European Review for Medical and Pharmacological Sciences 2019; 23: 3235-3242

LncSNHG14 promotes ovarian cancer by targeting microRNA-125a-5p

Y.-L. ZHAO[1], Y.-M. HUANG[2]

[1]Department of Obstetrics and Gynecology, Tengzhou Women and Children's Health Hospital in Shandong Province, Tengzhou, China
[2]Laboratory Medicine, Shandong Tengzhou Central People's Hospital, Tengzhou, China

Abstract. – OBJECTIVE: This study aims to investigate whether small nucleolar RNA host gene 14 (SNHG14) is involved in the development of ovarian cancer through affecting cell proliferation and cell cycle progression by regulating microRNA-125a-5p.

PATIENTS AND METHODS: We detected the mRNA expressions of SNHG14 and microRNA-125a-5p by quantitative Polymerase Chain Reaction (qPCR) in ovarian cancer tissues and normal ovarian tissues. Their expression levels in ovarian cancer cell lines were examined as well. Meanwhile, the regulatory effects of SNHG14 and microRNA-125a-5p on cell proliferation and cell cycle were detected by Cell Counting Kit-8 (CCK-8) and flow cytometry, respectively. The binding relationship between microRNA-125a-5p and SNHG14 was examined by the Luciferase reporter gene assay. It was further confirmed by recovery experiments whether SHHG14 can affect the proliferation and cycle of ovarian cancer cells by regulating microRNA-125a-5p.

RESULTS: SNHG14 was highly expressed in ovarian cancer tissues and cell lines relative to controls. The survival curve analysis showed that the AUC was 0.8681 and Cutoff value was 2.33. The five-year survival rate of the high SNHG14 expression group was markedly lower than that of the low SNHG14 expression group. In addition, we found that SNHG14 could accelerate cell proliferation and cell cycle progression of ovarian cancer cells. Dual-Luciferase reporter gene experiments indicated that SNHG14 could bind to microRNA-125a-5p, which was lowly expressed in ovarian cancer patients. However, the overexpression of microRNA-125a-5p reversed the promotive effect of SNHG14 on the proliferation and cell cycle of ovarian cancer cells. Dual-Luciferase reporter gene assay also indicated that DHX33 was a target gene of microRNA-125a-5p. The overexpression of DHX33 could attenuate the inhibitory effect of microRNA-125a-5p on cell proliferation and cell cycle in SKOV3 and OVCAR3 cells.

CONCLUSIONS: High expression of SNHG14 can promote the ovarian cancer cell proliferation and accelerate the cell cycle by sponging microRNA-125a-5p to regulate DHX33 expression.

Key Words
Ovarian cancer, SNHG14, MicroRNA-125a-5p, DHX33.

Introduction

As one of the most malignant diseases in gynecological malignancies, ovarian cancer has the highest morbidity and mortality[1]. The histological types of ovarian tumors are complex, including sex cord-stromal tumors and germ cell tumors along with epithelial tumors, of which epithelial sources ovarian cancer accounts for about 85% to 90% of ovarian malignancies[2]. Many cases are already in the late stage with extensive metastasis at the time of discovery, and half of the patients relapsed within 16 months, with a 5-year overall survival rate below 50%[3,4]. Therefore, exploring the mechanism of ovarian cancer and finding new therapeutic targets are of great significance for the diagnosis and treatment of ovarian cancer.

Long noncoding ribonucleic acid (10 ng noncoding ribonucleic acid, lncRNA) is a non-coding, single-stranded RNA of more than 200 nucleotides in length, which plays an important regulatory role in biological processes, such as tumor growth and metastasis[5]. LncRNA is involved in a variety of biological activities with different mechanisms, such as gene transcription, material synthesis, apoptosis, etc[6]. Huarte[7] have shown that lncRNAs play important regulatory parts in many tumors. For example, lncRNA UCA1 is up-regulated in bladder cancer tissues and cell line BLS-211. LncRNA UCA1 enhanced *in vitro* invasive ability of BLS-211 cells[8]. LncRNA H19 is upregulated in ovarian cancer tissues and cells, while silencing H19 inhibits ovarian cancer cell proliferation and induces apoptosis[9].

Studies[10,11] have found that lncRNA-small nucleolar RNA host gene 14 (SNHG14) promotes tumor development, including non-small cell lung cancer

Corresponding Author: Youmin Huang, BM; e-mail: rhwi49@163.com

and gastric cancer. However, the role of SNHG14 has not been reported in ovarian cancer. In this work, we aimed to investigate the possible role of SNHG14 in ovarian cancer and its potential mechanism.

