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Long noncoding RNA SNHG14 enhances migration and invasion of ovarian cancer by upregulating DGCR8

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Abstract. – OBJECTIVE: Ovarian cancer is the most common fatal gynecologic malignancy in females all over the world. Recently, long noncoding RNAs (lncRNAs) have been reported to exert pivotal functions in tumorigenesis. In this research, lncRNA SNHG14 was studied to identify its role in the metastasis of ovarian cancer.

PATIENTS AND METHODS: SNHG14 expression was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) in ovarian cancer specimens. Functional assays including wound healing assay, transwell assay, and Matrigel assay were performed to detect the effect of SNHG14 on the migration and invasion of ovarian cancer cells. In addition, the underlying mechanism was further explored through qRT-PCR and Western blot assay.

RESULTS: SNHG14 level was dramatically higher in ovarian cancer specimens. Moreover, cell migration and invasion were significantly attenuated via the inhibition of SNHG14, while enhanced via the SNHG14 overexpression. Besides, the expression of DGCR8 mRNA and protein was markedly downregulated after the knockdown of SNHG14, while upregulated after SNHG14 overexpression. Furthermore, the expression level of DGCR8 was increased in cancer tissues and positively related to the expression of SNHG14 in ovarian cancer tissues.

CONCLUSIONS: In summary, SNHG14 could enhance cell migration and invasion via upregulating DGCR8 in ovarian cancer.

Key Words:
Long noncoding RNA, SNHG14, Ovarian cancer, DGCR8.

Introduction

Ovarian cancer is the second most fatal gynecologic malignancy in females globally. It has been reported that nearly 22,500 patients were newly diagnosed with ovarian cancer and 14,100 women died of ovarian cancer in America in 2017[1]. As symptoms of ovarian cancer patients at early stage are atypical, most patients are often diagnosed at the advanced stage with the 5-year survival rate of only 30%[2,3]. Whereas, almost 80% of the patients develop resistance to chemotherapy or recurrence after surgery[4,5]. Therefore, it is urgent for early detection of these patients and the establishment of new therapeutic avenues for intervention.

It has been indicated that more than 90% of the mammalian genome is transcribed into noncoding RNAs (ncRNAs). Long noncoding RNAs (lncRNAs) are one subgroup of ncRNAs which are longer than 200 nt in length. Recently, it has been proved that lncRNAs are key regulators in many biological progressions, including carcinogenesis. For example, lncRNA MALAT1 was reported to promote tumorigenesis and metastasis in gastric cancer by regulating vasculogenic angiogenesis[6]. By negatively regulating miR-200b/a/429, lncRNA ILF3-AS1 could also enhance cell proliferation, migration, and invasion in melanoma[7]. LncRNA OGFRP1, as the sponge of miR-124-3p, participated in cell proliferation in the development of non-small cell lung cancer[8]. Furthermore, ZEB1-activated lncRNA HCCL5 has been shown to accelerate cell viability, cell migration, epithelial-mesenchymal transition, and the malignancy of hepatocellular carcinoma[9]. Besides, numerous lncRNAs have also been reported to function in the development and metastasis of ovarian cancer[10].

Recent researches have revealed that lncRNA SNHG14 functioned as a novel oncogene in tumorigenesis. However, the function of SNHG14 in ovarian cancer remains unknown. Our study

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indicated that the expression of SNHG14 was remarkably increased in ovarian cancer tissues. Moreover, knockdown of SNHG14 suppressed the migration and invasion of ovarian cancer cells *in vitro*. Furthermore, the underlying mechanism of SNHG14 in ovarian cancer metastasis was further studied.

Patients and Methods

Cell Lines and Clinical Samples

Totally 56 ovarian cancer patients were enrolled for human tissues who received surgery at the Zhoukou Central Hospital. No radiotherapy or chemotherapy was performed prior to surgery. Specimen harvested from the surgery was stored at –80°C immediately. Written informed consent was offered by the patients before the study. The Research Ethics Committee of Zhoukou Central Hospital approved this investigation. The protocol of the study was performed as the Declaration of Helsinki Principles required.

