# MALAT1 affects ovarian cancer cell behavior and patient survival

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**Abstract.** Epithelial ovarian cancer (EOC) is one of the most lethal malignancies of the female reproductive organs. Increasing evidence has revealed that long non-coding RNAs (lncRNAs) participate in tumorigenesis. Metastasis associated lung adenocarcinoma transcript 1 (MALAT1) is an lncRNA and plays a role in various types of tumors. However, the function of MALAT1 on cellular behavior in EOC remains unclear. The current study explored the expression of MALAT1 in ovarian cancer tissues and in EOC cell lines. Quantitative RT-PCR analysis revealed that the expression of MALAT1 was higher in human ovarian malignant tumor tissues and EOC cells than in normal ovarian tissues and non-tumorous human ovarian surface epithelial cells, respectively. By analyzing the online database Kaplan-Meier Plotter, MALAT1 was identified to be correlated with the overall survival (OS) and progression-free survival (PFS) of patients with ovarian cancer. Furthermore, knockdown of MALAT1 by small interfering RNA (siRNA) significantly decreased EOC cell viability, migration, and invasion. Finally, dual-luciferase reporter assays demonstrated that MALAT1 interacted with miR-143-3p, a miRNA that plays a role in EOC as demonstrated in our previous study. Inhibition of MALAT1 resulted in an increase of miR-143-3p expression, leading to a decrease of CMPK protein expression. In conclusion, our results indicated that MALAT1 was overexpressed in EOC. Silencing of MALAT1 decreased EOC cell viability and inhibited EOC cell migration and invasion. These data revealed that MALAT1 may serve as a new therapeutic target of human EOC.

## Introduction

Ovarian cancer (OC) is one of the most lethal malignancies of the female reproductive organs, in the world. The estimated number of new cases of OC was 22,440 and the estimated mortality was 14,080, accounting for ~5% of the 282,500 cancer-related deaths of females in the United States in 2017 (1). The most frequent type of OC is epithelial ovarian cancer (EOC), which accounts for ~85% of total OCs (2). In spite of developments in surgery, chemotherapy, and radiotherapy in the past decades, the overall survival (OS) rate of patients with EOC at the late stage is a consistently poor and unfavorable prognosis (3,4). It is also characterized by a high probability of drug resistance, leading to treatment failure and death in the majority of patients with distant metastasis (5). Nevertheless, the underlying molecular mechanisms for tumorigenesis, tumor progression, metastasis, and chemoresistance remain unclear. Therefore, it is necessary to acquire a better understanding of the targeted molecules involved in EOC and to find new therapeutic strategies for effective and sensitive intervention of EOC.

Long non-coding RNAs (lncRNAs), which are initially regarded as transcriptional junk, are functionally classified as transcripts over 200 nucleotides in length lacking evident protein-coding capacity (6-8). Previous studies have indicated that lncRNAs participate in different aspects of tumor development, including tumorigenesis, tumor progression, and metastasis (9,10). For instance, the lncRNA HOTAIR was revealed to be upregulated in breast cancer tissues and cell lines and was closely correlated with the survival and metastasis of patients (11). The lncRNA lncARSR is exosome-transmitted in renal cancer and was revealed to have a function on chemoresistance in patients with sunitinib treatment (12). The novel lncRNA UCC was found to be increased in colorectal cancer and could regulate cell growth, invasion, and tumor progression (13). The lncRNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1), first named in 2003, was revealed to be associated with the metastasis of patients with non-small cell lung cancer (NSCLC) (14).

Our previous analysis revealed the clinical value of MALAT1 (15) which may potentially be applied as a new prognostic marker. It has been revealed that MALAT1 is involved in the development of various cancers, including lung, renal, hepatic, bladder, pancreatic, gastric, colorectal, brain and breast cancers (16-24). Recently, several studies indicated that MALAT1 is associated with metastasis of patients with EOC (25,26). However, the effect of MALAT1 on cellular behavior in OC and the overall survival (OS) of patients with OC remain unclear. The present study aimed to examine MALAT1 expression in human EOC tissues and EOC cell

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lines and to analyze MALAT1 expression associated with the OS and progression-free survival (PFS) of patients with OC. Finally, a loss-of-function approach was used to explore the effect of MALAT1 on cellular behaviors in EOC cells.

## Materials and methods

*Cell line and cultivation*. Human EOC cells SK-OV-3, OVCAR-3, CAOV-3 and ES-2 were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and A2780 was obtained from the European Collection of Authenticated Cell Cultures (ECACC, Salisbury, UK). Human non-tumorous ovarian surface epithelial cells (HOSEpiC) were obtained from Guangzhou Jennio Biotech Co., Ltd. (Guangzhou, China). SK-OV-3 and CAOV-3 cells were cultured in DMEM (Corning Life Sciences, Manassas, VA, USA). A2780, OVCAR-3 and HOSEpiC cells were respectively cultured in RPMI-1640 media (Corning Life Sciences). ES-2 cells were cultured in McCoy's 5A medium (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). All media were supplemented with 10% fetal bovine serum (FBS; Gibco; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and were replaced with fresh medium every three days.