Patients and Methods

Tissue Specimens

Fresh ovarian cancer tissues and normal tissues were surgically collected from 24 patients with ovarian cancer. All clinical pathology information of the enrolled patients were collected, including gender, age, tumor size, and number of tumors. All patients were followed up. Patients denied family history and treatment history before surgery and were pathologically diagnosed with ovarian cancer. All the patients volunteered to participate in the study and signed the informed consent. This research was approved by the Ethics Committee of the Shandong Tengzhou Central People's Hospital. The collected specimens were stored in a liquid nitrogen tank.

Cell Culture

The normal cell line HOSEpiC and the ovarian cancer cell lines including C13K, SKOV3, 3AO and OVCAR3 were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Ovarian cancer cells used were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 100 U/mL penicillin and 0.1 mg/mL streptomycin and placed in an incubator with 5% CO_2 at 37°C.

Cell Transfection

Cells were seeded into a cell culture plate or culture flask. When the cell density reached 70%-80%, microRNA-125a-5p mimics, pcDNA-SNHG14, pcDNA-DHX33 or the relative Negative Controls were transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After mixing at room temperature for 20 min, the mixtures were slowly dripped into the culture medium. After co-culturing for 4 h, the culture medium was replaced with the fresh normal medium for 24 h, and the cells were collected for subsequent experiments.

Real Time-Quantitative Polymerase Chain Reaction (qPCR)

The total RNA was extracted by TRIzol (Invitrogen, Carlsbad, CA, USA). After determining RNA concentration and purity, complementary deoxyribose nucleic acid (cDNA) was synthesized by reverse transcription, and U6 was used as an internal reference. The SYBR Green master mix, template, upstream/downstream primer, and diethyl pyrocarbonate (DEPC) were formulated into a Polymerase Chain Reaction solution, and placed on a Real Time-PCR machine for PCR amplification reaction. The miRNA was reversely transcribed into cDNA using the miRNA RT Kit of Shanghai Tiangen Biochemical Co., Ltd. (Shanghai, China). PCR reaction and quantitative analysis of miRNA were carried out according to the MiRNA qPCR kit of Shanghai Tiangen Biochemical Co., Ltd. (Shanghai, China). The primer sequences were as follows: microRNA-125a-5p (F: 5' ACACTCCAGCTGGGTCCCTGAGACCCTTTAAC 3', R: 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTGGACACT-3') U6 (F: CTCGCTTCGGCAGCAGCACATATA, R: AAATATGGAACGCTTCACGA). SNHG14 (F: 5′-GGGTGTTTACGTAGACCAGAACC-3′, R: 5′-CTTCCAAAAGCCTTCTGCCTTAG-3′), DHX33 (F: 5'-TGCGTGAAGCAATTTCAGAC-3', R: 5′-AGGTCGACATCCATGGTAGC-3′)

Luciferase Assay

The transcript 3'untranslated region (3'UTR) sequence of the DHX33 gene was cloned into the vector pGL3 containing the Luciferase reporter gene. The control plasmid MUT3' UTR group was constructed by mutating the core region of the miRNA binding on the 3' UTR to a null binding sequence using a site-directed mutagenesis kit. At the same time, the pGL-3-SNHG14 WT and pGL-3-SNHG14 MUT groups were constructed. Renilla Luciferase internal reference plasmid and microRNA-125a-5p mimics were transfected into cells of each group. After 24 hours of transfection, the cell culture medium was discarded, and an appropriate amount of lysate was added to fully lyse the cells according to kit requirements. The Renilla Luciferase was used as an internal reference, and the RLU value obtained by the Firefly Luciferase measurement was divided according to the RLU value obtained by the Renilla Luciferase assay (Thermo Fisher Scientific, Waltham, MA, USA). The degree of activation of target reporter genes in different samples was compared based on the ratio obtained.

Cell Cycle

The cells were collected 72 h after transfection. After centrifugation, the cells were resuspended in 70% alcohol, transferred to the Eppendorf (EP) tube (Eppendorf, Hamburg, Germany)

and fixed at 4°C for at least 18 h. The cell number was adjusted to 10^6/mL, and 1 mL of cell suspension was resuspended in l mL of Propidium Iodide (PI) staining solution (BD Biosciences, Franklin Lakes, NJ, USA). After incubation at 37°C for 30 min, cell cycle was detected by flow cytometry analysis (Partec, Arlesheim, Switzerland).

Cell Proliferation

Cells were seeded into 96-well plates at 1×10^4/well and cultured for 6, 24, 48, 72, 96 h, respectively. 10 μL of Cell Counting Kit-8 (CCK-8) solution (Dojindo, Kumamoto, Japan) was added to each well, and incubation was continued for 1 hour at 37°C in the dark. The optical density (OD) value was measured at a 450 nm using a microplate reader. Five duplicate wells were set for each group.

