

# Long non-coding RNA SNHG16 promotes cell growth and metastasis in ovarian cancer

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**Abstract. – OBJECTIVE:** To investigate the expression of long non-coding RNA SNHG16 in ovarian cancer and to further investigate its role in the development of ovarian cancer as well as its potential regulatory mechanism.

**PATIENTS AND METHODS:** Quantitative Real-time polymerase chain reaction (qRT-PCR) was used to detect the expression of SNHG16 in 103 ovarian cancer tissues and normal tissues; the relationship between the expression of SNHG16 and the pathological parameters of ovarian cancer and the prognosis of patients was also analyzed. qRT-PCR was used to further verify the expression of SNHG16 in the ovarian cancer cells. After establishment of SNHG16 knock-down expression model in ovarian cancer cells SKOV-3 and HO8910 using small interfering RNA, the effect of SNHG16 on biological function of ovarian cancer cells was analyzed via cell counting kit-8 (CCK8), transwell invasion and migration assay. Finally, its potential mechanism was analyzed by Western Blot.

**RESULTS:** qRT-PCR results showed that the expression of SNHG16 in ovarian cancer was significantly higher than that in normal tissues, and the difference was statistically significant. Compared with patients with lower expression of SNHG16, patients with higher expression of SNHG16 had higher tumor stage, high rate of distant metastasis and low overall survival rate. Compared with the negative control si-NC group, the cell proliferation, invasion and migration ability in SNHG16 knockdown group (si-SNHG16) significantly decreased. Western Blot showed that after knockdown of SNHG16, expressions of P-AKT and MMP9 decreased significantly, while there was no significant change in the total AKT level.

**CONCLUSIONS:** SNHG16 was highly expressed in ovarian cancer, and was correlated with staging, distant metastasis and poor prognosis of ovarian cancer. SNHG16 may activate phosphorylation of AKT and upregulate the expression of MMP9 to promote cell proliferation, invasion and migration of ovarian cancer.

*Key Words:*

Long non-coding RNA, SNHG16, Ovarian cancer, Prognosis.

## Introduction

Ovarian cancer is one of the six common tumors in females. The malignant degree of ovarian cancer is the top three of the common tumors of female genital organs, which is lower than that of endometrial cancer and cervical cancer<sup>1,2</sup>. However, the mortality rate ranks first among gynecological tumors. Ovaries are located in the deep pelvic cavity, no specific clinical manifestations are presented in the early stage, and effective clinical screening tools are lacked, metastases are often accompanied when diagnosed<sup>3,4</sup>. All along, 5-year survival rate of patients with ovarian cancer was around 30%, 50% of patients relapsed or died within 2 years, and the incidence showed an increased trend year by year<sup>5,6</sup>. The etiology of ovarian cancer is still unknown, and may be related to the complicated ovarian embryonic development, tissue anatomy and endocrine function; it also may be related to the *in vivo* and *in vitro* environment<sup>7,8</sup>. Because most patients with ovarian cancer were diagnosed at the late stage of cancer, the best timing of treatment was missed, resulting in poor treatment, high postoperative recurrence rate, and poor prognosis. Therefore, searching for mechanisms of ovarian cancer, screening of ovarian cancer-specific tumor markers and drug treatment targets are currently the focuses of research on ovarian cancer<sup>9</sup>. Long non-coding RNA (lncRNA) is a type of non-coding protein, with over 200 bp in length<sup>10</sup>. Many investigations have reported that lncRNA played an important role in tumor progression. Studies have shown that lncR-

NA can play a tumor suppressor or cancer-promoting function through a variety of mechanisms<sup>11,12</sup>. LncRNA SNHG16, known as small nucleolar RNA host gene 1, was the earliest lncRNA found in neuroblastomas and has been shown to have a carcinogenic effect in colorectal and bladder cancers<sup>13-15</sup>. However, whether SNHG16 affected the progression and metastasis of ovarian cancer and its mechanism, have not been reported yet.

## Patients and Methods

### Patients and Tumor Samples

We collected 103 pairs of surgically resected ovarian cancer patients with tumor and corresponding non-tumor normal tissues samples, referred to ovarian cancer FIGO staging criteria in 2009, all included patients were confirmed as epithelial ovarian cancer by histopathological analysis, and without preoperative radiotherapy or chemotherapy and other anti-cancer treatment. The study was approved by the Ethical Committee; patients and their families have been fully informed that their specimens would be used for scientific research and signed the relevant informed consent.