*Clinical specimens*. All tissue samples (n=32) were derived from hospitalized patients between June 2012 to October 2016 at Jinshan Hospital, Fudan University. None of the patients with OC had received chemotherapy or radiotherapy prior to surgery. Control ovarian tissues were obtained from 12 patients with non-tumorous ovaries. The tumor and normal tissue specimens were frozen in liquid nitrogen after collection and stored at -80˚C until use. The present study was approved by the Ethics Committee of Jinshan Hospital and informed consent was obtained from each patient.

*Bioinformatics analysis*. The Kaplan-Meier Plotter database, an online bioinformatics tool (www.kmplot.com), is available to evaluate the effect of genes on survival information in OC (27). Before starting the use of the tool, the samples from patients were filtered by stage, histology, grade, and treatment elements containing debulking status and applied chemotherapy. To assess the clinical value of MALAT1, patients with OC were selected for the calculation of OS and PFS and were divided into two groups using the median, a group with low expression of MALAT1 and a group with high expression of MALAT1. The Kaplan-Meier survival curve was plotted. The hazard ratio (HR) with 95% confidence intervals (CIs) and the log-rank P-value were calculated. Online software LncBase Predicted v.2 was used to predict a candidate of miRNA that interacted with MALAT1 (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=lncbasev2%2Findex). Online software miRWalk 2.0 database was used to find a potential target of hsa-miR-143-3p (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/index.html) (28).

*Small interfering RNA (siRNA) transfection*. The MALAT1-siRNA (siMALAT1) and negative control-siRNA (siNC) were obtained from RiboBio Co., Ltd. (Guangzhou, China). The sequence of siMALAT1 was 5'-GCAAATGAAAGCTACC AAT-3'. Briefly, cells were seeded in a 6-well plate at a density of $2x10^5$ (OVCAR-3) or $1.5x10^5$ (SK-OV-3) cells/well. After culture for 24 h, the cells were transfected with siRNA using an X-treme GENE Transfection Reagent (Roche Applied Science, Indianapolis, IN, USA) according to the protocol recommended by the manufacturer. The cells were then collected for subsequent experiments. The knockdown efficiency of siMALAT1 was confirmed by qRT-PCR analysis.

*RNA isolation and quantitative real-time PCR*. Total RNA from tissues and cells was extracted using an Axygen Bioscience kit (Suzhou, China) according to the manufacturer's protocol. The cDNA was synthesized using a Transcriptor First Strand cDNA Synthesis kit (Roche Applied Science). The reaction conditions of reverse transcription were: 25˚C for 10 min, 50˚C for 60 min, 85˚C for 5 min, and 4˚C for 70 min. The qPCR experiments were conducted using a SYBR-Green Master kit (Roche Applied Science). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 18S and U6 served as a control for cells, tissues, and miRNAs, respectively. The primers were: MALAT1 forward, 5'-GTG TGCCAATGTTTCGTTTG-3' and reverse, 5'-AGGAGAAAG TGCCATGGTTG-3'; hsa-miR-143-3p forward, 5'-CTGAGA TGAAGCACTGTAGCTC-3' and reverse, 5'-GTGCAGGGT CCGAGGT-3'; GAPDH forward, 5'-GCACCGTCAAGGCTG AGAAC-3' and reverse, 5'-TGGTGAAGACGCCAGTGGA-3'; 18S forward, 5'-GACTCTGGCATGCTAACTAG-3' and reverse, 5'-GACATCTAAGGGCATCACAG-3'; and U6 forward, 5'-CTC GCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGA ATTTGCGT-3'. The expression of the target gene was analyzed by threshold cycle (Ct) $2^{-\Delta\Delta Ct}$ method obtained from Sequence Detection Software v1.4 (7300 Real-Time PCR System; Applied Biosystems; Thermo Fisher Scientific, Inc.). The assay was performed at least three times.

*Cell viability assessment*. In brief, cells were cultured in 96-well plates at a density of $6x10^3$ cells/100 $\mu$l medium/well overnight. After transfection and culture for 0, 24 and 48 h, the viability of cells was assessed using Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Kumamoto, Japan) according to the manufacturer's protocol. The optical density (OD) values at 450 nm were detected using a plate reader (Epoch; BioTek Instruments, Inc., Winooski, VT, USA). At least three independent experiments were conducted.

*Cell migration and invasion assays*. The cell migration and invasion capacities were evaluated by Transwell assays. Briefly, a Transwell chamber with a membrane of polycarbonate (6.5 mm in diameter with 8-$\mu$m pores; Corning Life Sciences) was placed into a well of a 24-well plate. In the lower chamber, 700 $\mu$l suitable medium containing 10% FBS was added. For the migration assay, cells were seeded in the upper chamber at a concentration of ~$6x10^4$/100 $\mu$l with serum-free culture medium. For the invasion assay, cells were seeded in the upper chamber with a Matrigel-coated membrane (BD Biosciences, Bedford, MA, USA) at a concentration of ~$8x10^4$ cells in a 100 $\mu$l volume of serum-free culture medium. After incubation for 48 h, the non-migrated or non-invaded cells on the upper chamber were carefully removed with a cotton swab and washed with phosphate-buffered saline (PBS). Migrated or invaded cells on the reversed membrane were fixed with 4% paraformaldehyde for 15 min, stained with crystal violet

(Sigma-Aldrich; Merck KGaA) for 30 min, photographed, and finally counted in three random fields under a light microscope (BX43; Olympus, Tokyo, Japan) at an x200 magnification. Experiments were conducted three times.

