



# LINC00641 inhibits the proliferation and invasion of ovarian cancer cells by targeting miR-320a

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**Background:** Ovarian cancer is a common malignancy of the female reproductive system, with one of the highest mortality rates among all malignant tumors. However, the pathogenesis of ovarian cancer has not been fully elucidated. This study investigated the role and molecular mechanism of LINC00641 in the development and progression of ovarian cancer.

**Methods:** Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expression level of LINC00641 in ovarian cancer tissue and adjacent normal tissue. Cell Counting Kit-8 (CCK-8), colony formation, and Transwell assays were used to detect the effects of LINC00641 overexpression on the proliferation and migration of ovarian cancer cells. Bioinformatics analysis and luciferase reporter gene assay were employed to detect the binding of LINC00641 to the downstream target molecule, microRNA-320a (miR-320a). Western blotting was used to determine the effect of miR-320a overexpression on the expression of proliferation-related proteins [Ki-67 and proliferating cell nuclear antigen (PCNA)] and invasion-related proteins (E-cadherin, N-cadherin, and vimentin) in overexpressed LINC00641 cells.

**Results:** qRT-PCR results showed that LINC00641 was under-expressed in ovarian cancer tissue compared to adjacent tissue. Cell function experiments showed that the overexpression of LINC00641 could significantly inhibit the proliferation and migration of ovarian cancer cells. The luciferase reporter gene assay showed that LINC00641 could bind to miR-320a, and the overexpression of LINC00641 could markedly inhibit the expression of miR-320a in ovarian cancer cells. Overexpression of miR-320a could significantly block the inhibitory effect of LINC00641 on the proliferation and migration of ovarian cancer cells.

**Conclusions:** As a tumor suppressor gene, LINC00641 can inhibit the proliferation and invasion of ovarian cancer cells by targeting miR-320a. The LINC00641/miR-320a axis may be a new target for the early diagnosis, treatment, or prognosis of ovarian cancer patients.

**Keywords:** LINC00641; miR-320a; ovarian cancer

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## Introduction

Ovarian cancer is a common malignant tumor of the female reproductive system, and its mortality rate ranks among the highest of all malignant tumors (1). With the continuous progress of medical technology, the treatment of ovarian cancer has been improved to a certain extent; the effective rate of surgical resection combined with chemotherapy for newly treated patients can reach more than 70% (2). However, the lack of sensitive indicators for the early diagnosis of ovarian cancer coupled with reduced patient sensitivity to chemotherapeutic drugs has led to a 5-year survival rate among ovarian cancer patients of less than 40% (3,4). Therefore, exploring new molecular markers of the occurrence and development of ovarian cancer is helpful to identify the causes of ovarian tumor tissue, and is of great significance for improving the diagnosis and treatment effect of ovarian cancer and prolonging the life cycle of patients.

Recent studies have shown that long non-coding RNA (lncRNA) is a kind of RNA fragment with a length of more than 200 nucleotides (nt), which does not have the ability to encode protein (5). lncRNA regulates downstream gene expression by regulating chromatin modification and transcription, and participates in many biological processes such as cell proliferation, invasion, and apoptosis (6,7). Numerous studies have shown that lncRNA not only involves the occurrence and progression of a variety of malignant tumors, but can also be used as a diagnostic marker for the early detection of tumors. For example, the expression of LINC00319 is up-regulated in ovarian cancer tissues and cell lines, and the overexpression of LINC00319 significantly promotes the proliferation and invasion of ovarian cancer cells; thus, it can be used as a potential prognostic biomarker for ovarian cancer patients in the future (8). lncRNA pro-transition associated RNA (PTAR) regulates zinc finger Ebox binding homeobox 1 (ZEB1) expression through competitive binding to miR-101-3p to promote epithelial-to-mesenchymal transition (EMT) as well as invasion and metastasis of serous ovarian cancer (9). In addition, studies have shown that the combined detection of plasma lncRNA regulator of reprogramming (ROR) and carbohydrate antigen 125 (CA125) is more valuable in the diagnosis of ovarian cancer than the detection of lncRNA ROR or CA125 alone (10). However, the biological functions of only a few lncRNAs have been verified by experiments so far. Therefore, identifying and clarifying the function of new lncRNAs in ovarian cancer has important

potential application prospects for later cancer treatment and diagnosis.

Long intergenic non-protein coding RNA 641 (LINC00641) is a newly identified non-coding RNA (ncRNA), which has been shown to play an important role in the occurrence and development of tumors in recent years. Studies have shown that LINC00641 is involved in the occurrence and progression of a variety of malignant tumors and plays various roles in different malignant tumors. For example, in non-small cell lung cancer, LINC00641 acts as a tumor suppressor gene and is down-regulated in tissues and cells, and LINC00641 overexpression significantly inhibits cell proliferation and induces apoptosis (11). In contrast, in acute myelocytic leukemia (AML), LINC00641 acts as a cancer promoting gene and is highly expressed in AML samples and cell lines; inhibiting LINC00641 significantly inhibits the proliferation, invasion, and cell cycle arrest of AML cells and induces cell apoptosis (12). However, the function and specific molecular mechanism of LINC00641 in ovarian cancer remains unclear. This study aims to investigate the expression of LINC00641 in ovarian cancer, examine the regulatory effect of LINC00641 on the biological function of ovarian cancer cells at the cellular level, and explore its possible regulatory mechanism. We present the following article in accordance with the MDAR reporting checklist (available at <https://dx.doi.org/10.21037/tcr-21-2314>).

