European Review for Medical and Pharmacological Sciences 2018; 22: 329-335

Role of long non-coding RNA SNHG1 in occurrence and progression of ovarian carcinoma

J. GE[1], X.-M. WU[1], X.-T. YANG[2], J.-M. GAO[3], F. WANG[4], K.-F. YE[1]

[1]Department of Gynecology, The First People's Hospital of Yunnan Province, Kunming,
[2]Department of Pathology, The First People's Hospital of Yunnan Province, Ku g, Chi
[3]Department of Basic Laboratory, The First People's Hospital of Yunnan Pro , Kunming,
[4]Department of Laboratory Medicine, The First People's Hospital of Yunnan nce, K ing, C

Abstract. – OBJECTIVE: To investigate the expression of human long non-coding ribonucleic acid (lncRNA) small nucleolar RNA host gene 1 (SNHG1) in epithelial ovarian carcinoma tissues and its effects on the *in vitro* proliferation, apoptosis, invasion and metastasis of ovarian carcinoma cells, and to investigate its possible mechanism.

PATIENTS AND METHODS: The expressions of SNHG1 in 20 pairs of epithelial ovarian carcinoma tissues and para-carcinoma normal tissues were detected by Real-time fluorescence quantitative polymerase chain reaction (qRT-PCR). The e sions of SNHG1 in normal ovarian epithel (IOSE25) and ovarian carcinoma cells (CA 3, SKOV-3, ES2 and A2780) were further dete The knockdown efficiency of SNHG1 small inter ing RNA (siRNA) in SKOV-3 cells detected v qRT-PCR. Moreover, the effect 1 knock down on proliferation, migra and a tosis of SKOV-3 cells were detecte cell co ng kit 8 (CCK8) proliferation assa e for transwell migration as a ometr nally, the expressio of apo related proteins, epithelial-m chymal tra (EMT)-related proteins atrix meta einases (MMPs) in co gr nd interfe ce group were detect by Weste tting.

RESUL he expressi el of lncRNA SNHG1 in rian carcinoma ues was signifi cantl gher t that in para-carcinoma normal ues. r lncRNA SNHG1 knockdown in S , the c roliferation and clone formati ilities e significantly inhibit- The osi ssay proved that inhibiting RNA could promote the apoptosis SKOV-3 s. Besides, Western blotting re that the expressions of pro-apoptotic terference group were significantupregulated compared with those in control up. Wound-healing assay and transwell mi- n assay showed that the down-regulation of RNA SNHG1 could inhibit the invasion and metastasis of SKOV-3 cells, whose mechanism was related to the inhibition of EMT process and down-regulation of expressions of MMPs.

CONCLUSIO LncRNA G1 ghly expressed in carcinoma can promote the w vasion and etastasis of ovarian c inoma s. The down-regulation of SNHG1 can inhibi roliferation, invasion and asis of SKO ells. Inhibiting the e ssion of SNHG1 may e a potentially effect- means of treating ovarian carcinoma.

K ords:
n carci a, lncRNA, SNHG1, Proliferation, Invas

Introduction

Ovarian carcinoma is a kind of common malignant tumor in women, accounting for 5% in systemic malignant tumors, whose incidence rate ranks third in gynecologic malignant tumors. Due to its concealed onset, atypical symptoms and a lack of effective early screening methods, 75% patients have been in the middle and advanced stage of ovarian carcinoma when treated, and they are mostly accompanied by intra-abdominal metastasis[1]. At present, the comprehensive treatment method of operation combined with radiotherapy and chemotherapy has a significant effect on ovarian carcinoma; 80% patients are sensitive to platinum/paclitaxel chemotherapy drugs, but the disease will relapse in most patients within a short period after treatment. Long-term data statistics show that the 5-year survival rate of patients with early ovarian carcinoma is as high as 80%, while that of patients with advanced ovarian carcinoma is just about 10-30%[2,3]. Therefore, searching effective molecular markers that can predict the metastasis and recurrence of ovarian carcinoma early, and investigating the molecular mechanisms of invasion and metastasis of ovarian carcinoma to obtain effective therapeutic targets, are important

