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SNHG7 Expression Is Upregulated in Ovarian Cancer and Promotes Cell Invasion and EMT Pathway

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OBJECTIVE: Long non-coding small nucleolar RNA host gene 7 (lncRNA SNHG7) has been identified as an oncogene in tumor progression. However, the role of SNHG7 in ovarian cancer remains unknown.

STUDY DESIGN: The expression of circ SNHG7 was detected in human ovarian cancer tissue and adjacent normal tissue samples using quantitative reverse transcription polymerase chain reaction (qRT-PCR) analyses. The chi-squared test was used to assess the association between expression of SNHG7 and clinical pathological parameters. Cell proliferation and invasion were performed by CCK8 and transwell assays. Western blot analysis was used to detect the protein expression.

RESULTS: We demonstrated that SNHG7 was significantly upregulated in human ovarian cancer tissues and cells. Higher SNHG7 expression was associated with clinical stage and lymph node metastasis. Furthermore, we demonstrated that knockdown of SNHG7 inhibited cell proliferation and cell invasion ability. Additionally, we observed that knockdown of SNHG7 suppressed the cell epithelial-mesenchymal transition (EMT) process by upregulating E-cadherin expression but downregulating MMP9 expression.

CONCLUSION: These results indicated that SNHG7 expression was higher in ovarian cancer tissues and promoted cell invasion and EMT process, which may

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serve as a target of ovarian cancer treatment. (Anal Quant Cytopathol Histpathol 2020;42:1–7)

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Ovarian cancer is one of the most common gynecological tumors.[1] Although the dramatic advances, including surgical resection and new chemotherapeutic methods, have been improved, the 5-year overall survival rate for ovarian cancer patients was less than 30%.[2] The molecular basis underlying ovarian cancer progression remains largely undefined.[3] Thus, it is necessary to identify reliable predictive biomarkers and therapeutic targets for ovarian cancer.

Long non-coding RNAs (lncRNAs) are a class of non-protein-coding RNAs that are aberrantly expressed and are critical in tumor progression.[4] Studies have identified some lncRNAs involved in ovarian cancer. The long non-coding RNA ANRIL promotes proliferation and cell cycle progression and inhibits apoptosis and senescence in epithelial ovarian cancer.[5] A new tumor suppressor lncRNA RP11-190D6.2 inhibits the proliferation, migration, and invasion of epithelial ovarian cancer cells.[6] Upregulation of the lncRNA MEG3 induces autophagy to inhibit tumorigenesis and progression of epithelial ovarian carcinoma by regulating activity of ATG3.[7] Long non-coding RNA CCAT1 promotes metastasis and poor prognosis in epithelial ovarian cancer.[8]

SNHG7 is reported to act as a tumor promoter in some tumors. For instance, lncRNA-SNHG7 promotes the proliferation, migration, and invasion and inhibits apoptosis of lung cancer cells by enhancing the FAIM2 expression.[9] LncRNA SNHG7 promotes the proliferation and inhibits apoptosis of gastric cancer cells by repressing the P15 and P16 expression.[10] However, the role of SNHG7 in ovarian cancer remains unknown.

In this study we demonstrated that it was significantly upregulated in human ovarian cancer tissues and cells. Moreover, knockdown of SNHG7 inhibited cell invasion and the epithelial-mesenchymal transition (EMT) process by upregulating E-cadherin expression but downregulating MMP9 expression. Thus, these results indicated that SNHG7 expression was higher in ovarian cancer tissues and promoted cell invasion and the EMT process, which may serve as target of ovarian cancer treatment.