Cell Culture

Human ovarian cancer cell lines (A2780, TOV112D, HO-8910, OVCAR-3, and SKOV3) and one normal ovarian cells (ISOE80) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Life Technologies, Gaithersburg, MD, USA) and 100 UI/μL penicillin in an incubator containing 5% CO_2 at 37°C.

Cell Transfection

Lentivirus expressing short-hairpin RNA (shRNA; Biosettia Inc., San Diego, CA, USA) targeting SNHG14 and negative control were cloned into the pLenti-EF1a-EGFP-F2A-Puro vector (Biosettia Inc., San Diego, CA, USA), which was then used for transfection of HO-8910 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Lentivirus against SNHG14 (SNHG14) and scramble vector, provided by GenePharma (GenePharma, Shanghai, China), were cloned into the pLenti-EF1a-EGFP-F2A-Puro vector (Biosettia Inc., San Diego, CA, USA), which was then used for transfection of A2780 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 24 h incubation, cells were harvested for further experiments.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was utilized to extract the total RNA from tissue and cultured cells. The RNA was reverse transcribed to cDNA using the reverse Transcription Kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). By using SYBR Green (TaKaRa, Dalian China) method, RT-qPCR was performed to detect the expression of SNHG14 in tumor and non-malignant tissues. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the internal reference in the quantitative analysis of the SNHG14 expression. The experiment was independently repeated 3 times. The primer sequences were as follows: SNHG14 forward 5′-GGGTGTTTACGTAGACCAGAACC-3′ and reverse 5′-CTTCCAAAAGCCTTCTGCCTTAG-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward 5′-CCAAAATCAGATGGGGCAATGCTGG-3′ and reverse 5′-TGATGGCATGGACTGTGGTCATTCA -3′. The thermal cycle was as follows: 30 sec at 95°C, 5 sec for 40 cycles at 95°C, 35 sec at 60°C.

Wound Healing Assay

After transferred into 6-well plates, cells were cultured in a DMEM medium overnight. Once scratched with a plastic tip, cells were continuously cultured in serum-free DMEM. Wound closure was viewed at 0 h and 24 h, respectively. Each assay was repeated in triplicate independently.

Transwell Assay

A total of 5×10^4 treated cells were transformed to the top chamber of an 8 μm pore size insert (Corning, New York, USA) added with 200 μL serum-free DMEM. DMEM containing FBS was added to the bottom chamber. After cultured for another 48 h, the top surface of chambers was wiped by cotton swab and immersed by precooling methanol for 20 min. Crystal violet was used for staining of the inserts.

Matrigel Assay

Totally 5×10^4 treated cells were transformed to top chamber of an 8 μm pore size insert (Corning, Corning, NY, USA) added with 200 μL serum-free DMEM. These inserts were previously coated with 50 μg Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). DMEM containing FBS was added to the bottom chamber. After cultured for another 48 h, the top surface of chambers was wiped by a cotton swab and immersed

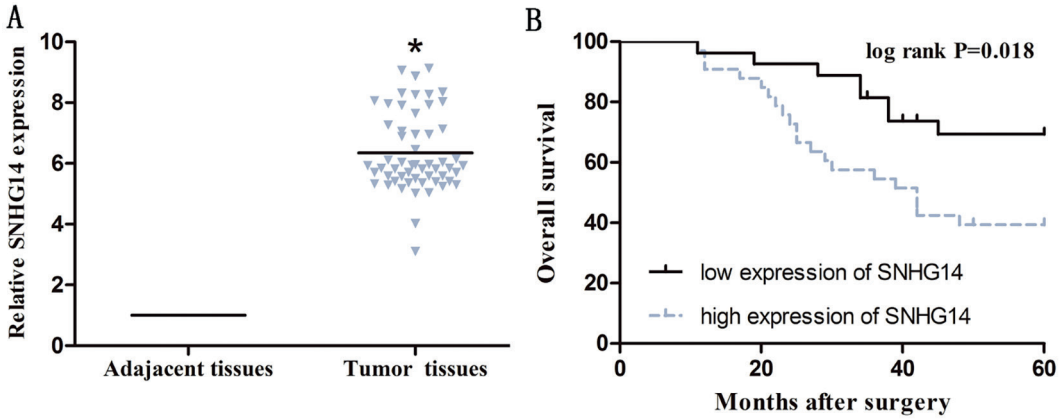

Figure 1. Expression of SNHG14 were increased in ovarian cancer tissues. **A,** SNHG14 expression was significantly increased in the ovarian cancer tissues compared with adjacent tissues. **B,** Expression of SNHG14 was negatively associated with patients' overall survival time. Data are presented as the mean ± standard error. *p<0.05, as compared with adjacent tissues.

by precooling methanol for 20 min. Crystal violet was used for staining the inserts.

