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ORIGINAL RESEARCH

LncRNA MALAT1 Promotes Survival of Epithelial Ovarian Cancer Cells by Downregulating miR-145-5p

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Ke Wang[1]
Ye Zhao[2,*]
Yi-Min Wang[3,*]

[1]Department of Gynaecology and Obstetrics, China-Japan Union Hospital of Jilin University, Changchun 130000, Jilin, People's Republic of China; [2]Department of Dermatology, China-Japan Union Hospital of Jilin University, Changchun, Jilin, 130000, People's Republic of China; [3]Central Research Room, China-Japan Union Hospital of Jilin University, Changchun 130000, Jilin, People's Republic of China

*These authors contributed equally to this work

Purpose: This paper was aimed at investigating the regulatory mechanism of long non-coding RNA metastasis-associated lung adenocarcinoma transcript-1 (MALAT1) in epithelial ovarian cancer (EOC).

Materials and Methods: MALAT1 and miR-145-5p expression in the tissues, serum, and EOC cell lines (TOV-112D, TOV-21G) of patients with EOC were detected. The two genes were transfected into the cells via upregulating or downregulating their expression. Levels of apoptosis-related proteins (Caspase-3, Bax, Bcl-2) were analyzed. Mechanisms of cell proliferation, invasion, and apoptosis were studied.

Results: MALAT1 was highly expressed in EOC tissues, while miR-145-5p was poorly expressed in them. The areas under the curves (AUCs) of the two genes for diagnosing EOC were greater than 0.85, and the two had a significantly negative correlation. According to multivariate Cox regression analysis, high MALAT1 expression, tumor size, degree of differentiation, clinical staging, and lymph node metastasis were the independent risk factors affecting prognosis. The 3-year overall survival rate (OSR) of patients with low MALAT1 expression was remarkably higher than that of those with high expression. Overexpressing miR-145-5p and silencing MALAT1 could inhibit EOC cells from proliferating and invading, increase the apoptotic rate, and improve levels of the apoptosis-related proteins. After co-transfection with MALAT1-inhibitor + miR-145-5p-inhibitor, the proliferation and invasion of TOV-112D and TOV-21G cells were inhibited and the apoptotic rate rose more obviously. Inhibiting MALAT1 could increase miR-145-5p expression, thus inhibiting EOC cells from proliferating and invading and thereby increasing their apoptotic rate.

Conclusion: MALAT1 promotes EOC cells' survival by downregulating miR-145-5p so it may become a new direction for EOC diagnosis and gene therapy.

Keywords: MALAT1, EOC, miR-145-5p, cell survival

Introduction

As a common cause of death of gynecologic cancers, epithelial ovarian cancer (EOC) is usually diagnosed when it is in the advanced stage.[1] Clinically, the disease is mostly treated by platinum-based chemotherapy and cytoreductive surgery. Although many patients with EOC have a high response rate after receiving chemotherapy for the first time, most patients with advanced EOC will be eventually resistant to chemotherapy.[2] Moreover, cancer cell metastasis is a major clinical problem, and ovarian cancer cells spreading outside the ovary leads to patients' death.[3] Therefore, we will continue to clarify the relevant mechanisms of EOC and to find its new and potential therapeutic targets, so as to improve the prognosis of patients with the disease.

Correspondence: Yi-Min Wang
Central Research Room, China-Japan Union Hospital of Jilin University, No. 126 Sendai Street, Nanguan District, Changchun 130000, Jilin, People's Republic of China
Tel +86-13265896584
Email Yiminwang@jlu.edu.cn

Ye Zhao
Department of Dermatological, China-Japan Union Hospital of Jilin University, No. 126 Sendai Street, Nanguan District, Changchun 130000, Jilin, People's Republic of China
Tel +86-13569874521
Email yezhao45@outlook.com