## Methods

### *Clinical sample collection*

This study was approved by the ethics committee of the First People's Hospital of Yancheng (No. 91921626), and the informed consent was signed by the patients or their families. In total, the tumor tissues and normal ovarian tissues of 24 patients with epithelial ovarian cancer were collected. The age range of the enrolled patients was 36–69 years, with an average age of  $54.5 \pm 7.6$  years. The tissue samples of all patients were diagnosed as epithelial ovarian cancer independently by two pathologists. All patients were initial operation patients. The patients did not receive any radiotherapy or other anti-tumor treatment preoperatively. The tissue messenger RNA (mRNA) was extracted and reverse transcribed into complementary DNA (cDNA) for preservation. At the same time, relevant clinical data of patients were collected.

All procedures performed in this study involving human

participants were in accordance with the Declaration of Helsinki (as revised in 2013).

### Cell culture

Human ovarian cancer cell lines (SKOV-3, OVCA-3, A2780, HO8910, and HEY) and normal ovarian epithelial cell (IOSE80) were purchased from the Cell Center of Chinese Academy of Medical Sciences (Beijing, China). The cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (10% fetal bovine serum) supplemented with 100 U/mL penicillin and 100 mg/mL streptomycin. The cells were cultured in a cell incubator with 5% CO<sub>2</sub> and 37 °C saturated humidity, and the cell culture medium was replaced every 2–3 days. The cells were sub-cultured at a rate of 1:2–1:3 based on cell growth conditions. The cells in the logarithmic growth phase were used for subsequent experiments and mRNA extraction.

### Real-time polymerase chain reaction (RT-PCR) assay

The total RNA of the cells was extracted using Trizol reagent (Source Leaf Biology Co., LTD, Shanghai, China). Ovarian cancer cells in the logarithmic phase were taken. One-milliliter Trizol reagent was added to the cells, blown evenly with a pipette, and placed on ice for 5 min to completely separate the nucleoprotein complex. The content and purity of the RNA were determined by NanoDrop spectrophotometer (Thermo Fisher Scientific Inc. USA). RNA purity was qualified when the OD260 nm/OD280 nm value was ranged between 1.8 and 2.0.

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using a two-step method. The reaction conditions were as follows: pre-denaturation at 95 °C for 30 s; 40 cycles of denaturation at 95.0 °C for 5 s and annealing and elongation at 60.0 °C for 30 s; and 72 °C for 30 s. After the reaction, the dissolution curve was generated by the built-in PCR amplifier software. The relative expression of the target gene in each group was analyzed using the  $2^{-\Delta\Delta C_t}$  method, where  $\Delta\Delta C_t = \Delta C_t$  (experimental group) –  $\Delta C_t$  (control group),  $\Delta C_t = C_t$  (target gene) –  $\Delta C_t$  [glyceraldehyde-3-phosphate dehydrogenase (GAPDH)], and  $C_t$  is the number of amplification cycles required for the fluorescence intensity to reach the threshold.

- ❖ LINC00641: forward, 5'-GTAACCTCTATGTACAACGTTAA-3'; reverse, 5'-TAGAAGTCAACTCAT

TATGCTGCTG-3';

- ❖ GAPDH: forward, 5'-GGAATCCA CTGGCGTCTTCA-3'; reverse: 5'-GGTTCACGCCCATCACAAAC-3';
- ❖ miR-320a: forward, 5'-GTTGGATCCGGCGTTTCCTTCCGACATG-3'; reverse, 5'-GCTGAATT CGTCCACTGCGGCTGTTC-3';
- ❖ U6: forward, 5'-CTCGCTTCGGCAGCACA-3'; reverse: 5'-AACGCTTCACGAATTTGCGT-3'.

### Transfection assay

The plasmids [pcDNA-641 and its empty vector (pcDNA-control), miR-320a mimics and its empty vector (control mimics)] were synthesized by Sangon Biotech (Shanghai, China). Ovarian cancer cells in the exponential growth stage were collected. After counting, the cells were seeded and inoculated in a six-well plate at a density of  $5 \times 10^5$  cells per well, and cultured in an incubator at 37 °C with 5% CO<sub>2</sub>. Transfection solution-1 was a mixture of 8 µL Lipofectamine 2000 and 100 µL RPMI-1640 medium, while transfection solution-2 was a mixture of 2 µg plasmid and 100 µL RPMI-1640 medium. After the preparation of transfection solutions 1 and 2, they were incubated at room temperature for 5 min, mixed, and then incubated at room temperature for 20 min. The old medium supernatant was discarded, and RPMI-1640 complete medium was added. Next, the cells were placed in a 37 °C incubator with 5% CO<sub>2</sub> for further culturing. After 4–6 h, the transfection mixture was removed and replaced with fresh Dulbecco's Modified Eagle Medium (DMEM) medium, and the culture plate was subsequently placed in the incubator for 48 h.

