Role of long non-coding RNA SNHG1 in occurrence and progression of ovarian carcinoma

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Abstract. – OBJECTIVE: To investigate the expression of human long non-coding ribonucleic acid (lncRNA) small nucleolar RNA host gene 1 (SNHG1) in epithelial ovarian carcinoma tissues and its effects on the in vitro proliferation, apoptosis, invasion and metastasis of ovarian carcinoma cells, and to investigate its possible mechanism.

PATIENTS AND METHODS: The expressions of SNHG1 in 20 pairs of epithelial ovarian carcinoma tissues and para-carcinoma normal tissues were detected by Real-time fluorescence quantitative polymerase chain reaction (qRT-PCR). The expressions of SNHG1 in normal ovarian epithelial cells (IOSE25) and ovarian carcinoma cells (CAOV-3, SKOV-3, ES2 and A2780) were further detected. The knockdown efficiency of SNHG1 small interfering RNA (siRNA) in SKOV-3 cells was detected via qRT-PCR. Moreover, the effects of SNHG1 knockdown on proliferation, migration and apoptosis of SKOV-3 cells were detected by cell counting kit 8 (CCK8) proliferation assay, clone formation assay, transwell migration assay and flow cytometry. Finally, the expressions of apoptosis-related proteins, epithelial-mesenchymal transition (EMT)-related proteins and matrix metalloproteinases (MMPs) in control group and interference group were detected by Western blotting.

RESULTS: The expression level of lncRNA SNHG1 in ovarian carcinoma tissues was significantly higher than that in para-carcinoma normal tissues. After lncRNA SNHG1 knockdown in SKOV-3 cells, the cell proliferation and clone formation abilities were significantly inhibited. The apoptosis assay proved that inhibiting lncRNA SNHG1 could promote the apoptosis of SKOV-3 cells. Besides, Western blotting revealed that the expressions of pro-apoptotic proteins in interference group were significantly upregulated compared with those in control group. Wound-healing assay and transwell migration assay showed that the down-regulation of lncRNA SNHG1 could inhibit the invasion and metastasis of SKOV-3 cells, whose mechanism was related to the inhibition of EMT process and down-regulation of expressions of MMPs.

CONCLUSIONS: LncRNA SNHG1 is highly expressed in ovarian carcinoma, which can promote the growth, invasion and metastasis of ovarian carcinoma cells. The down-regulation of SNHG1 can inhibit the proliferation, invasion and metastasis of SKOV-3 cells. Inhibiting the expression of SNHG1 may be a potentially effective means of treating ovarian carcinoma.

Key Words: Ovarian carcinoma, lncRNA, SNHG1, Proliferation, Invasion.

Introduction

Ovarian carcinoma is a kind of common malignant tumor in women, accounting for 5% in systemic malignant tumors, whose incidence rate ranks third in gynecologic malignant tumors. Due to its concealed onset, atypical symptoms and a lack of effective early screening methods, 75% patients have been in the middle and advanced stage of ovarian carcinoma when treated, and they are mostly accompanied by intra-abdominal metastasis. At present, the comprehensive treatment method of operation combined with radiotherapy and chemotherapy has a significant effect on ovarian carcinoma; 80% patients are sensitive to platinum/paclitaxel chemotherapy drugs, but the disease will relapse in most patients within a short period after treatment. Long-term data statistics show that the 5-year survival rate of patients with early ovarian carcinoma is as high as 80%, while that of patients with advanced ovarian carcinoma is just about 10-30%³. Therefore, searching effective molecular markers that can predict the metastasis and recurrence of ovarian carcinoma early, and investigating the molecular mechanisms of invasion and metastasis of ovarian carcinoma to obtain effective therapeutic targets, are important.
research topics in improving the prognosis of patients with ovarian carcinoma\textsuperscript{4,5}. Following extensive studies on micro ribonucleic acid (miRNA), the role of long non-coding RNA (IncRNA) with the transcript greater than 200 nt in length in the tumor has attracted much attention. Although IncRNA does not encode or only encodes a small number of proteins, its expressions in different tissues and developmental stages are specific and highly conserved in evolution process of mammals, suggesting that IncRNA may have important biological functions. In recent years, a series of studies have found that IncRNA participates in a series of important regulatory processes, such as X-chromosome silencing, chromatin modification, transcriptional interference and activation and intranuclear transport, through regulating the gene expression in the form of RNA. It is widely involved in almost all physiological and pathological processes in the human body, whose abnormal expression is closely related to the occurrence of a variety of tumors\textsuperscript{6,7}.