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According to recent reports, the identification and characterization of long non-coding RNAs (LncRNAs) have developed rapidly during many biological processes.[4,5] Ubiquitous in the genome, LncRNAs usually show cell types and the time-specific regulation of genes, and affect many cell processes via a variety of different mechanisms.[6,7] As one of the most abundant LncRNAs, MALAT1 is differentially expressed in various cancers,[8,9] with complex and extensive functions during the development and progression of the cancers.[10] Highly expressed in prostate cancer tissues and cell lines, it promotes the cells to proliferate and invade and inhibits their apoptosis in patients with prostate cancer.[11] In a study by Chen et al, the increasing expression of serum MALAT1 shortens the survival time of patients with EOC, so MALAT1 can be used as a biomarker for diagnosing metastasis.[12] miR-145-5p is a tumor suppressor that is associated with the metastasis of many different cancers, and its potential mechanism regulates cells' proliferation and migration through modulating various signal transduction pathways.[13] It downregulates in the serum of patients with EOC, thereby changing the clinicopathological features of the patients.[14] LncRNAs can act as sponges for miRNAs to reduce the regulatory effect of the latter.[15,16] According to Lu H and others, inhibiting the expression of serum MALAT1 can regulate miR-145-5p to reduce the formation of cancer cell colonies, and exert a synergistic effect on apoptosis in patients with cervical cancer.[17]

Therefore, the effects of MALAT1 regulating miR-145-5p on the cell biological functions of patients with EOC were explored in this study, so as to provide new therapeutic targets for the patients.

Materials and Methods

Cell Sources

EOC cells (TOV-112D, TOV-21G, CAOV3, OVCAR3) and human normal ovarian epithelial cells (IOSE80) were purchased from ATCC (BNCC510853, BNCC263069, BNCC267247, BNCC287[illegible], BNCC340318).

Reagents and Instruments

CCK-8 assay kits were purchased from Bestbio(BB-4202). Transwell kits were purchased from BioGenius, Shanghai (Transwell). qRT-PCR and reverse transcription kits were purchased from TransGen Biotech, Beijing, China (AQ141-01, AQ141-01). DMEM, phosphate buffer solution (PBS), fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Gibco, USA (1,142,802, 10,566,024, 10,010,023, 26,400,044, 15,070,063). RIPA reagents, BCA protein assay kits, ECL luminescence reagents, trypsin, and Lipofectamine™ 2000 Transfection Reagent were purchased from Thermo Scientific, USA (89,900, 23,250, 32,209, 90,059, 11,668,019). IGF-1 was purchased from Shanghai Hengfei Biotechnology Co., Ltd. (K002504P). β-actin primary antibody and goat anti-mouse IgG secondary antibody that was labeled by horseradish peroxidase (HRP) were purchased from R&D, USA (MAB8929, HAF007). Annexin V/PI apoptosis detection kits were purchased from Yisheng Biotechnology Co., Ltd., Shanghai, China (40302ES20). Dual luciferase reporter gene assay kits were purchased from Solarbio, Beijing, China (D0010). A PCR instrument was purchased from ABI, USA (7500). A flow cytometer was purchased from BD, USA (FACS Canto II). A multifunctional microplate reader was purchased from BioTek, USA (DLK00[illegible]622). All primers were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd.

Sample Collection

The cancer tissues and the normal adjacent tissues, which were resected during surgery, were collected from 105 patients with EOC and then stored in liquid nitrogen. Five mL of elbow venous blood was respectively drawn from the patients and from 74 healthy people who underwent physical examinations during the same period. The blood was centrifuged at 3000×g for 10 min, and the serum was stored for subsequent experiments. Both groups were informed of this study and they signed the informed consent form. This study was implemented after approved by the Medical Ethics Committee of China-Japan Union Hospital of Jilin University.

Cell Culture and Transfection

MALAT1 and miR-145-5p inhibitory plasmids (MALAT1-inhibitor, miR-145-5p-inhibitor), their overexpression plasmids (sh-MALAT1-mimics, miR-145-5p-mimics), and blank control groups (si-NC, miR-NC) were established, respectively. The established drug-resistant cell strains were transferred to a 24-well plate. Forty-eight hours later, the cell plasmids (100 nM overexpression, inhibitory, and blank controls) were transfected into the cells using the Lipofectamine 2000 kit, with operating steps strictly carried out based on the kit instruction.