Materials and Methods

Patient and Tissue Samples

A total of 68 paired ovarian cancer tissues and matched normal adjacent tissue samples were obtained from patients with ovarian cancer between July 2014 and April 2016 at the Department of Obstetrics and Gynecology, Chinese PLA General Hospital. Patients with ovarian cancer who received other therapeutic methods before surgery were excluded. After resection, the tissue samples were immediately snap-frozen in liquid nitrogen and stored at −80°C. The study was approved by the Ethics Committee of Chinese PLA General Hospital. Written informed consent was collected from all patients enrolled in the study. The clinical data are shown in Table I. Histopathological diagnoses were performed according to the criteria of the World Health Organization.[11]

Cell Line Culture

Two human ovarian cancer cell lines including SKOV3 and OVCAR3 and a HOSE human normal ovarian epithelial cell were purchased from the Cell Bank of the Chinese Academy of Sciences

Table I *Association Between SNHG7 Expression and Clinicopathological Factors*

	SNHG7 expression			
Variable	**Patients (n=68)**	**Higher (n=35)**	**Lower (n=33)**	**p Value**
Age				0.994
≤45	35	18	17	
>45	33	17	16	
Tumor size (cm)				0.475
≤4	32	15	17	
>4	36	20	16	
Differentiation				0.959
Well and moderate	41	21	20	
Poor	27	14	13	
Lymph node metastasis				0.002*
No	32	10	22	
Yes	36	25	11	
HPV 16/18 infection				0.105
Positive	45	20	25	
Negative	23	15	8	
FIGO stage				0.018*
I	35	12	23	
II	22	12	10	
III	11	9	2	

*p<0.05.

(Shanghai, China). Cells were grown in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA) and supplemented with 10% fetal bovine serum (FBS, Gibco; Thermo Fisher Scientific) at 5% CO_2 at 37°C in a humidified atmosphere.

Cell Transfection

The si-SNHG7-1, si-SNHG7-2, or si-negative control (si-NC) oligo was designed and synthesized by Gene Chem (Shanghai, China). The transfections were performed by Lipofectamine 3000 (Invitrogen, New York, USA) according to the manufacturer's instructions. At 48 hours after transfection the cells were harvested and used for the following experiments.

RNA Isolation and Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) Analysis

RNA was extracted from tissues and cells using Trizol reagent (Invitrogen; Thermo Fisher Scientific) according to the manufacturer's instructions. A 1 µg RNA was used to synthesize the complementary DNA (cDNA) using the Prime Script RT kit (Takara Bio, Inc., Otsu, Japan). QRT-PCR was performed using an ABI 7500 sequence detection system (Applied Biosystems; Thermo Fisher Scientific). The cDNA was amplified using the SYBR Premix Ex TaqII (Takara Bio). The conditions for qRT-PCR were as follows: 98°C for 10 minutes, followed by 45 cycles of 98°C for 10 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The mRNA expression was assessed by $2^{-\Delta\Delta Cq}$ method,[12] and GAPDH was used as an internal control.

Cell Viability Assay

Cell proliferation ability was detected by using a Cell Counting Kit-8 (CCK-8) assay (Beyotime Institute of Biotechnology, Haimen, China). Briefly, SKOV3 and OVCAR3 cells were plated onto 96-well plates at a density of 3,000 cells/well. Following culture for the indicated time-points (0, 24, 48, and 72 hours), 10 µL CCK-8 solution was added into each well and incubated at 37°C. After 2 hours, the absorbance was measured using a spectrophotometer (Thermo Fisher Scientific) at a wavelength of 450 nm.

Cell Invasion Assays

Transwell cell invasion assay was used to assess cell invasion ability using 8-µm pore size chambers (Becton Dickinson, San Jose, California, USA). The chamber was precoated with Matrigel (Corning Inc., Corning, New York, USA). A total of 1×10^5 cells were seeded into the upper chamber, and 500 µL medium containing 10% FBS was added to the lower chamber. In the lower chamber cells were fixed with methanol for 48 hours and stained with crystal violet (0.1%) for 10 minutes at room temperature. Cell invasion number was calculated by counting the cells in the lower chamber in 5 random fields.