### Cell Lines and Reagents

Four ovarian cancer cells (SKOV-3, ES2, HO8910, OMC685) and one ovarian normal epithelial cell line (IOSE-29) were purchased from American ATCC Company (Manassas, VA, USA). Dulbecco's Modified Eagle Medium (DMEM) medium and fetal bovine serum (FBS) were purchased from Life Technologies (Gaithersburg, MD, USA). Cell culture conditions: cells were incubated at 37°C, 5% CO<sub>2</sub>, and cultured in DMEM medium containing 10% fetal bovine serum (FBS).

### Transfection

Negative control (si-RNA) and siRNA containing SNHG16 interference sequence (si-SNHG16) were purchased from Shanghai Genechem Company (Shanghai, China). Cells were plated in a 6-well plate and cultured to a cell density of 70%. SiRNA transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer and cells were collected after 48 hours for qRT-PCR analysis and cell function assays.

### Cell Proliferation Assays

After 48 h of transfection, cells were collected and plated into a 96-well plate with 2000 cells per

well. After cells were cultured for 1, 2, 3 and 4 days, respectively, cell counting kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan) reagent was added and the incubation was continued for 2 h. Cells were placed in a microplate reader and the OD value of each well at 490 nm absorption wavelength was measured; data were analyzed.

### Transwell Cell Migration and Invasion Assay

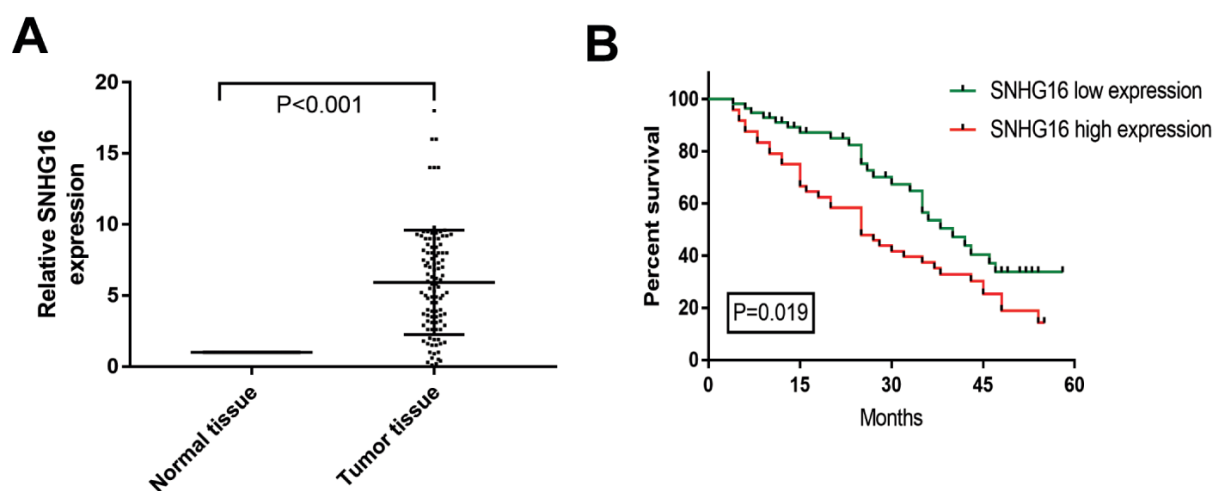
Transfected cells for 48 h were selected and trypsinized and resuspended in serum-free medium. The cell density was adjusted to  $2.0 \times 10^5$ /mL after cell counting. Transwell chambers containing Matrigel gel or not were placed in the 24-well plate, 200  $\mu$ L cell suspension were added to the upper chamber, and 500  $\mu$ L medium containing 10% fetal bovine serum (FBS) were added to the lower chamber. Cells were incubated at 37°C. Transwell chambers were removed after 48 h, fixed with 4% paraformaldehyde for 30 min, crystal violet staining for 15 min and washed with phosphate-buffered saline (PBS). The inner surface of the cell basement membrane was carefully clean and the inner cells were removed. Staining transmembrane cells in the outer layer were observed under the microscope, and five visual fields for cell count were randomly selected.