Detection of MALAT1 and miR-145-5p Expression (RT-PCR)

Glioma tissues and normal brain tissues were taken out from the liquid nitrogen container for grinding. Total RNA

in the tissues was extracted with Trizol reagents, and its purity and concentration were detected with an UV spectrophotometer. Then, the RNA (5 μg each) was taken out for the reverse transcription into cDNA based on the kit instruction. Parameters for the reaction were 37°C (15 min), 42°C (35 min), and 70°C (5 min). The amplification system for miR-145-5p was cDNA (1 μL), upstream and downstream primers (0.4 μL each), 2X TransScript® Tip Green qPCR SuperMix (10 μL), Passive Reference Dye (50X) (0.4 μL), and ddH2O that was finally added to supplement to 20 μL. Amplification conditions were pre-denaturation (94°C, 30 s), denaturation (94°C, 5 s), and annealing and extension (60°C, 30 s), which were cycled for 40 times. The amplification system for MALAT1 was cDNA (1 μL), upstream and downstream primers (0.4 μL each), 2X TransScript® Tip Green qPCR SuperMix (10 μL), Passive Reference Dye (50X) (0.4 μL), and Nuclease-free Water that was finally added to supplement to 20 μL. Amplification conditions were pre-denaturation (95°C, 30 s), denaturation (95°C, 10 s), and annealing and extension (60°C, 35 s), which were cycled for 40 times. There were 3 same wells for each sample. Three repeated experiments were performed. GADPH and U6 were internal references of MALAT1 and miR-145-5p, respectively. The data were analyzed by 2-ΔΔct.

Western Blotting (WB)

The cultured cells were lysed with RIPA buffer, and the protein concentration was detected by BCA protein assay. The protein was separated with 10% SDS-PAGE to transfer to the PVDF membrane (0.22 μm) and its concentration was adjusted to 4 μg/μL. The membrane was sealed in 5% skimmed milk (2 hours), and then sealed (4°C) all night with IGF-1 and β-actin primary antibodies (1: 1000). After cleaned to remove the antibodies, it was added with the HRP-labeled secondary antibody [goat anti-rabbit (1: 5000)], incubated at 37°C (1 hour), and then rinsed with PBS (5 min) for 3 times. Finally, it luminesced with the ECL luminescence reagents and was developed, after excess liquid on it was absorbed dry with filter papers. Protein bands were scanned and their gray values were analyzed in Quantity One, with GAPDH used as the internal reference.

Detection of Cell Proliferation (CCK-8)

After 24-hour transfection, the cells were collected, adjusted to $4*10^6$ cells/well, and then inoculated on a 96-well plate. After 24-, 48-, 72-, and 96-hour culture, each well was added with CCK-8 solution (10 μL) and basic medium (DMEM, 90 μL) for 2-hour culture (37°C). Optical density (OD) values at 570 nm in each group were detected using the microplate reader. Three repeated experiments were performed. The activity-time curves of the cells were plotted.

Detection of Cell Invasion (Transwell)

After 24-hour transfection, the cells were collected, then adjusted to $5*10^4$ cells/well, and finally inoculated on a 6-well plate. Next, they were washed with PBS for twice and inoculated on the upper chamber. This chamber was added with DMEM culture solution (200 μL), while the lower one was added with DMEM (500 mL; 20% FBS) for 48-hour culture at 37°C. The matrix and cells without penetrating the membrane surface in the upper chamber were wiped off. After the cells were cleaned with PBS (3 times), they were fixed with paraformaldehyde (10 min) and stained with crystal violet (15 min). Finally, the Transwell chamber was washed with PBS. Photographs of cell migration were collected under a 200-fold microscope, and 3 fields of view were randomly selected to calculate the number of cells, with the average value considered as the number of cells penetrating the membrane. Three repeated experiments were performed.

Detection of Apoptosis (Flow Cytometry)

After digested with 0.25% trypsin, the transfected cells were rinsed with PBS for twice, and then added with binding buffer (100 μL) to prepare a $1*10^6$ cells/mL suspension. After that, the suspension was sequentially added with AnnexinV-FITC and PI, and then incubated in the dark (room temperature, 5 min). The flow cytometer system was used for detection. Three repeated experiments were conducted to obtain the average value.

Detection of Target Genes

Starbase3.0 was used to predict the downstream target gene of MALAT1. SOX1-3′UTR wild type (Wt), SOX1-3′UTR mutant (Mut), MALAT1-mimics, and si-NC were transferred into the target cells using the Lipofectamine™ 2000 kits. After 48-hour transfection, their luciferase activities were determined by the dual luciferase reporter gene assay kits.

Follow-Ups

Through telephone, WeChat, and outpatient medical records, the patients were followed up for 5 years to record their

survival status, once every 4 months. The overall survival (OS) was recorded from the beginning of treatment to the death of patients or the end time of the last follow-up.

