European Review for Medical and Pharmacological Sciences 2019; 23: 4136-4142

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

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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 v* addition, the luciferase assays and RNA noprecipitation assay (RIP) were perform to investigate the underlying mechanism.

RESULTS: SNHG14 expression was remarka higher in ovarian cancer tissues th that of co responding normal tissues. S pressio was associated with patie over survival time as well. After SNHG1 s silenc in ovarian cancer cells, cell pro ion, m invasion were remark ly the expression of m 9a-5 significantly up-regulated after silence of 14. Further mechanism ass wed that mi -5p was a direct target N in ovarian c cer.

CONCLUSIONS: SN serves as a potential oncoc in ovarian er. In addition, it enhanc varian cell meta and prolifera-tion vi ongin miR-219a-5p.

Key
Long ding R NHG14, Ovarian cancer, 19a-5

Introduction

Ovarian cancer is one of the most common nancies in women worldwide. Around 22, new patients were diagnosed with ovarian cancer in America in 2017, with almost 14,100 deaths[1]. Due to unavailable tests for ovarian cancer, it i en diag d at e stage. This makes i of the lead es of cancer-related ong female he main intervention or ov cancer include surgery, chemotherapy, and r erapy. Currently, the sev ation unders the urgency of earl tection and new therapeutic treatment for ian cancer p ents.

ong non-co RNAs (lncRNAs) are one s up of non ding RNAs. Recent evidence has cRNAs play a vital role in the progress malignant tumors. For example, by regulating the expression of miR-335, lncRNA acts as an oncogene in gastric cancer and p motes cell proliferation[2]. LncRNA GIHCG promotes the development of ovarian cancer by regulating mi-429[3]. LncRNA PCAT-1 plays a vital role in tumorigenesis of hepatocellular carcinoma by modulating TP53-miR-215-PCAT-1-CRKL axis[4]. Besides, lncRNA PlncRNA-1 accelerates the progression of colorectal cancer cells via PI3K/Akt signaling pathway[5]. LncRNA RUNX1-IT1 suppresses the migration and proliferation of colorectal cancer cells[6]. 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

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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% CO_2 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 tracted using TRIzol Reagent (I en, Carl bad, CA, USA). Subsequen ed total RNA was reverse-transcri into c lemen-tary deoxyribose nucleic s (cDN reverse Transcription Kit ogy Co., Ltd., Dal Chin thermocycling conditions as follow -denaturation at 95°C f denaturatio 5°C for 10 s, and an ng a C for 30 s, for a total of 35 cycl Relative e ion was calculated by th $^{\Delta\Delta Ct}$ method. Pr equences used in thi dy we as follows: SNHG14 forward 5′-C TGTT GTAGACCAGAACC-3′ and reve TTCC AAGCCTTCTGCCT-TAG-3 in, fo d 5′-GATGGAAATC-AGA d reverse 5′-TGGCACT-TA TGGA C-3′.

Cell Proliferation Assay

2 ×10^3 transfected cells were first seeded into well plates. Cell proliferation was assessed at 4 h, 48 h, and 72 h after transfection by Cell Pro ration 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 s was detected according to the instruct f the EdU Kit (Roche, Basel, Switzerland). A r ntative photograph was taken by Axiop to microscope (Carl Zeiss, kochen, Ger

Transwell Assay

24 h after tran tio cells i 0 µL serum-free DM were t rme the upper chamber 8-µm cultu (Corning, Corning, N coated wit without 50 µg Matrig Matri tion (BD, Bedford, MA, USA). Meanwhile, 2 BS-DMEM was added t wer chambe ulture inserts. 24 h l the inserts were treated with methanol for in and stai with hematoxylin for 20 min. migrated a nvaded cells were observed u a Leica I4000B microscope (Leica Mic eidelberg, Germany). Three fields w domly selected for each sample, nd the number of migrated and invaded cells nted.

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 (6×10^5/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 viability 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 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 2E).

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 the 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 in 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. This sug-