### Quantitative Real-time PCR (qRT-PCR)

Total RNA was extracted from ovarian cancer cell lines and tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), then it was reverse transcribed into cDNA using Primescript RT Reagent (TaKaRa, Otsu, Shiga, Japan). The qRT-PCR reaction was performed using SYBR<sup>®</sup>Premix Ex Taq<sup>™</sup> (TaKaRa, Otsu, Shiga, Japan), and the StepOne Plus Real-time PCR system (Applied Biosystems, Foster City, CA, USA). The following primers were used for the qRT-PCR reaction: SNHG16: forward: CAGAATGCCATGGTTTCCCC, reverse: TGGCAAGAGACTTCCTGAGG; GAPDH: forward: CTCAGACACCATGGGAAGGTGA, reverse: ATGATCTTGAGGCTGTTGTCATA. Data were analyzed using ABI Step One software and relative mRNA levels were calculated using the 2<sup>- $\Delta\Delta$ Ct</sup> method.

### Western Blot

Transfected cells were lysed with lysis buffer, shaken on ice for 30 min, and centrifuged at 14000 g for 15 min at 4°C. Total protein concentration was calculated by the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL,



**Figure 1.** (A) The expression of SNHG16 in ovarian cancer tissue was significantly increased; (B) Kaplan-Meier survival curves of patients with ovarian cancer based on SNHG16 expression. Patients in the high expression group had a significantly more unfavorable prognosis than those in low expression group.

USA). The extracted protein was separated on a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Western blot analysis was performed according to standard procedures. Primary antibodies were P-AKT, AKT, MMP9 and GAPDH, secondary antibodies were anti-mouse and anti-rabbit, all were purchased from Cell Signaling Technology (Danvers, MA, USA).

#### Statistically Analysis

Data were processed by statistic package for social science (SPSS) 22.0 software (IBM, Armonk, NY, USA) and expressed as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ). Continuous variables were analyzed using  $t$ -test, categorical variables using  $\chi^2$ -test or Fisher's exact test. Kaplan-Meier method was used to assess the prognosis of patients and the Log-rank test was used to compare the differences between the curves.  $p < 0.05$  considered to be statistically significant.

## Results

### SNHG16 is Highly Expressed in Ovarian Cancer Tissues and Cell Lines

Expression of SNHG16 in 103 ovarian cancer tissues, para-cancerous tissues and ovarian cancer cell lines were detected by qRT-PCR. The results showed that the expression of SNHG16 in ovarian cancer tissues was significantly higher than

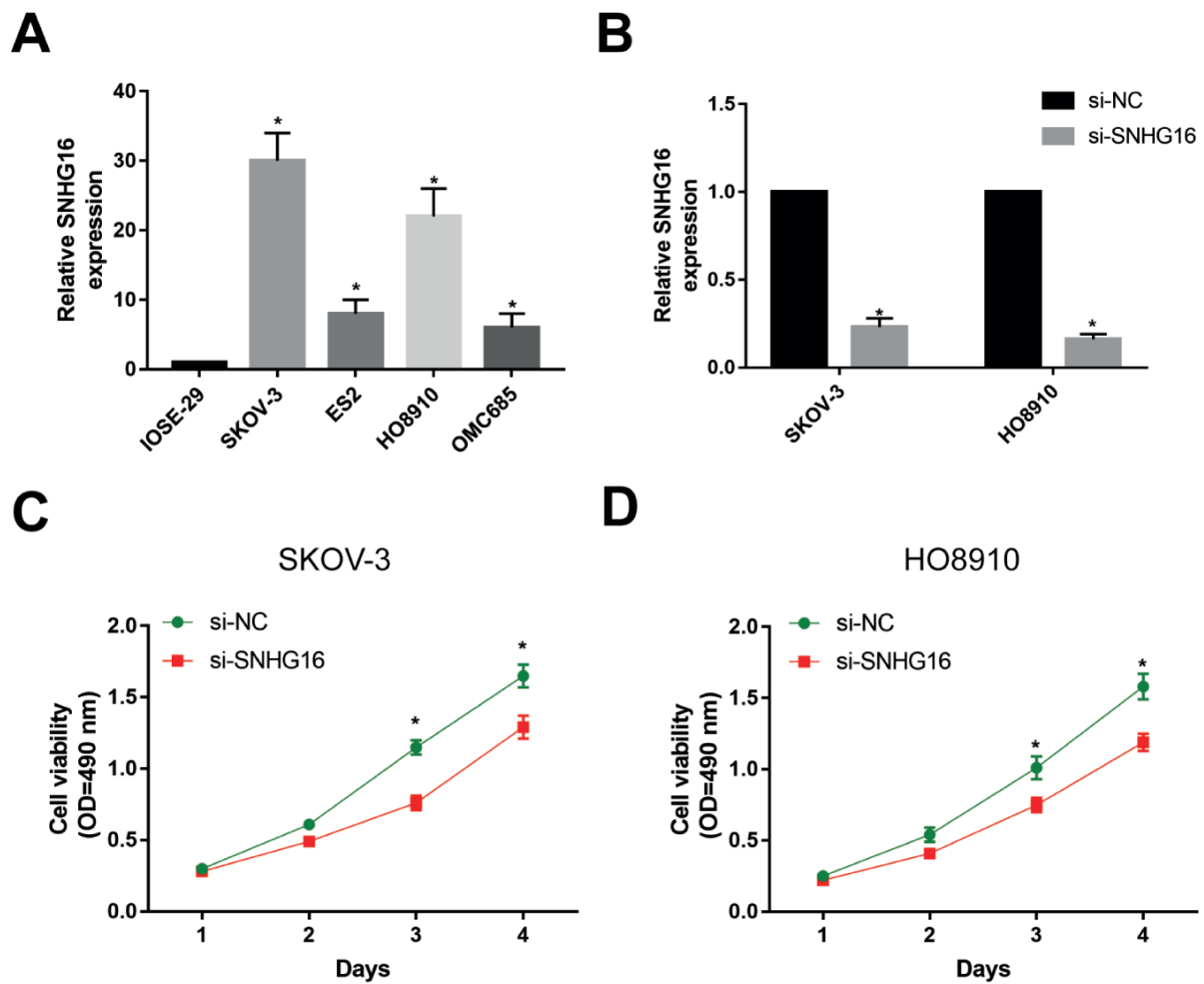
that in para-cancerous tissues; the difference was statistically significant (Figure 1A). Compared with normal ovarian epithelial cells (IOSE-29), SNHG16 was highly expressed in ovarian cancer cells; the difference was statistical significance (Figure 2A). Especially in SKOV-3 and HO8910 cell lines, SNHG16 expression was the highest, so these two cell lines were chosen for the following experiments.