Western Blot Analysis

SKOV3 and OVCAR3 cells were cultured into a six-well plate and transfected with specific si-SNHG7 or si-NC. Total proteins were extracted with RIPA buffer (Thermo Scientific, Rockford, Illinois, USA). An equal quantity of protein (40 µg) was separated on 10% SDS-PAGE and electrotransferred onto polyvinylidene fluoride (PVDF) membranes. Subsequently, the membranes were blocked with 5% skimmed milk for 2 hours at room temperature. Then the membranes were incubated with primary antibodies including E-cadherin (Santa Cruz Biotechnology, Inc., Dallas, Texas, USA), MMP9 (Santa Cruz Biotechnology), MMP2 (Santa Cruz Biotechnology), and GAPDH (Abcam, Cambridge, UK) at 4°C overnight. Following the incubation with horseradish peroxidase-conjugated goat anti-mouse IgG, they were incubated with the membrane for 2 hours at room temperature. The western blots were detected using ECL detection reagent (Amersham Biosciences, Castle Hill, Australia).

Statistical Analysis

All data were performed using SPSS software (SPSS Statistics for Windows, Version 17.0, SPSS Inc., Chicago, Illinois, USA). The results are presented as the mean ± standard deviation. The differences between groups were analyzed using two-sided Student's *t* test or one-way analysis of variance (ANOVA). Statistical significance was set at $p<0.05$.

Results

SNHG7 Expression Is Upregulated in Human Ovarian Cancer Tissues and Cells

In the study we examined the relative expression of SNHG7 in 68 paired ovarian cancer tissues and matched normal adjacent tissue samples. As shown in Figure 1A, the relative expression of

SNHG7 was upregulated in ovarian cancer tissues as compared to matched normal adjacent tissue samples ($p<0.05$). Furthermore, the expression of SNHG7 in SKOV3 and OVCAR3 cells was also upregulated as compared to that in HOSE human normal ovarian epithelial cells (Figure 1B). Moreover, we divided the patients into 2 groups according to mean expression of SNHG7 in tumor tissues. The clinicopathological correlation analysis was analyzed between SNHG7 expression and clinicopathological features of patients. The results indicated that high expression of SNHG7 was positively correlated with advanced tumor grade and lymph node metastasis (all $p<0.05$) (Table I). However, no correlation was found between SNHG7 expression and other factors of patients (all $p>0.05$) (Table I). Thus, these results indicated that SNHG7 expression was higher in ovarian cancer tissues and cells.

Knockdown of SNHG7 Expression Inhibits Cell Proliferation and Invasion of Ovarian Cancer

To identify the relative effects of SNHG7 expression in ovarian cancer, 2 siRNAs targeting SNHG7 were transfected into SKOV3 and OVCAR3 cells. We found that the si-SNHG7-1 showed a higher knockdown efficiency of SNHG7 and was therefore used for the following experiments (Figure 2A–B). The CCK8 cell proliferation showed that SNHG7 silencing obviously inhibited the proliferation of SKOV3 and OVCAR3 cells as compared to the si-NC group (Figure 2C–D). In addition, the cell invasion was assessed using transwell assays. The results showed that SNHG7 silencing obviously suppressed the invasion ability of SKOV3 and OVCAR3 cells as compared to the si-NC group (Figure 3A–B).

Knockdown of SNHG7 Expression Inhibits Cell EMT of Ovarian Cancer

Epithelial-mesenchymal transition (EMT) is identified as a crucial process for the initiation and progression of cancer metastasis, including ovarian cancer. In the study we found that SNHG7 silencing dramatically increased the expression of E-cadherin but inhibited the expression of MMP9