Statistical Analysis

Data were analyzed by Statistical Product and Service Solutions (SPSS) 13.0 (IBM, Armonk, NY, USA) software, and expressed as mean ± standard deviation (Mean ± SD). The *t*-test was used for comparison between the groups. $p<0.05$ was considered statistically significant.

Results

SNHG14 Was Upregulated in Ovarian Cancer

We detected SNHG14 expression in ovarian cancer tissues and found that SHHG14 was up-regulated in the ovarian cancer tissues compared with the normal tissues (Figure 1A). At the same time, we detected SNHG14 expression in ovarian cancer cells. The result showed that SNHG14 was also up-regulated in ovarian cancer cells (Figure 1B). Next, we performed the survival curve analysis of SNHG14 and found that the area under the curve was 0.8681 and the cutoff value was 2.33, indicating the diagnostic potential of SNHG14 in ovarian cancer (Figure 1C). In ad-

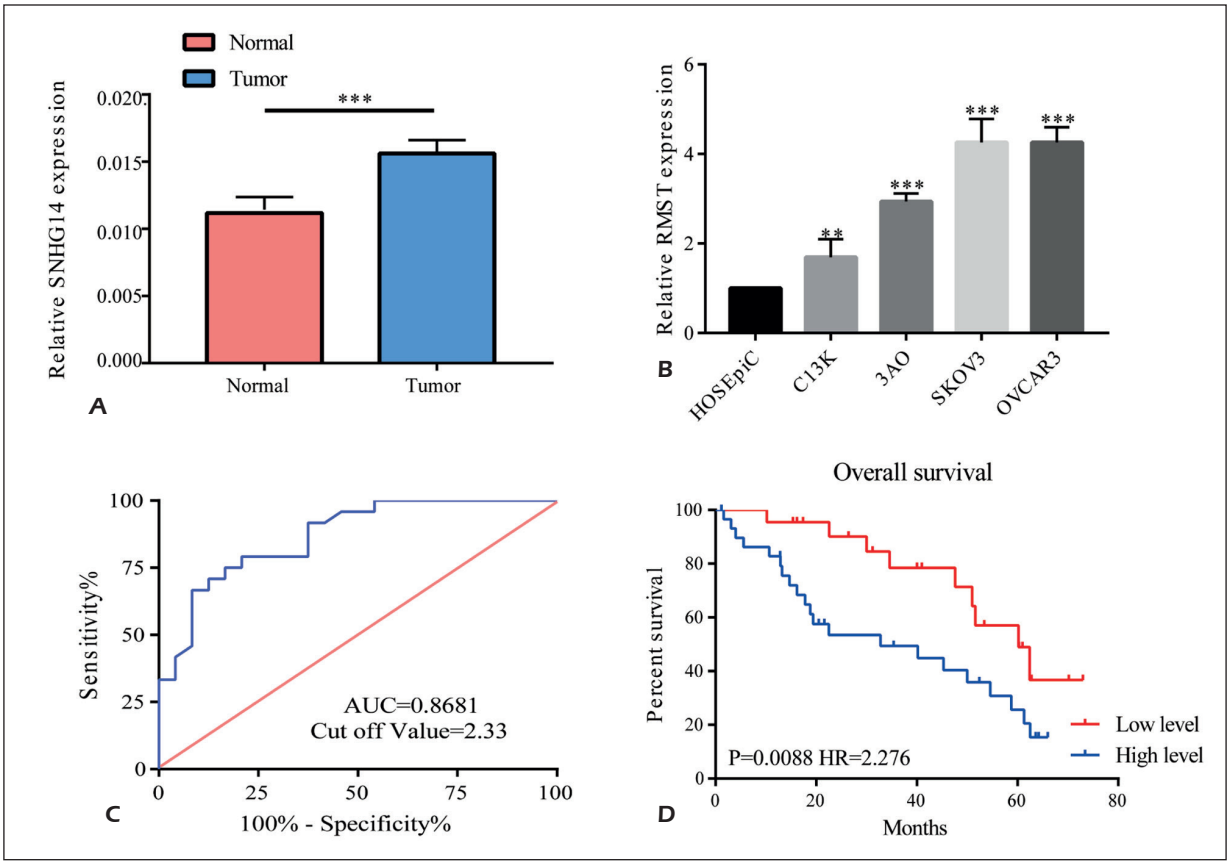

Figure 1. SNHG14 was highly expressed in ovarian cancer. **A**, SNHG14 was highly expressed in ovarian cancer tissues. **B**, SNHG14 was generally highly expressed in ovarian cancer cell lines. **C**, Survival analysis showed that the area under the curve was 0.8681 and the cutoff value was 2.33. **D**, The five-year survival rate was analyzed.

dition, we analyzed the five-year survival rates of ovarian cancer patients with high expression and low expression in SNHG14. The results showed that the five-year survival rates of patients with high SNHG14 expression group were markedly lower than that of the low expression group (Figure 1D).