Western Blot Analysis

Cell samples were washed with precooled phosphate-buffered saline (PBS) and then lysed with cell lysis solution (RIPA; Beyotime, Shanghai, China). The protein concentration was detected using bicinchoninic acid (BCA; Thermo Fisher Scientific Inc., Waltham, MA, USA) method. Proteins were transferred on to a polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) after separated by the polyacrylamide gel electrophoresis (PAGE) method. Tris-Buffered Saline and Tween (TBST) (25 mM Tris, 140 mM NaCl, and 0.1% Tween 20, pH 7.5) containing 5% skimmed milk was used to block the non-specific antigen for 2 h. Proteins were incubated with the primary antibody of target proteins including DGCR8 (Abcam Inc., Cambridge, MA, USA) and GAPDH (Abcam Inc., Cambridge, MA, USA) at 4°C overnight. After being washed (3×10 min) with TBST, the secondary antibody was added and protein samples were incubated at room temperature for 1 h. The results were analyzed by Image J software (NIH, Bethesda, MD, USA).

Statistical Analysis

Statistical analysis was conducted by GraphPad Prism 5.0 (La Jolla, CA, USA). Data were presented as mean ± SD (standard deviation). Student's t-test was performed to compare the difference between the two groups. A p-value less than 0.05 was considered statistically significant.

Results

SNHG14 Expression Level in Ovarian Cancer Tissues

RT-qPCR was conducted to detect SNHG14 expression in 56 patients' tissues. It showed that SNHG14 was remarkably increased in tumor tissue samples compared with adjacent tissues (Figure 1A). We then divided 56 patients into high SNHG14 level group and low SNHG14 level group according to their median expression. Kaplan-Meier analysis revealed that patients in high SNHG14 level group had poorer overall survival compared to those in low SNHG14 level group (Figure 1B).

Migration and Invasion of HO-8910 Cells were Inhibited by Knockdown of SNHG14

RT-qPCR was also performed to determine the SNHG14 expression in five ovarian cancer cell lines. Results showed that SNHG14 expression level in ovarian cancer cells was significantly higher than that in ISOE80 (Figure 2A). HO-8910 cell line was then selected for knockdown of SNHG14 and the transfection efficiency was detected by qRT-PCR (Figure 2B). Moreover, the results of wound healing assay indicated that knockdown of SNHG14 suppressed the migration of ovarian cancer cells (Figure 2C). The outcomes of transwell assay and Matrigel assay also revealed that the number of migrated cells and invaded cells was markedly reduced after SNHG14 was knocked down in ovarian cancer cells (Figures 2D and 2E).

