LncRNA-SNHG1 contributes to gastric cancer cell proliferation by regulating DNMT1

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Abstract

Objective: The objective of this study was to determine the expression of long-chain non-coding RNA SNHG1 (lncRNA-SNHG1) in gastric carcinoma and explore its function on cancer cell proliferation.

Methods: The expression of lncRNA-SNHG1 in tumor tissues and corresponding adjacent tissues from 50 patients with gastric cancer was detected with real-time-PCR. The relationships between the expression of lncRNA-SNHG1 and clinicopathological features of gastric cancer patients were analyzed. Survival analysis was performed to study the correlation between lncRNA SNHG1 expression and patient prognosis. To assess the effect of LncRNA SNHG1 on proliferation in cancer cells, cell viability and colony formation assays were conducted when lncRNA SNHG1 was upregulated or downregulated by Lentivirus or plasmid in gastric cancer cells. Furthermore, in vivo tumor assay was performed to confirm the impact of lncRNA SNHG1 on proliferation of gastric cancer.

Results: The expression of lncRNA SNHG1 in gastric cancer tissues was significantly higher than that in adjacent tissues and was correlated with TNM stage, T stage, and lymph node metastasis. The survival time of patients with higher expression level of lncRNA-SNHG1 was significantly lower than that of the lower expression level. LncRNA-SNHG1 accelerated the proliferation of gastric cancer cells obviously and increased the expression of DNMT1.

Conclusion: LncRNA SNHG1 promotes DNMT1 expression, which facilitates the gastric cancer proliferation.

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1. Introduction

Gastric cancer (GC) is considered one of the common cancers worldwide, and the third-highest cause of cancer deaths worldwide [1]. Although there have been numerous breakthroughs in pathogenesis and clinical studies of gastric cancer, the overall prognosis remains poor [2]. Recent studies have shown that the initiation and progression of gastric cancer is related to abnormal gene expression affecting cell proliferation, differentiation, and metastasis [3–6].

Human genome sequence data have revealed that only 2% of the genes encode for proteins, and thus most transcripts are considered non-coding RNAs (ncRNAs). Long-chain ncRNAs (lncRNAs), transcribed by RNA polymerase II, are longer than 200 nucleotides and do not encode proteins [7,8]. LncRNAs are involved in many important processes, such as signal transduction regulation, genomic imprinting, chromatin modification, transcription activation, post-transcription regulation, and protein function regulation [9,10].

Numerous previous oncology studies have focused on protein-encoding genes, but lncRNAs have recently attracted attention [11–13]. LncRNAs play vital roles in the development [14], drug-resistance [15], and metastasis [16] of tumor cells. Therefore, the molecular biological function of lncRNA in oncology should be examined.

LncRNA-SNHG1 (small nucleolar RNA host gene 1), located in chromosome 11, is expressed in many cancer tissues, including lung cancer and liver cancer. LncRNA-SNHG1 promotes the invasion and metastasis of hepatocellular carcinoma cells and is related to poor prognosis [17,18]. However, the expression of IncRNA-SNHG1 and its biological function and mechanism in gastric cancer remain unclear.

In this study, we explored the expression of lncRNA-SNHG1 in...
gastric cancer and its relationship with the prognosis and clinico-pathological features of gastric cancer. The goal of this study was to identify therapeutic targets and prognostic markers as well as provide a foundation for the treatment of gastric cancer.

2. Materials and methods

2.1. Patients and samples

Gastric cancer tissues and pair-matched adjacent normal tissues were obtained from 50 patients with basic clinical pathological information from the Department of General Surgery of Xiantao First People’s Hospital from January 2011 to January 2012. No patients had received preoperative chemotherapy, radiotherapy, or other anticancer therapy. All participants provided informed consent and the study was approved by the Ethics Committee of Xiantao First People’s Hospital.

2.2. Cell culture

Gastric cancer cells (NCI-N87 and MKN-45) and normal gastric cells (GES-1) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The cell lines were maintained in DMEM containing 10% fetal bovine serum at 37°C in a 5% CO2 humidified incubator.