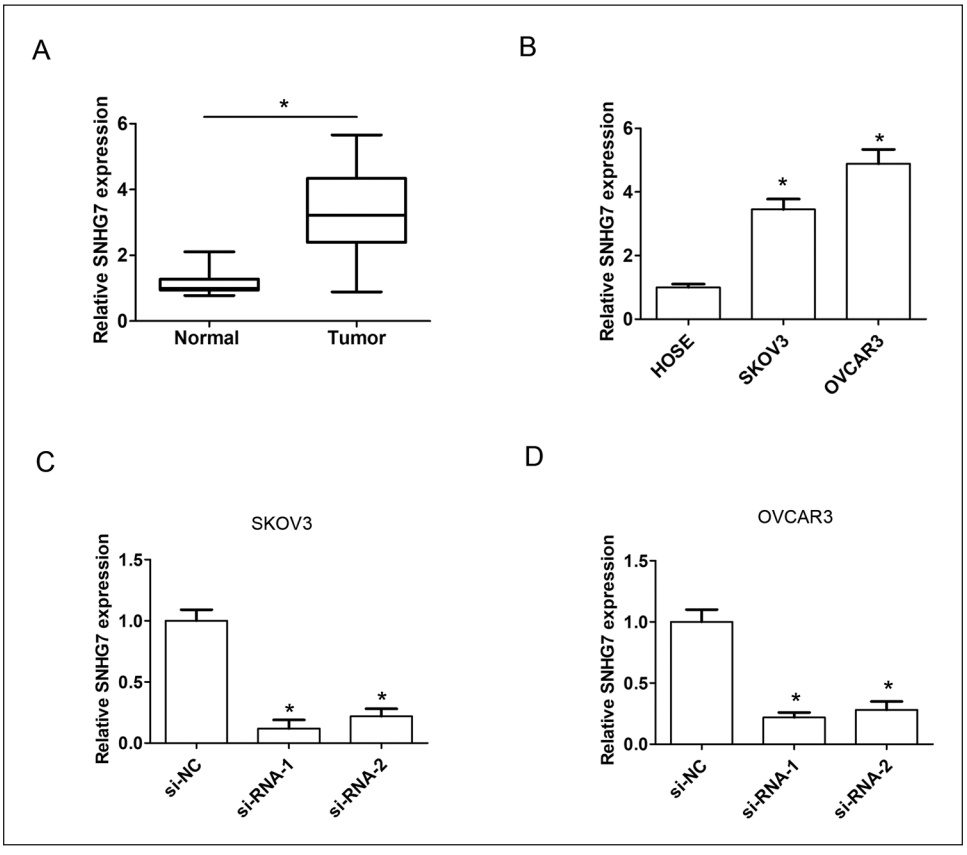

Figure 1
Relative SNHG7 expression is shown in different tissues and cells. (A) Note a higher SNHG7 expression in ovarian cancer tissues as compared to adjacent normal tissues with qRT-PCR analysis (n=68). (B) SNHG7 expression is higher in SKOV3 and OVCAR3 cells than that in HOSE cells by using qRT-PCR analysis. (C, D) Note a higher SNHG7 expression after downregulated SNHG7 in SKOV3 or OVCAR3 cells with qRT-PCR analysis. *$p<0.05$.

