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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 gel assay were performed to detect the [illegible] SNHG14 on the migration and invasion of [illegible]an cancer cells. In addition, the underlying me[illegible]nism was further explored through qRT-PCR [illegible] Western blot assay.

RESULTS: SNHG14 level [illegible]amatical[illegible]ly higher in ovarian cancer [illegible]. Moreover, cell migration and i[illegible]ion w[illegible] significantly attenuated via the [illegible]bition [illegible]NHG14 while enhanced via the S[illegible]4 ov[illegible]. Besides, the expres[illegible]n o[illegible]mRNA and protein was marke[illegible]down[illegible]d after the knockdown of SN[illegible]4, while up[illegible]ted after SNHG14 overex[illegible]on. Furtherm[illegible]he expression level[illegible] was increase[illegible] in cancer tissues a[illegible]positiv[illegible]lated to the expression of SN[illegible]G14 in ovaria[illegible]er tissues.

CONC[illegible]ONS: In summ[illegible]NHG14 could enhanc[illegible]ll mig[illegible]ation and inv[illegible]sion via upregulatin[illegible]GCR8 i[illegible]arian cancer.

Key
Long[illegible]ing RN[illegible]NHG14, Ovarian cancer, [illegible]R8.

[illegible]ntroduction

[illegible]varian cancer is the second most fatal gy[illegible]ic malignancy in females globally. It has been re[illegible]ed th[illegible]rly 22,500 patients were newly diagnosed wi[illegible]ian cancer and 14,100 wo[illegible]d of ovaria[illegible]cer in America in 2[illegible]. As symptoms of ovarian cancer patients at [illegible]y stage are a[illegible]pical, most patients are often [illegible]gnosed at the [illegible]vanced stage with the 5-year [illegible]ival rate of[illegible]ly 30%[2,3]. Whereas, almost 8[illegible] the pati[illegible] develop resistance to chemotherap[illegible]ence after surgery[4,5]. Therefore, it is urgent for early detection of these patients [illegible]d the establishment of new therapeutic avenues [illegible]ntion.

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 (A27 V112D, HO-8910, OVCAR-3, and SKOV nd one normal ovarian cells (ISOE80) were chased from the American Type Culture Col tion (ATCC; Manassas, VA, USA). Cells we maintained in Dulbecco's Mo le's Medium (DMEM; Gibco, Rock , MD A) containing 10% fetal bovine s n (FBS e Technologies, Gaithersburg, USA) µL penicillin in an in bat g 5% CO_2 at 37°C.

Cell Transfec

Lentivirus pres short-hairpin RNA (shRNA; Bi setta Inc., S go, CA, USA) targeting SN 14 and negative ol were cloned into t pLenti EF1a-EGFP-F A-Puro vector (Bios Inc., Diego, CA, USA), which was the for sfectio HO-8910 cells using Lipof 2000 (rogen, Carlsbad, CA, USA). Le s ag SNHG14 (SNHG14) and le ve ded by GenePharma (GenePh na, Shan , China), were cloned into the ti-EF1a-EGFP-F2A-Puro vector (Biosettia go, CA, USA), which was then for transfection of A2780 cells using Lipomine 2000 (Invitrogen, Carlsbad, CA, USA). A 4 h incubation, cells were harvested for furth experiments.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction

TRIzol reagent (Invitrogen, C ad, USA) was utilized to extract the RNA from tissue and cultured cells. The was reverse transcribed to cDNA using the e Transcription Kit (TaKaRa Bi hnolog Ltd Dalian, China). By usin BR Gree Ra, Dalian China) m d, RT- CR was formed to detect th pressi SNHG14 in tumor and non-mal tiss Glycer hyde 3-phosphate deh oge APDH) the internal referenc the qua e a sis of the SNHG14 ex ion. The exp was independently 3 times. The p mer sequences were follow HG14 forward 5′-GGGTGTTTACGTAGACC ACC-3′ and reverse 5′-G CAAAAGC CTGCCTTAG-3′; g aldehyde 3-phosphate dehydrogenase PDH), forw d 5′-CCAAAATCAGATGGCCAATGCTC ′ and reverse 5′-TGATGGGGACTGT TCATTCA -3′. The thermal c as as fo s: 30 sec at 95°C, 5 sec for 40 cycl sec at 60°C.