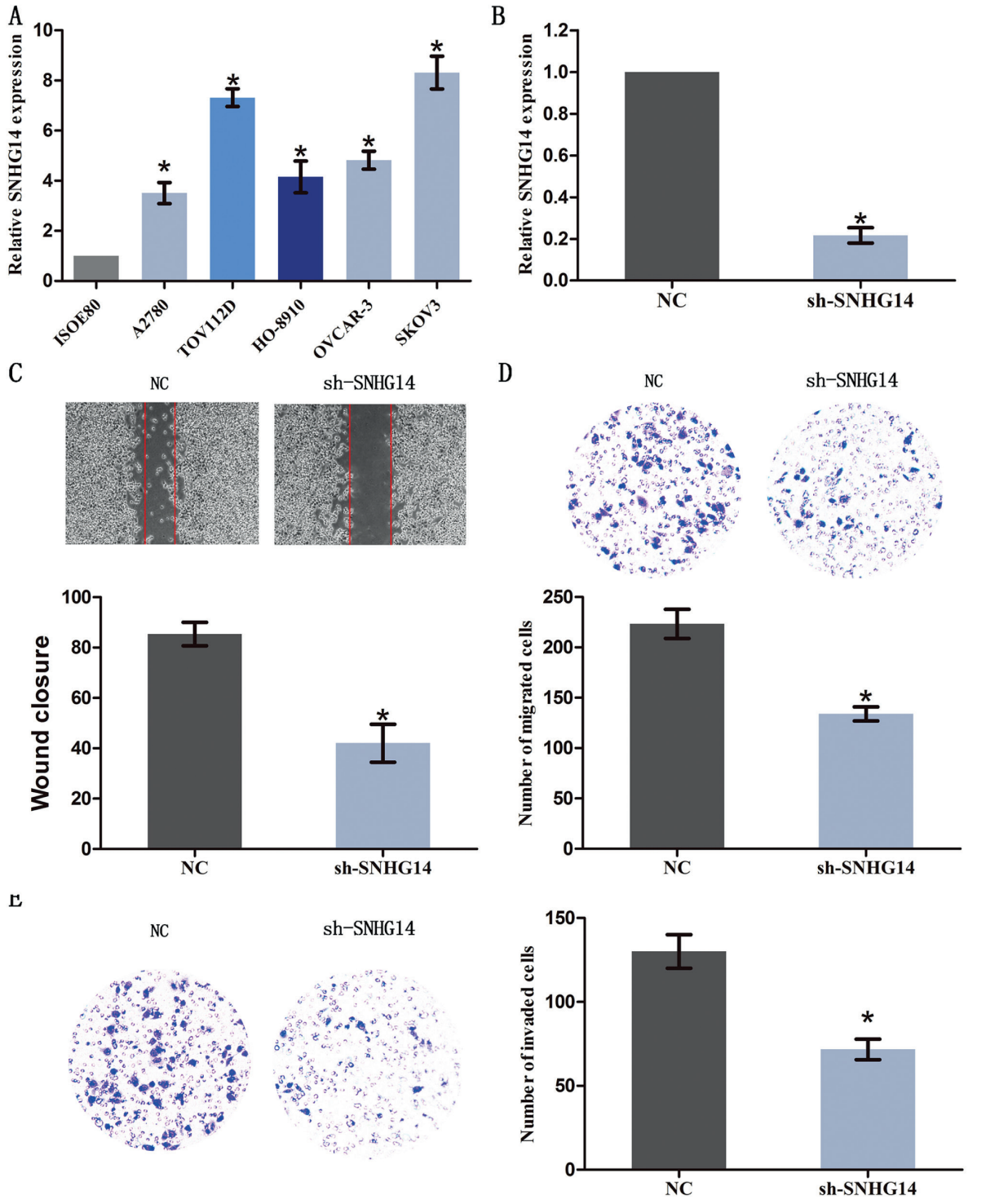

Figure 2. Migration and invasion of HO-8910 cells was inhibited by knockdown of SNHG14. ***A,*** Expression of SNHG14 relative to GAPDH were determined in the human ovarian cancer cell lines and ISOE80 by qRT-PCR. **B,** SNHG14 expression in ovarian cancer cells transduced with SNHG14 shRNA (sh-SNHG14) and the negative control (NC) was detected by qRT-PCR. GAPDH was used as an internal control. **C,** Wound healing assay showed that knockdown of SNHG14 significantly decreased cell migration in ovarian cancer cells (magnification: 40×). **D,** Transwell assay showed that the number of migrated cells was decreased *via* knockdown of SNHG14 in ovarian cancer cells (magnification: 40×). **E,** Matrigel assay showed that the number of invaded cells was decreased *via* knockdown of SNHG14 in ovarian cancer cells (magnification: 40×). The results represent the average of three independent experiments (mean ± standard error). *$p<0.05$, as compared with the control cells.