Statistical Analysis

In this experiment, SPSS 20.0 was used to statistically analyze the collected data and to plot the required figures. Count data were expressed as the number of cases/percentage [n(%)], and a chi-square test was used for their comparison between groups. Measurement data were expressed as mean ± standard deviation (mean±SD), and an independent samples *t*-test was used for their comparison between two groups. Receiver operating characteristic curves (ROC curves) were plotted to assess the diagnostic values of MALAT1 and miR-145-5p for EOC. Multivariate COX regression was conducted to analyze the prognosis of patients. The Kaplan-Meier method was used to plot the survival curves of 5-year OS, and the Log rank test was used for comparison. Pearson test was used for analyzing the correlation of MALAT1 with miR-145-5p in serum. The difference was statistically significant when $P<0.05$.

Results

MALAT1 and miR-145-5p Expression in Serum and Tissues

According to qRT-PCR, compared with the control group, relative MALAT1 expression in serum and tissues was remarkably higher in the study group, while relative miR-145-5p expression was remarkably lower in this group ($P<0.05$). See Figure 1.

Correlation of MALAT1 with Clinicopathological Features and Prognostic Survival

According to the multivariate Cox regression analysis of patients' pathological data and serum MALAT1, high MALAT1 expression, tumor size, degree of differentiation, case staging, and lymph node metastasis were the

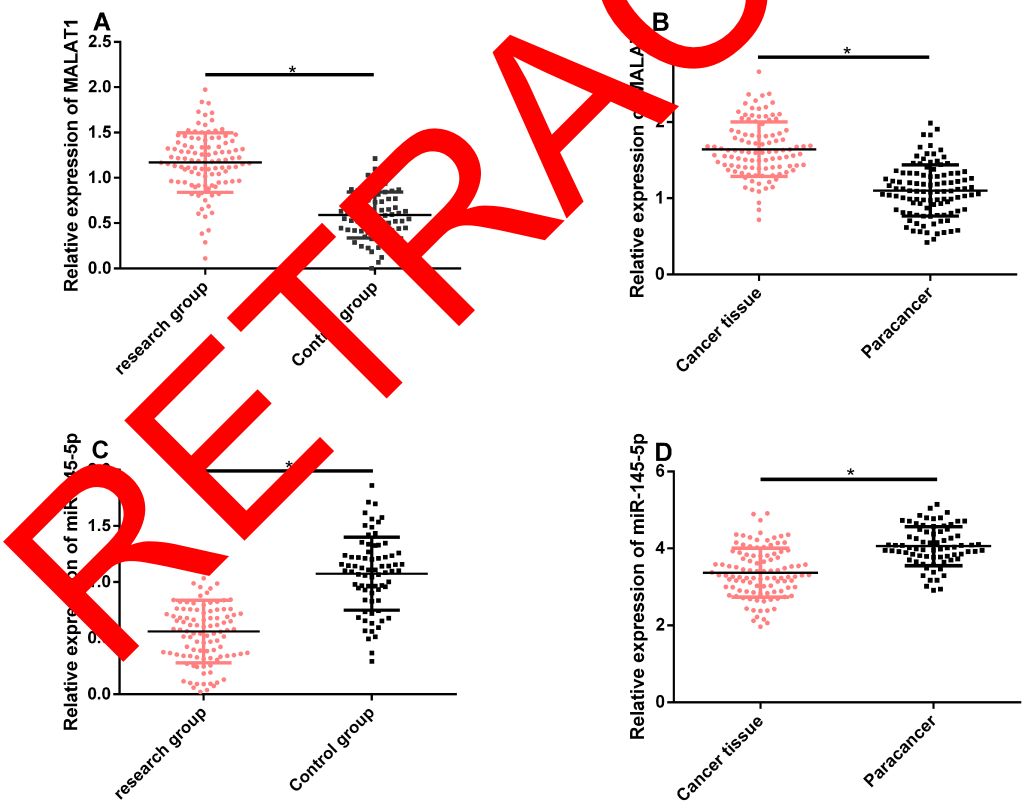

Figure 1 MALAT1 and miR-145-5p expression in serum and tissues. (**A** and **B**) MALAT1 expression remarkably rose in the serum and tissues of patients with EOC. (**C** and **D**) miR-145-5p expression remarkably rose in the serum and tissues of patients with EOC.
Note: *Indicates $P<0.05$.

