

Original Article

LINC01127 promotes the development of ovarian tumors by regulating the cell cycle

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Abstract: Background: Ovarian cancer is characterized by the high mortality rate and poor prognosis. Nevertheless, the oncogenesis mechanisms of ovarian cancer remain unclear. In our study, we focused on the potential role of lncRNA LINC01127 in the pathogenesis of ovarian cancer and its underlying mechanism. Methods: LINC01127, which may participate in the development of ovarian cancer, was screened out by bioinformatics analysis. GSEA was used to analyze the function of LINC01127. QRT-PCR was used to analyze the LINC01127 level in 72 cases of ovarian cancer tissues and 53 cases of normal ovarian tissues. LINC01127 level in ovarian cancer cell lines was also determined by qRT-PCR. Subsequently, the selected ovarian tumor cells were transfected with LINC01127 siRNA by Lipofectamine 2000, followed by cell cycle detection using flow cytometry. The regulatory effects of LINC01127 on tumor growth and cell cycle in nude mice were verified by tumor formation assay. The mechanism of LINC01127 involving in cell cycle regulation was further explored by Western Blot. Results: LINC01127 expression in ovarian cancer tissues was significantly higher than that in normal ovary tissues. The expression level of LINC01127 was negatively correlated with the prognosis of patients with ovarian cancer. GSEA analysis showed that LINC01127 was mainly enriched in the regulation of cell cycle. After transfection with LINC01127 siRNA, the proliferative abilities of SKOV3 and H08910 cells were inhibited and cell cycle was arrested at G1/G0 phase. Tumorigenicity assay in nude mice showed that low expression of LINC01127 inhibited the growth of ovarian tumors. Further study found that LINC01127 knockdown upregulated expression levels of Cyclin D, Cyclin E and CDK4, but dramatically upregulated expression levels of P16 and P21. Meanwhile, the AKT and ERK pathways were inhibited by LINC01127 knockdown. Conclusions: LINC01127 was up-regulated in ovarian cancer tissues. LINC01127 may be involved in the development of ovarian cancer by accelerating cell cycle progression through promoting the phosphorylation of ERK and AKT.

Keywords: Ovarian cancer, LINC01127, cell cycle, ERK, AKT

Introduction

Ovarian cancer is a gynecologic malignancy and the high metastatic rate of ovarian cancer cells results in a high mortality. Ovaries locate in the pelvic cavity with a deep anatomical position. Lack of special characteristics and effective laboratory hallmarks in the early stage of ovarian cancer makes it difficult to diagnose as early as possible [1-5]. Long-term survival of early-stage ovarian cancer (stages I and II) can reach to 80% to 95%, compared with only about 30% for advanced ovarian cancer (stages III

and IV). Ovarian malignancies have “two 70%”, indicating that more than 70% of patients with ovarian cancer have been diagnosed at advanced stage at the first diagnosis and about 70% of patients relapse within two years. Therefore, searching for early diagnosis and effective treatment is crucial for improving the survival rate of patients with ovarian cancer.

The “dualism model” of ovarian cancer believed that ovarian cancer can be divided into two categories, high-level and low-level. High-grade epithelial ovarian cancer accounts for 75% of

ovarian cancer incidence and 90% of ovarian cancer mortality. Its prognosis is poor because of the rapid onset, strong aggressiveness and widespread pelvic and abdominal metastasis and planting. The low-grade epithelial ovarian cancer develops slowly and often experiences three development processes, including benign, borderline and low-grade malignancy. Therefore, high-grade epithelial ovarian cancer is a malignant tumor that threatens the health of women due to its high incidence of invasion and metastasis [6-10]. However, the specific mechanism of high-grade epithelial ovarian cancer remains unclear.

With the recent development of high-throughput sequencing technologies, about 70-90% of genes in the human genome have been found to be able to transcribe into RNAs. However, only 1-2% of the RNAs can eventually be translated into proteins, that is, most of the RNAs do not have the ability of encoding protein. These RNAs without the potential of protein encoding are named as non-coding RNAs (ncRNAs). They were originally thought to be the noise or trash products produced by transcription. As the research progressed, people gradually came to realize that these ncRNAs show significant roles in multiple processes of pathophysiology. ncRNAs can be divided into two types according to their molecular size: short non-coding RNAs (such as miRNAs, siRNAs and piRNAs) and long non-coding RNAs (lncRNAs). In contrast to short ncRNAs, lncRNA has been started to explore only in recent years.

lncRNAs are a class of ncRNAs larger than 200 nt in length. lncRNA is capable of regulating expressions of downstream genes at the levels of chromatin modification, transcription or post transcription. Functionally, they could further participate in various biological processes such as cell proliferation, apoptosis and invasion, and more importantly, plays an important role in the occurrence and development of diseases such as tumors. Usually, differentially expressed lncRNAs in tumor tissues or cell lines are searched using gene chip or high-throughput sequencing technology. Yu et al found differentially expressed lncRNAs in the gene expression profiles of lncRNAs in renal clear cell carcinoma and para-cancerous tissues using gene chip, laying the foundation for subsequent studies [11]. Other scholars searched for differen-

tially expressed lncRNAs in liver cancer and gastric cancer using the similar methods as well [12, 13]. In recent years, differentially expressed lncRNAs in ovarian cancer have also been well explored.

In our study, four microarrays were jointly analyzed to screen out the differentially expressed lncRNAs between ovarian cancer and normal ovarian tissues. As a result, LINC01127 was selected for subsequent expression verification and mechanism studies.