Corresponding Author: Jing Ge, BM; e-mail: 1719971601@qq.com

research topics in improving the prognosis of patients with ovarian carcinoma[4,5]. Following extensive studies on micro ribonucleic acid (miRNA), the role of long non-coding RNA (lncRNA) with the transcript greater than 200 nt in length in the tumor has attracted much attention. Although lncRNA dose not encode or only encodes a small number of proteins, its expressions in different tissues and developmental stages are specific and highly conserved in evolution process of mammals, suggesting that lncRNA may have important biological functions. In recent years, a series of studies have found that lncRNA participates in a series of important regulatory processes, such as X-chromosome silencing, chromatin modification, transcriptional interference and activation and intranuclear transport, through regulating the gene expression in the form of RNA. It is widely involved in almost all physiological and pathological processes in the human body, whose abnormal expression is closely related to the occurrence of a variety of tumors[6,7].

LncRNA small nucleolar RNA host gene 1 (SNHG1) is located on 11q12.3 with 11 exons. More and more studies[5,8] have shown that it has the ... tion of promoting the occurrence and devel... of various tumors. Zhang et al[9] studied and s... ed that the SNHG1 expression is significantly up... ulated in hepatic carcinoma tissues, and SNH... can promote the occurrence a... ression ... hepatic carcinoma through in... ng ... 53 gene expression. Cui et al[10] fou... at, con... ed with that in normal lung tiss... he hi... of SNHG1 exists in ... -si... ng ca... . Moreover, relevant ... cal dat... also shown that the high exp... of SNHG1 ... ted to the later stage and ... nosis of n... all cell lung cancer. However, ... relationship between lncRNA S... G1 and ova... arcinoma has not been re... ed yet so far. Ther... , the expression and ... of lncR... SNHG1 in ovarian carcinoma we... estig... and its relevant mechanism was furthe... in this ... y.

Pat... s and Methods

...cinoma Specimen Collection

A total of 20 pairs of epithelial ovarian carci... a tissues and para-carcinoma normal tissues re... d via ovarian carcinoma operation in our hospital were collected. Patients received no radiotherapy and chemotherapy before operation. The specimens collected were all confirmed pathologically and stored in liquid nitrogen within 5 min after resection, so as to avoid RNA degradation affecting the experimental results. This study was approved by the Ethics Comm... of the First People's Hospital of Yunnan. Signed written informed consents w... obtained from the patients and/or guardian...

Materials

Anti-B-cell lymphoma-... l-2), anti-... sociated X protein (Bax)... i-Cas... e-9, an... ly-ADP-ribose polym... e (PA... , anti-E-cadherin, anti-N-cadhe... in, ... Vi... tin, an... atrix metalloproteinas... MMP... nti-M... 9 and anti-actin anti... es (Cell Si... hnology, Inc. Danver... SA); Hoech... 42 and Annexin V/p... dium ... de (PI) staining kits (BD); cell counting kit 8 (C... (Beyotime, Shanghai, Chi... ofectamine ... (Invitrogen, Carlsb... CA, USA); quantitative reverse transcript... polymerase ... ain reaction (qRT-PCR) kits ... gen, Hilden, ... rmany); si-NC and si-SNHG1 w... ynthesize... y Nanjing Genscript Biotech Co., ... , China), and other experimental reag... e purchased from Sigma-Aldrich (St. Louis, MO, USA).

...ture

IOSE25, CAOV-3, SKOV-3, ES2 and A2780 cells were purchased from American Type Cell Culture (ATCC, Manassas, VA, USA). Cells were incubated using the Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and 1% non-essential amino acids in an incubator with 5% CO_2 at 37°C.

qRT-PCR

The total messenger RNA (mRNA) of cells was extracted using TRIzol and reversely transcribed into complementary DNA (cDNA). Reverse transcription reaction conditions: 25°C for 10 min, 50°C for 30 min and 85°C for 5 min; the fluorescence quantitative PCR kit was used for detection. Primer sequences of SNHG1: forward primer: 5'-CCTAAAGCCACGCTTCTTG-3'; reverse primer: 5'-TGCAGGCTGGAGATCCTACT-3'. Primer sequences of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (internal reference): forward primer: 5'-GGTCTCCTCTGACTTCAACA-3'; reverse primer: 5'-AGCCAAATTCGTTGTCATAC-3'. Fluorescence quantitative PCR conditions: 95°C for 5 min, 95°C for 15 s, 60°C for 1 min, a total of 40 cycles.