### Cell Counting Kit-8 assay (CCK-8)

The cell proliferation activity of each group was detected by CCK-8. Cells in good condition were taken and washed twice with prechilled phosphate buffer saline (PBS) buffer. The cells were digested with 0.25% trypsin and made into single-cell suspension. After counting and adjusting the density, the cells were inoculated into a 96-well plate at a density of  $5 \times 10^3$  cells per well. Each well was set in quintuplicate, and 100 µL medium was added into each well. When the specified time was reached (1, 2, 3, and 4 days), 10 µL CCK-8 solution mixed with 100 µL medium was added into the 96-well plate and cultured for 2 h at 37 °C in dark. The procedure was performed in strict accordance with the instructions of the kit. The absorbance

(OD) at 450 nm was then measured in a microplate reader and the average value was taken. The cell growth curve of each group of cells was drawn based on the results.

### *Colony formation assay*

Cells in good condition were taken and washed twice with prechilled PBS buffer. The cells were digested with trypsin and made into a single-cell suspension. After counting, the cells were seeded into a 96-well plate at a density of 400 cells per well. The six-well plate was also prewashed with prechilled PBS buffer. The plate was shaken gently to disperse the cells evenly, and then placed in a 37 °C incubator with 5% CO<sub>2</sub>. The cell growth was observed on a regular basis and the medium was replaced on time. When visible cell colonies appeared, the culture was stopped. The original culture medium was discarded, and the cells were washed twice with PBS buffer, immersed with an appropriate amount of paraformaldehyde solution, fixed for 15 min, and stained with 4 mL of crystal violet. Next, the dye solution was recovered, and the six-well plate was washed with PBS buffer to remove the excess dye solution. After drying at room temperature, the plate was observed under a microscope, and the cell images were obtained for counting and analysis.

### *Transwell assay*

Cells in good condition were taken and washed twice with prechilled PBS buffer. The cells were digested with trypsin and made into a single-cell suspension with serum free medium. The cells were then transferred to a 15-mL centrifuge tube for counting. The upper chamber of the Transwell was added with 200 µL of cell suspension ( $2 \times 10^5$  cells per well) and serum-free DMEM. Each well was set in triplicate, and bubbles were avoided during the procedure. The Transwells were placed in incubator for further culturing. After 36 h (different culture time for different cells), the culture was stopped, the chamber was removed, and the supernatant and residual liquid was discarded. The plate was washed with PBS buffer three times, and the Matrigel in the upper chamber was removed using a cotton swab. The chambers were placed in a 24-well plate containing 700 µL methanol for 0.5 h. After fixation, the chambers were cleaned with PBS buffer three times. Crystal violet (0.1%) was added and dyed for approximately 30 min to ensure that the crystal violet dye solution could soak into the bottom of the chamber. The chambers were washed

with PBS buffer three times to clean the residual crystal violet dye as much as possible, and the cells were observed under an inverted microscope (Shenzhen Xingming Optical Instrument Co., Ltd., China). The number of cells stained with crystal violet in five visual fields was calculated.

### *Luciferase reporter gene assay*

Firstly, the target genes of LINC00641 were predicted by bioinformatics analysis. It was found that LINC00641 may regulate downstream miR-320a. Next, a dual luciferase assay was used to verify the targeting relationship between LINC00641 and miR-320a. The specific steps were as follows. The LINC00641 RNA with 3'-untranslated region (3'-UTR) was amplified, and the amplified fragment was inserted into the pGL3-promoter vector and co-transfected with the PRL-SV40 plasmid expressing renin fluorescence. The wild-type (Wt) plasmid was pGL3-Wt-LINC00641 3'UTR (LINC00641-Wt), and the mutant (Mut) plasmid was pGL3-Mut-LINC00641 3'UTR (LINC00641-Mut). The Wt plasmid and Mut plasmid were added to 50 µL of serum-free medium with miR-320a mimics and mimics control, respectively. After mixing, the luciferase recombinant vector was added at a ratio of 1:2. Dual luciferase detection was then performed using the dual-luciferase reporter assay system.

### *Western blot*

Cells in a good growth state were collected and added with pre-cooled 100 µL radioimmunoprecipitation assay (RIPA) lysis buffer. The culture bottle was placed on a 4 °C shaking table and gently shaken for 0.5 h. The cell suspension in the culture bottle was then transferred to a 2.5-mL centrifuge tube and centrifuged at 14,000 rpm at 4 °C for 10 min. The supernatant was transferred to a new centrifuge tube, sub packed, and stored in a refrigerator at -80 °C. The Bradford method was used to detect the protein concentration of the extracted total protein sample. Separation gel and concentrated gel were prepared using the conventional method. The samples were added into gel lanes with 60–80 per lane. Electrophoresis was performed using a constant voltage of 40 V (concentrated gel)/100 V (separation gel), until the bromophenol blue reached the edge of the gel. A polyvinylidene fluoride (PVDF) membrane was blocked with 5% skim milk powder at room temperature for 2 h, and the primary antibodies Ki-67 (1:1,000), GAPDH (1:1,000; internal reference),



proliferating cell nuclear antigen (PCNA) (1:800), E-cadherin (1:800), N-cadherin (1:1,000), and vimentin (1:1,000) diluted with 5% bovine serum albumin (BSA) were added respectively for incubation overnight at 4 °C. The membrane was washed four times with tris-buffered saline Tween (TBST), added with a secondary antibody (1:5,000) diluted with 5% BSA, and incubated at room temperature for 1.5 h. The membrane was washed four times again with TBST for 5 min each time, keeping the protein side downward. Liquid A and liquid B of electrochemiluminescence (ECL) developer were mixed in equal volumes and dropped into the PVDF membrane for development. Photographs were taken with the Tanon 5200 (Shenzhen Xingming Optical Instrument Co., Ltd., China).