SNHG14 Promoted Proliferation and Cycle of Ovarian Cancer Cells

Since SNHG14 was up-regulated in ovarian cancer, we further explored its possible role in ovarian cancer. In SKOV3 and OVCAR3 cell lines, we overexpressed SHNHG14 and verified its stable expression (Figure 2A). The effect of

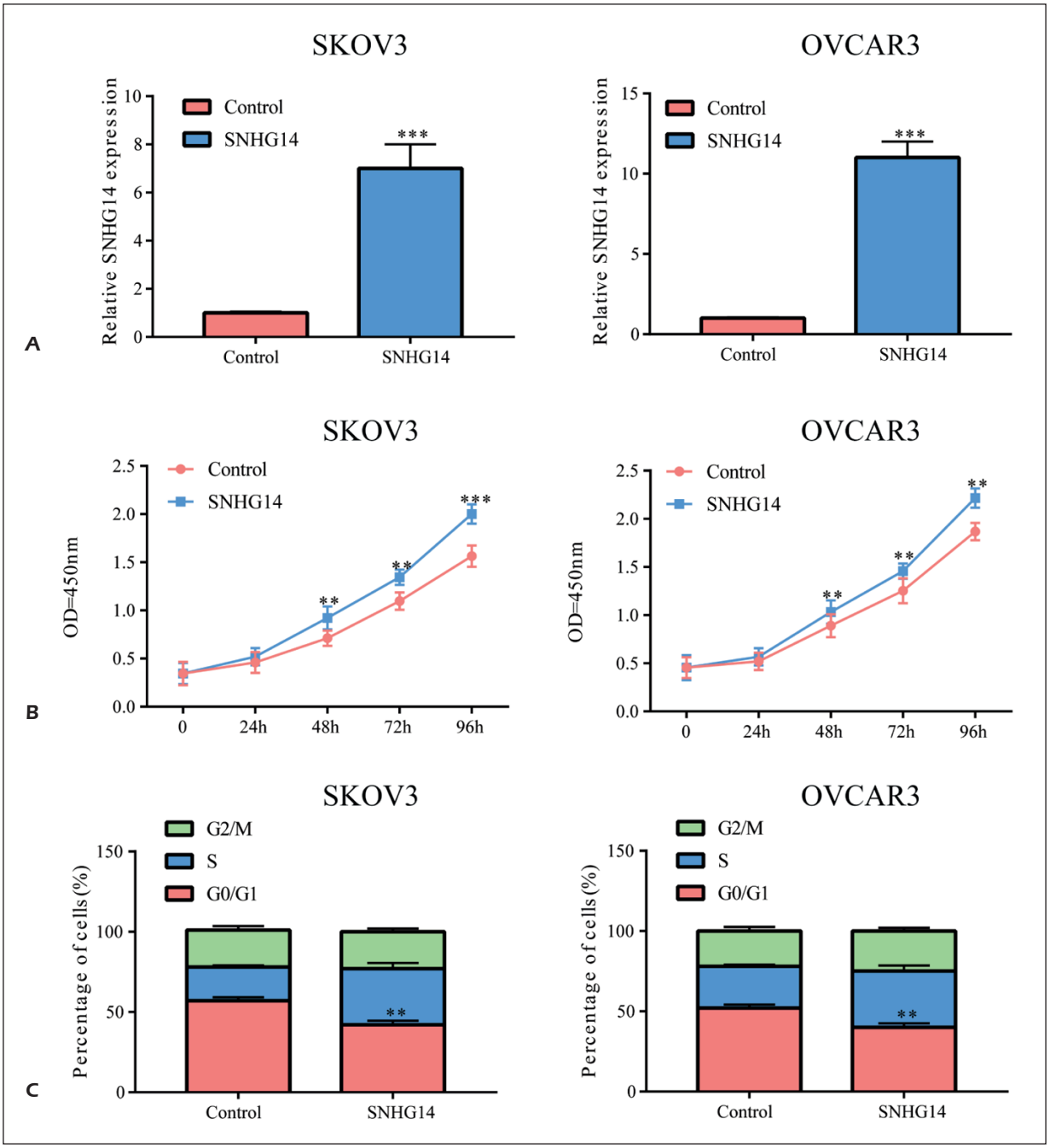

Figure 2. SNHG14 promoted proliferation and cycle of ovarian cancer cells. **A**, Transfection efficiency of SHNHG14 overexpression plasmid in SKOV3 and OVCAR3 cells. **B**, SNHG14 promoted the proliferation of SKOV3 and OVCAR3 cells. **C**, SNHG14 promoted the cycle of SKOV3 and OVCAR3 cells.