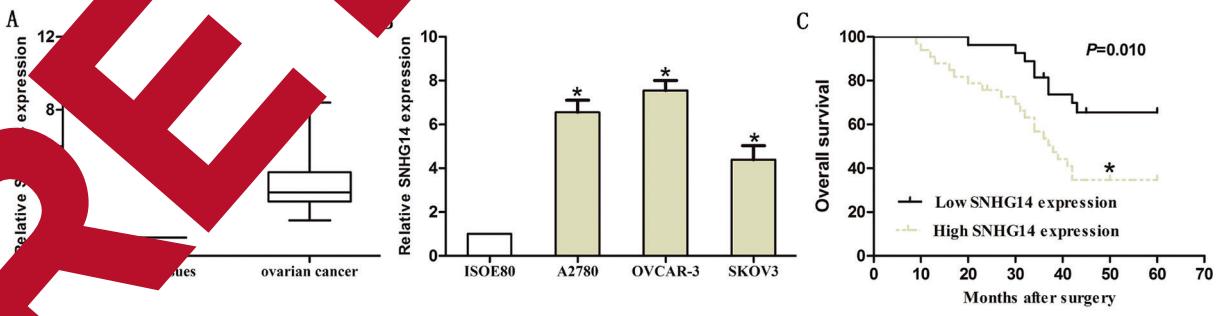

Figure 1. Expression level of SNHG14 increased significantly in ovarian cancer tissues and cell lines. ***A,*** SNHG14 expression significantly increased in ovarian cancer tissues compared to adjacent tissues. ***B,*** SNHG14 expression was associated with the overall survival time of ovarian cancer 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.

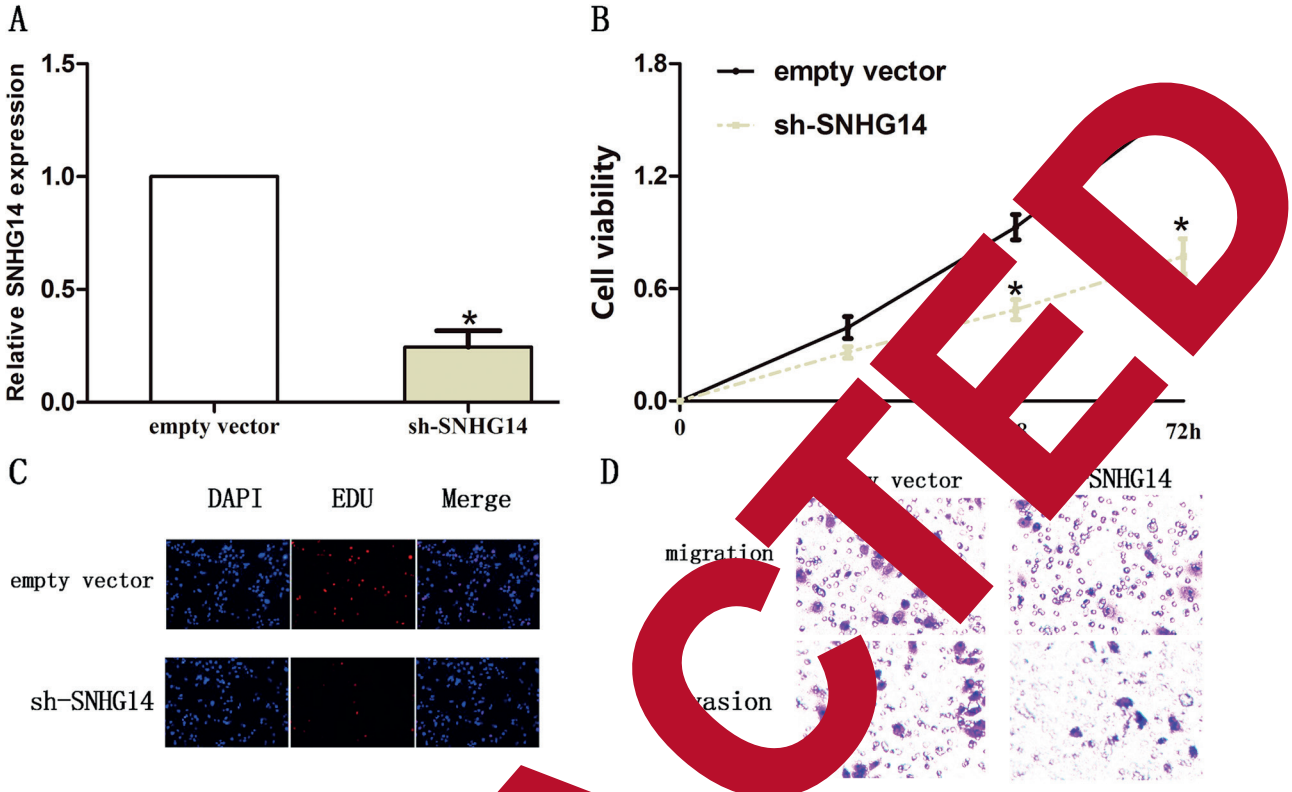

Figure 2. Knockdown of SNHG14 inhibited SK cell proliferation, migration and invasion. ***A,*** SNHG14 expression in SKOV3 ovarian cancer cells transduc h SN A (shRNA) and the empty vector was detected by RT-qPCR. β-actin was used as an internal control. ***B,*** ass t the knockdown of SNHG14 significantly inhibited the growth of SKOV3 ovarian cancer cells. ***C,*** EdU ion assay showed that EdU positive cells were significantly reduced after the knockdown of SNHG14 in SKOV3 cells nification×10). ***D,*** The transwell assay showed that the number of migrated and invaded cells signifi eased via down of SNHG14 in SKOV3 ovarian cancer cells (Magnification×20). The results represented aver f the thre pendent experiments (mean ± standard error of the mean). *$p<0.05$, as compared with cont ls.

gested that SNHG night work sponge of miR-219a-5p i cancer (Fi 3D). In summary, th ndi monstrated that miR-219a-5p w direct targ NHG14.