Hoechst 33342 Staining

SKOV-3 cells were transfected with si-NC and si-SNHG1. At 48 h after transfection, cells were collected and inoculated into a 12-well plate and cultured for 24 h. Then, the supernatant was discarded and cells were washed with phosphate-buffered saline (PBS) twice. 1 mL Hoechst 33242 fluorochrome was added, followed by incubation at 37°C for 15 min. Next, the fluorochrome was discarded, and cells were washed with PBS, observed and photographed under an inverted fluorescence microscope. The experiment was repeated for three times.

Apoptosis Detection

SKOV-3 cells were transfected with si-NC and si-SNHG1, collected at 48 h after transfection, and washed with PBS twice. 500 μL 1 × binding buffer was added to resuspend the cells and the cell suspension was added with 5 μL Annexin V-fluorescein isothiocyanate (FITC) reagent and mixed evenly, followed by incubation in a dark place at room temperature for 15 min. Next, 10 μL PI reagent was added and gently mixed, followed by incubation in a dark place at room temper for 5 min, and submission for detection w h. The experiment was repeated for three ti

Western Blotting

SKOV-3 cells were transfecte i-NC an si-SNHG1, and collected at ransfection. After cells were wa with -cooled PBS, they were fully ly ith r precipitation assay (R A) uffer ter centrifugation, t upernat as taken and quantified. Then g total pro as mixed with 5 × sodi l sulfonate S) protein loading b er, fo d by denaturation at 100°C for 5 min, loading in SDS-polyacrylamide gel electrophoresis (PAGE) and electrophoresis. The gel and activated polyvinylidene difluoride (PVDF) were placed on the membrane t for frame for membrane transfer under co rent for 2 h. PVDF membrane was oved and blocked with 5% skim milk pow r 1 h. The corresponding primary antibodies dded for incubation at 4°C overnight e mem was washed with Tris-Buffere ine and T (TBST), and added with corres nding h radish peroxidase (H abele condary an bodies for incubati a peratur 1 h. After washing w BST lor de pment, the gray valu s analyzed geJ software, and t e expressi el of target protein w rese s target protein/actin. The experiment was repe r three times.

Statistical Analysis

PSS 20.0 soft re (IBM, Armonk, NY, USA) used for sta cal analysis. All quantitative d ere expre d as mean ± standard deviation pari etween groups was done using One-wa VA test followed by Post Hoc Test (Least Significant Difference). p values < 0.05 nsidered statistically significant.

Results

SNHG1 was Highly Expressed in Ovarian Carcinoma Tissues and four Ovarian Carcinoma Cell Strains

Results of qRT-PCR showed that the expression of SNHG1 in epithelial ovarian carcinoma tissues was significantly higher than that in normal ovarian tissues, and the difference was

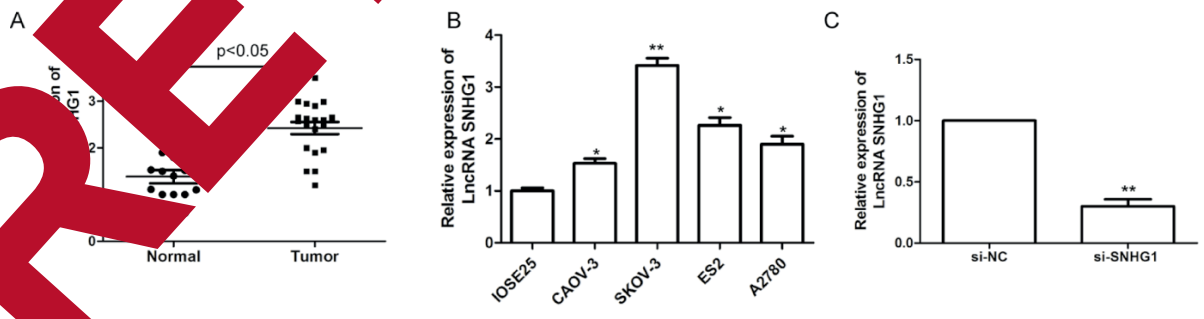

Figure 1. Relative SNHG1 expression levels in ovarian carcinoma tissues and cell lines. ***(A)*** qRT-PCR analysis of relative SNHG1 expression levels in 20 pairs of ovarian carcinoma tissues (Tumor) and adjacent non-cancerous tissues (Normal). ***(B)*** qRT-PCR analysis of relative SNHG1 expression levels in the IOSE25 cell line and four ovarian cancer cell lines. ***(C)*** Effective knockdown of SNHG1 in SKOV-3 epithelial ovarian cancer cells 48 h after siRNA treatment. ($^{*}p<0.05$, $^{**}p<0.01$).