2.3. Cell transfection

Lentivirus particles expressing sh-SNHG1 and plasmid pcDNA3.1-SNHG1 were purchased from Vigenebio (Jinan, China). Small interfering RNAs for SNHG1 and the negative control, siRNA were purchased from RiboBio (Guangzhou, China). Transfection with lentivirus or plasmid was performed according to the manufacturer’s instructions.

2.4. Quantitative reverse transcription-PCR

Total RNA was extracted from gastric cancer and adjacent cancer tissues, using Trizol reagent (Invitrogen, Carlsbad, CA, USA). RNA was reverse-transcribed into complementary DNA (cDNA), using the Reverse cDNA synthesis kit (Takara, Shiga, Japan). SYBR-Green qPCR mix (Toyobo, Osaka, Japan) was used for quantitative PCR.

2.5. Western blotting

Total protein in GC cells was extracted using NP40 lysate. In addition, equal amounts of protein were electrophoresed in a 10% SDS-polyacrylamide gel and then transferred onto a polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA). The membrane was incubated in primary antibodies at 4°C overnight. The membranes were then incubated in corresponding secondary antibodies for 1 h at room temperature. After washing with Tris-HCl-buffered saline solution, the membranes were visualized using the ECL kit (P0018, Beyotime, Beijing, China).

2.6. Cell viability assay

The CCK8 kit (Beyotime) was used to perform the cell viability assay. Cells were digested with trypsin and seeded in 96-well plates at 2000 cells per well. In addition, CCK8 reagent was added to each well and then incubated at 37°C for 4 h. The absorbance values at 490 nm were measured with a microplate reader. Absorbance values at 0, 24, 48, 72, and 96 h were recorded and used to represent cell viability. Each assay was performed in triplicate.

Fig. 1. Up-regulation of lncRNA SNHG1 in gastric cancer contributed to poor prognosis in gastric cancer patients. (A) The expression of SNHG1 in gastric cancer was calculated by realtime-PCR in gastric cancer tissues and adjacent tissues. (B–D) Expression of SNHG1 in human colorectal cancer primary tumors with TNM stages, Tumor invasion stages and lymph node metastasis. (E) The expression of SNHG1 in gastric cancer was calculated by realtime-PCR in gastric cancer cell lines and normal epithelial cell line. (F) The Kaplan-Meier method was used to analyze the overall survival by SNHG1 levels. Patients with high SNHG1 expression showed poorer survival compared with patients with low SNHG1 expression. (*p < 0.05, **p < 0.01, ***p < 0.001).
2.7. Colony formation

Cells were plated in 6-well plates and incubated in DMEM containing 10% fetal bovine serum at 37 °C. The number of cells in each well was 1000. Two weeks later, cells were washed with phosphate-buffered saline, fixed in methanol for 30 min, and stained with 1% crystal violet dye, after which the number of colonies was counted.

2.8. In vivo tumor assays

Four-week-old BALB/C-nu/nu nude mice were purchased from the Shanghai Laboratory Animal Center (China). NCI-N87 cells stably expressing shSNHG1 and control cells were subcutaneously injected into the flank area of nude mice. After 7 weeks, mice were sacrificed for further study.

2.9. Statistical analysis

SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analyses. Metrological data is expressed as the mean ± standard deviation (x ± s). Comparison of mean differences between groups was conducted by the paired t-test or independent sample t-test. Count data were analyzed by chi-square test. The Kaplan-Meier method was applied for survival analysis and log-rank comparison. Values with a p-value < 0.05 were considered significant.