Table 1 Correlation of MALAT1 with Clinicopathological Features [n(%)]

Factors	n	MALAT1		t value	P value
		High Expression Group (n=54)	Low Expression Group (n=54)		
Age (Years)				1.438	0.231
≥60	62	34 (54.84)	28 (45.16)		
<60	42	18 (42.86)	24 (57.14)		
Tumor size (cm)				5.992	0.014
<5	59	21 (35.59)	38 (64.41)		
≥5	49	29 (59.18)	20 (40.82)		
Degree of differentiation				9.[illegible]5	0.003
Lowly differentiated	56	34 (60.71)	22 (39.29)		
Moderately + highly differentiated	48	15 (31.25)	33 (68.75)		
Pathological types				7.829	0.005
Stages I + II	45	15 (33.33)	30 (66.67)		
Stages III+IV	59	36 (61.02)	23 (38.[illegible])		
Lymph node metastasis				[illegible]219	0.022
Yes	67	41 (61.19)	26 (38.[illegible])		
No	37	14 (37.84)	23 (62.16)		
Ascites				0.356	0.551
Yes	51	23 (45.10)	28 ([illegible].90)		
No	53	27 (50.94)	26 ([illegible].06)		
Tumor types				2.412	0.120
Serous carcinoma	64	34 ([illegible].13)	30 (46.88)		
Non-serous carcinoma	40	15 ([illegible]50)	25 (62.50)		

independent risk factors affecting the prognosis of patients with EOC. Subsequently, all of the patients were followed up for 5 years and their 5-year overall survival rate (OSR) was 65.2%. With the median expression of serum MALAT1 (1.[illegible]6) considered as the cut-off point, the 5-year OSR of patients with low expression was remarkably higher than that of those with high expression (P=0.49[illegible]). See Tables 1 and 2 and Figure 2.

Diagnostic Value of MALAT1 and miR-145-5p Expression

ROC curves of serum MALAT1 and miR-145-5p for diagnosing EOC were plotted. The area under the curve (AUC), sensitivity, specificity, and optimal cut-off value of serum MALAT1 were 0.897, 90.47%, 74.32%, and 0.768, respectively. The parameters of serum miR-145-5p were 0.878, 74.79%, 89.79%, and 0.803, respectively. According to the Pearson test,

Table 2 COX Regression Analysis

Factors	Univariate Cox			Multivariate Cox		
	P value	HR	95CI%	P value	HR	95CI%
Age	0.022	1.012	0.511–2.024	0.009	1.713	0.817–3.426
Tumor size	0.025	2.726	1.363–5.452	0.039	2.453	1.227–4.906
Degree of differentiation	0.018	2.318	1.159–4.636	0.026	3.325	1.663–6.550
Pathological types	0.005	2.317	1.159–4.634	0.043	1.876	0.938–3.752
Lymph node metastasis	0.041	1.273	0.637–2.546	0.037	1.479	0.739–2.968
Ascites	0.083	1.135	0.568–2.270	–	–	–
Histological types	0.126	0.978	0.489–1.956	–	–	–

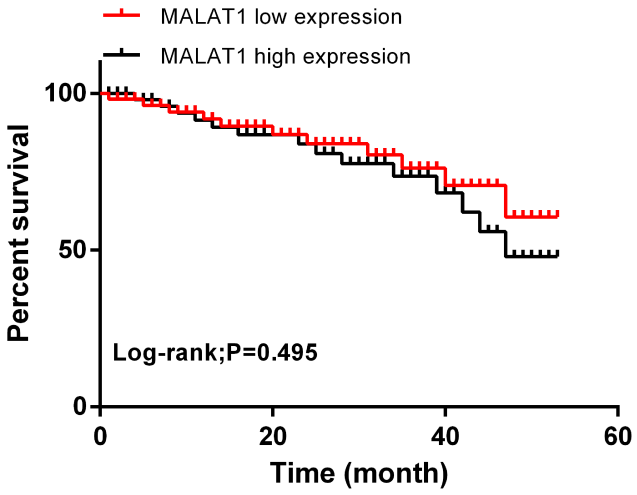

Figure 2 Correlation of MALAT1 with prognostic survival. The correlation of high and low MALAT1 expression with the 5-year OSR of patients with EOC.

serum MALAT1 and miR-145-5p expression was negatively correlated in patients with EOC (P<0.05). See Table 3 and Figure 3.