SNHG14 on cell proliferation and cell cycle was evaluated. Both CCK-8 assay and flow cytometry analysis indicated that the up-regulation of SNHG14 could promote cell proliferation (Figure 2B) and accelerated cell cycle (Figure 2C). These above results indicated that SNHG14 might play its role by enhancing proliferation and cycle of ovarian cancer cells.

SNHG14 Regulated Ovarian Cancer Through MicroRNA-125a-5p

Through bioinformatics prediction and analysis, we found that there may be a binding site for SNHG14 and microRNA-125a-5p, which was further confirmed by the Dual-Luciferase reporter gene assay (Figure 3A). We subsequently detected an especially low level of microRNA-125a-5p in ovarian cancer tissues (Figure 3B). To explore whether SNHG14 functioned through microRNA-125a-5p, we performed a series of recovery experiments. First, microRNA-125a-5p overexpression was achieved by transfection of microRNA-125a-5p mimics (Figure 3C). We then overexpressed SNHG14 and microRNA-125a-5p in both SKOV3 and OVCAR3 cells. The results showed that the up-regulation of microRNA-125a-5p partially reversed the promotive effect of SNHG14 on cell proliferation (Figure 3D, 3E). In the same situation, simultaneous up-regulation of microRNA-125a-5p partially reversed promotive role of