### Statistical analysis

The data were analyzed using SPSS 19.0. Measurement data were shown as mean  $\pm$  standard deviation (mean  $\pm$  SD); measurement data with a normal distribution were compared using the *t*-test or analysis of variance (ANOVA), while those did not follow a normal distribution were tested using the non-parametric rank sum test. The Chi-square test was used for counting data comparison.  $P < 0.05$  was considered statistically significant.

## Results

### *LINC00641 is under-expressed in ovarian cancer tissues and cell lines*

The expression of LINC00641 in ovarian cancer tissues was analyzed using The Cancer Genome Atlas (TCGA) database. The results showed that LINC00641 was under-expressed in ovarian cancer tissues compared to adjacent tissues (*Figure 1A*). Further analysis showed that the expression of LINC00641 decreased with the increase of pathological grade (*Figure 1B*). Additionally, the expression of LINC00641 in 24 pairs of ovarian cancer tissues and adjacent tissues was detected by qRT-PCR. The detection results were consistent with the TCGA database analysis; that is, LINC00641 was under-expressed in ovarian cancer tissues (*Figure 1C*). It was found that the expression level of LINC00641 in ovarian cancer cells was significantly lower than that in normal ovarian epithelial cells IOSE80, and the expression level in SKOV-3 and HEY cells was the lowest (*Figure 1D*). Therefore, ovarian cancer cell lines SKOV-3 and HEY were selected for subsequent functional

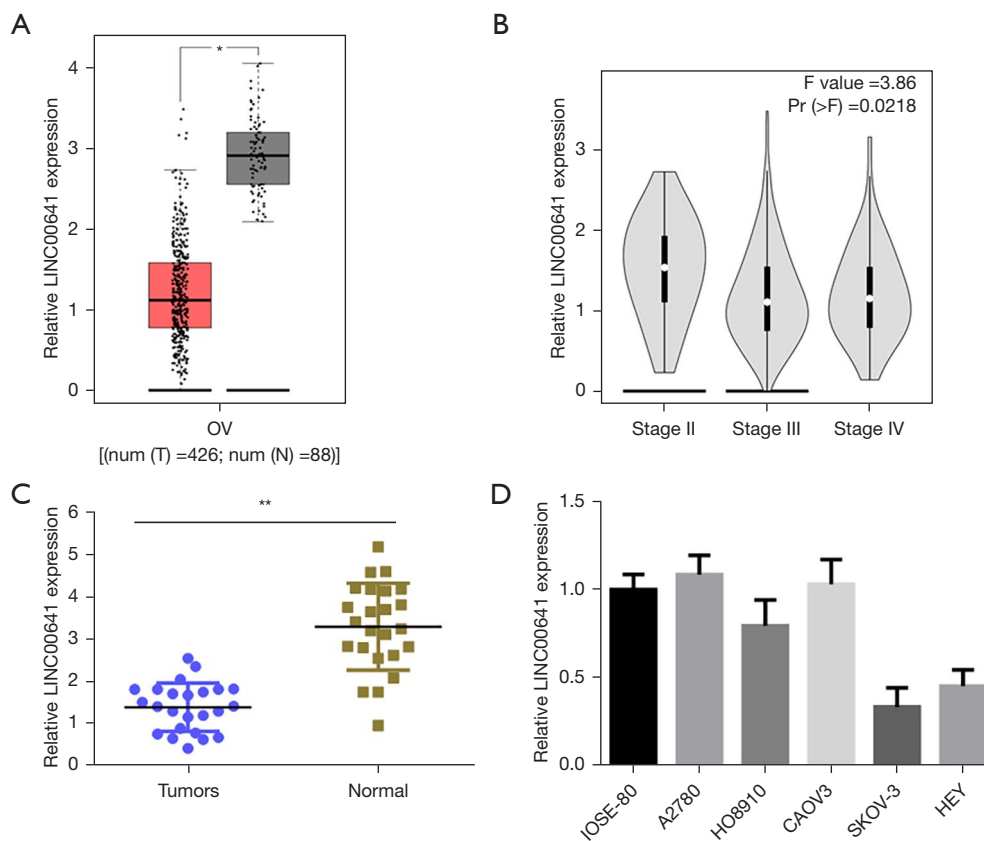
experiments and molecular mechanism studies.

