IncrNA SNHG1 suppresses gastric cancer cell proliferation and promotes apoptosis via Notch1 pathway

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Summary

Purpose: To explore the influence of long non-coding RNA small nucleolar RNA host gene 1 (SNHG1) on the proliferation and apoptosis of gastric cancer cells.

Methods: SNHG1 was knocked down using small-interfering RNAs (siRNAs) in gastric cancer cell line MGC-803 and then the changes in the expression levels of SNHG1 and Notch1 in each group of cells were evaluated via quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Results: After intervention of SNHG1 by siRNAs, compared with NC-siRNA group, SNHG1-siRNA group exhibited notably lowered RNA and messenger RNA (mRNA) levels of SNHG1 and Notch1 (p<0.05), a substantially lowered proliferation rate (p<0.05), a remarkably raised apoptosis rate (p<0.05) and markedly decreased protein expression levels of Notch1 and Bax (p<0.05). Additionally, the cells treated with Notch1 inhibitor had the proliferation substantially inhibited (p<0.05), while there was no notable change in the RNA expression of SNHG1 in the cells.

Conclusions: IncrNA SNHG1 can depend on the Notch1 pathway to suppress the proliferation of gastric cancer cells and promote their apoptosis.

Key words: IncrNA SNHG1, Notch1, gastric cancer cells, proliferation, apoptosis

Introduction

The morbidity and mortality rates of gastric cancer remain stubbornly high worldwide, and this malignancy has become a leading factor for cancer-induced deaths in China [1]. In treating early-stage gastric cancer, surgery is still the most direct and effective approach, but most patients are in advanced stage when diagnosed, losing the best opportunity for surgical treatment [2]. Therefore, the main therapeutic means available for the majority of patients include chemotherapy, targeted therapy and immunotherapy. Although these therapies are effective to a certain degree, they have considerable side effects with a very low 5-year survival rate, often seriously affecting the patient quality of life [3]. Hence, it is of great significance to explore the pathogenesis of gastric cancer and search for more effective treatments.

As a highly conserved signaling regulator, the Notch signaling pathway plays an important role in physiological processes such as cell proliferation, differentiation and maturation [4]. Currently, a considerable number of studies have demonstrated that Notch1 gene is important in the occurrence and development of tumors, but no exact conclusion has been reached on whether it functions as an oncogene or a cancer suppressor gene in the pathogenesis of gastric cancer [5]. In addition, there are increasingly more reports on long non-coding
ribonucleic acids (lncRNAs) that are unable to code proteins, but are physiologically versatile players [6]. At present, the roles of lncRNAs in tumors have attracted increasing attention from researchers [7,8]. According to the existing findings, small nucleolar RNA host gene 1 (SNHG1) is highly expressed in lung cancer cells and tissues [9], and it plays a role as an oncogene in cervical cancer and liver cancer as well [10]. The role and regulatory mechanism of SNHG1 in gastric cancer, however, remain to be clarified.

The present study detected the expression of IncRNA SNHG1 in MGC-803 gastric cancer cells and explored the related mechanism, by which IncRNA SNHG1 affects the proliferation and apoptosis of the cells via regulating the Notch1 pathway, to corroborate the research prospects and significance for the clinical diagnosis and treatment of gastric cancer.

**Methods**

**Drugs, reagents and instruments**

Cell counting kit-8 (CCK-8) and Notch1 inhibitor DAPT (MSU, Sigma, St. Louis, MO, USA), RNA extraction kit (Invitrogen, Carlsbad, CA, USA), quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), primer synthesis kit, Ki67, AV/PI and bicinchoninic acid (BCA) protein assay kits and cell lysate (Hanbio Biotechnology Co., Ltd., Shanghai, China), and Notch1, B-cell lymphoma 2 (Bcl-2) associated X protein (Bax) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Cell Signaling Technology, Inc., Danvers, MA, USA).

**Cell culture and transfection**

The human gastric cell line MGC-803 was cultured in an incubator at 37°C with 5% CO₂ until about 80% confluence. Then, they were digested using trypsin, made into single cell suspension and inoculated into the corresponding culture plates for subsequent experiments. When grown to 60-70% confluence, the cells were transfected with SNHG1-small-interfering RNA (siRNA) and NC-siRNA, separately or treated with the inhibitor DAPT and incubated in the incubator.

**Detection of cell proliferation activity via CCK-8 assay**

At 48 h after transfection, the proliferation activity of cells was detected via CCK-8 assay. The cells were firstly seeded into 96-well plates at a concentration of 1×10⁵ cells/mL and 100 μL/well, with triplicate wells set in each group. After incubation at 37°C with 5% CO₂ for 48 h, the RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) was replaced. Then, each well of cells was added with 10 μL of CCK-8 assay solution, and 30 min later, the optical density (OD) at the wavelength of 562 nm was measured using a microplate reader in each well.