### SNHG16 Expression is Correlated with Clinical Stage, Distance Metastasis and Overall Survival in Ovarian Cancer Patients

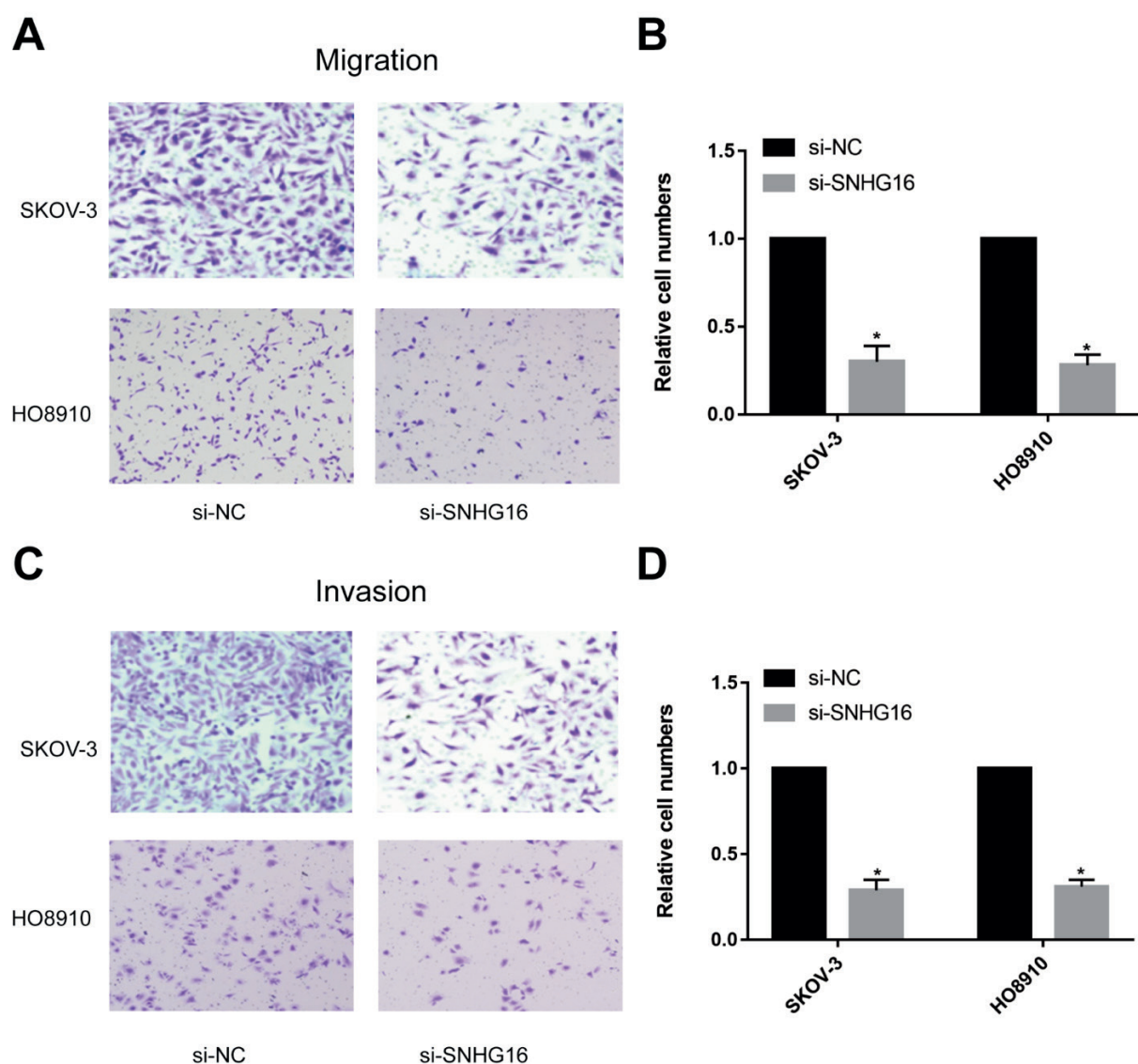
According to the results of qRT-PCR of SNHG16 expression in 103 pairs of ovarian cancer tissues and para-cancerous tissues, SNHG16 expression was divided into high expression group and low expression group, and the patient number of each group was counted. The relationship between SNHG16 expression and age, tumor size, clinical stage and distant metastasis in patients with ovarian cancer was analyzed by  $\chi^2$ -test. High expression of SNHG16 was positively correlated with clinical stage, tumor size, lymph node metastasis and distant metastasis of ovarian cancer, but not correlated with age and gender (Table I). In addition, in order to explore the relationship between the expression of SNHG16 and the prognosis of patients with ovarian cancer, relevant follow-up data were collected. Kaplan-Meier survival curves showed that high expression of SNHG16 was significantly associated with poor prognosis of ovarian cancer, and the higher