Wound Healing Assay

ansferred into 6-well plates, cells were ltur 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

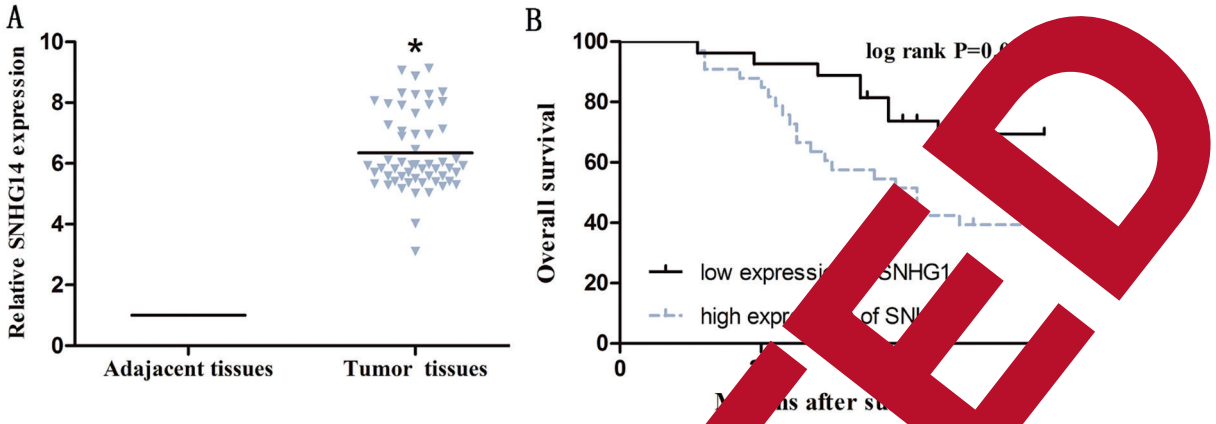


Figure 1. Expression of SNHG14 were increased in ovarian cancer tissues. [illegible]14 expression w[illegible]gnificantly increased in the ovarian cancer tissues compared with adjacent tissues. **B,** Expr[illegible]n of [illegible]14 was negatively associated with patients' overall survival time. Data are presented as the mean ± standard er[illegible] *p<0.05, [illegible]pared 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 [illegible]d with cell lysis solution (RIPA; Beyotime, Shanghai, China). The protein concentration was [illegible]ct-ed using bicinchoninic acid (BCA; Thermo [illegible]er Scientific Inc., Waltham, MA, USA) meth[illegible] Proteins were transferred on to a polyvinylidene difluoride (PVDF) membran[illegible]ore, Billerica, MA, USA) after se[illegible]ed b[illegible]e polyacrylamide gel electroph[illegible]s (PAG[illegible]method. Tris-Buffered Saline and[illegible]n (TB[illegible] Tris, 140 mM NaCl, a[illegible]l 0.[illegible]20, pH 7.5) containing 5% skim[illegible] milk [illegible]ed to block the non-specific a[illegible]n for 2 h. Pr[illegible]were incubated with th[illegible] antibody o[illegible]get proteins includin[illegible]GCR[illegible]am Inc., Cambridge, MA, USA) and GAPDH ([illegible] Inc., Cambridge, MA, USA) at 4°C overnight. [illegible]being washed (3×10 [illegible] with TBST, the secondary antibody was [illegible]d and [illegible]in samples were incubated at roo[illegible]pera[illegible] for 1 h. The results were analyzed [illegible]J softw[illegible](NIH, Bethesda, MD, USA).

Sta[illegible]cal Ana[illegible]s

S[illegible]tical analysis was conducted by GraphPa[illegible]La Jolla, CA, USA). Data were [illegible]ented as mean ± SD (standard deviation). Stu[illegible]'s *t*-test was performed to compare the differe[illegible]tween the two groups. A *p*-value less than 0.05[illegible]s considered statistically significant.