### *Overexpression of LINC00641 inhibits the proliferative and migrative capacity of ovarian cancer cells*

To further study the function of LINC00641 in the occurrence and development of ovarian cancer, the LINC00641 overexpression vector (pcDNA-641) and control vector (pcDNA-control) were constructed first, which were then transfected into SKOV-3 and HEY cells to detect the overexpression efficiency of LINC00641. The results showed that compared to the control vector group (pcDNA-control), transfection of pcDNA-641 significantly increased the expression level of LINC00641 in ovarian cancer cells (SKOV-3 and HEY) (*Figure 2A*). Subsequently, the effect of LINC00641 overexpression on the proliferation of SKOV-3 and HEY in ovarian cancer cells was detected by CCK-8. The results showed that compared to the control vector group (pcDNA-control), overexpression of LINC00641 significantly inhibited the proliferation of ovarian cancer cells (SKOV-3 and HEY) (*Figure 2B*). The colony formation assay showed that compared to the control vector group (pcDNA-control), overexpression of LINC00641 significantly inhibited the colony formation ability of ovarian cancer cells (SKOV-3 and HEY) (*Figure 2C*). In addition, the effect of LINC00641 expression on the migrative ability of ovarian cancer cells was further detected by Transwell assay. The results showed that, compared to the control vector group (pcDNA-control), overexpression of LINC00641 markedly inhibited the migrative ability of ovarian cancer cells (SKOV-3 and HEY) (*Figure 2D*). These results indicated that LINC00641 overexpression significantly repressed the proliferation and migration of ovarian cancer cells.

### *LINC00641 directly regulates miR-320a expression in ovarian cancer cells*

The bioinformatics online prediction software (Starbase, Guangdong, China, <https://starbase.sysu.edu.cn/starbase2/index.php>) found that LINC00641 sequence contains the binding site with miR-320a (*Figure 3A*). Based on the predicted LINC00641 binding sequence with miR-320a, the vector containing the normal sequence (LINC00641-Wt) and the vector containing the mutant base (LINC00641-Mut) were constructed. These were respectively co-transfected into the cells with blank control group (control mimics) and miR-320a mimics to detect luciferase



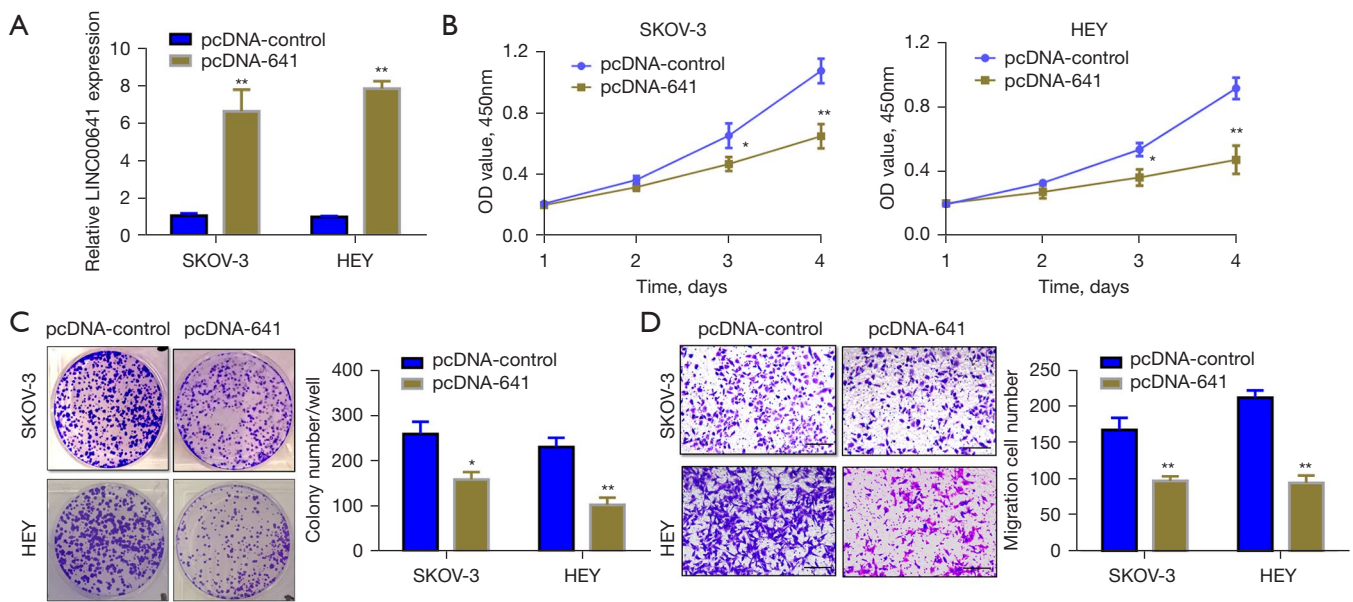
**Figure 1** LINC00641 was under-expressed in ovarian cancer tissues and cell lines. (A) LINC00641 expression in ovarian cancer tissue and adjacent tissue analyzed using TCGA database; (B) correlation between LINC00641 expression and pathological grade of ovarian cancer; (C) LINC00641 expression in 24 ovarian cancer patients and paired adjacent tissue samples detected by qRT-PCR; (D) LINC00641 expression in ovarian cancer cell lines and normal gastric fibroblasts detected by qRT-PCR. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ . TCGA, The Cancer Genome Atlas.