Materials and methods

Data acquisition and analysis

Data of four expression profiling microarrays concerning ovarian cancer (GSE18520, GSE38666, GSE40595, GSE52037) were obtained from the Gene Expression Omnibus database (GEO, <http://www.ncbi.nlm.nih.gov/geo>). GSE18520 contains 53 cases of ovarian tumor tissues and 10 cases normal tissues [14]; GSE38666 contains 20 normal tissues and 25 ovarian tumor tissues [15]; GSE40595 contains 8 normal ones and 69 ovarian cancer ones [16]; GSE52037 contains 10 normal ones and 10 cases of ovarian cancer tissues [17]. Differently expressed lncRNAs between ovarian cancer tissues and normal tissues were screened using Limma R package allowing the comparisons of two sets of samples under the same experimental conditions [18]. Adjusted *P* values (adj.*P*) were used to correct the false-positive results using Benjamini and Hochberg methods. $P < 0.05$ and $|\log FC| \geq 1$ were assigned as the cut-off values. The RankProd method was used to integrate the four chips analysis.

Tissue collection

From 2012 to 2017, a total of 125 participants were analyzed in this study. Among them, 72 underwent resection of primary ovarian cancer at the First Affiliated Hospital of Nanjing Medical University, while the rest were participants without ovarian cancer. The ovarian tumor tissues and the normal tissues were immediately frozen in the liquid nitrogen and further stored at -80°C . Tumor staging and grading were in accordance with the FIGO guidelines. Complete follow-up data were obtained from each participant for calculating the survival time from the

day of the first surgery until their death or the last day of follow-up. This investigation was approved by the Ethics Committee of First Affiliated Hospital of Nanjing Medical University. The written informed consent had been signed by all patients.

RNA extraction and real-time quantitative PCR

Total RNA of tissues or cultured cells were extracted by the TRIzol reagent. The cDNA was synthesized by reverse transcription according to Takara PrimeScript RT Master Mix instructions. QRT-PCR was performed according to the manufacturer's instructions. GAPDH was used as the internal control. The primers were listed below: GAPDH (forward): 5'-CACCACTCCTC-CACCTTTG-3'; GAPDH (reverse): 5'-CCACCAC-CCTGTTGCTGTAG-3'; LINC01127 (forward): 5'-GCTTTTCTCGCTATGC TGCC-3'; LINC01127 (reverse): 5'-GTTTGCCATTGGGTGGTCC-3'.

TCGA database

The lncRNA sequencing data for ovarian cancer was downloaded at TCGA, containing a total of 379 ovarian cancer samples. The dataset types were Illumina HiSeq mRNASeq (isoforms) and Illumina HiSeq RNASeqV2 (genes), and all data were normalized by the MD Anderson Cancer Center. The clinical data of ovarian cancer patients with stage 3 were downloaded from the TCGA database data.

Gene enrichment analysis

Gene set enrichment analysis (GSEA) was performed using GSEA software (version 2.2.1). The c2.cp.kegg.v5.1.symbols.gmt dataset was obtained from the GSEA website MsigDB database. Enrichment analysis was performed by default weighted enrichment statistics, with the random combinatorial count set as 1,000. Gene sets were judged as significantly enriched by $P < 0.05$ as well as false discovery rates (FDR) < 0.25 in GSEA.

Cell culture

Human ovarian cancer cell lines IOSE-386, OMC685, SKOV3 and H08910 were purchased from ATCC. Cells were cultured in DMEM containing 10% FBS, 1 units/ml penicillin and 100 μ l/ml streptomycin at 37°C in a 5% CO₂ incubator.

Transfection of cells

Cells were seeded into 6-well plates one day prior to transfection. Until 60-80% of cell confluency, small interfering RNA and Lipofectamine 2000 were diluted in opti-MEM for 5 min. Diluted reagents were gently mixed and maintained for another 20 min. After that cells were added with the mixture and incubated for 4-6 hours. Cells were harvested for further analyses 24-48 h after transfection. Sequences of siRNAs were listed below: si-LINC01127-1: GCUUGAAUGAGCAUGGAAUTT; si-LINC01127-2: GGAGCUCGAUCAGGCAAUUTT; si-LINC01127-3: GCACUUGAGUUUACACCUUTT.

Flow cytometry

Cells were digested, washed with cold PBS, fixed with 70% cold ethanol and stored at -20°C for at least 48 h. Before flow cytometry determination, fixed cells were washed and resuspended in 1 ml PBS containing 10 mg/ml RNase A, and then incubated for 1 hour at 37°C. Cell suspensions were stained with propidium iodide solution (100 μ g/ml) in dark for 30 min. For each sample, 10000 events were acquired, and cell cycle was determined by a FACS flow cytometer.

Tumor formation experiment

SKOV3 cells were stably transfected with sh-RNA or negative control, followed by PBS wash twice and preparation of single cell suspension at 1.5×10^8 cells/ml. Cells were injected into the loose connective tissues in the subcutaneous layer of the left lumbar hip of nude mice (100 μ l). The sh-LINC01127 targeting sequence was GGAAGTCTGTCAAGTGCATTC. One week after injection, visible tumors could be observed. The maximum and the minimum diameter of the tumor were calculated and represented as a and b respectively, on the 6th, 9th, 12th, 15th, 18th and 21st days after subcutaneous injection using a vernier caliper. Tumor volume was calculated according to the formula $V = 1/2ab^2$ and the subcutaneous xenograft tumor growth curve in nude mice was drawn.