*Dual-luciferase reporter assay*. 293T cells were cultured in a 24-well plate and 70-80% confluency was reached prior to the experiment. Cells were co-transfected with 0.4 $\mu$g luciferase reporter vector (pmirGLO-MALAT1-wt or pmirGLO-MALAT1-mut) and 50 nM miRNA (miR-143-3p mimics or miR-negative control (miR-Ctrl; RiboBio Co., Ltd.) using the X-tremeGENE Transfection Reagent (Roche Applied Science) and cultured for 24 h. Firefly and *Renilla* luciferase activities were detected using Luc-Pair™ Duo-Luciferase Assay Kit 2.0 (GeneCopoeia, Inc., Rockville, MD, USA) following co-transfection according to the instructions recommended by the manufacturer. The relative firefly luciferase activity was corrected in accordance with the *Renilla* luciferase activity.

*Western blot analysis*. SK-OV-3 and OVCAR-3 cells were lysed in SDS buffer with a phosphatase inhibitor (Nanjing KeyGen Biotech Co., Ltd., Nanjing, China). The protein concentration was determined using a BCA Protein Assay kit (Thermo Fisher Scientific, Inc.). After separation on SDS-PAGE, total proteins were transferred to a PVDF membrane (EMD Millipore, Billerica, MA, USA) and incubated with either mouse anti-CMPK (cytidine monophosphate kinase; Cell Signaling Technology, Inc., Danvers, MA, USA) or rabbit anti-GAPDH (Abcam, Cambridge, UK) primary antibody at 4˚C overnight. After incubation with horseradish peroxidase (HRP)-conjugated goat anti-mouse or anti-rabbit IgG (Cell Signaling Technology) for 1 h at room temperature, the signals were detected using Tanon-4500 Gel Imaging System (Tanon Science and Technology Co., Ltd., Shanghai, China) with an Immobilon™ Western Chemiluminescent HRP Substrate (EMD Millipore).

*Statistical analysis*. SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze the collected data. For comparison between two groups, a Student's t-test was used. The survival curve was evaluated with a log-rank test. All data are displayed as the mean ± the standard error of the mean (SEM) from three independent experiments. A P-value <0.05 was considered to indicate a statistically significant difference.

## Results

*MALAT1 is upregulated in human epithelial ovarian cancer tissues and cell lines*. In order to identify functional MALAT1 relevant to the progression of ovarian tumors, we performed qRT-PCR to evaluate the expression of MALAT1 in human ovarian normal tissues (n=12), benign tumors (n=8), and malignant tumors (n=12, serous adenocarcinoma). The results revealed that the expression level of MALAT1 was significantly higher in ovarian malignant tumors than normal ovarian tissues and ovarian benign tumors (P<0.01) (Fig. 1A). In addition, the expression of MALAT1 between diverse OC cell lines and normal ovarian HOSEpiC cells was detected by qRT-PCR. The expression level of MALAT1 was high in EOC cell lines SK-OV-3, OVCAR-3, CAOV-3 and A2780 cells compared to normal ovarian HOSEpiC cells and ovarian clear cell carcinoma ES-2 cells (P<0.05) (Fig. 1B).

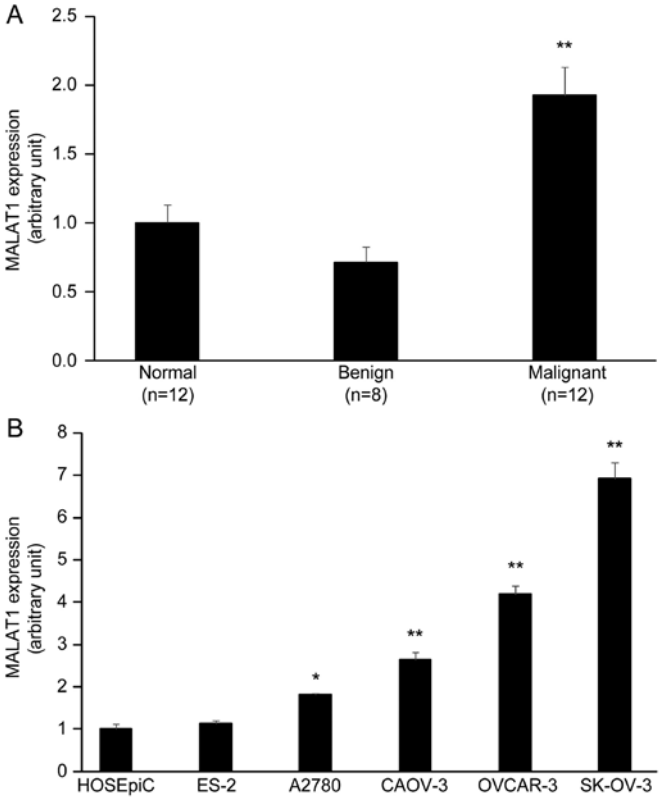


Figure 1. MALAT1 expression in human ovarian cancer. (A) Relative expression of MALAT1 was detected by qRT-PCR in the normal ovarian tissues (n=12), ovarian benign (n=8), and malignant (n=12) tumors. (B) The expression of MALAT1 was detected by qRT-PCR in diverse cell lines, including HOSEpiC, ES-2, A2780, CAOV3, SK-OV-3 and OVCAR-3 cells (n=3 repeats for each cell line). The results are presented as the mean ± SEM. $^{*}$P<0.05; $^{**}$P<0.01. MALAT1, metastasis associated lung adenocarcinoma transcript 1.