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W ed the e of SNHG14 in tumor formati *vivo*. T r size in shRNA group ignifi r when compared with the em vector g Figure 4A). Subsequently, the exp ion level f SNHG14 and miR-219a-5p in g tissues were detected by RT-qP- as well. he results showed that SNHG14 was ly expressed in the shRNA group when com- with the empty vector group (Figure 4B). Ho er, 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[7]. 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[8]. Through regulation of epithelial-mesenchymal transition (EMT), the down-regulation of lncRNA SPRY4-IT1 enhances metastasis of ovarian cancer[9]. Furthermore, lncRNA CCAT1 promotes EMT, migration, and invasion of epithelial ovarian cancer cells[10].

LncRNA small nucleolar RNA host gene 14 (SNHG14), a novel lncRNA, is located in 15q11.2. Recent studies[11,12] have shown that SNHG14 is significantly up-regulated in various cancers. For instance, SNHG14 enhances chemoresistance to trastuzumab in breast cancer[13]. SNHG14 promotes cell proliferation by targeting miR-206/YWHAZ in cervical cancer[14]. Meanwhile, SNHG14 functions as an oncogene by sponging miR-340 in non-small cell lung cancer[15]. Wang et al[16] 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 [illegible]. The above results suggested that SN[illegible]14 cou[illegible] promote tumorigenesis of ovarian [illegible]er.

To identify the underlying [illegible]anism of SNHG14 function in ovarian cance[illegible] tumorigenesis and metastasis, th[illegible]informa[illegible]al ysis, and functional assa[illegible]edicted and v[illegible] that miR-219a-5p was [illegible]ential l[illegible]ng mic[illegible]NA of SNHG14. Pre[illegible]stud[illegible]ave fo[illegible]d that miR-219a-5p is k[illegible]n [illegible]or sup[illegible]or in many carcinom[illegible]d regu[illegible]liver[illegible]ological processes. Fo[illegible]mple, miR-[illegible]suppresses migration [illegible]ncer cell via[illegible]ersing EMT process[17]. [illegible]iR-21[illegible] suppresses metastasis of osteosarcoma by [illegible]ting EYA2 *in vitro*[18]. Mo[illegible]miR-219-5p i[illegible]s cell proliferation a[illegible]nvasion via regulating calcyphosin in col-[illegible]tal cancer[19]. A recent report[20] has revealed