Immunohistochemistry

Slides of the xenograft tumors were pretreated for staining. Briefly, slides were blocked with goat serum and incubated with diluted primary

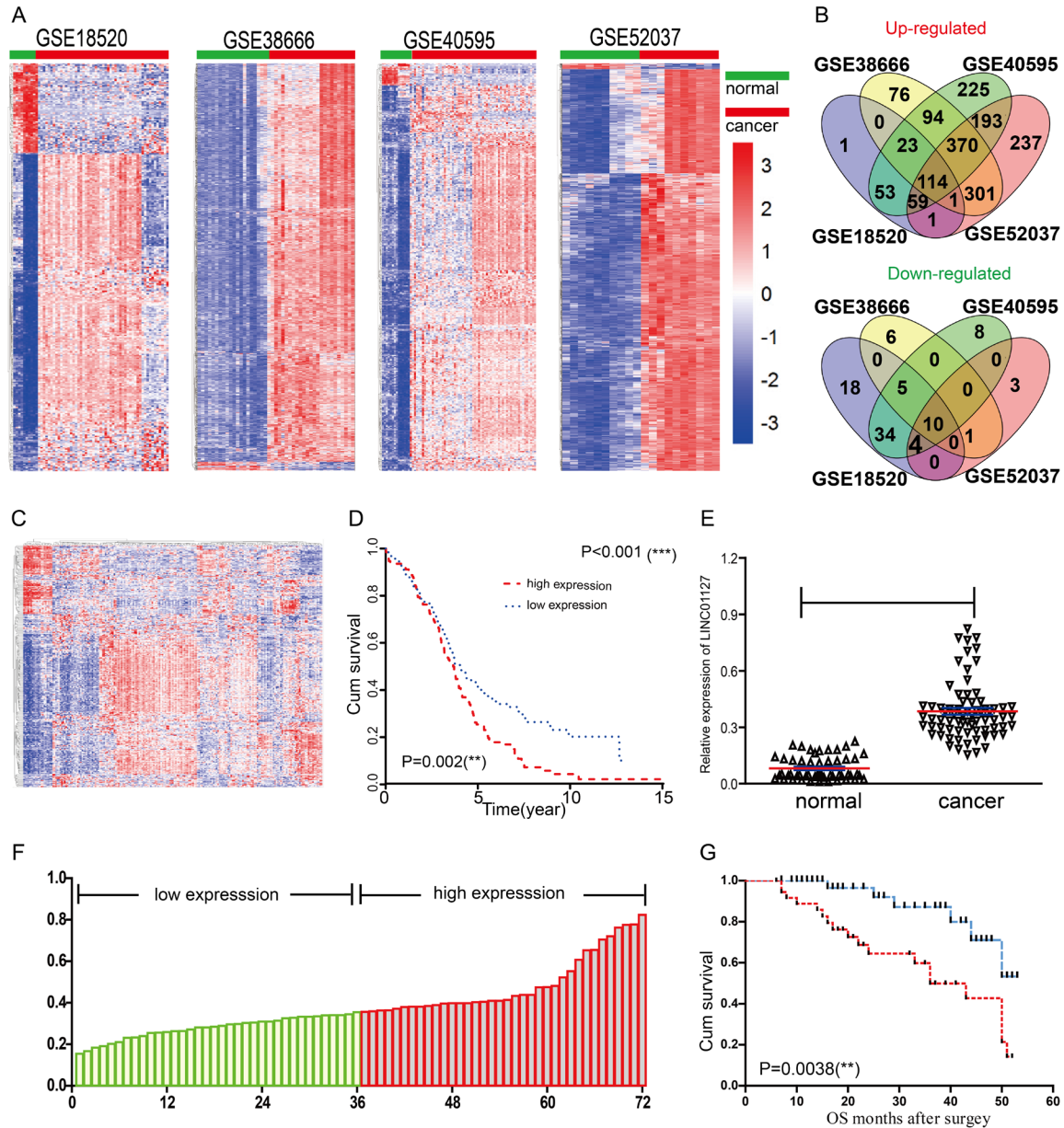


Figure 1. LINC01127 is highly expressed in ovarian tumors and is negatively correlated with the prognosis. A. Hierarchical clustering analysis of lncRNAs that were differentially expressed (fold change >2 ; $P<0.05$) in ovarian tumors and normal tissues in GEO datasets. B. Co-upregulated and co-downregulated long non-coding RNAs in GEO datasets. C. Integration analysis of the 4 databases was performed using the Rank Prod method. D. Analysis of TCGA database showed that patients with high levels of LINC01127 expression showed worse prognosis (log-rank test). E. QRT-PCR of clinical samples showed that the expression levels of LINC01127 in ovarian tumor tissues were significantly higher than those in non-tumorous tissues. F. Ovarian tumor patients were divided into high expression group and low expression group according to the median of LINC01127 expression. G. LINC01127 expression was negatively correlated with the prognosis of patients with ovarian cancer. The mean values and SD were calculated from triplicates of a representative experiment, $*P<0.05$.

antibodies overnight at 4°C. In the next day, the slides were washed with PBS, incubated with secondary antibodies for 2 h in a 37°C incubator. Subsequently, slides were stained with DAB for 1-5 min and washed in distilled water. After that, the slides were re-stained with the hema-

toxylin solution for 3-5 min, and dehydrated by gradient concentrations of ethanol. Lastly, the slides were soaked in the xylene solution twice for 10 min and sealed. The images were observed and photographed with a digital slice scanner.

Statistical analysis

SPSS software was used to analyze statistical data. Chi-square test analysis and Fisher exact probability method were used for the correlation analysis of clinicopathological parameters and difference between groups. Survival analysis using Kaplan-Meier and Log-rank test. $P < 0.05$ was considered statistically significant.

Results

LINC01127 is aberrantly expressed in ovarian tumors and negatively correlated with the prognosis of ovarian cancer

The expression of long non-coding RNAs in 157 cases of ovarian cancer patients were detected according to ovarian cancer expression data downloaded from gene expression omnibus accession GSE18520, GSE38666, GSE40595 and GSE52037. Through lncRNA microarray analysis, the differently expressed lncRNAs in these databases were searched (**Figure 1A**). By the Wayne Map analysis, we found 114 co-upregulated lncRNAs and 10 co-downregulated lncRNAs (**Figure 1B**). In addition, we integrated the four databases through the RankProd method analysis (**Figure 1C**). Combined with the co-altered lncRNAs, LINC01127 was finally selected for further study.