**Table 1.** Association of SNHG16 expression with clinicopathological characteristics of ovarian cancer.

Parameters	Number of cases	SNHG16 expression		p-value
		Low (%)	High (%)	
Age (years)				0.969
< 60	48	26	22	
≥ 60	55	30	25	
Clinical stage				0.013
I-II	30	22	8	
III-IV	73	34	39	
Tumor size (cm)				0.017
≤10	57	25	32	
>10	46	31	15	
Tumor Grade				0.002
G1	36	27	9	
G2-G3	67	29	38	
Distance metastasis				0.006
No	65	42	23	
Yes	38	14	24	



**Figure 2.** (A) qRT-PCR analysis of SNHG16 expression in ovarian cell lines and ovarian epithelial cells OMC685; (B) qRT-PCR were used to verify the efficiency of SNHG16 knockdown. (C-D) Growth curve analysis shows the cell growth of SKOV-3 and HO8910 cells with SNHG16 knockdown.



**Figure 3.** (A-B) SKOV-3 and HO8910 cells transfected with si-SNHG16 displayed significantly lower migration capacity. (C-D) SKOV-3 and HO8910 cells transfected with si-SNHG16 displayed significantly lower invasion capacity.

the SNHG16 expression, the worse the prognosis ( $p=0.019$ ; Figure 1B). This result suggested that SNHG16 may be used as a new biological indicator to predict the prognosis of ovarian cancer.

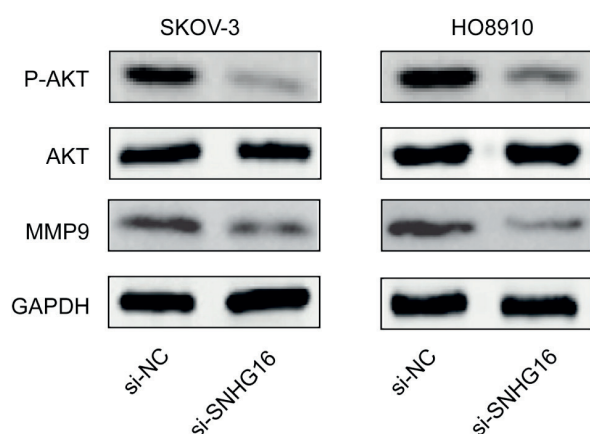
#### **Knockdown of SNHG16 Inhibits Cell Proliferation**

To explore the effect of SNHG16 on the proliferation of ovarian cancer cells, we first successfully constructed the SNHG16 interference expression model (Figure 2B) and detected the proliferation of cells transfected with si-NC and si-SNHG16 using CCK8. As shown in Figures 2C and 2D, the cell proliferation rate was significant-

ly decreased in the si-SNHG16 group compared to the si-NC group.