3. Results

3.1. Up-regulation of lncRNA SNHG1 in gastric cancer contributed to poor prognosis in gastric cancer patients

To determine the function of IncRNA-SNHG1 in gastric cancer, real-time PCR was performed to examine the levels of IncRNA-SNHG1 in 50 paired gastric cancer tissues and adjacent tissues. The results showed that IncRNA-SNHG1 was up-regulated in gastric cancer (Fig. 1A). In addition, we investigated the relationship between IncRNA-SNHG1 and clinicopathological features in gastric cancer. The results showed that IncRNA-SNHG1 expression was associated with TNM stage, T stage, and lymph node metastasis (Fig. 1B-D). To confirm these results, we detected the expression of IncRNA-SNHG1 in gastric cancer cells and normal gastric cells. Similarly, expression of IncRNA-SNHG1 was significantly higher in gastric cancer cells than in normal gastric cells (Fig. 1E). Finally, we analyzed the role of IncRNA-SNHG1 in the prognosis of gastric cancer patients. A Kaplan-Meier survival curve was used to compare patients with high and low levels of IncRNA-SNHG1. Patients with high IncRNA-SNHG1 levels showed shorter survival compared to the low-expression group, indicating that up-regulation of IncRNA-SNHG1 resulted in poor prognosis in gastric cancer patients (Fig. 1F). These data suggest that IncRNA-SNHG1 plays an important role in the development and progression of gastric cancer.

Fig. 2. lncRNA-SNHG1 promoted cell proliferation and metastasis in gastric cancer cell. (A) SNHG1 knockdown by shRNA in NCI-N87 cells were examined via real-time PCR. (B) CCK8 assay was used to reveal that knockdown of SNHG1 inhibited proliferation of NCI-N87 cells. (C) Cell cycle assay was used to reveal that knockdown of SNHG1 inhibited proliferation of NCI-N87 cells. (D) Colon formation assay was used to reveal that knockdown of SNHG1 inhibited proliferation of NCI-N87 cells. (E) SNHG1 up-regulation by pcDNA3.1-SNHG1 in MKN45 cells were examined via real-time PCR. (F) CCK8 assay was used to reveal that up-regulation of SNHG1 promoted proliferation of MKN45 cells. (G) Cell cycle assay was used to reveal that up-regulation of SNHG1 promoted proliferation of MKN45 cells. (H) Colon formation assay was used to reveal that up-regulation of SNHG1 promoted proliferation of MKN45 cells. (I) SNHG1 knockdown by siRNA in NCI-N87 cells were examined via real-time PCR. (J) CCK8 assay was used to reveal that knockdown of SNHG1 inhibited proliferation of NCI-N87 cells. (K) Cell cycle assay was used to reveal that knockdown of SNHG1 inhibited proliferation of NCI-N87 cells.
3.2. Knockdown of lncRNA-SNHG1 inhibited gastric cancer cell proliferation

Lentivirus particles expressing sh-SNHG1 were used to knock down the expression of lncRNA-SNHG1 in gastric cancer cells NCI-N87, which was confirmed by real-time PCR (Fig. 2A). In addition, the CCK8 assay results showed that the proliferation ability of gastric cancer cells transfected with sh-SNHG1 was significantly decreased in a time-dependent manner (Fig. 2B). Moreover, cell cycle assay demonstrated that SNHG1 down-regulation induced significant cell-cycle arrest at G0/G1 phases. Finally, the colony formation assay revealed that knockdown of SNHG1 in gastric cancer cells decreased colony formation ability (Fig. 2D). In order to avoid the off-target effects, siRNA was used to decrease the expression of lncRNA-SNHG1 (Fig. 2I). The CCK8 assay showed that silence of lncRNA-SNHG1 inhibited the proliferation ability in NCI-N87 cells (Fig. 2J). The cell cycle assay showed that silence of lncRNA-SNHG1 induced cell-cycle arrest at G0/G1 phases (Fig. 2K). These data indicate that knockdown of lncRNA-SNHG1 suppressed tumor proliferation in human gastric cancer cells.