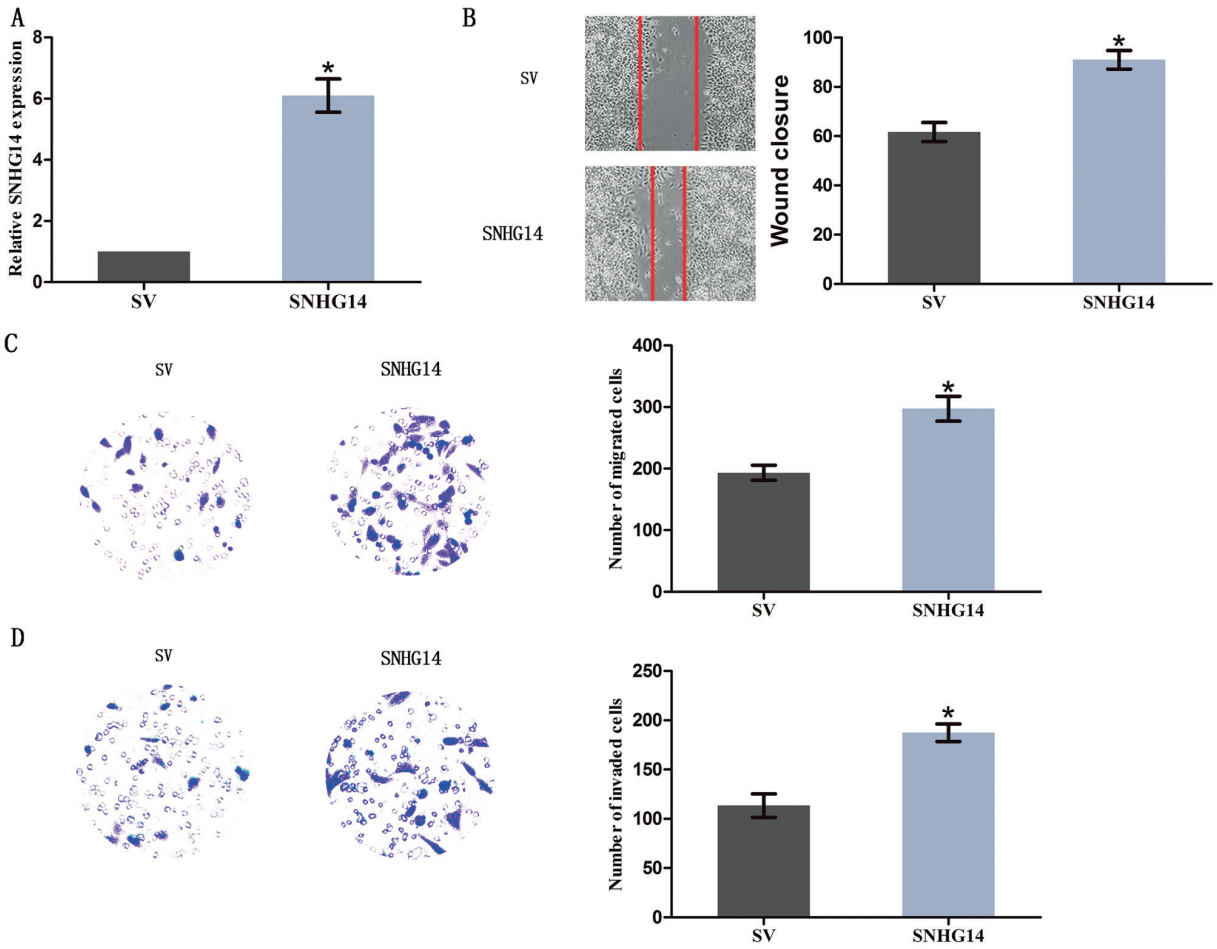

Figure 3. Migration and invasion of A2780 cells was promoted by overexpression of SNHG14. **A,** SNHG14 expression in ovarian cancer cells transduced with SNHG14 lentivirus (SNHG14) and the scramble vector (SV) was detected by qRT-PCR. GAPDH was used as an internal control. **B,** Wound healing assay showed that overexpression of SNHG14 enhanced cell migration in ovarian cancer cells (magnification: 40×). **C,** Transwell assay showed that number of migrated cells was increased *via* overexpression of SNHG14 in ovarian cancer cells (magnification: 40×). **D,** Matrigel assay showed that the number of invaded cells was decreased *via* overexpression of SNHG14 in ovarian cancer cells (magnification: 40×). The results represent the average of three independent experiments (mean ± standard error). *$p<0.05$, as compared with the control cells.