#### **Knockdown of SNHG16 Inhibits Cell Migration and Invasion**

Transwell migration invasion assay was used to explore the effect of SNHG16 on migration and invasion ability of ovarian cancer cells. Migration assay (Figure 3A and 3B) showed that the number of cells penetrating the transwell chamber was significantly reduced after knockdown of SNHG16 compared to the si-NC group, indicating that migration capacity was inhibited. The results of the invasion assay were consistent (Figure 3C and 3D).



**Figure 4.** Knockdown of SNHG16 expression significantly decreased the expression of P-AKT and MMP9.

#### **Knockdown of SNHG16 Inhibits the Expression of P-AKT and MMP9**

To further investigate the role of SNHG16 in migration and invasion ability of ovarian cancer cells, SNHG16 was knockdown in SKOV-3 and HO8910 via transfection of si-SNHG16. Meanwhile, the expressions of P-AKT, AKT and MMP9 were detected by using Western blot, respectively. The results showed that the expression of P-AKT and MMP9 decreased significantly after SNHG16 knockdown, while the total AKT level did not change significantly (Figure 4).

### **Discussion**

Most of the patients were already in the advanced stage when they were diagnosed. Therefore, looking for early diagnosis and prognostic markers has become an important method to improve the diagnosis and treatment of ovarian cancer<sup>16</sup>. In recent years, more and more lncRNAs have been proved to play crucial roles in tumor formation, invasion, and metastasis. Meantime, lncRNAs, as new tumor markers and therapeutic targets, showed a good clinical application in the diagnosis and treatment of cancers<sup>17</sup>.

Previous studies have shown that lncRNA expression was increased in neuroblastoma, bladder cancer and colorectal cancer and played a role in promoting cancer<sup>14,15</sup>. In this study, we first detected the expression of SNHG16 in 103 pairs of ovarian cancer tissues and para-cancerous tissues. The results showed that SNHG16 expression was significantly up-regulated and positively correlated with the stage, distant meta-

stasis and poor prognosis of ovarian cancer. Thus, we believed that SNHG16 may play a role in promoting ovarian cancer. To further investigate the effect of SNHG16 on the biological function of ovarian cancer, we constructed SNHG16 knockdown expression model using small interfering RNA. CCK8, invasion and migration assay results showed that SNHG16 can promote the occurrence and development of ovarian cancer. PI3K/AKT was a classical signal transduction pathway, which can resist cell apoptosis and promotes cell survival, playing a crucial role in tumor invasion and metastasis of tissues<sup>18,19</sup>. Invasion and metastasis are two important characteristics of ovarian cancer. At present, the research on the mechanism of invasion and metastasis of ovarian cancer cells were not fully complete. Among the mechanisms of invasion and metastasis of malignant tumors, matrix metalloproteinases (MMPs) are a series of proteolytic enzymes that have been found to be closely related to degradation of the extracellular matrix<sup>20</sup>. MMP9 is an important member of the MMP family, the main role of which is to degrade the extracellular matrix, to help tumor cells break through the tissue barrier, the tumor invasion or with the blood circulation to other tissues and organs to achieve the purpose of distant metastasis<sup>21</sup>. To explore whether SNHG16 can promote the development of ovarian cancer by regulating the PI3K/AKT pathway and MMP9, we detected the expression of P-AKT, AKT and MMP9 in cells after knockdown of SNHG16 by Western Blotting. The results showed that after the knockdown of SNHG16, expressions of P-AKT and MMP9 decreased significantly, while the total AKT level did not change significantly. This suggested that SNHG16 can promote the development of ovarian cancer by activating phosphorylated AKT and up-regulating the expression of MMP9.

### **Conclusions**

SNHG16 expression was significantly increased in ovarian cancer, and was correlated with ovarian cancer staging, distant metastasis and poor prognosis. SNHG16 may activate phosphorylation of AKT and upregulate the expression of MMP9 to promote cell proliferation, invasion and migration of ovarian cancer.

#### **Conflict of Interest**

The Authors declare that they have no conflict of interest.

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