Analysis of TCGA database including the clinical information and lncRNA expressions of 379 patients with ovarian cancer showed that LINC01127 was associated with the prognosis of this disease. LINC01127 expression was negatively correlated to survival time of patients with ovarian cancer (**Figure 1D**). In addition, we detected the expression of LINC01127 in ovarian cancer and non-tumor ovarian tissues. QRT-PCR results indicated that the expressions of LINC01127 in ovarian cancer tissue were significantly higher than those in normal ovarian tissues (**Figure 1E**). Based on the median value of LINC01127 level, ovarian cancer samples were divided into high and low expression groups (**Figure 1F**). The result indicated that the LINC01127 high expression group had shorter survival time than the low expression group ($P = 0.0038$) (**Figure 1G**). These results demonstrated that LINC01127 was highly expressed in ovarian tumors and its expression

was negatively correlated with the prognosis of ovarian tumors.

LINC01127 mainly regulates cell cycle in vitro

To further investigate the function of LINC01127 in ovarian cancer, the GSE18520, GSE38666, GSE40595 and GSE52037 from GEO (including 48 normal tissues and 157 ovarian cancer tissues) were merged by the InslicoMerging R package. GSEA analysis was applied to investigate the relationship between LINC01127 and gene signatures in those above data. Results showed that cell cycle-related genes were significantly changed in the LINC01127 high expression group (GEO analysis in **Figure 2A** and **2B**; TCGA analysis in **Figure 2C** and **2D**), suggesting that LINC01127 might regulate the cell cycle *in vitro*.

Interference with LINC01127 blocks the cell cycle in G0/G1 phase

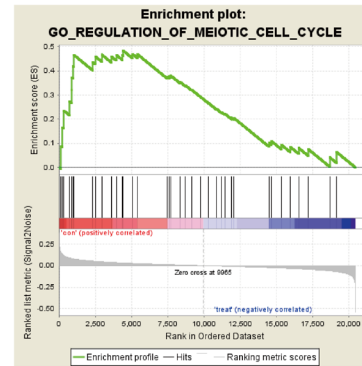
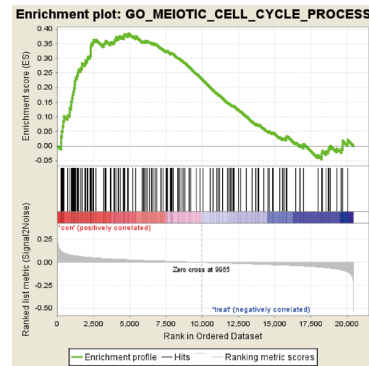
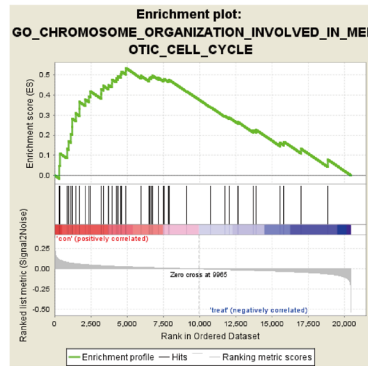
We detected LINC01127 level in ovarian cancer cell lines IOSE-386, OMC685, SKOV3 and HO8910 by qRT-PCR and found that LINC01127 was highly expressed in SKOV3 and HO8910 cell lines (**Figure 3A**). Therefore, SKOV3 and HO8910 were selected for subsequent *in vitro* analysis. To explore the role of LINC01127 in ovarian tumors, siRNA was used for exogenously knockdown of LINC01127 in SKOV3 and HO8910 cell lines (**Figure 3B**). Flow cytometry was applied to detect the cell cycle after LINC01127 knockdown in SKOV3 and HO8910 cells. As shown in **Figure 3C** and **3D**, cells were blocked in G0/G1 phase after interfering with LINC01127. Besides, LINC01127 knockdown decreased the cells ratio in the S phase (**Figure 3C** and **3D**). These data demonstrated that LINC01127 could block the cell cycle in the G0/G1 phase.

LINC01127 regulates the tumor growth in vivo

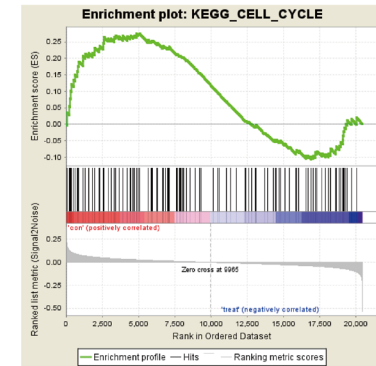
To further explore whether LINC01127 could regulate tumorigenesis of ovarian cancer *in vivo*, SKOV3 cells stably transfected with sh-LINC01127 or empty vector were injected to nude mice, respectively. The subcutaneous xenograft tumor growth curve in nude mice was drawn according to the tumor volume recorded on different time points after subcutaneous injection. Results showed that the growth of subcutaneous xenografts in nude mice with