Migration and Invasion of A2780 Cells was Promoted by Overexpression of SNHG14

A2780 cell line was selected for overexpression of SNHG14 and the transfection efficiency was confirmed by qRT-PCR (Figure 3A). Moreover, wound healing assay showed that knockdown of SNHG14 promoted the migration in ovarian cancer cells (Figure 3B). Transwell assay and Matrigel assay further demonstrated that the number of migrated cells and invaded cells was increased after SNHG14 was overexpressed in ovarian cancer cells (Figures 3C and 3D).

The Interaction Between DGCR8 and SNHG14 in Ovarian Cancer

DGCR8 was predicted as one of the potential targets of SNHG14 through Starbase v2.0 (http://starbase.sysu.edu.cn/starbase2/rbpLncRNA.php). The mRNA and protein level of DGCR8 in ovarian cancer cells was significantly reduced in SNHG14 shRNA (sh-SNHG14) group compared with that in negative control (NC) group (Figure 4A). Meanwhile, overexpression of SNHG14 significantly upregulated the mRNA and protein level of DGCR8 (Figure 4B). Furthermore, enhanced DGCR8 expression was also observed in ovarian cancer tis-

sues compared with adjacent tissues (Figure 4C). In addition, there was a positive association between DGCR8 expression level and SNHG14 expression in ovarian cancer tissues (Figure 4D).

Discussion

Previous studies have indicated that lncRNAs serve as important regulators in the progression of various diseases, including ovarian cancer, which convinced lncRNAs potential biomarkers and therapeutic targets of ovarian cancer. For instance, lncRNA BACE1-AS inhibited the proliferation and invasion of ovarian cancer stem cell[11], and thus functioned as a novel target for ovarian cancer. Also, through the regulation of epithelial-mesenchymal transition, the downregulation of lncRNA SPRY4-IT1 enhanced cell metastasis of ovarian cancer[12]. In addition, lncRNA ElncRNA1, activat-