3.3. Overexpression lncRNA-SNHG1 promoted gastric cancer cell proliferation

We transfected pcDNA3.1-SNHG1 into gastric cancer cells MKN-45 to up-regulate the expression of lncRNA-SNHG1, which was verified by real-time PCR (Fig. 2E). Moreover, the CCK8 assay showed that the proliferation ability of gastric cancer cells transfected with pcDNA3.1-SNHG1 was significantly increased, with the most obvious effect at 72 h (Fig. 2F). Similarly, cell cycle assay demonstrated that SNHG1 up-regulation decreased significant cell-cycle arrest at G0/G1 phases (Fig. 2G). Finally, the colony formation assay showed that overexpression of SNHG1 promoted colony formation ability in gastric cancer cells (Fig. 2H). These data indicate that up-regulation of lncRNA-SNHG1 promoted tumor proliferation in human gastric cancer cells.

3.4. LncRNA-SNHG1 regulated expression of DNMT1 to promote tumor progression

In order to reveal the mechanism of SNHG1, TANRIC database was used in the present study, which is a user-friendly, open-access database.
webapp for interactive exploration of lncRNAs in cancer [19]. Using the database, we analyzed the differences in IncRNA-SNHG1 expression in different molecular types. The results showed that the expression of IncRNA-SNHG1 in gastric cancer tissues with EBV-positivity, microsatellite instability, and chromosomal instability was significantly higher than that in gastric cancer tissues with genomic stability (Fig. 3A). In addition, we found that IncRNA-SNHG1 and DNMT1 expression are positively correlated, with a 0.675 correlation coefficient (Fig. 3B). To further confirm the relationship, we calculated the correlation between IncRNA-SNHG1 and DNMT1 expression in tissues. Notably, there was also a strong correlation in tissues, which was consistent with the TANRIC database (Fig. 3C). Thus, IncRNA-SNHG1 may play a role in gastric cancer by affecting epigenetic regulation. To evaluate our hypothesis, we transfected lentivirus particles expressing sh-SNHG1 into NCI-N87 cells. The results showed that the expression of DNMT1 was down-regulated both at RNA and protein levels (Fig. 3D-F). Next, we observed that DNMT1 was up-regulated when IncRNA-SNHG1 was up-regulated via transfecting pcDNA3.1-SNHG1 into gastric cancer cells MKN-45 cells (Fig. 3D-F). Finally, we performed a rescue assay to confirm that SNHG1 induced gastric cancer progression in a DNMT1-dependent manner. Si-DNMT1 and pcDNA3.1-SNHG1 were simultaneously transfected into MKN-45 cells (Fig. 3G). The CCK8 assay revealed that knockdown of DNMT1 decreased the cell growth caused by SNHG1 up-regulation (Fig. 3H). In addition, the cell cycle analysis revealed that the silencing of DNMT1 inhibited the changes in cell cycle arrest caused by SNHG1 up-regulation (Fig. 3I). These data revealed that IncRNA-SNHG1 could regulate DNMT1 to promote the proliferation in gastric cancer cells.

3.5. Knockdown of IncRNA-SNHG1 inhibited gastric cancer cell proliferation in vivo

Finally, xenograft assays were used to reveal the effects of IncRNA-SNHG1 on tumor growth in vivo. Tumors with the NCI-N87-shSNHG1 cells showed a more slowly growth speed than tumors derived from the NCI-N87-shNC cells (Fig. 4A). Furthermore, we estimated the weight and size of the tumors after 28 days. We found that tumors derived from NCI-N87-SNHG1 cells were obviously smaller than those derived from the control group (Fig. 4B-C). The expression of SNHG1 and DNMT1 was significantly lower in the xenograft tumors from the shSNHG1 group than in those from the control group (Fig. 4D-E).

4. Discussion

Gastric cancer is a common malignant tumor worldwide, particularly in China [20]. Despite multiple measures of treatment for GC cancer, it is also critical to identify new biomarkers and therapies to enhance treatment outcomes. In recent years, an increasing number of studies has confirmed that lncRNAs are involved in tumor growth signal regulation [21,22], but most of the biological functions of lncRNAs remain unclear. The functions of lncRNA in cancer development have attracted attention. Wu et al. found that lncTGF7 affects the invasion and metastasis of hepatocellular carcinoma by regulating the epithelial mesenchymal transition [23].