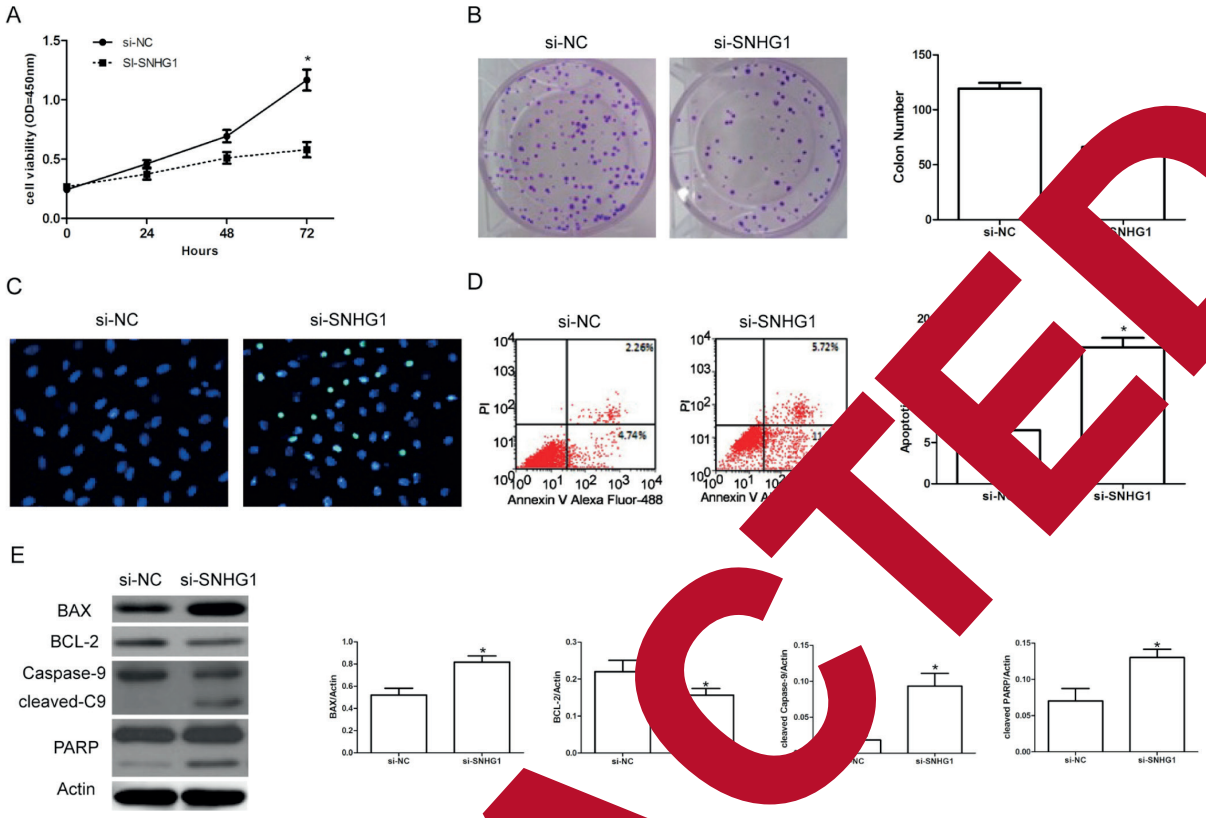

Figure 2. Effects of SNHG1 on SKOV-3 epithe vari cells proliferation and cell apoptosis *in vitro*. (A) CCK-8 cell proliferation assays were performed t ermi -transfected SKOV-3 cells. (B) Colony-forming assays were conducted to determine the prolifer SNHG1 transfected SKOV-3 cells. (C,D) Hoechst 33342 staining and Flow cytometry were conducted to deteri apoptosis of si-SNHG1-transfected SKOV-3 cells. (E) The apoptosis related proteins levels we ined by rn blotting in SKOV-3 cells transfected with si-SNHG1 or si-NC. (*p<0.05).