*MALAT1 is involved in the development of ovarian cancer*. Based on the Kaplan-Meier Plotter online database, we further analyzed the effect of MALAT1 on the OS and PFS of patients with OC. The survival plots revealed that the expression levels of MALAT1 were correlated with OS and PFS. The patients with high MALAT1 expression had low OS as shown in Fig. 2A (Affymetrix ID: 226675_s_at) and Fig. 2B (Affymetrix ID: 224567_x_at) and PFS as shown in Fig. 2C (Affymetrix ID: 226675_s_at) and Fig. 2D (Affymetrix ID: 224567_x_at) (all P<0.05).

*Knockdown of MALAT1 decreases ovarian cancer cell viability*. In order to investigate the potential function of MALAT1 on the biological behaviors of OC cells, we conducted a loss-of-function assay. Knockdown of MALAT1 by MALAT1-siRNA (siMALAT1) was confirmed by qRT-PCT in OVCAR-3 and SK-OV-3 cells (Fig. 3A and B). Cells transfected with siMALAT1 had a low expression of MALAT1 compared with cells transfected with negative control-siRNA (siNC) and blank control (Blank). Next, we assessed cell viability using a CCK-8 assay. We found that the knockdown of MALAT1 significantly inhibited OC cell viability after 48 h of transfection with siMALAT1 (Fig. 3C and D).