LncRNA-SNHG1, a new lncRNA located on chromosome 11 and containing 11 exons, can predict poor prognosis in hepatocellular carcinoma, promote hepatocellular carcinoma growth [24], and accelerate the proliferation of non-small cell lung cancer [18]. LncRNA-SNHG1 may also serve as a predictor of high-risk neuroblastoma [25]. Additionally, lncRNA-SNHG1 was found to be involved in the effects of ionizing radiation on cells [26]. However, no studies have examined the expression and mechanism of IncRNA-SNHG1 in gastric cancer.

Our data showed that IncRNA-SNHG1 expression in gastric cancer was significantly up-regulated compared to that in adjacent tissues, suggesting that IncRNA-SNHG1 plays an important role in the development of gastric cancer. These results were consistent with those of previous studies of hepatocellular carcinoma and lung cancer. Based on clinical pathological data of IncRNA-SNHG1 and gastric cancer, IncRNA-SNHG1 was associated with gastric.

Fig. 4. Knockdown of IncRNA-SNHG1 inhibited gastric cancer cell proliferation in vivo. (A) Photographs of tumors excised 7 weeks after inoculation of stably shSNHG1 transfected NCI-N87 cells into nude mice. (B–C) Tumor weight and volume were measured after injection of NCI-N87 cells stably transfected with shSNHG1 or Con. (D) Real-time-PCR was used to analysis the expression of SNHG1 and DNMT1 in transplantable tumor. (\*p < 0.05, \**p < 0.01, \***p < 0.001).
cancer T stage, TNM stage, and lymph node metastasis. Specifically, the degree of malignancy of gastric cancer increased with lncRNA-SNHG1 levels, indicating that this lncRNA promotes the malignant progression of gastric cancer, which is similar to findings in other tumors [13, 18, 25]. Survival analysis of patients showed that the median survival time of patients expressing different levels of lncRNA-SNHG1 was significant, and the prognosis of patients with high levels of lncRNA-SNHG1 was significantly poorer than that of the low-expression group. This indicated that lncRNA-SNHG1 could be used as a marker for predicting the prognosis of gastric cancer. In summary, lncRNA-SNHG1 was highly expressed in gastric cancer tissues and is related to prognosis and clinicopathological parameters including TNM staging T stage, and lymph node metastasis. It may play a role in the progression of gastric cancer and can be used as a marker for early diagnosis of gastric cancer.

Furthermore, the proliferation of gastric cancer cells were significantly enhanced after lncRNA-SNHG1 levels were increased. LncRNA-SNHG1 promoted the progression of gastric cancer, which was similar to the role of lncRNA-SNHG1 in non-small cell lung cancer [18]. To further clarify the specific mechanism by which lncRNA-SNHG1 exerts its ability to promote the proliferation of gastric cancer, we analyzed the expression of lncRNA-SNHG1 and its possible target genes, using the TANRIC database. The results showed that lncRNA-SNHG1 was positively correlated with the expression of DNMT1, and expression of lncRNA-SNHG1 was significantly higher in tissues with EBV-positivity, microsatellite instability, and chromosomal instability than that in tissues with genomic stability. Thus, lncRNA-SNHG1 may play a role in gastric cancer by affecting epigenetic regulation. To evaluate our hypothesis, we examined the expression DNMT1 when lncRNA-SNHG1 was up-regulated or silenced in gastric cancer cells. Interestingly, the results revealed that lncRNA-SNHG1 could regulate DNMT1 expression in both RNA and protein level. Furthermore, a rescue assay was performed to confirm that SNHG1 induced gastric cancer progression in a DNMT1-dependent manner. Finally, the results of xenograft assays showed that silence of lncRNA-SNHG1 inhibited the tumor growth in vivo, which was consistent with the results in vitro.

In summary, lncRNA SNHG1 was up-regulated in gastric cancer and promoted DNMT1 expression, which facilitated the gastric cancer proliferation. Thus, lncRNA-SNHG1 may serve as a novel predictor of gastric cancer prognosis and potential therapeutic target for gastric cancer treatment.

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References

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