Effects of MALAT1 Expression on Biological Functions of Transfected Cells

According to the detection, compared with IOSE cells, MALAT1 expression remarkably rose in TOV-112D, TOV-21G, CAOV3, and OVCA cells (P<0.05). TOV-112D and TOV-21G cells with the greatest difference were selected for transfection. After the transfection with MALAT inhibitor, MALAT1 expression in the MALAT1-inhibitor group was remarkably lower than that in the si-NC group (P<0.05). According to the detection of cell proliferation, invasion, and apoptosis after transfection, the cells in the MALAT1-inhibitor group had remarkably inhibited proliferation and invasion, but a remarkably higher apoptotic rate (P<0.05). Additionally, we detected the effects of MALAT1 on apoptosis-related proteins, and found that inhibiting this gene could increase Caspase-3 and Bax levels but reduce Bcl-2 levels. See Figure 4.

Effects of miR-145-5p Expression on Biological Functions of Transfected Cells

According to the detection, compared with IOSE80 cells, miR-145-5p expression remarkably reduced in TOV-112D, TOV-21G, CAOV3, and OVCAR3 cells (P<0.05). TOV-112D and TOV-21G cells with the greatest difference were selected for transfection. After the transfection with miR-145-5p-mimics, miR-145-5p expression in the miR-145-5p-mimics group was remarkably lower than that in the miR-NC group (P<0.05). According to the detection of cell proliferation, invasion, and apoptosis after transfection, the cells in the miR-145-5p-mimics group had inhibited proliferation and invasion, but a higher apoptotic rate (P<0.05). Additionally, we detected the effects of miR-145-5p on apoptosis-related proteins, and found that overexpressing this miR could increase Caspase-3 and Bax levels but reduce Bcl-2 levels. See Figure 5.

Gene Identification of MALAT1

For further verifying the correlation of MALAT1 with miR-145-5p, the downstream target gene of MALAT1 was first predicted by starbase3.0, and a targeted binding site was found between the two. According to the dual luciferase reporter gene assay, miR-145-5p overexpression could remarkably reduce MALAT1-3′UTR Wt luciferase activity (P<0.05), but it had no effect on MALAT1-3′UTR Mut luciferase activity (P>0.05). According to PCR, the transfection of MALAT1-mimics could remarkably reduce miR-145-5p expression in TOV-112D and TOV-21G cells, while that of MALAT1-inhibitor could remarkably increase the expression in the cells (P<0.05). See Figure 6.

Rescue Experiment

TOV-112D and TOV-21G cells were co-transfected with MALAT1-inhibitor + miR-145-5p-mimics, and their biological functions were detected. The cells in the MALAT1-inhibitor + miR-145-5p-inhibitor group were not different from those in the si-NC group with respect to their proliferation, invasion, and apoptosis, but they had remarkably

Table 3 Diagnostic Value of MALAT1 and miR-145-5p Expression

Diagnostic Indicators	AUC	95% CI	Standard Error	Cut-Off Value	Sensitivity (%)	Specificity (%)
MALAT1	0.897	0.853–0.941	0.022	0.768	90.47	74.32
miR-145-5p	0.878	0.832–0.923	0.023	0.803	74.79	89.79