statistically significa (p igure), suggesting that SN 1 plays tain role in promoting the c ence and d pment of ovarian carcin rther valida esults *in vitro*, the exp sions c HG1 in normal ovarian epith cells (IOS and four ovarian carcino cell strains (CA , SKOV-3, ES2 and 0) we mpared. Compared with that in al o n epithelial cells, the expressions 1 in f varian carcinoma cell ins w nifi y higher. Compared with in the ee cell strains, the expressio vel of S 1 in SKOV-3 was the highest, an ifference was statistically significant ure 1B). In this study, SKOV-3 lls with the highest expression of SNHG1 were ted for subsequent transfection and study. T hibition efficiency at 48 h after transfection with si-SNHG1 was verified via qRT-PCR. As shown in Figure 1C, si-SNHG1 was effective in inhibiting the expression of SNHG1 in SKOV-3 cells, and there was a statistically significant difference compared with that in negative control group (p<0.01), so si-SNHG1 was used for transfection in subsequent experiments.

Down-Regulation of SNHG1 Expression Inhibited the in vitro Proliferation of SKOV-3 Cells

In this study, the effect of inhibiting SNHG1 on the *in vitro* proliferation of SKOV-3 cells was verified via CCK8 and clone formation assays. SKOV-3 cells were cultured for 24, 48 and 72 h after interference with si-SNHG1 and si-NC. Compared with that in control group, the optical density (OD) value in experimental group was decreased in different degrees, indicating that the inhibition of SNHG1 expression reduced the SKOV-3 cell growth rate (Figure 2A). Similarly, results of clone formation assay showed that the number of cell clone in si-SNHG1 interference group was significantly decreased compared with

that in si-NC group (p<0.01, Figure 2B), suggesting that the inhibition of SNHG1 expression can significantly inhibit the cell clone formation.

Inhibition of SNHG1 Expression Promoted the SKOV-3 Cell Apoptosis

To further investigate the mechanism of inhibiting SNHG1 in inhibiting the SKOV-3 cell proliferation, the effect of SNHG1 small interfering RNA (siRNA) on apoptosis was verified. First, the change in the proportion of apoptotic cells after interference with SNHG1 siRNA was observed via Hoechst 33342 staining. The results showed that the proportion of apoptotic cells in experimental group was significantly increased compared with that in control group at 48 h after interference in SKOV-3 cells with SNHG1 siRNA (Figure 2C). The results of flow cytometry showed that the total apoptosis rate in experimental group was obviously increased compared with that in control group (p<0.05, Figure 2D), indicating that inhibiting SNHG1 expression promotes the apoptosis. In order to further explore the mechanism of inhibiting SNHG1 expression in promoting the apoptosis, the changes in Bax, Bcl-2, cleaved Caspase-9, [illegible] and cleaved PARP were detected. As sh[illegible] Figure 2E, the expression of Bax protein wa[illegible]g-nificantly increased but the expression of Bcl-2 [illegible] decreased after interference with SNHG1 siR[illegible] Moreover, cleaved Caspase-9 a[illegible]ved PAR[illegible] were significantly increased [illegible]SN[illegible] siRNA interference group. The ab[illegible] results[illegible]eal that inhibiting the expression [illegible]NHG1[illegible]if[illegible] cantly up-regulate the [illegible]io[illegible]-2, th[illegible]y increasing the exp[illegible]ons of [illegible]d Caspase-9 and cleaved PAR[illegible]d ultimately [illegible]oting the cell apoptosis.