activity. The results showed that transfection of miR-320a mimics and the vector containing the normal sequence (LINC00641-Wt) significantly inhibited the luciferase activity of cells, while the mutant type (LINC00641-Mut) did not have a notable effect (Figure 3B). qRT-PCR was also used to detect the effect of LINC00641 overexpression on the expression of miR-320a. The results showed that compared to the control vector group (pcDNA-control), the expression level of miR-320a was increased in LINC00641-overexpressing ovarian cancer cells (SKOV-3 and HEY) (Figure 3C). In addition, the expression level of miR-320a in ovarian cancer tissue and para-cancer tissue was further detected, and the results showed that miR-320a was highly expressed in ovarian cancer tissue compared to para-cancer tissue (Figure 3D). The above results showed that LINC00641 could directly regulate the expression of miR-320a in ovarian cancer cells.

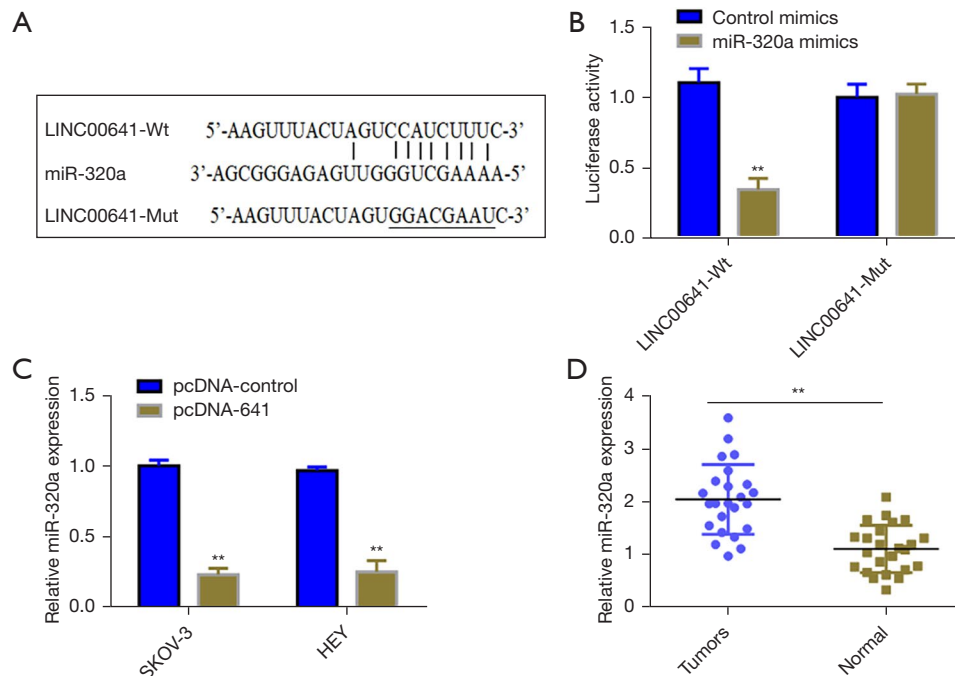
#### ***LINC00641 regulates the proliferation and migration of ovarian cancer cells by targeting miR-320a***

To further determine the effect of LINC00641 on the proliferative and migrative ability of ovarian cancer cells (SKOV-3) by targeting miR-320a expression, miR-320a mimics were transfected into overexpressed LINC00641 ovarian cancer cells, and cell activity was then detected by CCK-8. The results showed that LINC00641 overexpression significantly inhibited the proliferation of ovarian cancer cells (SKOV-3), while miR-320a overexpression (miR-320a mimics) markedly blocked the inhibitory effect of LINC00641 overexpression on the proliferation of ovarian cancer cells (Figure 4A).