LINC01127 promotes tumorigenesis of ovarian tumors

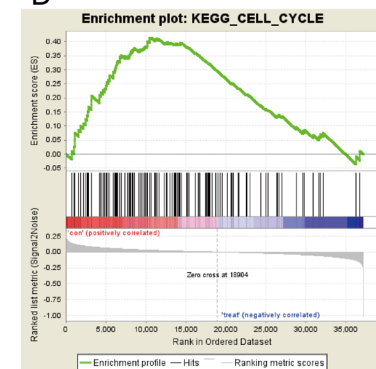
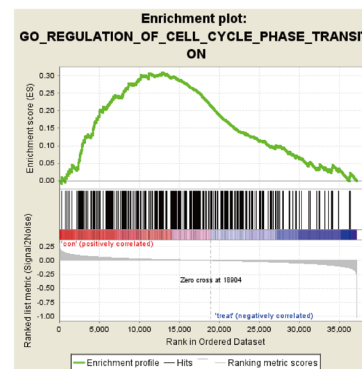
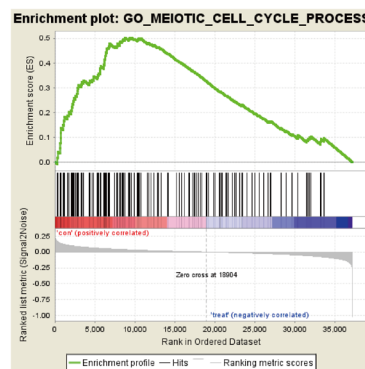
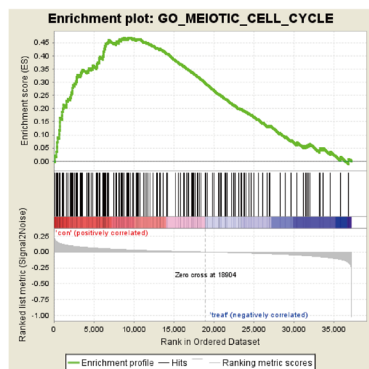
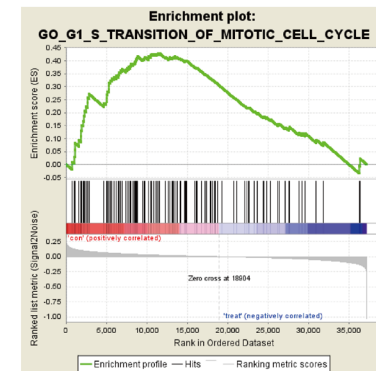
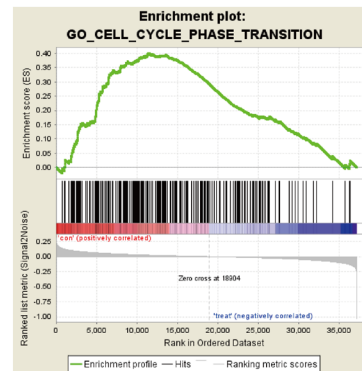
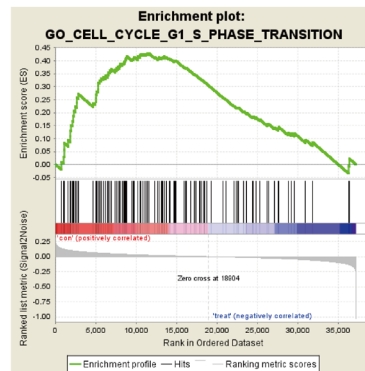
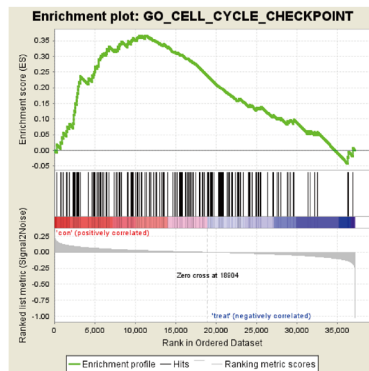
A



B



C



D

LINC01127 promotes tumorigenesis of ovarian tumors

Figure 2. LINC01127 is associated with ovarian cancer cell cycle. A and B. GSEA analysis of integrated GSE18520, GSE38666, GSE40595 and GSE52037 through the InslicoMerging method indicated that the main regulatory biological process of LINC01127 was the cell cycle; C and D. GSEA analysis based on the TCGA database also indicated that the main regulatory biological process of LINC01127 was the cell cycle. The enrichment score (ES, green line) indicates the degree to which the gene set is over represented at the top or bottom of the ranked list of genes.