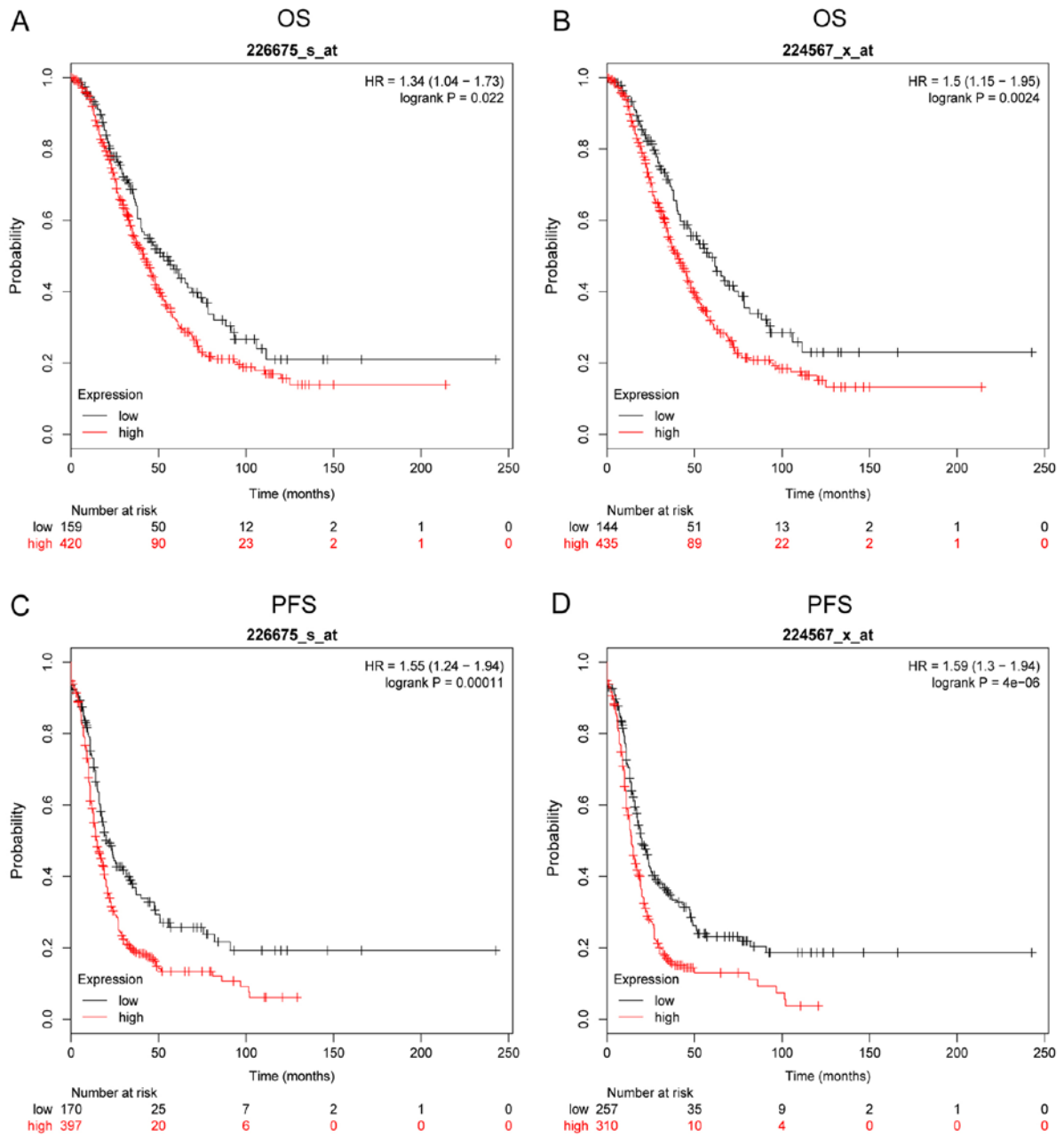


Figure 2. Survival plots. (A and B) Kaplan-Meier OS curves and (C and D) PFS curves are presented. All patients were divided into two groups based on the expression of MALAT1: A high expression group and a low expression group. (A) OS curves derived from a microarray (Affymetrix ID: 226675_s_at). (B) OS curves derived from a microarray (Affymetrix ID: 224567_x_at). (C) PFS curves derived from a microarray (Affymetrix ID: 226675_s_at). (D) PFS curves derived from a microarray (Affymetrix ID: 224567_x_at). The HR with 95% CIs, and log-rank P for OS and PFS were calculated, respectively. OS, overall survival; PFS, progression-free survival; MALAT1, metastasis associated lung adenocarcinoma transcript 1; HR, hazard ratio; CIs, confidence intervals.

*Downregulation of MALAT1 expression suppresses ovarian cancer cell migration and invasion*. Next, we investigated the effect of MALAT1 knockdown on OC cell migration and invasion. Using Transwell migration assays, we determined that the number of migrated cells of OVCAR-3 (Fig. 4A and B) and SK-OV-3 (Fig. 4C and D) was significantly decreased after MALAT1-siRNA transfection (siMALAT1) for 48 h compared with non-transfected cells (Blank) and negative control-siRNA (siNC) transfected cells. Furthermore, the knockdown of MALAT1 also significantly decreased the number of invaded cells of OVCAR-3 (Fig. 5A and B) and SK-OV-3 (Fig. 5C and D).

*MALAT1 interacts with miR-143-3p*. One of the functions of lncRNAs is to regulate RNA expression. The regulatory mechanism of lncRNAs on miRNAs is that lncRNAs can act as sponges to influence the function of miRNAs (29). Using online software LncBase Predicted v.2, we predicted a candidate of miRNA hsa-miR-143-3p that is a potential target of MALAT1. Using the loss-of-function approach, we demonstrated for the first time that MALAT1 expression was correlated with miR-143-3p expression. The expression of miR-143-3p was significantly increased in OVACR-3 (Fig. 6A) and SK-OV-3 (Fig. 6B) cells after MALAT1 knockdown (siMALAT1) for 48 h compared with the controls (Blank

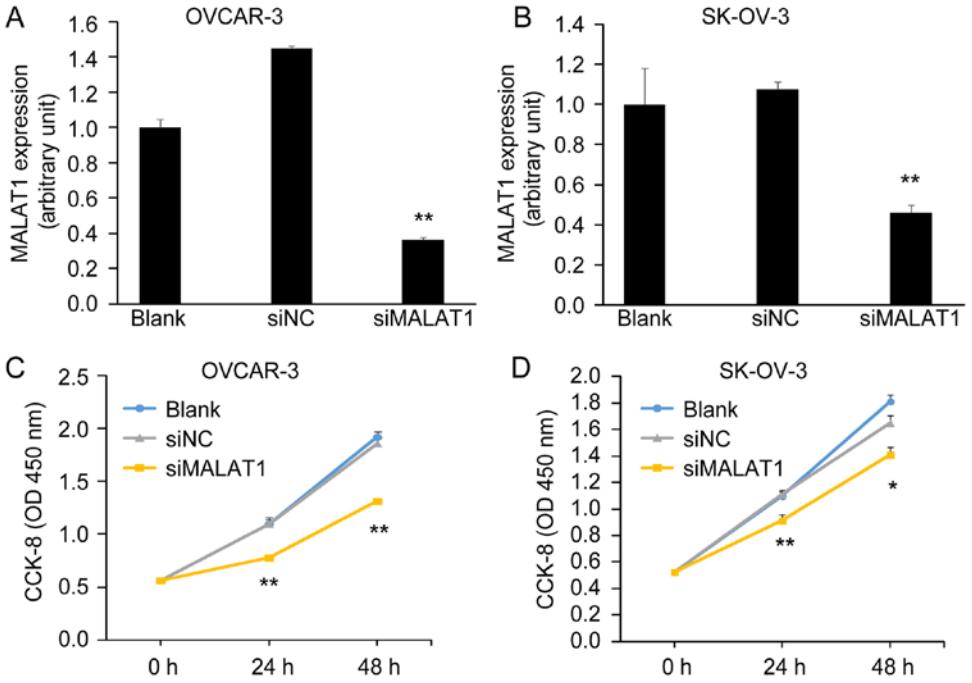


Figure 3. Effect of MALAT1-siRNA on ovarian cancer cell viability. (A and C) OVCAR-3 and (B and D) SK-OV-3 cells were transiently transfected with MALAT1-siRNA (siMALAT1) or negative control-siRNA (siNC). Non-transfected cells were used as a blank control (Blank). (A and B) MALAT1 expression was detected by qRT-PCR at 48 h post-transfection. (C and D) A time-course study revealing EOC cell viability as detected by CCK-8 assay. The results are presented as the mean ± SEM. $^{*}$P<0.05; $^{**}$P<0.01; n=3 independent experiments. MALAT1, metastasis associated lung adenocarcinoma transcript 1; EOC, epithelial ovarian cancer.