LncRNA small nucleolar RNA host gene 1 (SNHG1) is located on 11q12.3 with 11 exons. More and more studies\textsuperscript{5,8} have shown that it has the function of promoting the occurrence and development of various tumors. Zhang et al\textsuperscript{9} studied and showed that the SNHG1 expression is significantly up-regulated in hepatic carcinoma tissues, and SNHG1 can promote the occurrence and progression of hepatic carcinoma through inhibiting the p53 gene expression. Cui et al\textsuperscript{10} found that, compared with that in normal lung tissues, the high expression of SNHG1 exists in non-small cell lung cancer. Moreover, relevant clinical data have also shown that the high expression of SNHG1 is related to the later stage and poor prognosis of non-small cell lung cancer. However, the relationship between IncRNA SNHG1 and ovarian carcinoma has not been reported yet so far. Therefore, the expression and role of IncRNA SNHG1 in ovarian carcinoma were investigated and its relevant mechanism was further studied in this study.

Patients and Methods

Ovarian Carcinoma Specimen Collection

A total of 20 pairs of epithelial ovarian carcinoma tissues and para-carcinoma normal tissues resected via ovarian carcinoma operation in our hospital were collected. Patients received no radiotherapy and chemotherapy before operation. The specimens collected were all confirmed pathologically and stored in liquid nitrogen within 5 min after resection, so as to avoid RNA degradation affecting the experimental results. This study was approved by the Ethics Committee of the First People's Hospital of Yunnan Province. Signed written informed consents were obtained from the patients and/or guardians.

Materials

Anti-B-cell lymphoma-2 (Bcl-2), anti-Bcl-2 associated X protein (Bax), anti-Caspase-9, anti-poly-ADP-ribose polymerase (PARP), anti-E-cadherin, anti-N-cadherin, anti-Vimentin, anti-matrix metalloproteinase (MMP)-2, anti-MMP-9 and anti-actin antibodies (Cell Signaling Technology, Inc. Danvers, MA, USA); Hoechst 33342 and Annexin V/propidium iodide (PI) staining kits (BD); cell counting kit 8 (CCK8) (Beyotime, Shanghai, China); Lipoctamine 2000 (Invitrogen, Carlsbad, CA, USA); quantitative reverse transcription polymerase chain reaction (qRT-PCR) kits (Qiagen, Hilden, Germany); si-NC and si-SNHG1 were synthesized by Nanjing Genscript Biotech Co., Ltd. (Nanjing, China), and other experimental reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell Culture

IOSE25, CAOV-3, SKOV-3, ES2 and A2780 cells were purchased from American Type Cell Culture (ATCC, Manassas, VA, USA). Cells were incubated using the Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and 1% non-essential amino acids in an incubator with 5% CO\textsubscript{2} at 37°C.

qRT-PCR

The total messenger RNA (mRNA) of cells was extracted using TRIzol and reversely transcribed into complementary DNA (cDNA). Reverse transcription reaction conditions: 25°C for 10 min, 50°C for 30 min and 85°C for 5 min; the fluorescence quantitative PCR kit was used for detection. Primer sequences of SNHG1: forward primer: 5'-CCTAAAGCCACGCTTCTTG-3'; reverse primer: 5'-TGCAGGCTGGAGATCCTACT-3'. Primer sequences of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (internal reference): forward primer: 5'-TGCAGGCTGGAGATCCTACT-3'; reverse primer: 5'-GTTGTCATACCTCCATTCTC-3'. Fluorescence quantitative PCR conditions: 95°C for 5 min, 95°C for 15 s, 60°C for 1 min, a total of 40 cycles.
**Hoechst 33342 Staining**