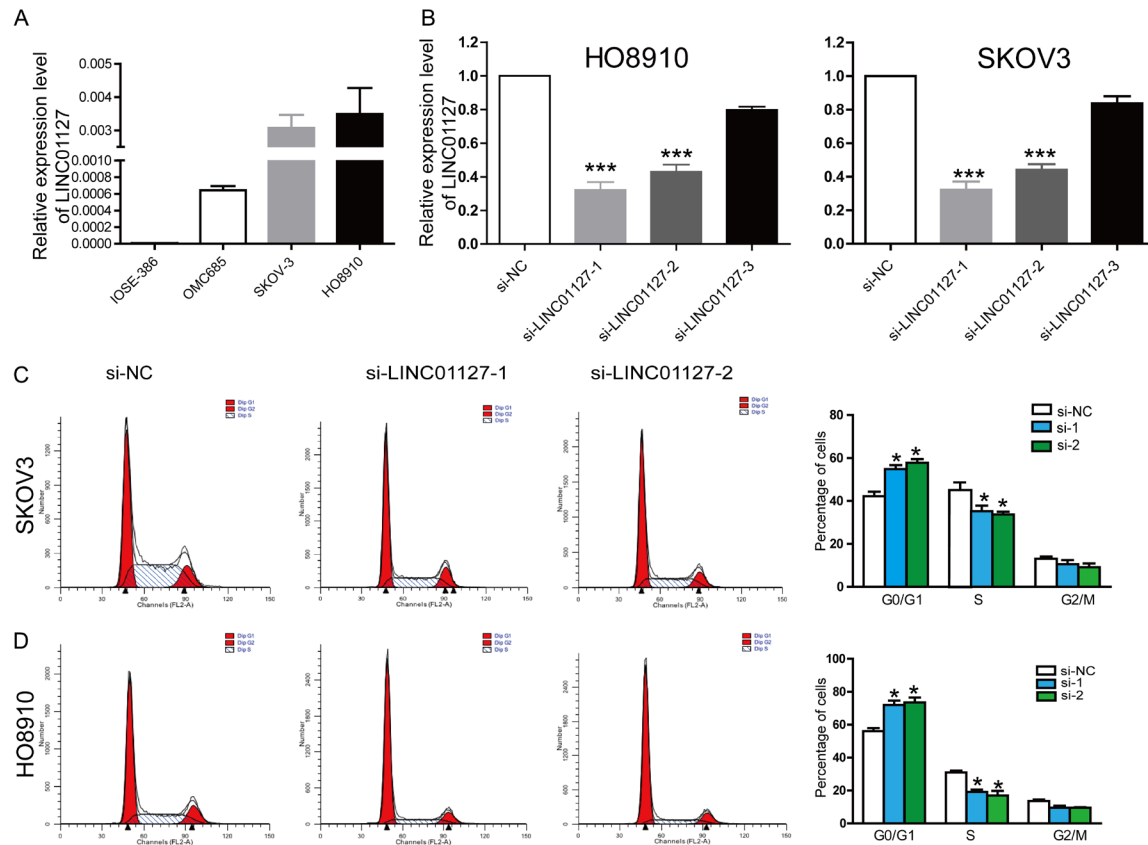


Figure 3. Interference with LINC01127 blocks the ovarian cancer cell cycle in G0/G1 phase. A. QRT-PCR analysis showed that LINC01127 was overexpressed in ovarian tumor cell lines compared with the normal cell lines; B. The efficiency of small interference RNAs was detected by sequences qRT-PCR; C and D. The cell cycle distribution of HO8910 and SKOV3 cells after LINC01127 interference was analyzed by flow cytometry. The mean values and SD were calculated from triplicates of a representative experiment, * $P < 0.05$.

LINC01127 knockdown was markedly slower compared to that of the control group (Figure 4A and 4B). Mice were then sacrificed for measuring the volume of tumor tissues. It was found that the volume of the tumor in the sh-LINC01127 group was significantly smaller than the control group. Meanwhile, tumor weight in the sh-LINC01127 group was markedly lower than the control group (Figure 4C). QRT-PCR analysis confirmed that LINC0027 level in the tumor tissues of the sh-LINC0027 group was significantly lower than that in the control group (Figure 4D). The xenograft tumor tissues were sectioned for immunohistochemistry analysis and the results revealed that the tumor proliferation markers PCNA and Ki-67 significantly

decreased in the LINC0027 interference group. Besides, expression levels of cell cycle promoting factors Cyclin D, Cyclin E and CDK4 in LINC01127 knockdown group significantly decreased, while expression levels of cell cycle inhibiting factor P16 and P21 markedly increased compared the control group (Figure 4E). In sum, these findings indicated that LINC01127 could regulate the tumor growth *in vivo*.

The regulatory mechanism of LINC01127 in cell cycle

To validate the effects of LINC01127 on cell cycle and its underlying mechanism, SKOV3 and HO8910 cells were transfected with si-