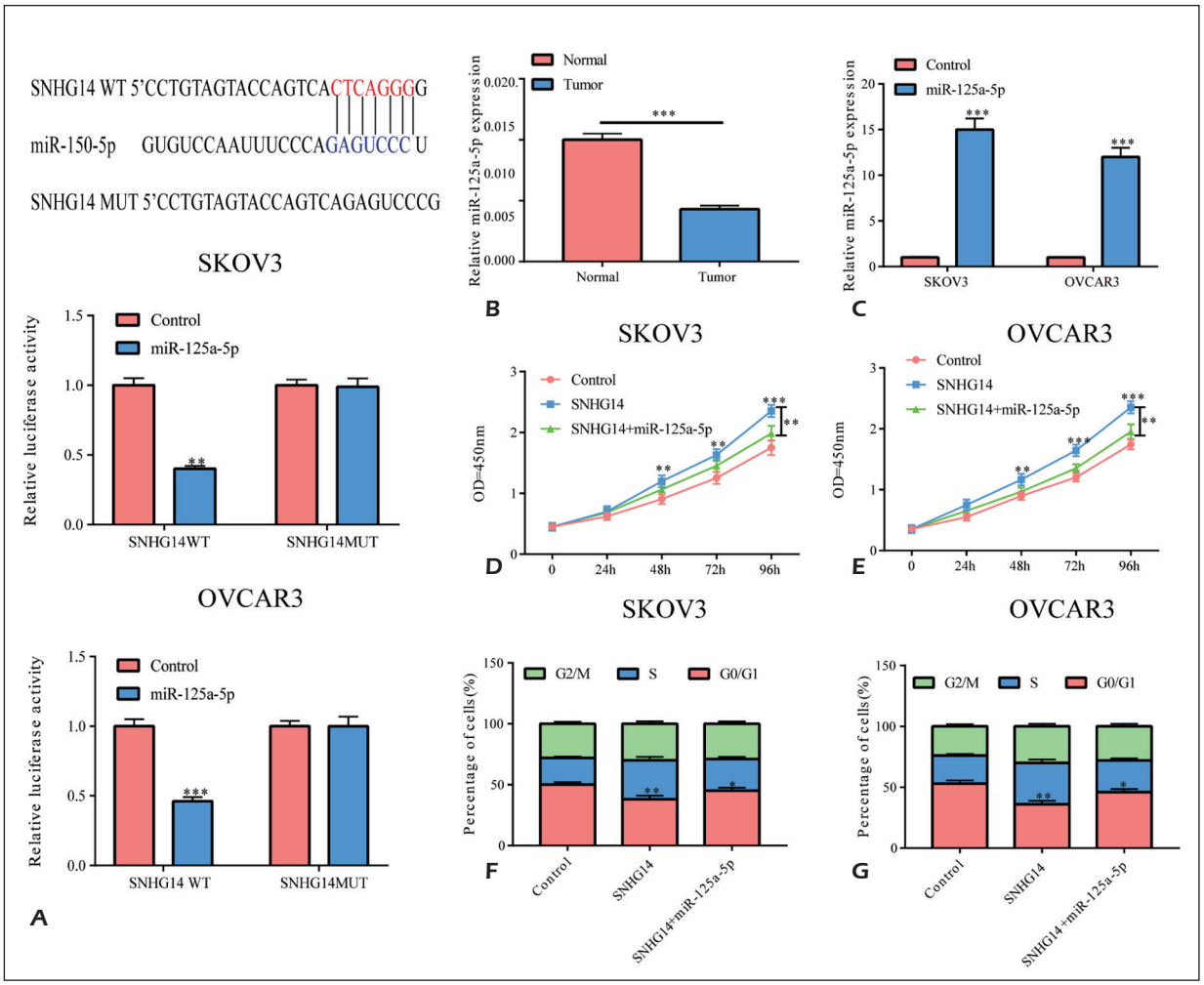

Figure 3. SNHG14 regulated ovarian cancer by microRNA-125a-5p. **A**, Luciferase reporter gene results showed that SNHG14 had a binding site with microRNA-125a-5p. **B**, MicroRNA-125a-5p had a significantly decreased expression of in ovarian cancer. **C**, MicroRNA-125a-5p expression in SKOV3 and OVCAR3 cells. **D**, Transfection efficiency of microRNA-125a-5p mimics. **E**, The overexpression of microRNA-125a-5p in SKOV3 and OVCAR3 cells reversed the promoting effect of SNHG14 on cell proliferation. **F-G**, The overexpression of microRNA-125a-5p in SKOV3 and OVCAR3 cells reversed the promoting effect of SNHG14 on cell cycle.

SNHG14 in the cell cycle (Figure 3F, 3G), which further confirmed our suggestion that SNHG14 could regulate ovarian cancer through microRNA-125a-5p.

DHX33 Was the Downstream Target Gene of MicroRNA-125a-5p

Since miRNAs usually act by their target genes, we predicted that DHX might be a potential downstream target of microRNA-125a-5p through the biological website. We indicated the binding relationship between them by Luciferase reporter gene assay (Figure 4A). Furthermore, we examined DHX33 expression in ovarian cancer tissues and found that it was up-regulated in ovarian cancer tissues (Figure 4B). Next, we verified the transfection efficiency of DHX33 overexpression plasmid (Figure 4C). Then, we examined the effects of simultaneous upregulation of microRNA-125a-5p and DHX33 on cell proliferation and cycle. The result showed that the up-regulation of DHX33 in SKOV3 cells partially reversed the inhibitory effect of microRNA-125a-5p on cell proliferation and cycle (Figure 4D, 4E). This was consistent in OVCAR3 cells (Figure 4F, 4G). These results suggested that SNHG14 affected ovarian cancer cell proliferation and cycle *via* regulating microRNA-125a-5p.

Discussion

In 2011, Salmena et al[12] proposed the hypothesis of competitive endogenous RNA (ceRNA), suggesting that mRNA, lncRNA and pseudogene can competitively bind to miRNA through the