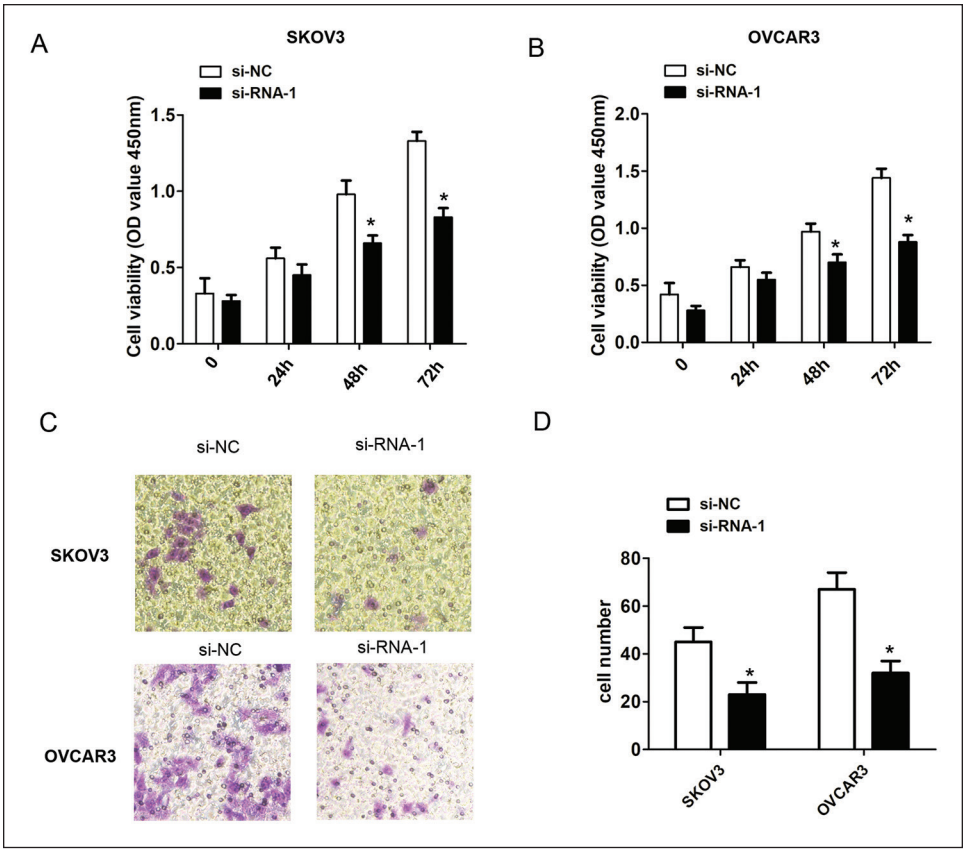

Figure 2
Inhibition of cell proliferation and invasion in SKOV3 and OVCAR3 cells after knockdown of SNHG7. (A, B) Cell viability shown by CCK8 assays after cells were transfected with si-NC or si-SNHG7 in SKOV3 or OVCAR3 cells following culture for the indicated time-points (0, 24, 48, and 72 h). (C, D) Cell proliferation ability is shown transfected with si-NC or si-SNHG7 in SKOV3 or OVCAR3 cells as images (magnification 200×) and graphic. *p<0.05.

using western blot analysis in SKOV3 and OVCAR3 cells. Thus, these results indicated that knockdown of SNHG7 expression inhibited cell EMT of ovarian cancer.

Discussion

Accumulating findings have shown that lncRNAs are essential participants in tumor biology, including cell proliferation, migration, invasion, and metastasis.[4] Some studies revealed that the lncRNASNHG7 has high expression in various tumor tissues and cells.[9,10,13] In the study, our results showed that SNHG7 expression was upregulated in ovarian cancer tissues as compared to matched normal adjacent tissue samples. Furthermore, SNHG7 expression positively correlated with advanced tumor grade and lymph node metastasis. These results indicated that SNHG7

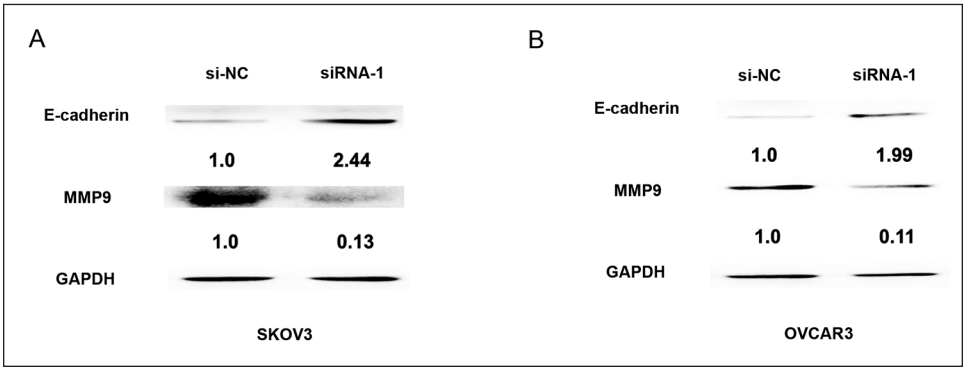

Figure 3
Knockdown of SNHG7 inhibited cell EMT process in SKOV3 and OVCAR3 cells. (A) Western blot analysis was used to detect the expression of E-cadherin and MMP9 after knockdown of SNHG7 expression in SKOV3 cells. (B) Note the expression of E-cadherin and MMP9 after knockdown of SNHG7 expression in VCAR3 cells by western blot analysis.

expression may be involved in tumor biological function of ovarian cancer.