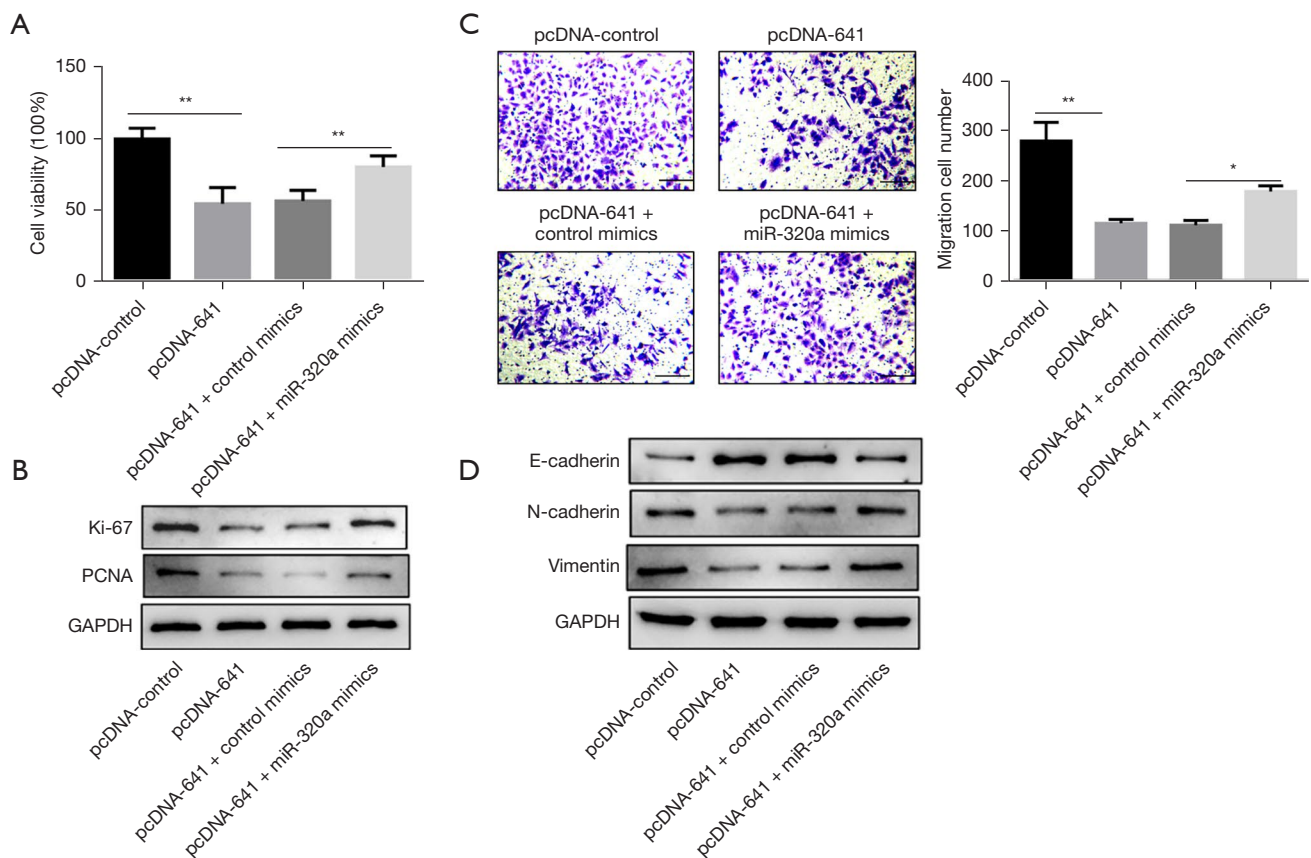
The expression levels of proliferation-related proteins (Ki-67 and PCNA) were detected by western blot, and the results showed that LINC00641 overexpression



**Figure 2** LINC00641 overexpression significantly repressed the proliferation and migration of ovarian cancer cells. (A) qRT-PCR detected LINC00641 overexpression in ovarian cancer cells; (B) CCK-8 detected the effect of LINC00641 overexpression on the proliferation of ovarian cancer cells; (C) colony formation assay detected the effect of LINC00641 overexpression on the colony formation ability of ovarian cancer cells (crystal violet staining); (D) Transwell assay detected the effect of LINC00641 overexpression on the migrative ability of ovarian cancer cells (crystal violet staining). Scale bar: 200  $\mu$ m. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ . qRT-PCR, quantitative real-time polymerase chain reaction.



**Figure 3** LINC00641 targets miR-320a expression. (A) Prediction of regional binding sites between LINC00641 and miR-320a; (B) the luciferase reporter gene detected the binding of LINC00641 to miR-320a; (C) qRT-PCR detected the effect of LINC00641 overexpression on miR-320a expression level; (D) qRT-PCR detected miR-320a expression in 24 ovarian cancer patients and paired adjacent tissue samples. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ . qRT-PCR, quantitative real-time polymerase chain reaction.



**Figure 4** LINC00641 regulates the proliferation and migration of ovarian cancer cells (SKOV-3) by targeting miR-320a expression. (A) CCK-8 detected the effect of miR-320a overexpression on the proliferation of ovarian cancer cells that overexpressed LINC00641; (B) Western blot detected the effect miR-320a overexpression on the expression of Ki-67 and PCNA in ovarian cancer cells that overexpressed LINC00641; (C) Transwell detected the effect of miR-320a overexpression on migration of ovarian cancer cells overexpressing LINC00641 (crystal violet staining, scale bar: 200  $\mu$ m); (D) Western blot detected the effect of miR-320a overexpression on the expression of E-cadherin, N-cadherin, and vimentin in ovarian cancer cells that overexpressed LINC00641. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ . GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PCNA, proliferating cell nuclear antigen.

significantly inhibited the expression levels of Ki-67 and PCNA in ovarian cancer cells (SKOV-3), while miR-320a overexpression (miR-320a mimics) notably blocked the inhibitory effect of LINC00641 overexpression on the expression of Ki-67 and PCNA proteins in ovarian cancer cells (Figure 4B). In addition, the Transwell assay showed that LINC00641 overexpression significantly inhibited the migration of ovarian cancer cells (SKOV-3), and miR-320a overexpression (miR-320a mimics) substantially blocked the inhibitory effect of LINC00641 overexpression on the migration of ovarian cancer cells (Figure 4C). The expression levels of migration-related proteins (E-cadherin, N-cadherin, and vimentin) were further detected by western blot. The results showed that LINC00641

overexpression significantly promoted the expression of E-cadherin and inhibited the expression of N-cadherin and vimentin; meanwhile, miR-320a overexpression (miR-320a mimics) markedly blocked the up-regulation of E-cadherin expression by LINC00641 overexpression and inhibited the expression levels of N-cadherin and vimentin (Figure 4D). The above experimental results indicated that LINC00641 can regulate the proliferation and invasion of ovarian cancer cells by targeting miR-320a.