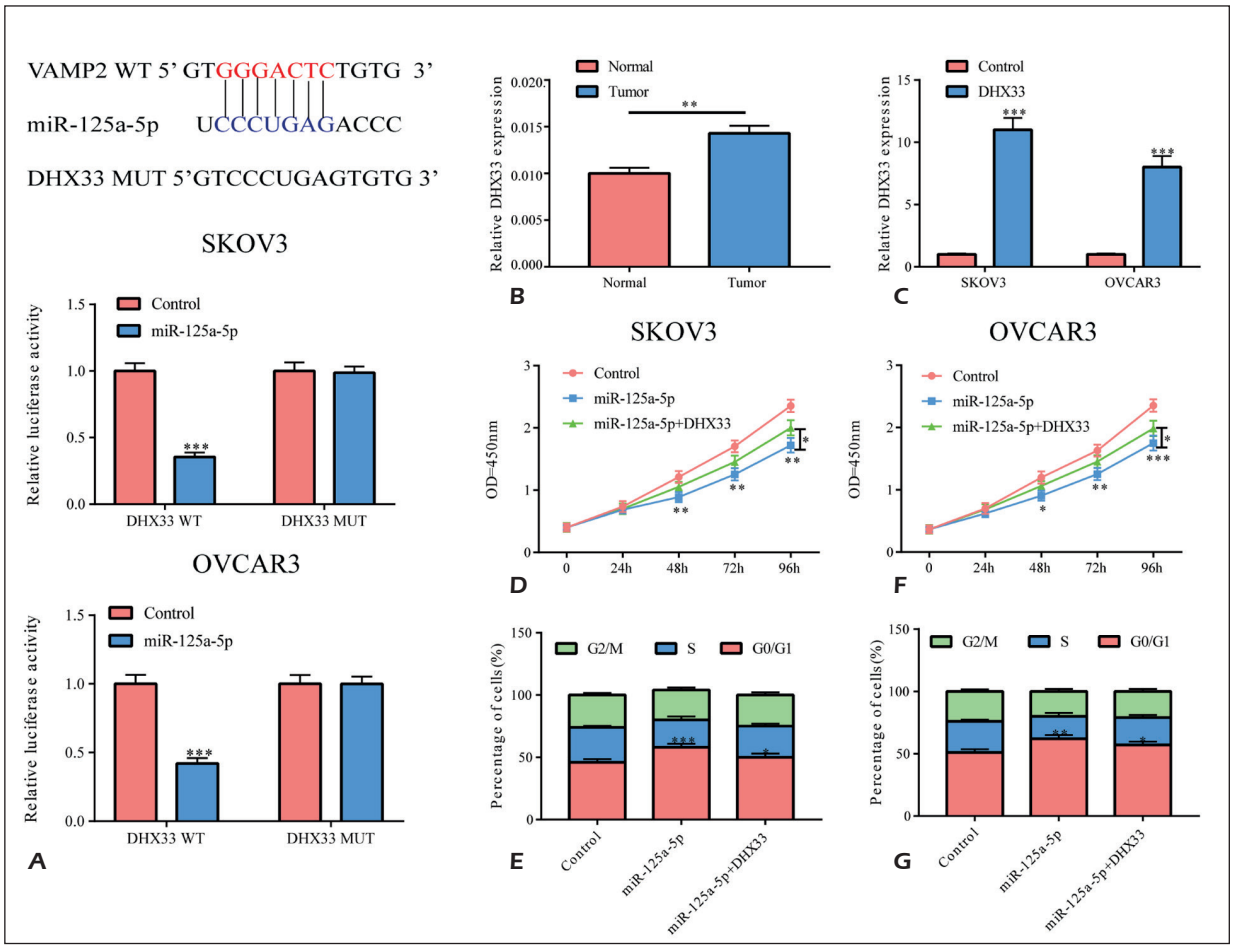

Figure 4. DHX33 was the target gene of microRNA-125a-5p. **A**, Luciferase reporter gene results showed that DHX33 and microRNA-125a-5p had binding sites. **B**, DHX33 expression was significantly increased in ovarian cancer. **C**, DHX33 expression in SKOV3 and OVCAR3 cells. **D**, DHX33 overexpression plasmid transfecting efficiency. **E**, The overexpression of DHX33 in SKOV3 and OVCAR3 cells reversed the inhibiting effect of microRNA-125a-5p on cell proliferation. **F-G**, The overexpression of DHX33 in SKOV3 and OVCAR3 cells reversed the inhibiting effect of miR-125a-5 on cell cycle.

extensively existed MRE (miRNA response element) to indirectly regulate gene expression. At present, experimental studies have found that the occurrence and development of various tumors, such as prostate cancer, renal clear cell carcinoma, breast cancer, liver cancer and endometrial cancer, are closely related to the change of ceRNA expression level[13,14]. In this work, we found that SNHG14 was up-regulated in ovarian cancer and promoted ovarian cancer cell proliferation and cycle. Through bioinformatics analysis, we found that microRNA-125a-5p was a potential target of SNHG14, and further results of Dual-Luciferase reporter gene experiments verified their binding. Therefore, we hypothesized that SNHG14 may function by acting as a ceRNA to target microRNA-125a-5p. Studies[15,16] have shown that microRNA-125a-5p can inhibit tumor progression in a variety of tumors. In this work, we demonstrated that microRNA-125a-5p was lowly-expressed in ovarian cancer. In addition, microRNA-125a-5p was found to partially reverse the promotive effect of SNHG14 on proliferation and cell cycle of ovarian cancer cells after microRNA-125a-5p overexpression, suggesting that SNHG14 may function by combining with miRNA-125a-5p.

Since ceRNA usually plays a part in regulating downstream gene expression by sponging miRNA, we predicted through bioinformatics website that DHX33 might be a potential target of microRNA-125a-5p and verified this by Luciferase reporter gene experiment. Studies[17,18] have shown that in liver cancer, DHX33 expression is closely related with the prognosis of liver cancer, and the high expression of DHX33 often suggests a poor prognosis. We showed that the expression of DHX33 was markedly up-regulated in ovarian cancer. Further analysis indicated that DHX33 up-regulation could partially reverse the inhibitory effect of microRNA-125a-5p on ovarian cancer cells. The ceRNA expression varies in different tumors. Changes in ceRNA can be used as a diagnostic or prognostic indicator of tumors. For example, the expression levels of CNOT6L and VAPA decrease in prostate cancer[19], while the expression of PTENP1 is down-regulated in renal clear cell carcinoma[20]. Besides, overall survival is often correlated with changes in expression levels of some genes. High expression of pseudogene OCT4-p94 is in association with the poor prognosis of patients with hepatocellular carcinoma[21]. ceRNA acts as an anti-cancer or cancer-promoting gene in tumors, thus playing a vital role in guiding the clinical diagnosis. In this work, we found that SNHG14 could act as a ceRNA in ovarian cancer through the SNHG14/microRNA-125a-5p/DHX33 pathway, thus providing a new target for the treatment of ovarian cancer.