In the previous study, SNHG7 was found to affect cell proliferation and invasion in tumors. LncRNA-SNHG7 promotes the proliferation, migration, and invasion and inhibits apoptosis of lung cancer cells by enhancing the FAIM2 expression.[9] SNHG7 is upregulated in gastric cancer tissues and cells and partially contributes to tumor progression of gastric cancer through regulation of p15 and p16 expressions.[10] SNHG7 promoted the proliferation, migration, and invasion of GBM cells through the inhibition of miR-5095 and concomitant activation of Wnt/β-catenin signaling pathway.[14] Long noncoding RNA SNHG7 accelerates prostate cancer proliferation and cycle progression through cyclin D1 by sponging miR-503.[15] In our results we found that SNHG7 silencing inhibited cell proliferation and invasion ability.

Ovarian cancer is the most lethal of all gynecological malignancies, primarily due to the sloughing-off of highly metastatic cells from primary tumors and their subsequent spread throughout the peritoneal cavity.[16] Epithelial-mesenchymal transition (EMT) is identified as a crucial process for cancer metastasis of ovarian cancer.[17] In the study we found that SNHG7 silencing dramatically increased the expression of E-cadherin but inhibited the expression of MMP9 using western blot analysis in SKOV3 and OVCAR3 cells. E-cadherin and MMP9 are involved in the EMT process,[18] such as, MiR-5692a promotes the invasion and metastasis of hepatocellular carcinoma via MMP9.[19] Wang et al showed that melatonin inhibits epithelial-to-mesenchymal transition in gastric cancer cells via attenuation of IL-1β/NF-κB/MMP2/MMP9 signaling.[20] Vitamin K3 (menadione) suppresses epithelial-mesenchymal-transition and Wnt signaling pathway in human colorectal cancer cells by increasing the expression of E-cadherin but inhibiting the expression of MMP9.[21] Our results showed that SNHG7 knockdown affects the E-cadherin and MMP9 expression. Thus, these results indicated that knockdown of SNHG7 expression inhibited cell EMT of ovarian cancer.