SKOV-3 cells were transfected with si-NC and si-SNHG1. At 48 h after transfection, cells were collected and inoculated into a 12-well plate and cultured for 24 h. Then, the supernatant was discarded and cells were washed with phosphate-buffered saline (PBS) twice. 1 mL Hoechst 33242 fluorochrome was added, followed by incubation at 37°C for 15 min. Next, the fluorochrome was discarded, and cells were washed with PBS, observed and photographed under an inverted fluorescence microscope. The experiment was repeated for three times.

**Apoptosis Detection**

SKOV-3 cells were transfected with si-NC and si-SNHG1, collected at 48 h after transfection, and washed with PBS twice. 500 μL 1 × binding buffer was added to resuspend the cells and the cell suspension was added with 5 μL Annexin V-fluorescein isothiocyanate (FITC) reagent and mixed evenly, followed by incubation in a dark place at room temperature for 15 min. Next, 10 μL PI reagent was added and gently mixed, followed by incubation in a dark place at room temperature for 5 min, and submission for detection within 1 h. The experiment was repeated for three times.

**Western Blotting**

SKOV-3 cells were transfected with si-NC and si-SNHG1, and collected at 48 h after transfection. After cells were washed with pre-cooled PBS, they were fully lysed with radioimmunoprecipitation assay (RIPA) cell lysis buffer. After centrifugation, the supernatant was taken and quantified. Then, 20 μg total protein was mixed with 5 × sodium dodecyl sulfonate (SDS) protein loading buffer, followed by denaturation at 100°C for 5 min, loading in SDS-polyacrylamide gel electrophoresis (PAGE) and electrophoresis. The gel and activated polyvinylidene difluoride (PVDF) were placed on the membrane transfer frame for membrane transfer under constant current for 2 h. PVDF membrane was removed and blocked with 5% skim milk powder for 1 h. The corresponding primary antibodies were added for incubation at 4°C overnight. The membrane was washed with Tris-Buffered Saline and Tween 20 (TBST), and added with the corresponding horse-radish peroxidase (HRP)-labeled secondary antibodies for incubation at room temperature for 1 h. After washing with TBST and color development, the gray value was analyzed using ImageJ software, and the relative expression level of target protein was presented as target protein/actin. The experiment was repeated for three times.

**Statistical Analysis**

SPSS 20.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. All quantitative data were expressed as mean ± standard deviation. Comparison between groups was done using One-way ANOVA test followed by Post Hoc Test (Least Significant Difference). *p* values < 0.05 were considered statistically significant.

**Results**

**SNHG1 was Highly Expressed in Ovarian Carcinoma Tissues and four Ovarian Carcinoma Cell Strains**

Results of qRT-PCR showed that the expression of SNHG1 in epithelial ovarian carcinoma tissues was significantly higher than that in normal ovarian tissues, and the difference was...
statistically significant ($p<0.05$) (Figure 1A), suggesting that SNHG1 plays a certain role in promoting the occurrence and development of ovarian carcinoma. To further validate results in vitro, the expressions of SNHG1 in normal ovarian epithelial cells (IOSE25) and four ovarian carcinoma cell strains (CAOV-3, SKOV-3, ES2 and A2780) were compared. Compared with that in normal ovarian epithelial cells, the expressions of SNHG1 in four ovarian carcinoma cell strains were significantly higher. Compared with those in the other three cell strains, the expression level of SNHG1 in SKOV-3 was the highest, and the difference was statistically significant ($p<0.05$) (Figure 1B). In this study, SKOV-3 cells with the highest expression of SNHG1 were selected for subsequent transfection and study. The inhibition efficiency at 48 h after transfection with si-SNHG1 was verified via qRT-PCR. As shown in Figure 1C, si-SNHG1 was effective in inhibiting the expression of SNHG1 in SKOV-3 cells, and there was a statistically significant difference compared with that in negative control group ($p<0.01$), so si-SNHG1 was used for transfection in subsequent experiments.