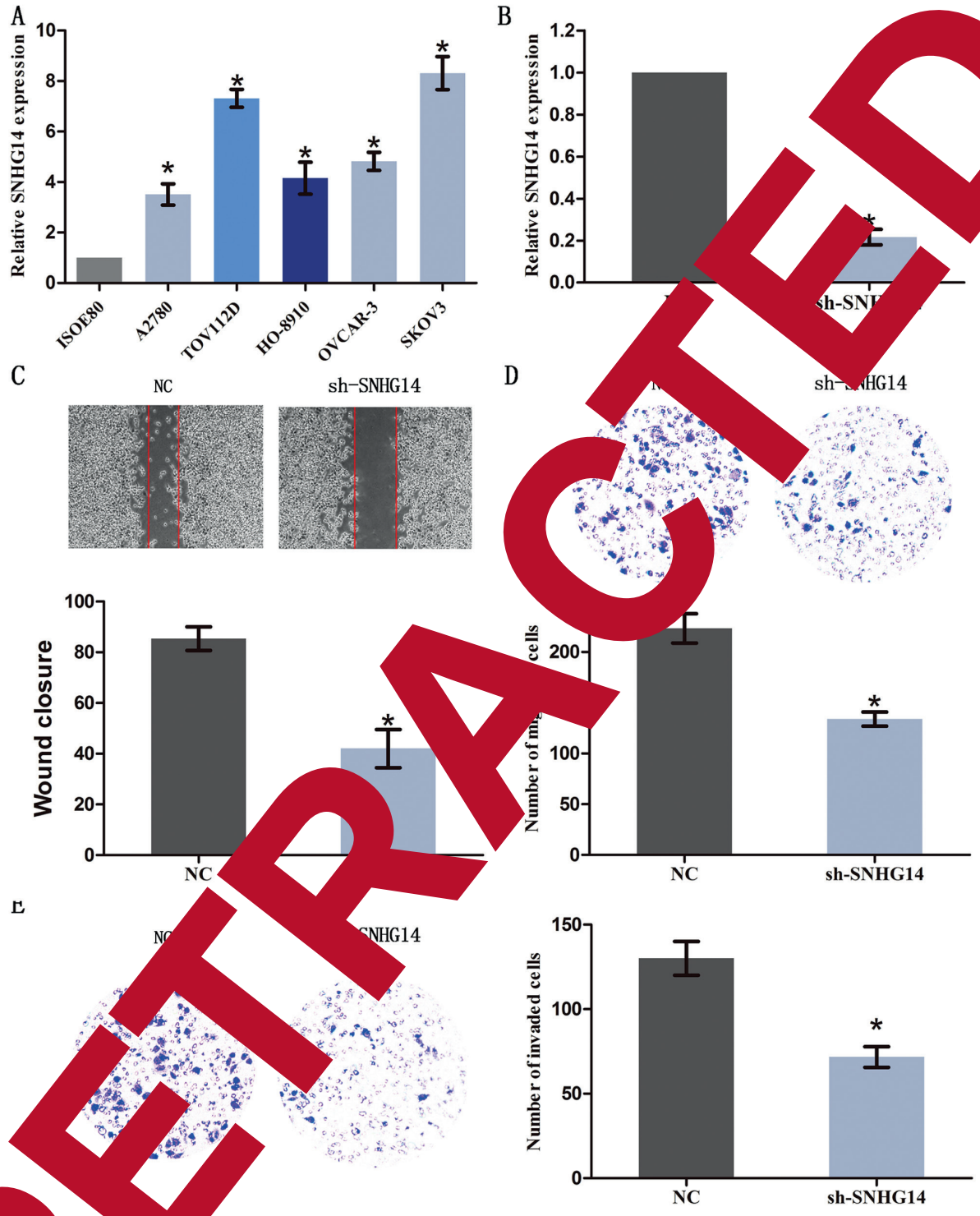
Results

[illegible]HG14 Expr[illegible]ion Level in Ovarian C[illegible]r Tissu[illegible]

R[illegible] conducted to detect SNHG14 expression in 56 patients' tissues. It showed that SNHG14 was remarkably increased in tumor [illegible]ples compared with adjacent tissues ([illegible]gu[illegible]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).