In conclusion, our results suggested that SNHG7 expression is higher in ovarian cancer. Functional analyses have shown that degradation of SNHG7 inhibits cell proliferation, invasion, and the EMT process of ovarian cancer. Therefore, these results indicate, for the first time, that SNHG7 plays an important role in ovarian cancer.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D: Global cancer statistics. CA Cancer J Clin 2011;61:69-90
2. Cannistra SA: Cancer of the ovary. N Engl J Med 2004;351: 2519-2529
3. Schwartz PE, Zheng W: Neoadjuvant chemotherapy for advanced ovarian cancer: The role of cytology in pretreatment diagnosis. Gynecol Oncol 2003;90:644-650
4. Atkinson SR, Marguerat S, Bahler J: Exploring long non-coding RNAs through sequencing. Semin Cell Dev Biol 2012; 23:200-205
5. Qiu JJ, Wang Y, Liu YL, Zhang Y, Ding JX, Hua KQ: The long non-coding RNA ANRIL promotes proliferation and cell cycle progression and inhibits apoptosis and senescence in epithelial ovarian cancer. Oncotarget 2016;7:32478-32492
6. Tong W, Yang L, Yu Q, Yao J, He A: A new tumor suppressor lncRNA RP11-190D6.2 inhibits the proliferation, migration, and invasion of epithelial ovarian cancer cells. Onco Targets Ther 2017;10:1227-1235
7. Xiu YL, Sun KX, Chen X, Chen S, Zhao Y, Guo QG, Zong ZH: Upregulation of the lncRNA Meg3 induces autophagy to inhibit tumorigenesis and progression of epithelial ovarian carcinoma by regulating activity of ATG3. Oncotarget 2017; 8:31714-31725
8. Cao Y, Shi H, Ren F, Jia Y, Zhang R: Long non-coding RNA CCAT1 promotes metastasis and poor prognosis in epithelial ovarian cancer. Exp Cell Res 2017;359:185-194
9. She K, Huang J, Zhou H, Huang T, Chen G, He J: lncRNA-SNHG7 promotes the proliferation, migration and invasion and inhibits apoptosis of lung cancer cells by enhancing the FAIM2 expression. Oncol Rep 2016;36:2673-2680
10. Wang MW, Liu J, Liu Q, Xu QH, Li TF, Jin S, Xia TS: LncRNA SNHG7 promotes the proliferation and inhibits apoptosis of gastric cancer cells by repressing the P15 and P16 expression. Eur Rev Med Pharmacol Sci 2017;21:4613-4622
11. Morgan RJ Jr, Alvarez RD, Armstrong DK, Burger RA, Chen LM, Copeland L, Crispens MA, Gershenson DM, Gray HJ, Hakam A, Havrilesky LJ, Johnston C, Lele S, Martin L, Matulonis UA, O'Malley DM, Penson RT, Powell MA, Remmenga SW, Sabbatini P, Santoso JT, Schink JC, Teng N, Werner TL, Dwyer MA, Hughes M; National Comprehensive Cancer Networks: Ovarian cancer, version 2.2013. J Natl Compr Canc Netw 2013;11:1199-1209
12. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25:402-408
13. Chaudhry MA: Expression pattern of small nucleolar RNA host genes and long non-coding RNA in X-rays-treated lymphoblastoid cells. Int J Mol Sci 2013;14:9099-9110
14. Ren J, Yang Y, Xue J, Xi Z, Hu L, Pan SJ, Sun Q: Long noncoding RNA SNHG7 promotes the progression and growth of glioblastoma via inhibition of miR-5095. Biochem Biophys Res Commun 2018;496:712-718

15. Qi H, Wen B,Wu Q, Cheng W, Lou J, Wei J, Huang J, Yao X, Weng G: Long noncoding RNA SNHG7 accelerates prostate cancer proliferation and cycle progression through cyclin D1 by sponging miR-503. Biomed Pharmacother 2018;102: 326-332
16. Wang L, Mezencev R, Svajdler M, Benigno BB, McDonald JF: Ectopic over-expression of miR-429 induces mesenchymal-to-epithelial transition (MET) and increased drug sensitivity in metastasizing ovarian cancer cells. Gynecol Oncol 2014; 134:96-103
17. Chen J, Wang L, Matyunina LV, Hill CG, McDonald JF: Overexpression of miR-429 induces mesenchymal-to-epithelial transition (MET) in metastatic ovarian cancer cells. Gynecol Oncol 2011;121:200-205
18. Son H, Moon A: Epithelial-mesenchymal transition and cell invasion. Toxicol Res 2010;26:245-252
19. Sun SJ, Wang N, Sun ZW, Chen J, Cui HW: MiR-5692a promotes the invasion and metastasis of hepatocellular carcinoma via MMP9. Eur Rev Med Pharmacol Sci 2018;22: 4869-4878
20. Wang X, Wang B, Xie J, Hou D, Zhang H, Huang H: Melatonin inhibits epithelial-to-mesenchymal transition in gastric cancer cells via attenuation of IL-1β/NF-κB/MMP2/MMP9 signaling. Int J Mol Med 2018;42(4):2221-2228
21. Kishore C, Sundaram S, Karunagaran D: Vitamin K3 (menadione) suppresses epithelial-mesenchymal-transition and Wnt signaling pathway in human colorectal cancer cells. Chem Biol Interact 2019;309:108725