LINC01127 promotes tumorigenesis of ovarian tumors

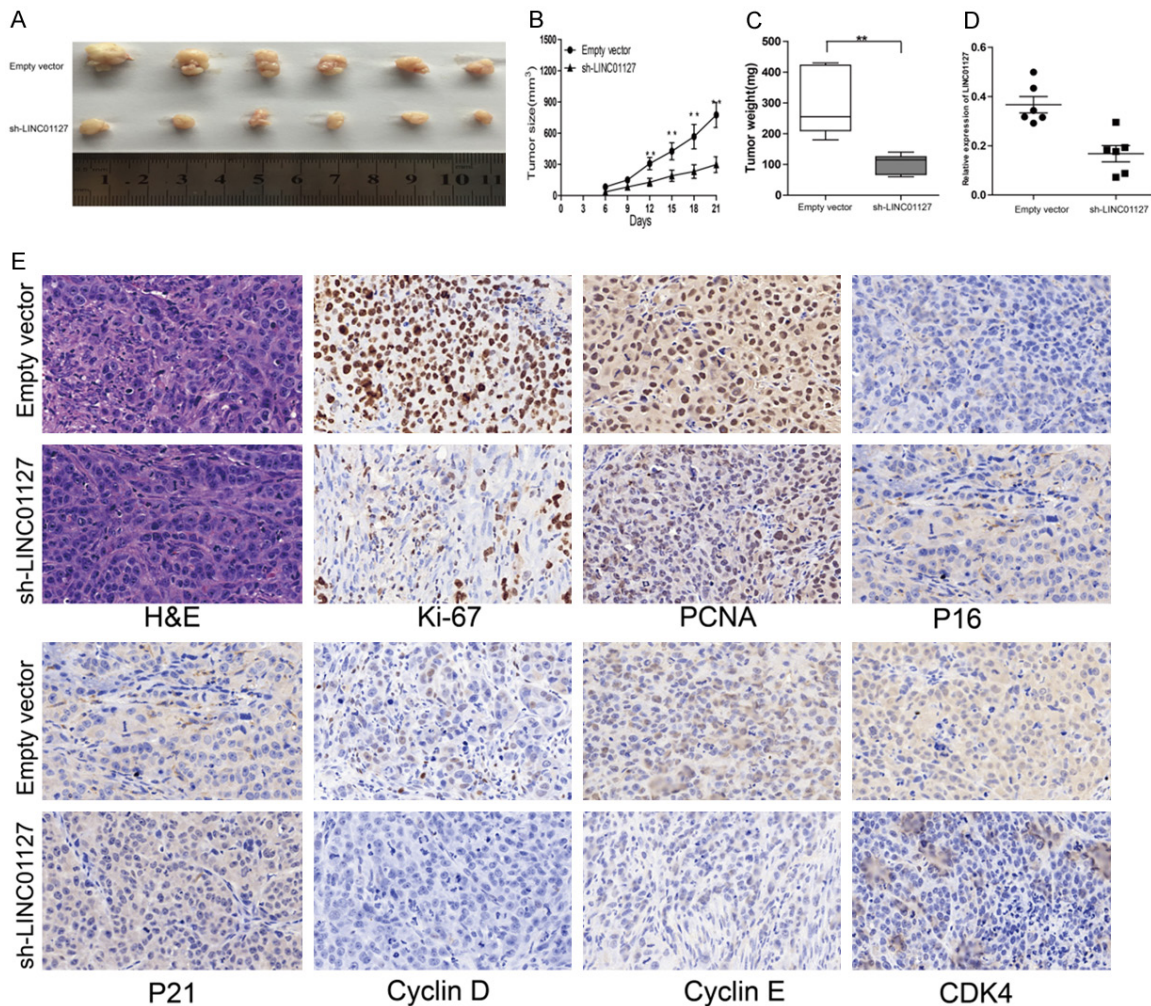


Figure 4. LINC0027 regulates the ovarian cancer cell growth *in vivo*. A and B. sh-LINC01127 and empty vector were stably transfected into the ovarian cells and then injected to nude mice respectively. The subcutaneous xenograft tumor growth curve in nude mice was drawn according to the tumor volume recorded on the 6th, 9th, 12th, 15th, 18th and 21st days after subcutaneous injection. C. Tumor weights were represented. D. The expression level of LINC01127 in both groups were detected by qRT-PCR; E. The tumor sections of each group were stained with tumor proliferation markers Ki-67 and PCNA, cell cycle promoting proteins Cyclin D, Cyclin E and CDK4 and cell cycle inhibiting proteins P16 and P21 by Immunohistochemistry (magnification 400 ×). The mean values and SD were calculated from triplicates of a representative experiment, **P*<0.05.

LINC01127. Expressions of Cyclin D, Cyclin E, CDK4 as well as P16, P21 were detected. The results showed that LINC01127 interference markedly decreased the expressions of cell cycle promoting factors Cyclin D, Cyclin E and CDK4, while the expressions of cell cycle inhibiting factors P16 and P21 were significantly upregulated (Figure 5A). It has been reported that AKT and ERK pathways play important roles in tumorigenesis and cell cycle regulation. Therefore, we detected the expressions of phosphorylated AKT and ERK as well as total AKT and ERK after LINC01127 knockdown. The results showed that AKT and ERK pathways

were significantly inhibited in the LINC01127 interference group, suggesting that LINC01127 may regulate the cell cycle and participate in the ovarian cancer through the AKT and ERK pathways (Figure 5B). Our *in vitro* data therefore demonstrated that LINC01127 regulated the cell cycle of ovarian cancer through the AKT and ERK pathways.

Discussion

Ovarian cancer ranked the fifth of the cancer-related deaths in women [19]. It is expected that the new cases of ovarian cancer account-

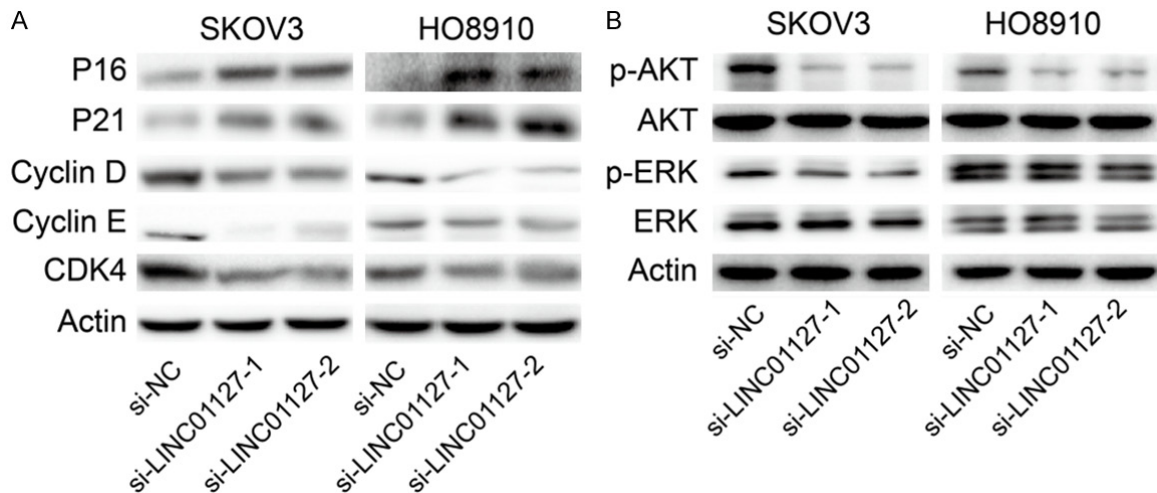


Figure 5. The regulatory mechanism of LINC01127 in ovarian cancer cell cycle. A. The expressions of Cyclin D, Cyclin E and CDK4 as well as P16 and P21 after LINC01127 interference by siRNA were detected by Western blot; B. The expressions of phosphorylated AKT and ERK as well as total Akt and ERK pathways were detected after LINC01127 interference by siRNA were detected by Western blot. The mean values and SD were calculated from triplicates of a representative experiment, * $P < 0.05$.

ed for 2.63% of all new cases of female tumors in the United States in 2017. In addition, the incidence of ovarian cancer accounted for 20.9% of the female reproductive system tumors and 44.6% of the tumor-related deaths of the reproductive system [20], which make it a serious threat to women's life and health. Most ovarian cancer cells are phenotypically distinct from normal cells of ovary. Epithelial tumors account for 85-90% of ovarian malignancies. The types and origins of ovarian cancer are diverse and pose challenges to the experimental study of its pathogenesis. The most common precursor cells of ovarian tumor do not exclusively originate from ovarian cells. Lack of obvious symptoms and signs or effective screening and diagnostic methods in the early stage of epithelial ovarian cancer makes more than 60% of patients who have been diagnosed in advanced stage at the first time of diagnosis. The 5-year survival rate of advanced ovarian cancer was only 27%. More seriously, there is rare improvement in the overall survival rate for decades, posing great threatens on females.