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

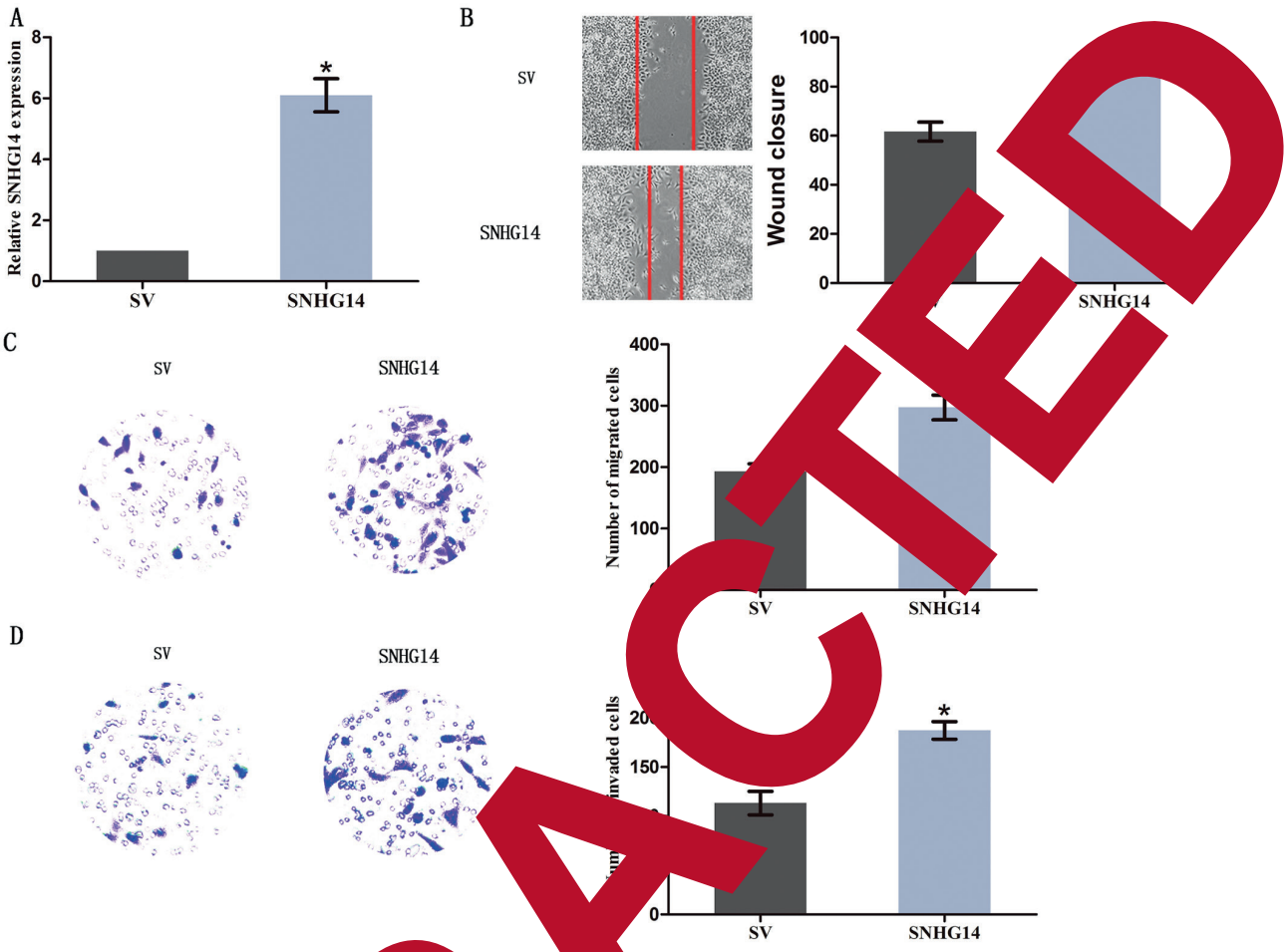


Figure 3. Migration and invasion ... ls was pro... by overexpression of SNHG14. **A,** SNHG14 expression in ovarian cancer cells transduced w... NHG1... ivirus (SN... 4) and the scramble vector (SV) was detected by qRT-PCR. GAPDH was used as an internal ... ol. **B,** W... healing assa... showed that overexpression of SNHG14 enhanced cell migration in ovarian cancer cells (m... cation: 4... assay showed that number of migrated cells was increased *via* overexpression of SNHG14 in o... an... ns (ma... cation: 40×). **D,** Matrigel assay showed that the number of invaded cells was decreased *via* ov... pres... NG14 in ovarian cancer cells (magnification: 40×). The results represent the average of three independen... eriments (... standard error). *p<0.05, as compared with the control cells.

Migra... and Invasion of A2780 Cell... s Pro...ed by Overexpression of ...G14

A2... le was ...ted for overexpression of SNHG... d the ...nsfection efficiency was ...ned b... R (Figure 3A). Moreover, wou... healing a... showed that knockdown of SNH... 4 promoted the migration in ovarian cancer ce... 3B). Transwell assay and Matrigel assay further demonstrated that the number of migrated cells and invaded cells was increased a... HG14 was overexpressed in ovarian cancer c... (Figures 3C and 3D).