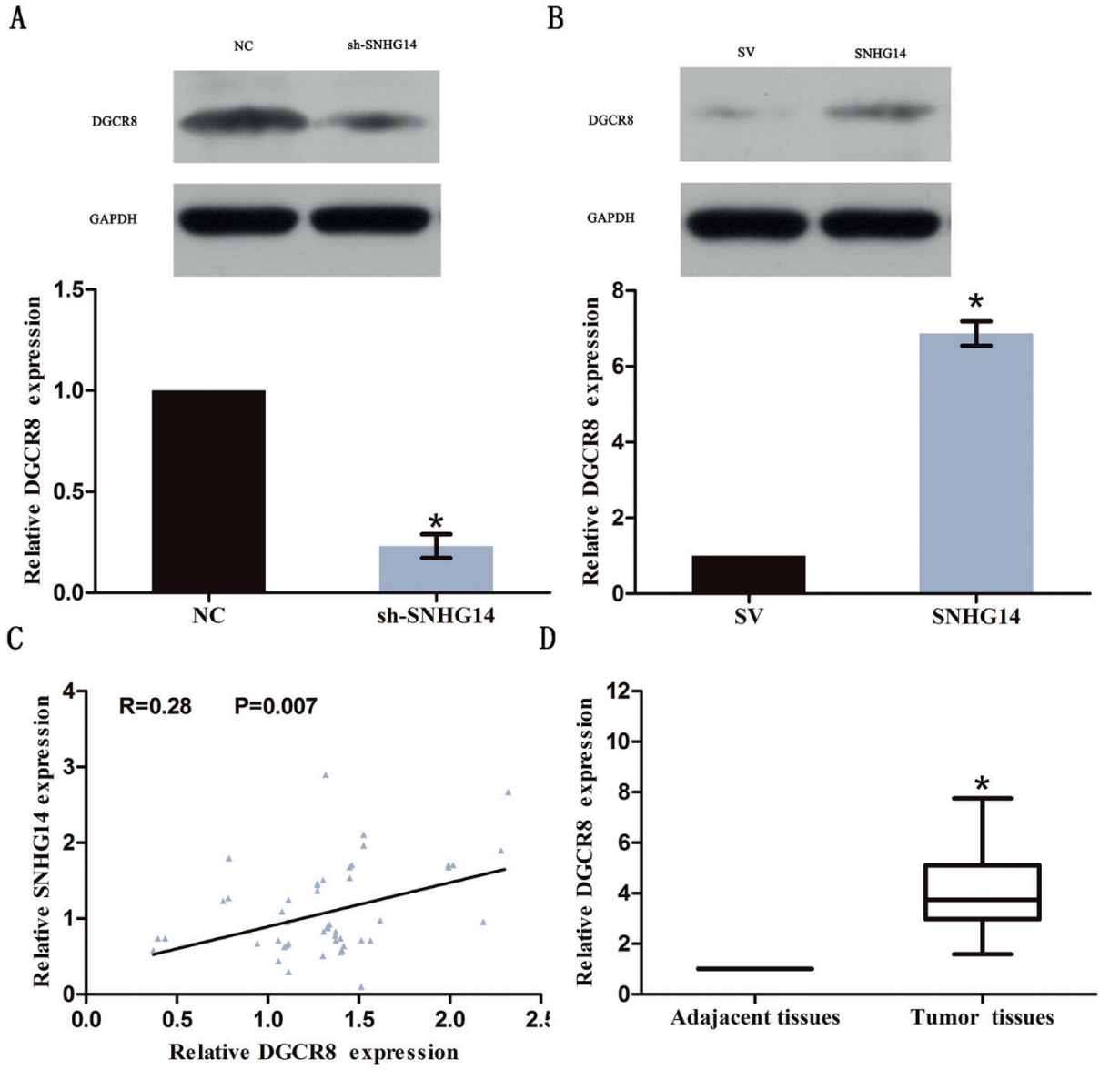

Figure 4. Interaction between SNHG14 and DGCR8. **A,** RT-qPCR and Western blot assay results showed that DGCR8 expression was downregulated in sh-SNHG14 group compared with the NC group. **B,** RT-qPCR and Western blot assay results revealed that DGCR8 protein expression was upregulated in SNHG14 group compared with the SV group. **C,** DGCR8 was significantly upregulated in ovarian cancer tissues compared with adjacent tissues. **D,** Linear correlation between the expression of DGCR8 and SNHG14 in ovarian cancer tissues. The results represent the average of three independent experiments Data are presented as the mean ± standard error. *p<0.05, as compared with the control cells.

ed by Oestrogen, functions as an oncogene in the proliferation of epithelial ovarian cancer cell[13].

Small nucleolar RNA host gene 14 (SNHG14) is located on chromosome 15q11.2 and is a novel identified lncRNA which exhibited oncogenic activity in various human malignancies. Ji et al[14] reported that SNHG14 facilitated the progression of cervical cancer by regulating miR-206/YWHAZ signaling pathway. SNHG14 could also enhance the development of bladder cancer by targeting miRNA-150-5p[15]. Through the H3K27 acetylation, SNHG14 contributed to trastuzumab resistance in breast cancer by regulating the expression of PABPC1[16]. Moreover, SNHG14 promoted cell apoptosis and suppressed cell proliferation and invasion in glioma via sponging miR-92a-3p[17]. In this study, we found that SNHG14 was significantly increased in ovarian cancer samples and was closely associated with patients' prognosis. Besides, the knockdown of SNHG14 repressed cell migration and invasion in ovarian cancer, while overexpression of SNHG14 promoted cell migration and invasion. Collectively, SNHG14 enhanced tumor metastasis in ovarian cancer and might act as an oncogene in ovarian cancer.

To further explore the underlying mechanism of SNHG14 in ovarian cancer, bioinformatics analysis was utilized to predict the potential targets of SNHG14. DGCR8 was then identified due to its vital function in tumorigenesis[18]. DGCR8 has been pointed out to inhibit tumor progression in prostate cancer[19]. It is discovered that knockdown of DGCR8 suppressed cell proliferation, cell migration, and cell invasion in ovarian cancer[20]. In this work, we first explored the interaction between DGCR8 and SNHG14. Results demonstrated that the DGCR8 expression could be downregulated by knockdown of SNHG14. Furthermore, DGCR8 expression in ovarian cancer tissues showed a positive association with SNHG14 expression.

Conclusions

The results of this study showed that SNHG14 could promote cell migration and invasion in ovarian cancer by upregulating DGCR8, which offers a new sight for the treatment of ovarian cancer.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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