## Discussion

Ovarian cancer is among the most common malignant tumors of the female reproductive system. It ranks third



(after cervical cancer and endometrial cancer) in terms of incidence, and this trend is increasing every year; however, it has the highest mortality rate (1). Therefore, further elucidation of the formation and metastasis mechanisms of ovarian cancer as well as improving the early diagnosis, targeted therapy, and prognosis of ovarian cancer is the focus of future research. A large number of recent studies have shown that the maladjustment of lncRNAs is closely associated to the occurrence and progression of tumors, which indicates that lncRNAs are expected to become new biomarkers for the early diagnosis, prognosis, and treatment of tumors (6,13). The present study showed that LINC00641 was lowly expressed in ovarian cancer and significantly correlated with the TNM stage of ovarian cancer patients. *In vitro* function experiments showed that LINC00641 overexpression markedly suppressed the proliferation and migration of ovarian cancer cells. Molecular mechanism studies showed that LINC00641 could bind miR-320a, and miR-320a overexpression could notably block the inhibitory effect of LINC00641 overexpression on the proliferation and migration of ovarian cancer cells.

lncRNAs are a class of endogenous ncRNAs with a length of more than 200 nucleotides, accounting for 70% of ncRNAs (14). They were previously considered insignificant, but in recent years, an increasing number of studies have shown that lncRNA is closely associated to the occurrence and development of human diseases, especially the cell processes related to tumor proliferation, differentiation, invasion, and metastasis (15). For example, lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) promotes the proliferation and metastasis of ovarian cancer cells via the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway (16). lncRNA TP73-AS1 promotes the proliferation and metastasis of ovarian cancer cells by regulating the expression of matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9 (MMP9) (17). In this study, it was found that LINC00641 was lowly expressed in ovarian cancer and was closely related to the TNM stage of ovarian cancer patients. Cell function experiments showed that LINC00641 overexpression could significantly inhibit the proliferative and migrative capacity of ovarian cancer cells. Based on the above experimental results, it is inferred that LINC00641 is a tumor suppressor gene in ovarian cancer. However, the underlying molecular mechanism of LINC00641's biological function remains unclear.

Previous studies have shown that lncRNA has no

ability to encode proteins, but is involved in the regulation of protein encoding genes at various levels (epigenetic, transcriptional, and post-transcriptional levels) in the form of RNA (18,19). For example, LINC00641 mainly affects the expression of downstream target genes by regulating the expression of intermediate miRNA; that is, binding miRNA like a sponge, so as to prevent the binding of miRNA with its target molecule mRNA. In gastric cancer, LINC00641 activates oxaliplatin resistance in gastric cancer cells by regulating the expression of miR-582-5p (20). LINC00641 inhibits the proliferation and invasion of glioma cells by targeting the miR-4262/recombinant neurogranin (NRGN) axis, which provides a new potential therapeutic target for the treatment of glioma (21). The present study confirmed that LINC00641 can bind miR-320a to regulate its expression through bioinformatics and luciferase reporter gene; that is, LINC00641 overexpression significantly reduced the expression of miR-320a in ovarian cancer cells. Studies have shown that miR-320a plays an important regulatory role in a variety of tumors as a tumor suppressor or promoter. For example, miR-320a is lowly expressed in melanoma tissues and cells, and miR-320a overexpression can markedly inhibit cell proliferation and migration (22). In gastric cancer, miR-320a inhibits the proliferation of gastric cancer cells by targeting RAB14 (23). However, miR-320a is highly expressed in retinoblastoma cell lines, and significantly inhibits cell proliferation and migration (24). Another study showed that miR-320a is highly expressed in ovarian cancer, and miR-320a overexpression significantly blocks the inhibitory effect of LINC00641 overexpression on the proliferation and migration of ovarian cancer cells (23). The results of the present study are consistent with the previous research. Since miRNA does not encode protein and participates in biological function by regulating the expression of target genes, the downstream genes of miR-320a will be explored in subsequent studies.

In conclusion, LINC00641 was lowly expressed in ovarian cancer and was closely related to the TNM stage. LINC00641 overexpression could significantly inhibit the proliferative and migrative capacity of ovarian cancer cells. Molecular mechanism studies showed that LINC00641 suppressed the proliferation and migration of ovarian cancer cells by targeting miR-320a expression, and miR-320a overexpression significantly blocked the inhibitory effect. Therefore, LINC00641/miR-320a axis may be a new target for the early diagnosis, treatment, or prognosis of ovarian cancer patients.

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## Footnote

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the ethics committee of the First People's Hospital of Yancheng (No. 91921626), and the informed consent was signed by the patients or their families. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013).

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