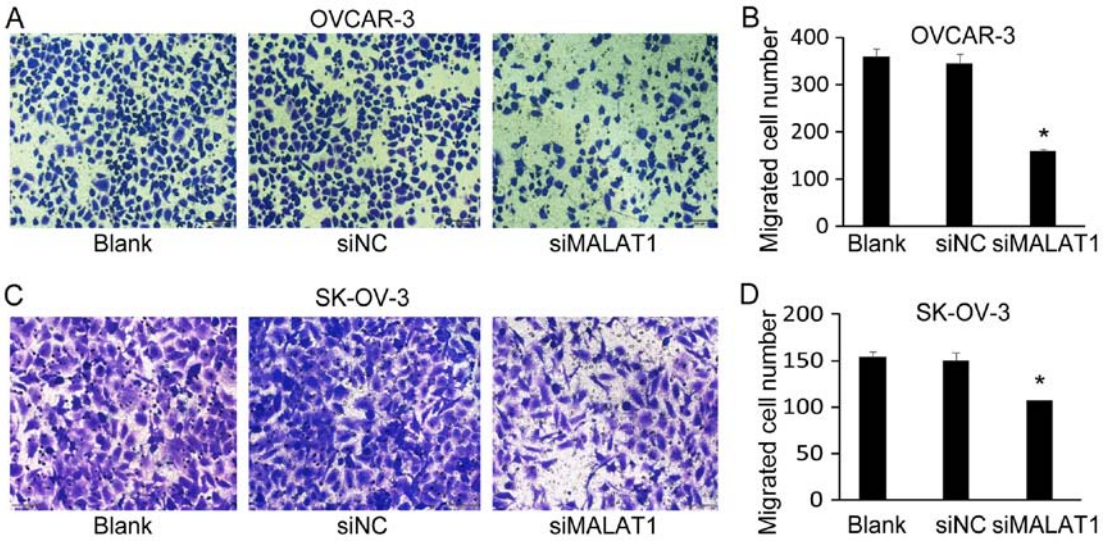


Figure 4. Effect of MALAT1-siRNA on cell migration. (A and B) OVCAR-3 and (C and D) SK-OV-3 cell migration was performed using Transwell migration assays. The cells were transfected with MALAT1-siRNA (siMALAT1) or negative control-siRNA (siNC). Cells without transfection were used as a blank control (Blank). The effective migrating cells were counted and photographed. Original amplification, x200; scale bar, 100 $\mu$m. The histogram displays the quantitative analysis of migrated cells. The results are presented as the mean ± SEM. $^{*}$P<0.05; n=3 independent experiments. MALAT1, metastasis associated lung adenocarcinoma transcript 1.

and siNC) (P<0.05) as detected by qRT-PCR. Next, we used a dual-luciferase reporter assay to confirm the interaction between MALAT1 and miR-143-3p. Three sequences are shown in Fig. 6C: hsa-miR-143-3p, a wild-type MALAT1 containing a miR-143-3p binding site (position at 3990-3997 of MALAT1), and a mutated MALAT1 in which the binding site was changed. The Dual-Luciferase reporter assay confirmed that wild-type MALT1, not mutated MALAT1, bound to miR-143-3p in 293T cells after 72 h of co-transfection (Fig. 6D). These data revealed that MALAT1 could directly bind to miR-143-3p. Using miRWalk 2.0 database, we found that miR-143-3p potentially targets CMPK, a molecule previously demonstrated to play a role in EOC (30). Western blotting revealed that treating OVCAR-3 and SK-OV-3 cells with miR-143-3p mimics significantly decreased CMPK protein expression (Fig. 6E and F).