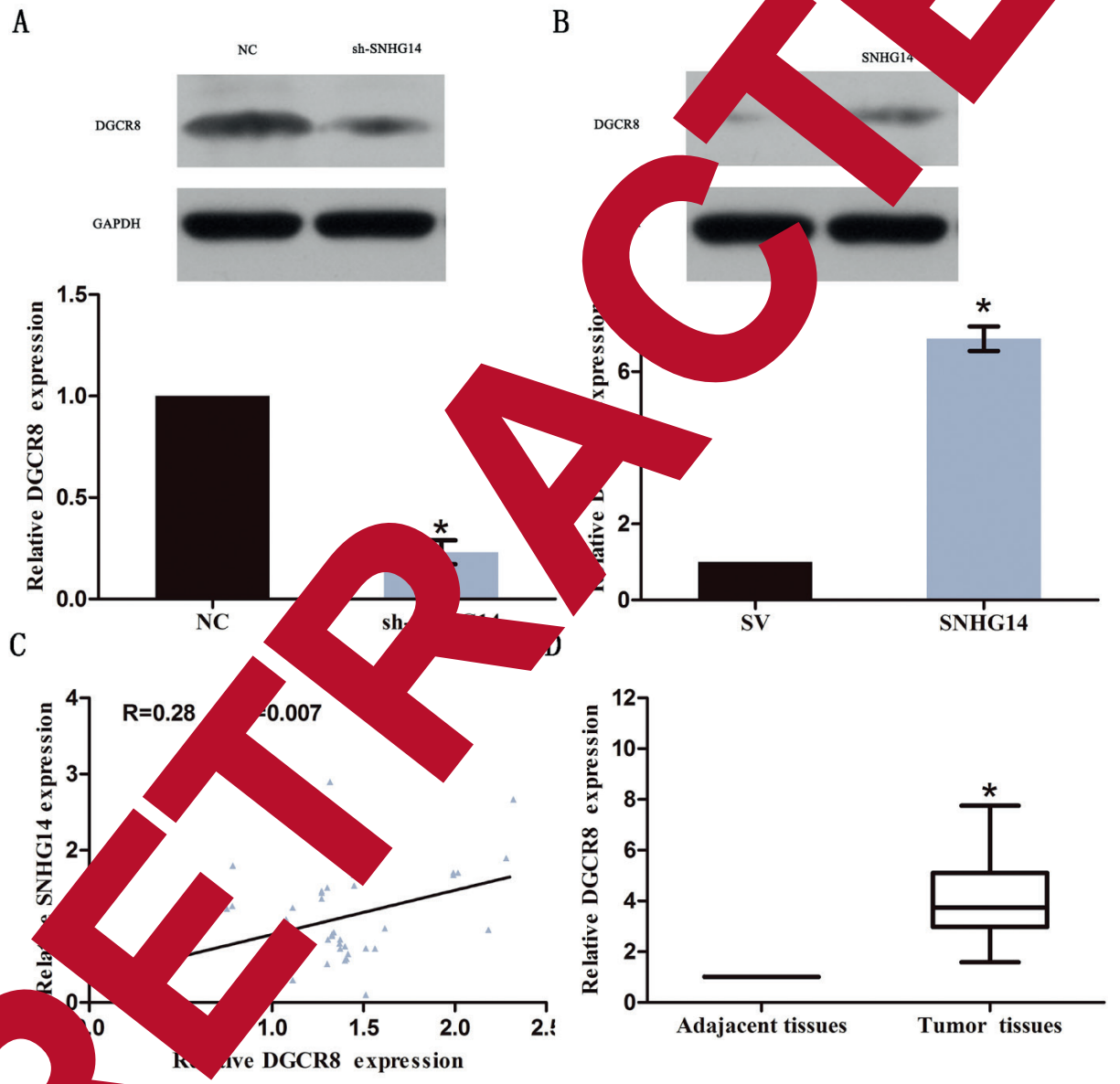
The Interaction Between DGCR8 and SNHG14 in Ovarian Cancer

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 biomarks apeutic targets of ovarian cancer. Fo cRNA BACE1-AS inhibited the invasion of ovarian cancer ste functioned as a novel target for Also, through the regulati chymal transition, the do SPRY4-IT1 enhanced cancer[12]. In addition



Fi on between SNHG14 and DGCR8. **A,** RT-qPCR and Western blot assay results showed that DGCR8 expression was down-regulated 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 oncog in ovarian cancer.

To further explore the underlying mec m of SNHG14 in ovarian cancer, bioinform analysis was utilized to predict the potential gets of SNHG14. DGCR8 was the identified d to its vital function in tumor DGCR8 has been pointed out to inhi mor ression in prostate cancer[19]. It is overed knockdown of DGCR8 supp cell cell migration, and c inv varian cancer[20]. In this work first e d the interaction between R8 and SN Results demonstrated t GCR8 expre n could be downregu by kdown of SNHG14. Furthermor DGCR8 ex n in ovarian cancer tissu owed a positi ociation with SNHG pressi n.

Con ions

result tudy showed that SNHG14 cou omote c igration and invasion in ovarian c r by upregulating DGCR8, which offers a ne treatment of ovarian cancer.

t of Interests

The rs declare that they have no conflict of interests.

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