-transfected cells was significantly lower than that in the control group (Figure 4C). Next, xenograft tumors were fixed and sliced for immunohistochemistry assay, HE staining and Ki67 staining. HE staining results showed that the xenograft tumor was successfully established, and the Ki67 staining results indicat-
ed that in xenograft tumors formed by injection of sh-SNHG7-transfected cells, the positive rate of Ki67 was remarkably lower than that in the control group (Figure 4D).

**SNHG7 Regulated Expressions of p15 and p16**

According to the literatures\textsuperscript{12,13}, in a variety of tumors, p15 and p16 act as tumor-suppression genes, and their expressions are inhibited. However, IncRNA, as the major regulator, is involved in the regulation of p15 and p16 expressions. Su et al\textsuperscript{14} reported that in colorectal cancer, IncRNA BLACAT1 can epigenetically silence the expression of p15, thereby facilitating the proliferation of colorectal cancer cells. In breast cancer, IncRNA PANDAR can inhibit the expression of p16, further regulating the cell cycles in tumor\textsuperscript{15}. In this study, we firstly found that interference on expression of SNHG7 could promote the expressions of p15 and p16 (Figure 5).
Pathogenesis of gastric cancer remains unclear despite that various oncogenes, tumor-suppressor and tumor-associated signal pathways have been discovered and confirmed in recent years. Currently, molecules, regulation, and intervention mechanism in association with the gastric cancer, have become the hotspots.

At first, IncRNA was believed to be the “noise” in transcription of human genome without any biological effect in human. However, with the development in science and technology, scientists have found that in growth, development and many diseases, IncRNAs, though without any protein-encoding function, exhibit critical effect in genetic regulation; particularly in the diseases with complicated pathogenesis and mechanism, such as tumor, the regulatory effect of IncRNA is more important. In recent years, there emerge new studies reporting the correlations of IncRNAs with proliferation, apoptosis, invasion, metastasis and resistance to chemotherapeutics in gastric cancer. For instance, Hu et al. found that IncRNA SNHG1 can promote the proliferation of gastric cancer cells through regulating the expression of...

Figure 2. Effect of SNHG7 on proliferation of gastric cancer cells. A and B, CCK8 experiment result shows that after interference on SNHG7 expression, the proliferation of gastric cancer cells is significantly inhibited. C and D, Clone formation assay shows that in gastric cancer cells, after interference on SNHG7 expression, cell proliferation is decreased (**p < 0.01; *p < 0.05).
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DNAmethyltransferase 1 (DNMT1). LncRNA 00152 can promote the invasion and metastasis of gastric cancer cells through regulating the epithelial-mesenchymal transition (EMT). However, in this study, we firstly found the relatively high expression of SNHG7 in gastric cancer tissues and cells, and discovered that after interference on SNHG7 expression, proliferation and apoptosis of tumor cells were inhibited and facilitated respectively, and arrest of cell cycle was induced; the experiment of tumor formation in nude mice confirmed that knockdown of SNHG7 expression could inhibit the in vivo tumorigenesis.

In many tumors, p15 and p16 have been confirmed as tumor suppressor. Through the Western blotting assay, we found that inference on SNHG7 could increase the expressions of p15 and p16, suggesting that SNHG7 could promote the development and progression of gastric cancer partially through regulation of p15 and p16. However, the potential molecular mechanism was not investigated in this study. It is reported that downregulation of p15 and p16 in tumors is closely correlated with the methylation of gene promoters. LncRNA, as a key gene regulator, can deliver the epigenetic compound to the promoter of target gene, thereby suppressing the transcrip-

Figure 3. Effect of SNHG7 on cell cycle and apoptosis in gastric cancer. A and B, FCM result shows that the interference on SNHG7 expression in gastric cancer cells can facilitate cell apoptosis. C and D, FCM result shows that after interference on SNHG7 expression in gastric cancer cells, cell cycle is arrested in G1-G0 phase \( *p < 0.05 \).
Zhang et al. found that lncRNA 00668 can inhibit the expressions of epigenetically silenced p15 and p16 through binding to polycomb repressive complex 2 (PRC2). Next, we will investigate whether SNHG7 can inhibit the transcription of p15 and p16 through PRC2 epigenetic compound.

**Conclusions**

Upregulation of SNHG7 in gastric cancer contributes to the development and progression of gastric cancer via targeting p15 and p16. Thus, inhibition of SNHG7 can partially reverse the malignant phenotype of gastric cancer.

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**Conflict of Interest**

The Authors declare that they have no conflict of interests.

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Figure 5. SNHG7 inhibits the expressions of p15 and p16. A and B Detecting the mRNA expressions of p15 and p16 in SGC-7901 and AGS cells through qRT-PCR after interference on SNHG7 expression. C and D, Detecting the protein expressions of p15 and p16 in SGC-7901 and AGS cells through Western blotting assay after interference on SNHG7 expression.


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