Long noncoding RNA SNHG14 promotes ovarian cancer cell proliferation and metastasis via sponging miR-219a-5p

L. LI, R. ZHANG, S.-J. LI

Department of Gynecology, Weifang People’s Hospital, Weifang, China

Abstract. – OBJECTIVE: The ovarian cancer is one of the most common fatal cancers. Recently, the role of long noncoding RNAs (lncRNAs) in tumor progression has attracted much attention in researchers. The aim of this study was to investigate the role of lncRNA SNHG14 in the progression of ovarian cancer and to explore the possible mechanism.

PATIENTS AND METHODS: Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was utilized to detect SNHG14 expression in ovarian cancer tissues. To identify the function of SNHG14 in ovarian cancer, functional experiments were conducted in vitro and in vivo. In addition, the luciferase assays and RNA immunoprecipitation assay (RIP) were performed to investigate the underlying mechanism.

RESULTS: SNHG14 expression was remarkably higher in ovarian cancer tissues than that of corresponding normal tissues. SNHG14 expression was associated with patients’ overall survival time as well. After SNHG14 was silenced in ovarian cancer cells, cell proliferation, migration, and invasion were remarkably inhibited. In addition, the expression of miR-219a-5p was significantly up-regulated after the silence of SNHG14. Further mechanism assays showed that miR-219a-5p was a direct target of SNHG14 in ovarian cancer.

CONCLUSIONS: SNHG14 serves as a potential oncogene in ovarian cancer. In addition, it enhances ovarian cell metastasis and proliferation via sponging miR-219a-5p.

Key Words: Long noncoding RNA, SNHG14, Ovarian cancer, MiR-219a-5p.

Introduction

Ovarian cancer is one of the most common malignancies in women worldwide. Around 22,500 new patients were diagnosed with ovarian cancer in America in 2017, with almost 14,100 deaths. Due to unavailable tests for ovarian cancer, it is often diagnosed at a late stage. This makes it one of the leading causes of cancer-related deaths among females. The main interventions for ovarian cancer include surgery, chemotherapy, and radiotherapy. Currently, the severe situation underscores the urgency of early detection and new therapeutic treatment for ovarian cancer patients.

Long non-coding RNAs (lncRNAs) are one subgroup of non-coding RNAs. Recent evidence has proved that lncRNAs play a vital role in the progression of malignant tumors. For example, by regulating the expression of miR-335, lncRNA MSTO2P acts as an oncogene in gastric cancer and promotes cell proliferation. LncRNA GIHCG promotes the development of ovarian cancer by regulating mi-429. LncRNA PCAT-1 plays a vital role in tumorigenesis of hepatocellular carcinoma by modulating TP53-miR-215-PCAT-1-CRKL axis. Besides, lncRNA PlncRNA-1 accelerates the progression of colorectal cancer cells via PI3K/Akt signaling pathway. LncRNA RUNX1-IT1 suppresses the migration and proliferation of colorectal cancer cells. Currently, lncRNA SNHG14, known as a novel lncRNA, has been found to exert crucial roles in malignancies. Therefore, the aim of this study was to investigate whether SNHG14 participated in the proliferation and metastasis of ovarian cancer, and to explore its potential mechanism.

Patients and Methods

Tissue Specimens

Paired ovarian cancer tissues and corresponding normal tissues were sequentially collected from 64 ovarian cancer patients undergoing surgery in Weifang People’s Hospital from January 2016 to December 2017. This study was approved by the Ethics Committee of Weifang People’s
LncRNA SNHG14 promotes ovarian cancer cell proliferation and metastasis

Hospital. The informed consent was obtained from each patient before the study.

**Cell Culture**

The human ovarian cancer cell lines (A2780, OVCAR-3, and SKOV3) and normal ovarian cell line (ISOE80; Shanghai Model Cell Bank, Shanghai, China) were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), penicillin, and streptomycin. All cells were maintained in a humidified incubator with 5% CO₂ at 37°C.

**Lentivirus Expressing Short-Hairpin RNA (shRNA)**

The shRNA directed against SNHG14 was synthesized by GenePharma (Shanghai, China). Complementary DNA encoding SNHG14 was then amplified and inserted into pcDNA3.1 (GenePharma, Shanghai, China). Subsequently, they were transfected into OVCAR-3 ovarian cancer cells according to the instructions of polybrene (GenePharma, Shanghai, China).