Conclusions

We demonstrated that high expression of SNHG14 can promote the ovarian cancer cell proliferation and accelerate cell cycle by sponging microRNA-125a-5p to regulate DHX33 expression.

Conflict of Interests

The authors declared no conflict of interest.

References

1) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
2) Coleman RL, Monk BJ, Sood AK, Herzog TJ. Latest research and treatment of advanced-stage epithelial ovarian cancer. Nat Rev Clin Oncol 2013; 10: 211-224.
3) Berek JS, Crum C, Friedlander M. Cancer of the ovary, fallopian tube, and peritoneum. Int J Gynaecol Obstet 2015; 131 Suppl 2: S111-S122.
4) Jelovac D, Armstrong DK. Recent progress in the diagnosis and treatment of ovarian cancer. CA Cancer J Clin 2011; 61: 183-203.
5) Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet 2009; 10: 155-159.
6) Cech TR, Steitz JA. The noncoding RNA revolution-trashing old rules to forge new ones. Cell 2014; 157: 77-94.
7) Huarte M. The emerging role of lncRNAs in cancer. Nat Med 2015; 21: 1253-1261.
8) Wang F, Li X, Xie X, Zhao L, Chen W. UCA1, a non-protein-coding RNA up-regulated in bladder carcinoma and embryo, influencing cell growth and promoting invasion. FEBS Lett 2008; 582: 1919-1927.
9) Zhu Z, Song L, He J, Sun Y, Liu X, Zou X. Ectopic expressed long non-coding RNA H19 contributes to malignant cell behavior of ovarian cancer. Int J Clin Exp Pathol 2015; 8: 10082-10091.
10) Liu Z, Yan Y, Cao S, Chen Y. Long non-coding RNA SNHG14 contributes to gastric cancer development through targeting miR-145/SOX9 axis. J Cell Biochem 2018; 119: 6905-6913.
11) Zhang Z, Wang Y, Zhang W, Li J, Liu W, Lu W. Long non-coding RNA SNHG14 exerts oncogenic functions in non-small cell lung cancer through

acting as a miR-340 sponge. Biosci Rep 2018; BSR20180941.

12) Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 2011; 146: 353-358.

13) Tay Y, Kats L, Salmena L, Weiss D, Tan SM, Ala U, Karreth F, Poliseno L, Provero P, Di Cunto F, Lieberman J, Rigoutsos I, Pandolfi PP. Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. Cell 2011; 147: 344-357.

14) Jeyapalan Z, Deng Z, Shatseva T, Fang L, He C, Yang BB. Expression of CD44 3'-untranslated region regulates endogenous microRNA functions in tumorigenesis and angiogenesis. Nucleic Acids Res 2011; 39: 3026-3041.

15) Tong Z, Liu N, Lin L, Guo X, Yang D, Zhang Q. miR-125a-5p inhibits cell proliferation and induces apoptosis in colon cancer via targeting BCL2, BCL2L12 and MCL1. Biomed Pharmacother 2015; 75: 129-136.

16) Zhang Y, Zhang D, Lv J, Wang S, Zhang Q. MiR-125a-5p suppresses bladder cancer progression through targeting FUT4. Biomed Pharmacother 2018; 108: 1039-1047.

17) Tian QH, Zhang MF, Luo RG, Fu J, He C, Hu G, Zeng JS. DHX33 expression is increased in hepatocellular carcinoma and indicates poor prognosis. Biochem Biophys Res Commun 2016; 473: 1163-1169.

18) Zhang CZ, Cao Y, Fu J, Yun JP, Zhang MF. miR-634 exhibits anti-tumor activities toward hepatocellular carcinoma via Rab1A and DHX33. Mol Oncol 2016; 10: 1532-1541.

19) Sumazin P, Yang X, Chiu HS, Chung WJ, Iyer A, Llobet-Navas D, Rajbhandari P, Bansal M, Guarnieri P, Silva J, Califano A. An extensive microRNA-mediated network of RNA-RNA interactions regulates established oncogenic pathways in glioblastoma. Cell 2011; 147: 370-381.

20) Yu G, Yao W, Gumireddy K, Li A, Wang J, Xiao W, Chen K, Xiao H, Li H, Tang K, Ye Z, Huang Q, Xu H. Pseudogene PTENP1 functions as a competing endogenous RNA to suppress clear-cell renal cell carcinoma progression. Mol Cancer Ther 2014; 13: 3086-3097.

21) Wang L, Guo ZY, Zhang R, Xin B, Chen R, Zhao J, Wang T, Wen WH, Jia LT, Yao LB, Yang AG. Pseudogene OCT4-pg4 functions as a natural micro RNA sponge to regulate OCT4 expression by competing for miR-145 in hepatocellular carcinoma. Carcinogenesis 2013; 34: 1773-1781.