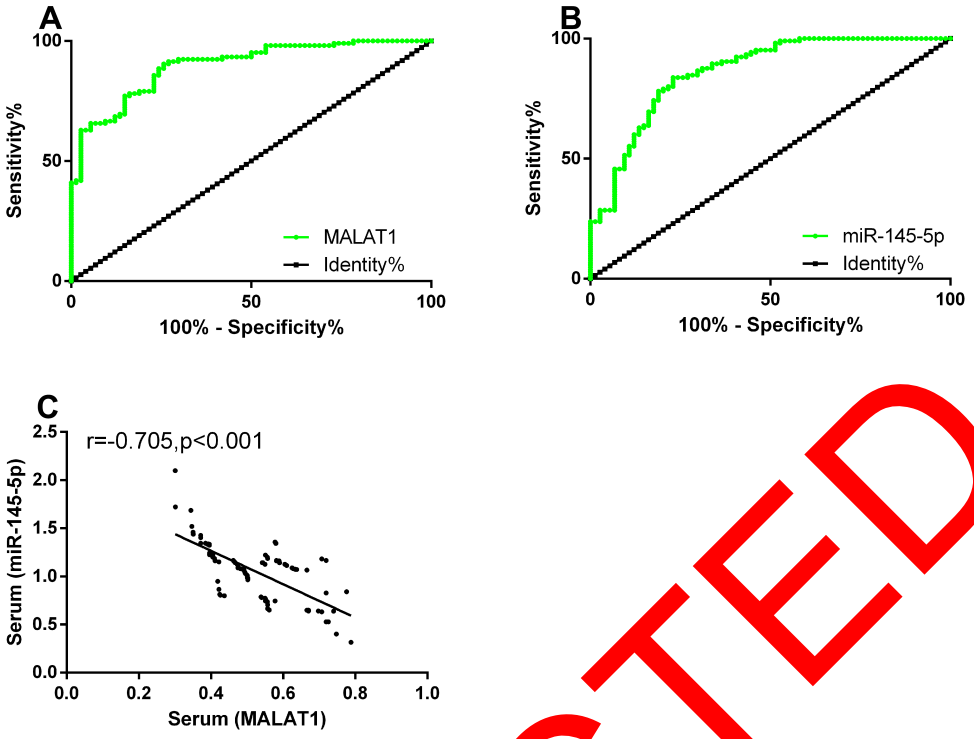

Figure 3 Diagnostic value of MALAT1 and miR-145-5p expression. (**A**) The sensitivity and specificity of serum MALAT1 for diagnosing EOC were 90.47% and 74.32%, respectively. (**B**) The sensitivity and specificity of serum miR-145-5p for diagnosing EOC were 74.79% and 89.79%, respectively. (**C**) Serum MALAT1 expression was negatively correlated with miR-145-5p expression in the study group (r=0.618, P<0.001).

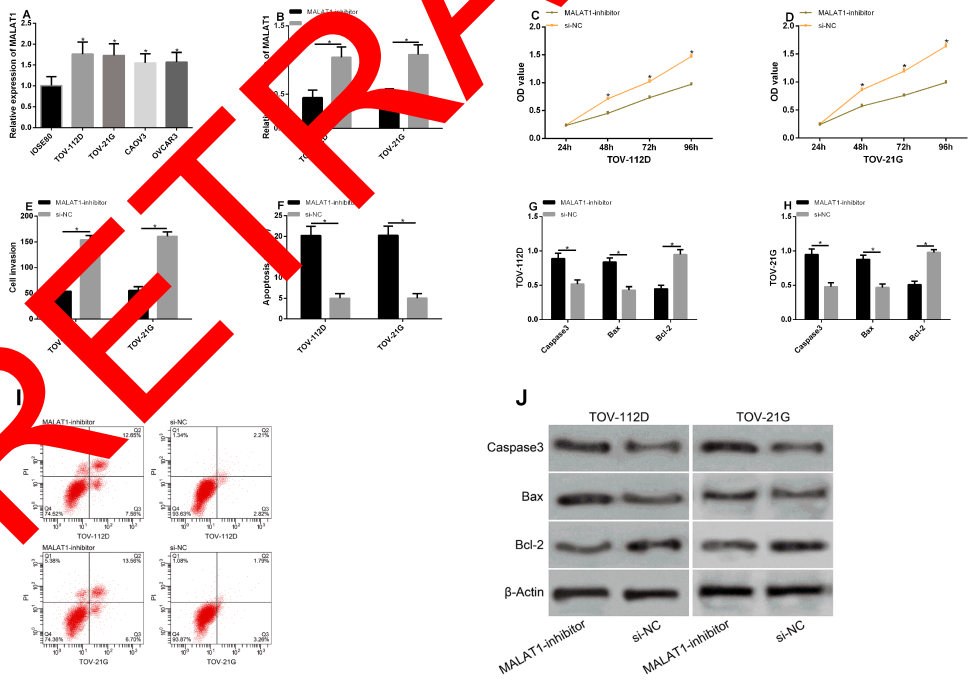

Figure 4 Effects of MALAT1 expression on biological functions of transfected cells. (**A**) MALAT1 expression in the cell lines of patients with EOC. (**B**) MALAT1 expression in TOV-112D and TOV-21G cells after transfection. (**C**) The proliferation of TOV-112D cells after transfection. (**D**) The proliferation of TOV-21G cells after transfection. (**E**) The invasion of TOV-112D and TOV-21G cells after transfection. (**F**) The apoptosis of TOV-112D and TOV-21G cells after transfection. (**G** and **H**) The effects of inhibiting MALAT1 on apoptosis-related proteins in TOV-112D and TOV-21G cells. (**I**) Flow cytometry maps. (**J**) Apoptosis-related protein maps.
Note: *Indicates P<0.05.