**RNA Extraction and Real Time-Quantitative Polymerase Chain Reaction (RT-qPCR)**

The total RNA in tissues and cells were extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Subsequently, extracted total RNA was reverse-transcribed into complementary deoxyribose nucleic acids (cDNAs) through reverse Transcription Kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). The thermocycling conditions were as follows: pre-denaturation at 95°C for 5 min, denaturation at 95°C for 10 s, and annealing at 60°C for 30 s, for a total of 35 cycles. Relative expression was calculated by the 2^ΔΔCt method. Primer sequences used in this study were as follows: SNHG14 forward 5’-GGGTGTTTACGTAGACCAGAACC-3’ and reverse 5’-CTTCCAAAAGCCTTCTGCTTAG-3’; β-actin, forward 5’-GATGGAAATCGTACAGGCT-3’ and reverse 5’-TGGCACCTAGTTGGAAATGC-3’.

**Cell Proliferation Assay**

2 ×10³ transfected cells were first seeded into 96-well plates. Cell proliferation was assessed at 0 h, 24 h, 48 h, and 72 h after transfection by Cell Proliferation Reagent Kit I (MTT; Roche, Basel, Switzerland), respectively. Absorbance at 490 nm was assessed by using an enzyme-linked immunosorbent assay (ELISA) reader system (Multiskan Ascent, LabSystems, Helsinki, Finland).

**Ethynyl Deoxyuridine (EdU) Incorporation Assay**

The proliferation of transfected cells was detected according to the instructions of the EdU Kit (Roche, Basel, Switzerland). A representative photograph was taken by Zeiss AxioPhot Photomicroscope (Carl Zeiss, Oberkochen, Germany).

**Transwell Assay**

24 h after transfection, 2 ×10⁵ cells in 100 µL serum-free DMEM were transformed to the upper chamber of an 8-µm culture insert (Corning, Corning, NY, USA) coated with or without 50 µg Matrigel Matrix dilution (BD, Bedford, MA, USA). Meanwhile, 20% FBS-DMEM was added to the lower chamber of culture inserts. 24 h later, the inserts were treated with methanol for 30 min and stained with hematoxylin for 20 min. The migrated and invaded cells were observed under a Leica DM4000B microscope (Leica Microsystems, Heidelberg, Germany). Three fields were randomly selected for each sample, and the number of migrated and invaded cells was counted.

**Luciferase Assay and RNA Immunoprecipitation Assay (RIP)**

The 3’-UTR of SNHG14 was first cloned into the pGL3 vector (Promega, Madison, WI, USA) as wild-type (WT) 3’-UTR. Site-direction mutagenesis of the miR-219a-5p binding site in SNHG14 3’-UTR was conducted through quick-change site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA), namely mutant (MUT) 3’-UTR. Then, they were transfected into ovarian cancer cells. A luciferase assay was conducted by the Dual-Luciferase reporter assay system (Promega, Madison, WI, USA).

RIP assay was performed according to the instructions of Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA). Finally, RT-qPCR was used to detect Co-precipitated RNAs.

**Xenograft Model**

After transfection, OVCAR-3 cells (6×10⁵/mL) were injected into NOD/SCID mice (6 weeks old) subcutaneously. Tumor diameters were detected every 5 days. The tumor volume was calculated as the following formula: volume = length × width² × 1/2. After 4 weeks, the tumors were extracted.
This experiment was approved by the Animal Ethics Committee of Weifang People’s Hospital.

**Statistical Analysis**

GraphPad Prism 5.0 (La Jolla, CA, USA) was used for all statistical analysis. The Student’s t-test was applied to compare the difference between the two groups. $p<0.05$ was considered statistically significant.

**Results**

**SNHG14 Expression Level in Ovarian Cancer Tissues and Cells**

RT-qPCR was first conducted to detect SNHG14 expression in 64 patients’ tissues and 3 ovarian cancer cell lines. The results showed that SNHG14 expression was significantly up-regulated in ovarian cancer tissues (Figure 1A). Meanwhile, SNHG14 expression was associated with the overall survival of ovarian cancer patients (Figure 1B). In addition, the expression level of SNHG14 in ovarian cancer cells was significantly higher than that of ISOE80 cells (Figure 1C).