**Down-Regulation of SNHG1 Expression Inhibited the in vitro Proliferation of SKOV-3 Cells**

In this study, the effect of inhibiting SNHG1 on the in vitro proliferation of SKOV-3 cells was verified via CCK8 and clone formation assays. SKOV-3 cells were cultured for 24, 48 and 72 h after interference with si-SNHG1 and si-NC. Compared with that in control group, the optical density (OD) value in experimental group was decreased in different degrees, indicating that the inhibition of SNHG1 expression reduced the SKOV-3 cell growth rate (Figure 2A). Similarly, results of clone formation assay showed that the number of cell clone in si-SNHG1 interference group was significantly decreased compared with
In this study, the expressions of IncRNA SNHG1 in 20 pairs of ovarian carcinoma tissues and para-carcinoma tissues were detected via RT-PCR. The results showed that the expression level of SNHG1 in ovarian carcinoma was significantly increased compared with that in para-carcinoma tissues.

**Discussion**

In this study, the expressions of IncRNA SNHG1 in 20 pairs of ovarian carcinoma tissues and para-carcinoma tissues were detected via RT-PCR. The results showed that the expression level of SNHG1 in ovarian carcinoma was significantly increased compared with that in para-carcinoma tissues. Further *in vitro* cell experiments also revealed that compared with that in...
Figure 3. Knockdown of SNHG1 inhibits SKOV-3 cells migration and invasion in vitro. (A-B) Wound-healing and (C-D) transwell assays showing the migratory abilities and invasive capacities of SKOV-3 cells transfected with si-SNHG1 or si-NC. (*p<0.05).

Figure 4. Knockdown of SNHG1 inhibits cell migration and invasion in vitro. (A) The expression levels of target proteins (E-cadherin, N-cadherin, Vimentin, MMP-2 and MMP-9) measured by Western blotting in SKOV-3 cells transfected with si-SNHG1 or si-NC. (B) Data shown are representative of 3 independent experiments. Data are presented as means ± SD (n = 3). (*p<0.05, **p<0.01)
normal ovarian epithelial cells, lncRNA SNHG1 was highly expressed in different ovarian carcinoma cells. These results strongly suggest that lncRNA SNHG1 also promotes the occurrence and development of ovarian carcinoma. In CCK8 and clone formation assays, it was found that after knockdown of SNHG1 in SKOV-3 cells, the cell proliferation rate was significantly reduced. Interestingly, it was found in the apoptosis assay that the proportion of apoptotic SKOV-3 cells with SNHG1 knockdown was significantly increased. Besides, results of Western blotting confirmed that when the expression of SNHG1 was inhibited, the expressions of pro-apoptotic proteins in mitochondrial apoptosis pathway were upregulated and activated, while the anti-apoptosis proteins were inhibited, thereby activating the mitochondrial apoptosis pathway and inducing apoptosis. These results also partially clarify the pro-apoptotic mechanism after SNHG1 is inhibited.

Conclusions

We found that SNHG1 can promote the proliferation of ovarian carcinoma cells, and inhibiting the expression of SNHG1 can partially reverse its tumor-promoting effect. The underlying mechanism is related to the activation of mitochondrial apoptotic pathway after the inhibition of SNHG1. In addition, SNHG1 can also promote the metastasis of ovarian carcinoma cells through the regulation of EMT and MMPs. There are some limitations in this study, and the specific mechanism of SNHG1 in regulating EMT and MMPs needs to be further studied. This study provides a new horizon for the mechanism of occurrence and development of ovarian carcinoma and a new potential target for the treatment of ovarian carcinoma in the future.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References