LncRNA is a relatively new research field compared with the small molecule non-coding RNAs such as miRNAs, siRNAs and piRNAs. The number of lncRNA is very large and is estimated to exceed that of protein-coding genes. Accumulating literatures have confirmed that

lncRNAs are involved in several biological processes such as cell proliferation, apoptosis and invasion. They are related to the occurrence and development of diseases such as tumors. It was reported that HOST2 [22], PVT1 [23], CCAT2, NBAT-1 [25] and some other lncRNAs are involved in the development of ovarian cancer. In our study, we analyzed the published microarray data of ovarian cancer and LINC01127 was found to be possibly related to the occurrence and development of ovarian cancer. LINC01127 was found to be negatively correlated with its prognosis. According to GSEA analysis, LINC01127 was mainly enriched in the regulation of cell cycle. Additionally, a series of experiments confirmed that downregulation of LINC01127 was able to inhibit tumor growth.

The cell cycle is the process of eukaryocyte continues to divide from the end of one mitosis to the next. It is a cycle of cell material accumulation and cell division. Abnormal division cycles are frequently seen in cancerous cells [25]. Therefore, regulation of tumor cell cycle is an important strategy and target of tumor therapy. Cyclin-dependent kinases (CDKs) are major factors in the regulation of cell cycle progression and mainly involved in the regulation of DNA replication as well as chromosome segregation during cell cycle progression [27]. Cyclin is an evoked factor in the cell cycle and can

activate the activities of the corresponding downstream ligands, which prompts cells to pass through each checkpoint in cell cycle to form abnormally separated conditions. Cyclin D1 and Cyclin E1 are key checkpoint proteins from G1 phase to S phase in cell cycle, and can target the downstream pRB protein thus regulating its expression [28]. During DNA replication, Cyclin E1 is a key protein in the process where MCMs anchor into the DNA replication complex to promote DNA replication. MCMs anchoring is abnormal in Cyclin E1-deficient fibroblasts, leading to cell cycle arrest in G1 phase [29]. Cyclin D1 plays a major role in transcriptional regulation and DNA repair during cell cycle progression [30]. Cyclin D1, on the one hand, can activate transcription mediated by non-estrogen-dependent estrogen receptor mainly through the activation of the protease A-dependent signaling pathway. On the other hand, it can inhibit NRF-mediated transcription by regulating the activities of CDK4 and CDK6 [31]. In this study, we found that LINC01127 knockdown in ovarian cancer cells can significantly induce the cell cycle arrest at G1 phase, thereby suppressing the cell proliferation. In a preliminary study about the molecular mechanism, we found that the expressions of Cyclin E1 and Cyclin D1 were significantly inhibited by the downregulation of LINC01127 expression. Our results were consistent to the fact that they are the key checkpoint proteins in the process of cells entering the S phase from G1 phase.

Extracellular signal-regulated kinase (ERK), a member of the mitogen-activated protein kinase (MAPK) family, controls multiple biological reactions including cell proliferation and differentiation, cell morphology, apoptosis and malignant transformation [32]. ERK over-activation can be found in many human malignancies, accepting signals such as growth factors, mitogen, environmental stimuli. Nuclear translocation of ERK upon activation by the ERK signaling cascade further regulates gene expressions through acting on nuclear transcription factors such as transcription factor AP-1, NF- κ B and other regulatory genes. The ERK pathway is regarded as a new target for cancer therapy [33, 34]. In this study, the results of Western blot showed that low expression of LINC01127 could reduce the expression of p-ERK protein. Therefore, it was estimated that the low expression of LINC01127 may inhibit the phosphorylation of ERK protein in ovarian cancer cells,

thereby inducing cell cycle arrest in G1 phase and then inhibiting cell proliferation.

The PI3K/Akt/mTOR signaling pathway has aroused wide concern nowadays. The family of phosphoinositide-3-kinase (PI3K) in this pathway involves extensively in several cellular functional activities including growth, proliferation, migration and survival. Akt, an evolutionarily conserved serine/threonine kinase, is one of the major protein kinases that is highly activated in human cancer. mTOR is the primary regulator of cellular breakdown and anabolism and can be inhibited via combined effects of starvation and molecular targeting agents. Subsequently, co-regulation by inactivation of p70 S6K and decreased phosphorylated level of 4E-BP1 (a major downstream inhibitor of mTOR) induce cell cycle arrest in G1 phase [35]. In this study, the decreased expression of LINC01127 may inhibit the proliferation of ovarian tumor cells by suppressing the phosphorylation of AKT protein and inducing the cells arrest in G1 phase.

In summary, our research for the first time confirmed that down-regulation of LINC01127 expression can cause cell cycle arrest of ovarian tumor cells. The mechanism of its regulation may be through cell cycle arrest in the G1/G0 phase by inhibition of phosphorylated ERK and AKT, so as to participate in the development of ovarian cancer.

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Disclosure of conflict of interest

None.

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