**Knockdown of SNHG14 Inhibited the Proliferation of OVCAR-3 Ovarian Cancer Cells**

In our study, OVCAR-3 cells were chosen for knockdown of SNHG14 in vitro. RT-qPCR was then utilized to detect SNHG14 expression (Figure 2A). MTT assay showed that the growth ability of OVCAR-3 cells was significantly suppressed after SNHG14 was knocked down (Figure 2B). Furthermore, EdU incorporation assay showed that EdU positive cells were significantly reduced after the knockdown of SNHG14 in OVCAR-3 cells (Figure 2C).

**Knockdown of SNHG14 Inhibited the Migration and Invasion of OVCAR-3 Ovarian Cancer Cells**

A transwell assay was then conducted to explore whether the knockdown of SNHG14 affected the migrated and invaded abilities of OVCAR-3 cells. The results revealed that after SNHG14 was knocked down in ovarian cancer cells, the number of migrated and invaded cells remarkably decreased (Figure 2D).

**The Interaction between MiR-219a-5p and SNHG14 in Ovarian Cancer Cells and Tissues**

Starbase v2.0 (http://starbase.sysu.edu.cn/mirLncRNA.php) was used to search for miRNAs that contained complementary bases with SNHG14. MiR-219a-5p was a tumor suppressor and was able to suppress cancer cell proliferation. Therefore, we focused on miR-219a-5p among these miRNAs interacting with SNHG14 (Figure 3A). Subsequent RT-qPCR assay showed that the expression of miR-219a-5p was significantly higher in SNHG14/shRNA cells than that of control cells (Figure 3B). Furthermore, the luciferase assay revealed that the co-transfection of SNHG14-WT and miR-219a-5p significantly decreased the luciferase activity. However, no significant changes were observed in the luciferase activity after the co-transfection of SNHG14/MUT and miR-219a-5p (Figure 3C). Meanwhile, the RIP assay results demonstrated that miR-219a-5p could be remarkably enriched in SNHG14 group when compared with the control group.

![Figure 1](image1.png)

*Figure 1.* Expression level of SNHG14 increased significantly in ovarian cancer tissues and cell lines. A, SNHG14 expression significantly increased in ovarian cancer tissues compared to adjacent tissues. B, SNHG14 expression was associated with the overall survival time of ovarian cancer patients. C, The expression levels of SNHG14 relative to β-actin in human ovarian cancer cell lines and normal ovarian cell line (ISOE80) were determined by RT-qPCR. The data were presented as mean ± standard error of the mean. *$p<0.05$. 

4138
LncRNA SNHG14 promotes ovarian cancer cell proliferation and metastasis

Figure 2. Knockdown of SNHG14 inhibited SKOV3 ovarian cancer cell proliferation, migration and invasion. A, SNHG14 expression in SKOV3 ovarian cancer cells transduced with SNHG14 shRNA (shRNA) and the empty vector was detected by RT-qPCR. β-actin was used as an internal control. B, MTT assay showed that the knockdown of SNHG14 significantly inhibited the growth of SKOV3 ovarian cancer cells. C, EdU incorporation assay showed that EdU positive cells were significantly reduced after the knockdown of SNHG14 in SKOV3 cells (Magnification×10). D, The transwell assay showed that the number of migrated and invaded cells significantly decreased via knockdown of SNHG14 in SKOV3 ovarian cancer cells (Magnification×20). The results represented the average of the three independent experiments (mean ± standard error of the mean). *p<0.05, as compared with control cells.

SNHG14 Knockdown Inhibited Tumor Formation In Vivo

We also explored the role of SNHG14 in tumor formation in vivo. Tumor size in shRNA group was significantly smaller when compared with the empty vector group (Figure 4A). Subsequently, the expression levels of SNHG14 and miR-219a-5p in generated tumor tissues were detected by RT-qPCR as well. The results showed that SNHG14 was lowly expressed in the shRNA group when compared with the empty vector group (Figure 4B). However, miR-219a-5p was highly expressed in shRNA group when compared with the empty vector group (Figure 4C). The above results suggested that SNHG14 could induce tumor formation in vivo.