Down-r[illegible]ation of S[illegible]1 Expression Inhibi[illegible] the SKOV-3 Ce[illegible]igration and [illegible]asion

[illegible]migra[illegible]abilities of cells in two groups were [illegible]via wo[illegible]healing assay first, and [illegible]degre[illegible]scrat[illegible]aling were observed at 24 [illegible] h af[illegible]atch model was established. The[illegible]sults sho[illegible] that after 24 and 48 h, the mi-gr[illegible]istance of cells in SNHG1 knockdown [illegible]p [illegible]gnificantly shorter than that in con-[illegible]l group. With the prolongation of healing time, [illegible] difference in migration distance was further e[illegible]ed between two groups, and the difference was statistically significant (p<0.05, Figure 3A-B), indicating that inhibiting the SNHG1 expression can significantly inhibit the wound healing ability of SKOV-3 cells. Next, transwell migration assay was conducted, and the consistent results to wound healing assay were obtained. After SNHG1 knockdown, the number of SKOV-3 [illegible]lls passing through the filter membrane i[illegible] chamber was significantly decreas[illegible]ompared with that in control group, and th[illegible]erence was statistically significant (p<0.01, Fig[illegible]C-D).

Knockdown of SNHG[illegible]pression Inhibited EMT Proce[illegible]nd M[illegible]P-2 an[illegible] MMP-9 Expressio[illegible] SKO[illegible] Cells

To further invest[illegible]g[illegible]m[illegible]anism [illegible]ock-down of SNHG1[illegible]ressi[illegible]nhibit[illegible]ne mi-gration and in[illegible]n abilities [illegible]C[illegible] cells, the changes in r[illegible]lecules wer[illegible]er detected via Weste[illegible]blotti[illegible]s well known that invasion and metastasis are c[illegible]eristics of tumors, and its m[illegible]sm is closely [illegible]d to epithelial-mes-e[illegible]mal transition (EMT)[illegible][12]. Therefore, chang-[illegible] expression l[illegible]els of EMT-related proteins in [illegible]V-3 cells aft[illegible]nockdown of SNHG1 expres-si[illegible]ere furthe[illegible]ected. E-cadherin expression was [illegible]ifica[illegible]up-regulated, but N-cadherin and Vi[illegible]expressions were down-regulated [illegible]ignificantly after SNHG1 knockdown (Figure 4), [illegible]ing that down-regulating the SNHG1 ex-[illegible]es[illegible]can inhibit the EMT process of SKOV-3 cells. It is all known that extracellular hydrolase is necessary for tumor cells passing through the basement membrane. The extracellular matrix degradation is required for tumor cells in invasion to surrounding tissues, passing through the vascular wall and formation of distant metastasis, in which MMPs play key roles[13,14]. The results (Figure 4) showed that after SNHG1 knockdown, the expressions of MMP-2 and MMP-9 in SKOV-3 cells could also be down-regulated. The above results reveal that after the down-regulation of SNHG1 expression, the invasion and metastasis of cells can be inhibited through the regulation of EMT and MMPs.

Discussion

In this study, the expressions of lncRNA SNHG1 in 20 pairs of ovarian carcinoma tissues and para-carcinoma tissues were detected via RT-PCR. The results showed that the expression level of SNHG1 in ovarian carcinoma was significantly increased compared with that in para-carcinoma tissues. Further *in vitro* cell experiments also revealed that compared with that in