**Detection of proliferation activity of MGC-803 cells via flow cytometry**

Upon 60-70% confluence, the MGC-803 cells in 6-well plates were transfected with SNHG1-siRNA or NC-siRNA for 48 h, treated with trypsin and harvested. The resulting cells were lysed using 1% Triton and labeled with Ki67. Finally, the proliferation of the cells was detected via flow cytometry.

**Detection of MGC-803 cell apoptosis via TUNEL assay**

The cells were fixed in 4% paraformaldehyde at room temperature for 40 min and then in 70% ethanol at -20°C for 1 h, incubated in 5% H₂O₂ in methanol at room temperature for 10 min and permeated by 0.1% Triton X-100 in 0.1% sodium citrate on ice in a refrigerator at 4°C for 3.5 min. Subsequently, the cells were incubated with TUNEL reaction mixture in a wet box at 37°C for 90 min and with peroxidase in a wet box at 37°C for 40 min. Finally, they were stained with 100 μL of diaminobenzidine (DAB) at room temperature for 10 min and with hematoxylin for 2 min. After each step above, the resulting cells were washed using phosphate buffered saline (PBS) for 3 times for 5 min.

**Determination of the expression levels of the related RNAs via qRT-PCR**

After being treated, the cells in 6-well plates were exposed to TRIzol (500 μL/well) (Invitrogen, Carlsbad, CA, USA), let stand for 5 min and added with chloroform. Then, the mixture was intensely shaken for 15 s and let stand for 15 min, and the water phase was transferred into another batch of new Eppendorf (EP) tubes, added with isopropanol and overturned for several times. After centrifugation, the white deposits at the bottom of tubes were RNAs. Subsequently, the RNAs were washed and dissolved in water, and their concentration and purity

*Table 1. QRT-PCR primer sequence*
were measured, with the $A_{260}/A_{280}$ ratio of 1.8-2.0 as the eligibility criterion. After the RNAs were reversely transcribed into complementary deoxyribose nucleic acids (cDNAs), the messenger RNA (mRNA) expression level was analyzed using real-time fluorescence qRT-PCR. The primer sequences are shown in Table 1. The reaction was performed under the following conditions: 94°C for 5 min, 94°C for 30 s, 57°C for 30 s, 72°C for 30 s for 40 amplification cycles and 72°C for 5 min. The data were processed using the Microsoft Excel software, and with GAPDH gene as the reference gene, the relative expression level was calculated based on the formula $2^{-\Delta\Delta C_{T}}$ (where $\Delta C_{T}$ represents the difference in the threshold cycle between the target and reference genes).

Western blotting

The cells treated in 6-well plates were exposed to radioimmunoprecipitation assay (RIPA) lysis buffer (100 μL/well) (Beyotime, Shanghai, China) and lysed on ice for 20 min, scraped and centrifuged at 12,000 rpm and 4°C for 10 min, and the supernatant was collected and stored in new EP tubes. Then, the concentration of proteins extracted was determined and quantified using the BCA kit, and 20 μg of proteins were taken for Western blotting, electrophoretically separated and transferred onto polyvinylidene fluoride (PVDF) membranes (Milipore, Billerica, MA, USA) in electrophoresis buffer solution. The membranes were sealed in 5% skim milk at room temperature for 2 h, washed on a shaker and incubated in the antibody incubator containing the primary antibody (1:1,000) at 4°C overnight. After being fully washed using tris buffered saline-tween (TBST), the membranes were incubated with the secondary antibody (1:5,000) at room temperature for 1 h, and development was performed by the electrochemiluminescence (ECL) method in the dark. Finally, the images were scanned and recorded using the gel imager (Bio-Rad Laboratories, Hercules, CA, USA), and the gray scale was analyzed and compared, with GAPDH as the internal reference.

Statistics

All the data were expressed as mean±standard deviation, and processed using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). Comparison between multiple groups was done using one-way ANOVA followed by post hoc test (Least Significant Difference). $P<0.05$ denoted that the difference was statistically significant.

Results

Influence of SNHG1 on Notch1 after siRNA intervention

As shown in Figure 1, compared with that in NC-siRNA group, after intervening in SNHG1, the RNA level of SNHG1 was substantially lowered ($p<0.01$), and the same held true for the mRNA level of Notch1 ($p<0.01$), indicating that SNHG1 may positively regulate the expression of Notch1.