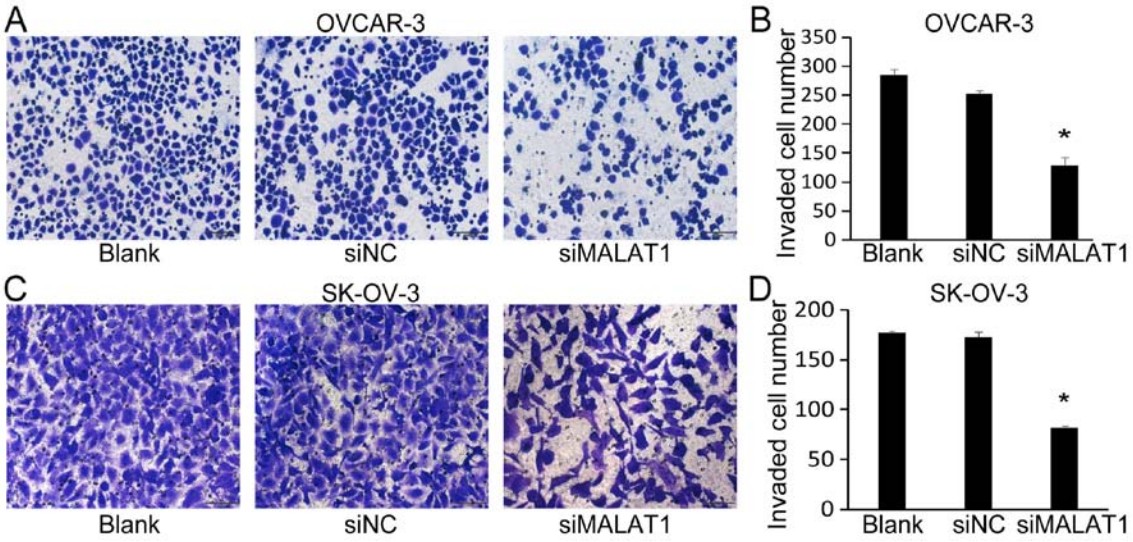


Figure 5. Effect of MALAT1-siRNA on cell invasion. (A and B) OVCAR-3 and (C and D) SK-OV-3 cell invasion was performed using Transwell invasion assays. The cells were transfected with MALAT1-siRNA (siMALAT1) or negative control-siRNA (siNC). Cells without transfection were used as a blank control (Blank). The effective invaded cells were counted and photographed. Original amplification, x200; scale bar, 100 $\mu$m. The histogram displays the quantitative analysis of invaded cells. The results are presented as the mean ± SEM. $^{*}$P<0.05; n=3 independent experiments. MALAT1, metastasis associated lung adenocarcinoma transcript 1.

## Discussion

The present study demonstrated that MALAT1 was overexpressed in human ovarian malignant tissues and influenced the survival of patients with OC. MALAT1, an lncRNA, plays a role in the regulation of miRNAs, which further affect downstream gene regulation.

It has been revealed that MALAT1 is upregulated in several malignant tumors such as breast (24,31), bladder (32), pancreatic (20,33) and colorectal (22) cancers. Our present data revealed a similar result. MALAT1 was overexpressed in ovarian malignant tumors compared with benign tumors and normal ovarian tissue. Moreover, we also observed the high expression of MALAT1 in several EOC cell lines compared with non-tumorous human ovarian surface epithelial cells (HOSEpiC). These data indicated that MALAT1 plays a role in EOC.

Through an online bioinformatics tool, we determined that MALAT1 could be a predictive biomarker of the survival of patients with OC. The available survival curves from the Kaplan-Meier Plotter database were analyzed and they revealed that a high expression of MALAT1 was associated with poor OS and PFS in OC patients. Previous studies from us and other research groups have revealed the clinical significance of MALAT1 (15,32). The overexpression of MALAT1 was correlated with a decrease of disease-specific survival of patients with breast cancer (24). Elevated plasma MALAT1 was associated with distant metastasis in patients with EOC (25). However, the possible regulation mechanism remains unclear.

Our functional assays revealed that the knockdown of MALAT1 significantly inhibited OC cell viability, migration, and invasion. Similar results have been reported by other research groups. For instance, the inhibition of MALAT1 expression decreased OC cell proliferation, migration and invasion (34,35). MALAT1 induced EOC cell proliferation via the PI3K/Akt signaling pathway (26). These data indicated that MALAT1 may play a role in OC cell behavior.

MALAT1 can act as a regulator of the expression of other RNAs such as miRNAs and forms a molecular interaction network in different types of cancer (36,37). Recent studies have revealed that MALAT1 can target various miRNAs, which partly explains the mechanism of MALAT1 which plays a role in disease processes (38,39). For instance, miR-200s was revealed to be sponged by MALAT1 in clear cell kidney carcinoma (40). miR-206 was determined to be negatively regulated by MALAT1 in gallbladder cancer (41). Using online software, we identified miR-143-3p as a possible target of MALAT1. Our Dual-Luciferase reporter assay demonstrated the interaction between MALAT1 and miR-143-3p, indicating that MALAT1 is capable of functioning as a molecular sponge to adsorb miR-143-3p and subsequently regulate OC cell behaviors. Indeed, inhibition of MALAT1 resulted in an increase of miR-143-3p. Due to the size of MALAT1 which is over 8,000 nt in length (14), we were not able to obtain a full-length clone. Currently, we are unable to do a gain-of-function assay.

Several recent studies have revealed that miRNAs, a class of non-coding RNAs ~22 nt in length, are subject to the regulation of various biological processes as part of an integrated pathophysiological response to various stimuli (42,43). It has been revealed that miR-143-3p could play a role in tumorigenesis and function as a tumor suppressor gene in breast cancer and esophageal squamous cell carcinoma (44,45). We recently revealed that CMPK plays a role in ovarian tumorigenesis (30). Based on the bioinformatics analysis and our further experiments, we demonstrated that CMPK was one of the targets of miR-143-3p. It may be considered that miR-143-3p is a tumor-inhibitory factor by targeting CMPK in OC. These results ascertained that MALAT1 negatively regulated miR-143-3p via a sponge-like function, and in turn,