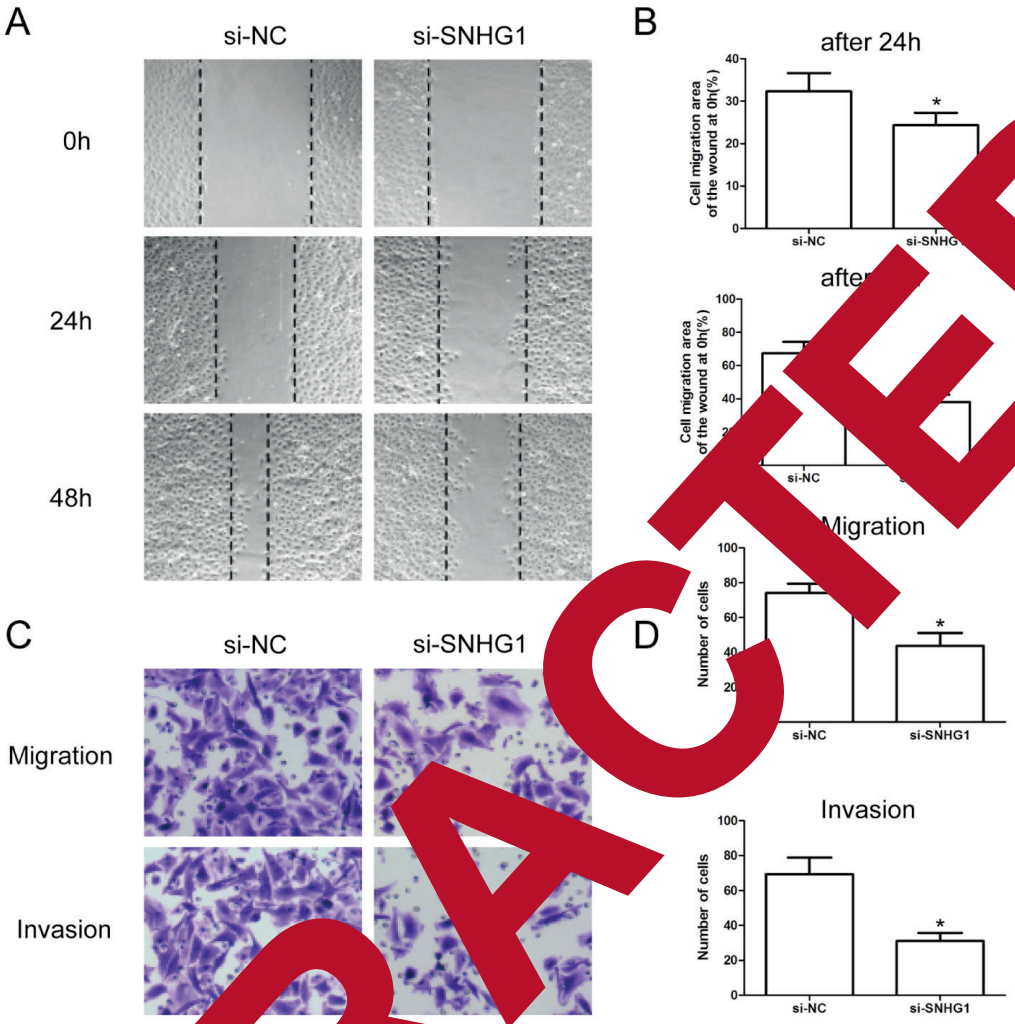

Figure 3. Knockdown of SNHG1 inhibits cell migration and invasion *in vitro*. ***(A-B)*** Wound-healing and ***(C-D)*** transwell assays showing the migratory abilities and invasive capacities of SKOV-3 cells transfected with si-SNHG1 or si-NC. ($^{*}p<0.05$).

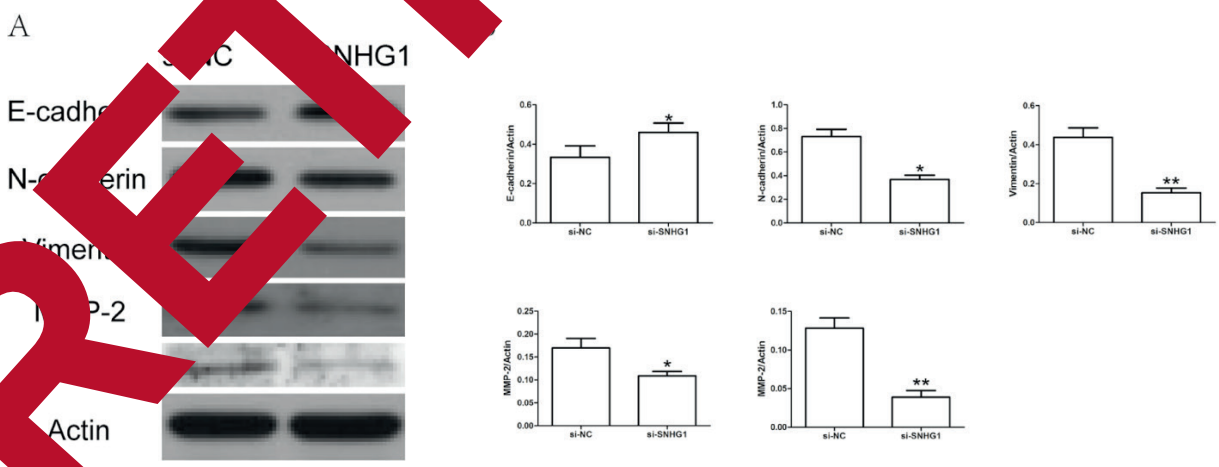

Figure 4. Knockdown of SNHG1 inhibits cell migration and invasion *in vitro*. ***(A)*** The expression levels of target proteins (E-cadherin, N-cadherin, Vimentin, MMP-2 and MMP-9) measured by Western blotting in SKOV-3 cells transfected with si-SNHG1 or si-NC. ***(B)*** Data shown are representative of 3 independent experiments. Data are presented as means ± SD (n = 3). ($^{*}p<0.05$, $^{**}p<0.01$)