Impact of SNHG1 on cell proliferation activity after siRNA intervention detected via Ki67 assay

Compared with that in NC-siRNA group, the number of MGC-803 cells in the proliferation phase notably declined after interfering in SNHG1 ($p<0.01$) (Figure 3).
Impact of SNHG1 on cell apoptosis after intervention with siRNA detected via TUNEL assay

Compared with that in NC-siRNA group, the number of MGC-803 cells in the apoptosis phase was notably raised after interfering in SNHG1, suggesting that SNHG1 is significantly correlated with the apoptosis of MGC-803 cells (Figure 4).

Influences of SNHG1 on Notch1 and Bax proteins after siRNA intervention detected via Western blotting

The Western blotting results (Figure 5) revealed that compared with those in NC-siRNA group, the protein expression level of Notch1 was substantially lowered (p<0.01), but that of Bax was notably raised in gastric cancer MGC-803 cells after interfering in SNHG1 (p<0.01).

Impacts of Notch1 inhibitor treatment on SNHG1 and cell proliferation activity

As shown in Figure 6, after treating gastric cancer MGC-803 cells with the Notch1 inhibitor, the expression of SHNG1 did not significantly differ between control group and DAPT group, while the cell proliferation activity was notably weakened in DAPT group (p<0.01).

Influence of Notch1 inhibitor treatment on Bax protein detected via Western blotting

According to the Western blotting results (Figure 7), compared with that in the control group,
the protein expression level of Bax was remarkably raised in gastric cancer MGC-803 cells treated with Notch1 inhibitor.

**Discussion**

As one of the malignant tumors in the digestive system, gastric cancer's morbidity and mortality are increasing year by year. The factors for this disease involve multi-gene regulation, in which the Notch1 signaling pathway has been extensively concerned and researched and plays an important role in the occurrence and development of gastric cancer as well as in its invasion and metastasis [11]. The regulation on the Notch1 signaling pathway in gastric cancer is not very clear.

LncRNA SNHG1 shows a high expression level in multiple cancer tissues or cells, for example, liver tissues and cells, and it can regulate miR-195 in a targeted manner, thus accelerating the proliferation and metastasis of cancer cells [12,13]. Besides, another study report manifested that the expression of SNHG1 is remarkably raised in colon cancer cells, and that after intervention of SNHG1, the proliferation ability of colon cancer cells is notably weakened due to cell apoptosis [14]. Another study has also found that the expression of SNHG1 is positively correlated with the size of lung tumors, their distant metastasis and TNM stage by the related mechanism that may be associated with the up-regulated protein expression level of CDK4 and the down-regulated protein expression level of p27kip1. As negative regulators of cell cycle, both CDK4 and p27kip1 can weaken the biological activity of several intracellular kinases, further prolonging the normal cell cycle process [16].

The Notch signaling pathway acts as a highly conserved signaling regulation system in maintaining evolutionary process and plays an important role in the normal proliferation and differentiation of cells [17]. The Notch signaling pathway is mainly composed of Notch1-4, among which, under the synergy of multiple enzymes, Notch1, a major member in the Notch family, binds to the intracellular active fragment NICD and further forms the transcription initiation complex, thereby regulating the proliferation and homeostasis of cells [18]. An increasing number of reports has suggested that the protein expression level of Notch1 is notably elevated in gastric cancer tissues, which is significantly correlated with the progression and metastasis of tumors [19]. However, little is still known about the regulatory relationship between LncRNA SNHG1 and the Notch signaling pathway.
and their influences on the occurrence and development of gastric cancer.

This study took gastric cancer MGC-803 cell line as the study object and knocked down SNHG1 using siRNAs in MGC-803 cell line. According to the results, compared with NC-siRNA group, SNHG1-siRNA group exhibited notably lowered RNA and mRNA expression levels of SNHG1 and Notch1, a substantially lowered proliferation rate, a remarkably raised apoptosis rate and markedly decreased protein expression levels of Notch1 and Bax. Additionally, the cells treated with Notch1 inhibitor had substantially inhibited proliferation ($p<0.05$), while no notable change in the RNA expression of SNHG1 was seen. Similarly, a study revealed that SNHG1 is highly expressed in glioma, and the higher its expression, the poorer the prognosis [20], while the proliferation of cervical cancer cells is obviously inhibited after intervening in SNHG1 [21], which is consistent with the conclusion in the present study.

**Conclusions**

In conclusion, lncRNA SNHG1 suppresses the proliferation of gastric cancer cells and promotes their apoptosis via the Notch1 pathway.

**Conflict of interests**

The authors declare no conflict of interests.

**References**