Discussion

Recently, a high rate of therapy resistance and metastasis occur in almost 80% of ovarian cancer patients, eventually contributing to high mortality for ovarian cancer. Evidence has proved that lncRNA is an important factor in the development of ovarian cancer and can be used as potential indicators. For instance, LncRNA HOXA11as facilitates the proliferation and migration of serous ovarian cancer cells. Meanwhile, it is associated with the prognosis of patients as well. The proliferation and migration of ovarian cancer cells are significantly inhibited after the knockdown of LncRNA MNX1-AS1. This indicates that MNX1-
AS1 can be used as a potential target for ovarian cancer. Through regulation of epithelial-mesenchymal transition (EMT), the down-regulation of lncRNA SPRY4-IT1 enhances metastasis of ovarian cancer. Furthermore, lncRNA CCAT1 promotes EMT, migration, and invasion of epithelial ovarian cancer cells.

LncRNA small nucleolar RNA host gene 14 (SNHG14), a novel lncRNA, is located in 15q11.2. Recent studies have shown that SNHG14 is significantly up-regulated in various cancers. For instance, SNHG14 enhances chemoresistance to trastuzumab in breast cancer. SNHG14 promotes cell proliferation by targeting miR-206/YWHAZ in cervical cancer. Meanwhile, SNHG14 functions as an oncogene by sponging miR-340 in non-small cell lung cancer. Wang et al. has indicated that SNHG14 acts as a sponge for miR-92a-3p and induces apoptosis of glioma. In this work, we found that SNHG14 was up-regulated in ovarian cancer tissues and was correlated with poor prognosis of patients. Our further functional investigations revealed that after SNHG14 was knocked down, the ovarian cancer cell proliferation and metastasis were significantly inhibited. The above results suggested that SNHG14 could promote tumorigenesis of ovarian cancer.

To identify the underlying mechanism of SNHG14 function in ovarian cancer cell tumorigenesis and metastasis, the bioinformatics analysis, and functional assays predicted and verified that miR-219a-5p was a potential binding microRNA of SNHG14. Previous studies have found that miR-219a-5p is known as a tumor suppressor in many carcinomas and regulates diverse biological processes. For example, miR-219a-5p suppresses migration of breast cancer cell via reversing EMT process. MiR-219a-5p suppresses metastasis of osteosarcoma by regulating EYA2 in vitro. Moreover, miR-219-5p inhibits cell proliferation and invasion via regulating calcyphosin in colorectal cancer. A recent report has revealed

![Figure 3](image)

**Figure 3.** Reciprocal repression between SNHG14 and miR-219a-5p. **A,** The binding sites of miR-219a-5p on SNHG14. **B,** MiR-219a-5p expression significantly increased in sh-SNHG14 group compared with empty vector group. **C,** Co-transfection of miR-219a-5p and SNHG14-WT strongly decreased luciferase activity. Co-transfection of miR-control and SNHG14-WT did not change luciferase activity. Meanwhile, co-transfection of miR-219a-5p and SNHG14-MUT did not change the luciferase activity either. **D,** RIP assay results demonstrated that miR-219a-5p could be remarkably enriched in SNHG14 group compared with control group. The results represented the average of three independent experiments. The data were presented as mean ± standard error of the mean. *p<0.05.
LncRNA SNHG14 promotes ovarian cancer cell proliferation and metastasis

that miR-219-5p acts as a tumor suppressor in ovarian cancer. In our research, the miR-219a-5p expression was significantly up-regulated after the knockdown of SNHG14. Subsequent luciferase assay demonstrated that miR-219a-5p could directly bind to SNHG14. RIP assay indicated that miR-219a-5p was significantly enriched by SNHG14. All the results above suggested that SNHG14 might promote tumorigenesis of ovarian cancer via sponging miR-219a-5p. The tumorigenesis assay revealed that the knockdown of SNHG14 and miR-219a-5p expression in those extracted tumors, we found that SNHG14 was down-regulated, while miR-219a-5p was up-regulated in nude mice treated with SNHG14 shRNA.

Conclusions

We showed that SNHG14 enhanced ovarian cancer cell proliferation, migration, and invasion by sponging miR-219a-5p. Our findings implied that LncRNA SNHG14 could be used as a prospective therapeutic target for ovarian cancer.

Conflict of interest

The authors declare no conflicts of interest.

References


9) Yu J, Han Q, Cui Y. Decreased long non-coding RNA SPRY4-IT1 contributes to ovarian cancer cell metastasis partly via affecting epithelial-mesenchymal transition. Tumour Biol 2017; 39: 1010428317709129.