normal ovarian epithelial cells, lncRNA SNHG1 was highly expressed in different ovarian carcinoma cells. These results strongly suggest that lncRNA SNHG1 also promotes the occurrence and development of ovarian carcinoma. In CCK8 and clone formation assays, it was found that after knockdown of SNHG1 in SKOV-3 cells, the cell proliferation rate was significantly reduced. Interestingly, it was found in the apoptosis assay that the proportion of apoptotic SKOV-3 cells with SNHG1 knockdown was significantly increased. Besides, results of Western blotting confirmed that when the expression of SNHG1 was inhibited, the expressions of pro-apoptotic proteins in mitochondrial apoptosis pathway were upregulated and activated, while the anti-apoptosis proteins were inhibited, thereby activating the mitochondrial apoptosis pathway and inducing apoptosis. These results also partially clarify the pro-apoptotic mechanism after SNHG1 is inhibited.

Conclusions

We found that SNHG1 can promote the
eration of ovarian carcinoma cells, and inh g
the expression of SNHG1 can partially revers
tumor-promoting effect. The underlying mec
nism is related to the activation chondri
apoptotic pathway after the i SNHG1.
In addition, SNHG1 can promo he me-
tastasis of ovarian carci cells
regulation of EMT a M e are
limitations in this y, and ecific mech-
anism of SNHG gulating E d MMPs
needs to be fu ed. This st provides
a new horizo or the anism of occurrence
and devel ent of ovaria cinoma and a new
potenti get for the treatm f ovarian carci-
noma the fut

flict teres

hors d they have no conflict of interest.

References

1) Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.
2) Banerjee S, Gore M. Recent advances ic treatments for ovarian cancer. Ca imagi 2012; 12: 305-309.
3) Su Z, Graybill WS, Zhu Y. Detecti monitoring of ovarian cancer. Clin Chim Acta 15: 341-345.
4) Davidson B, Trope CG. an cancer: tic, biological and p stic as cts. Wo Health (Local) 2014 519-53
5) Caiola E, Broggi M, Genetic rkers for prediction eati tcomes varian cancer. Pha ogenom 014: 01-410.
6) Kondo Y, K, Katsushima non-coding RNAs etic regulat human cancers. cer So 108: 1927-1933.
7) Z M, Wu WB, ZW, Wang XH. LncRNA s closely relate progression of breast ancer via promoting p iferation and EMT. Eur Rev Med Pharm col Sci 2017; 21: 1020-1026.
Li J, Zhang Z ng L, Guo C, Jiang T, Zeng L, Li Wang J. S G1 lncRNA negatively regulates 199a-3 enhance CDK7 expression and oliferation in prostate cancer. Biochem phys Res Commun 2017; 487: 146-152.
Zhang M, Wang W, Li T, Yu X, Zhu Y, Ding F, Li ng T. Long noncoding RNA SNHG1 predicts or prognosis and promotes hepatocellular carcinoma tumorigenesis. Biomed Pharmacother 2016; 80: 73-79.
10) Cui Y, Zhang F, Zhu C, Geng L, Tian T, Liu H. Upregulated lncRNA SNHG1 contributes to progression of non-small cell lung cancer through inhibition of miR-101-3p and activation of Wnt/beta-catenin signaling pathway. Oncotarget 2017; 8: 17785-17794.
11) Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell 2011; 144: 646-674.
12) Kudo-Saito C, Shirako H, Takeuchi T, Kawakami Y. Cancer metastasis is accelerated through immunosuppression during Snail-induced EMT of cancer cells. Cancer Cell 2009; 15: 195-206.
13) Deryugina EI, Quigley JP. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev 2006; 25: 9-34.
14) Al-Alem L, Curry TJ. Ovarian cancer: involvement of the matrix metalloproteinases. Reproduction 2015; 150: R55-R64.