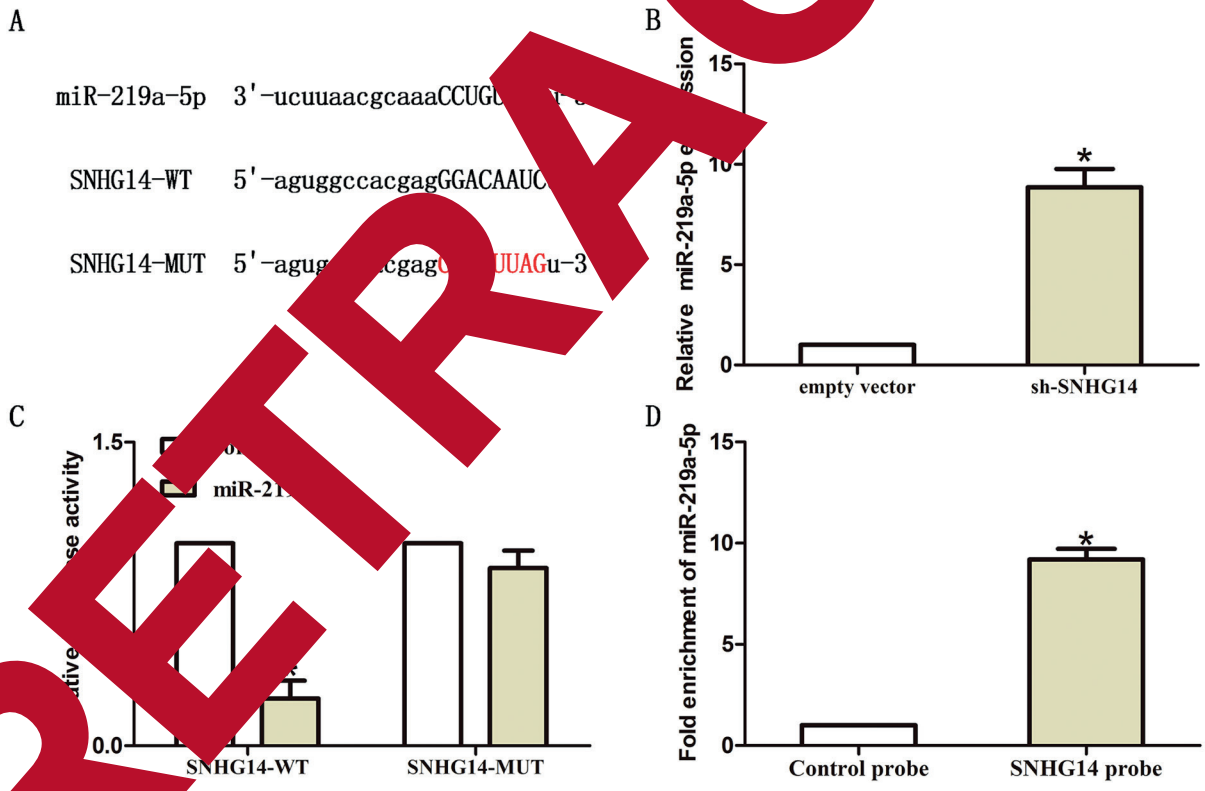

Figure 3. Reciprocal repression between SNHG14 and miR-219a-5p. ***A,*** The binding sites of miR-219a-5p on SNHG14. ***B,*** miR-219a-5p expression significantly increased in sh-SNHG14 group compared with empty vector group. ***C,*** Co-transfection of miR-219a-5p and SNHG14-WT strongly decreased luciferase 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. *$p<0.05$.

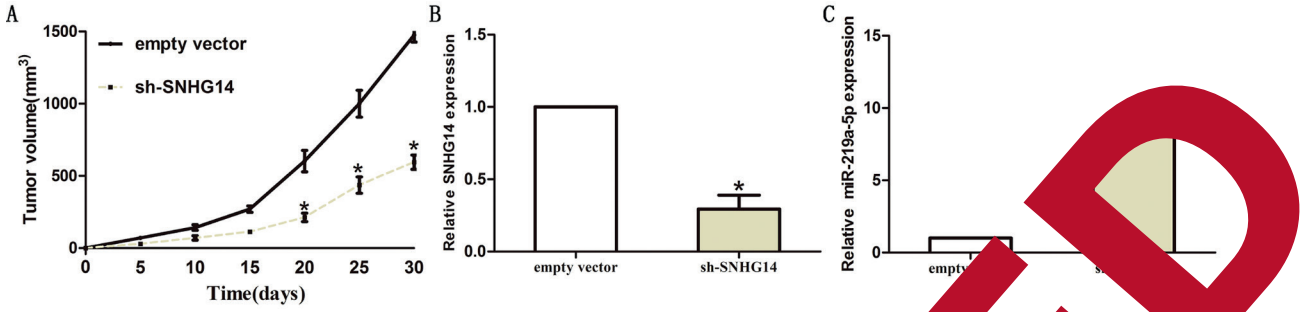

Figure 4. Knockdown of SNHG14 inhibited tumor formation *in vivo*. ***A,*** After tumor extra[illegible] the tu[illegible]lume in em[illegible]y vector or sh-SNHG14 group was calculated, respectively. ***B,*** The relative expression of [illegible]14 in [illegible]cted tu[illegible]rs was examined by RT-qPCR. ***C,*** The relative expression of miR-219a-5p in tumors was exam[illegible]d by [illegible]. The dat[illegible]e presented as mean ± SD of three independent experiments. *$p<0.05$.

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 [illegible]at SNHG14 might promote tumorigenesis [illegible] ian cancer via sponging miR-219a-5p. The [illegible]n-origenesis assay revealed that the knockdow[illegible]f SNHG14 could inhibit tumor formation *in vi*[illegible] Through the detection of SNHG1[illegible] miR-219[illegible] 5p expression in those extract[illegible] [illegible]e found that SNHG14 was down-r[illegible]ated, [illegible]e miR-219a-5p was up-regulate[illegible] nude [illegible]ted with SNHG14 shRNA.

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We sho[illegible]d that SNH[illegible] enhanced ovarian cancer c[illegible]roliferation, mi[illegible]n, and invasion by sp[illegible]ng miR-219a-5p. Our findings implied that [illegible]NA S[illegible]G14 could be used as a prospective [illegible]arget f[illegible]varian cancer.

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The [illegible]ors declare [illegible]o conflicts of interest.

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