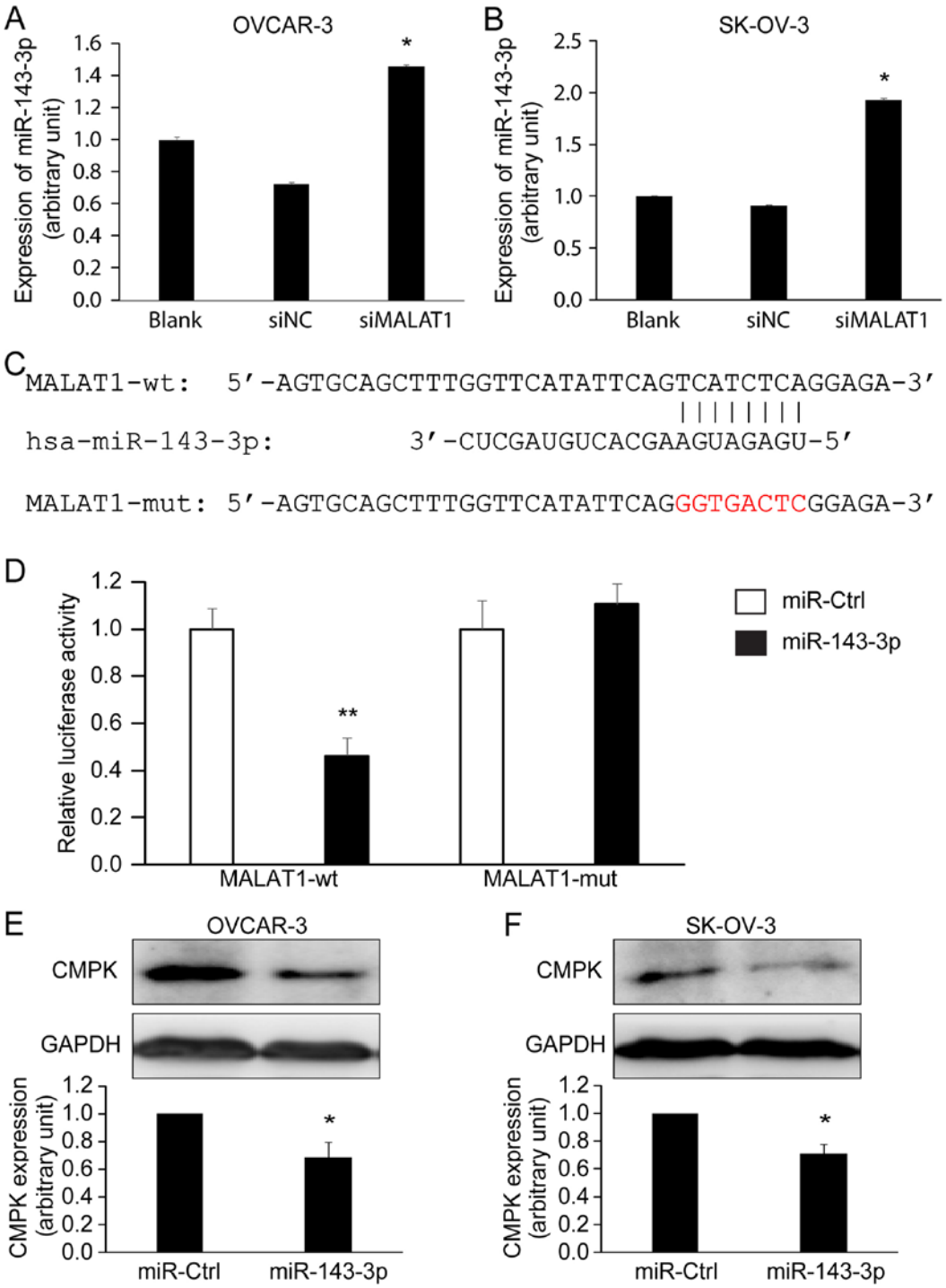


Figure 6. Effect of MALAT1 on miR-143-3p expression. (A and B) Expression of miR-143-3p was detected by qRT-PCR in (A) OVCAR-3 and (B) SK-OV-3 cells transfected with MALAT1-siRNA (siMALAT1) or negative control-siRNA (siNC). Cells without transfection were used as a blank control (Blank). (C) Sequences revealing specific binding sites between MALAT1 and miR-143-3p. MALAT1-wt, wild-type MALAT1; MALAT1-mut, mutated MALAT1. (D) Dual-Luciferase reporter assay. 293T cells were respectively co-transfected with MALAT1-wt/-mut plasmids and miR-143-3p/negative control. (E and F) CMPK protein expression was detected by western blotting. (E) OVCAR-3 and (F) SK-OV-3 cells were transfected with either miR-143-3p or control miRNA (miR-Ctrl). The histogram reveals the quantitative analysis of the gels. The results are presented as the mean ± SEM. Ctrl, control; $^{*}$P<0.05; $^{**}$P<0.01; n=3 independent experiments. MALAT1, metastasis associated lung adenocarcinoma transcript 1.

released the suppression of miR-143-3p to CMPK inhibition, leading to the progression of OC development.

In conclusion, our findings indicated that MALAT1 is overexpressed in ovarian malignant tumors and influences the survival of patients with OC. Knockdown of MALAT1 affected OC cell behavior. MALAT1 functions as a tumor enhancer by interacting with miR-143-3p and may promote the development OC. Therefore, it is speculated that MALAT1 may serve as a therapeutic target for the treatment of patients with OC. However, an understanding of concrete and extensive mechanisms underlying regulation of MALAT1 in OC needs to be further investigated.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Author's contributions**

QL conducted experiments and performed data analysis, Figure generation and manuscript writing. WG and WR contributed to the collection of clinical samples and pathological diagnosis. WG, LZ and JZ performed part of the experiments and bioinformatics analyses. GX contributed to the experimental design, data analysis, Figure generation and manuscript writing. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**Ethics approval and consent to participate**

The present study was approved by the Ethics Committee of Jinshan Hospital.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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