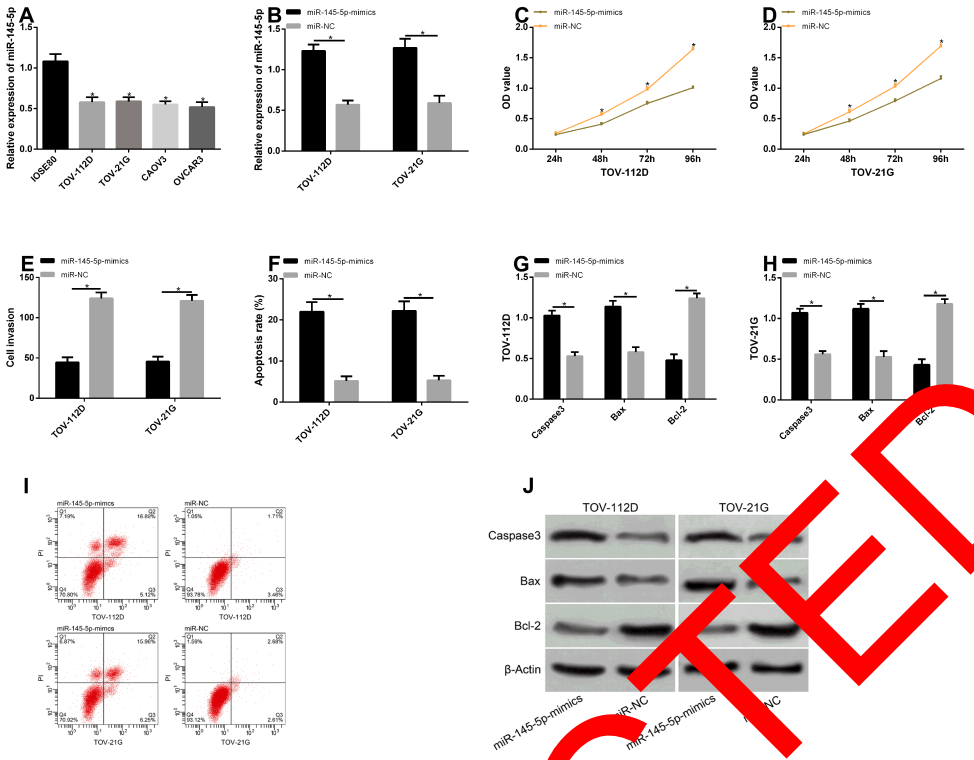

Figure 5 Effects of miR-145-5p expression on biological functions of transfected cells. (**A**) miR-145-5p expression in the cell lines of patients with EOC. (**B**) miR-145-5p expression in TOV-112D and TOV-21G cells after transfection. (**C**) The proliferation of TOV-112D cells after transfection. (**D**) The proliferation of TOV-21G cells after transfection. (**E**) The invasion of TOV-112D and TOV-21G cells after transfection. (**F**) The apoptosis of TOV-112D and TOV-21G cells after transfection. (**G** and **H**) The effects of overexpressing miR-145-5p on apoptosis-related proteins in TOV-112D and TOV-21G cells. (**I**) Flow cytometry maps. (**J**) Apoptosis-related protein maps.
Note: * indicates P<0.05.

increasing proliferation and invasion and a remarkably lower apoptotic rate when compared with those in the MALAT1-inhibitor group (P<0.05). See Figure 7.

Discussion

As the fifth leading cause of cancer death among women and one of the most lethal gynecologic malignant tumors in clinical practice,[18] EOC has different causes, pathogenesis, and prognosis.[19] Despite its standard therapeutic methods, its mortality rate remains high and has been not significantly improved.[20] Therefore, finding biomarkers that affect the prognosis of patients with the disease is particularly significant for improving the prognosis.

It has been confirmed that LncRNAs exert a great function in a variety of cancer biology,[21] and affect tumor progression by regulating cell cycles and immune responses.[22] MALAT1 expression has been determined to upregulate in various tumors. Moreover, this gene can affect the proliferation, migration, invasion, and apoptosis of tumor cells, so it is considered as a potential biomarker for diagnosing or predicting cancers and as a therapeutic target for treating specific tumors.[23] According to Li X and others, MALAT1 is associated with diabetic nephropathy, and its expression remarkably rises in patients with the disease. Inhibiting its expression can lower levels of apoptosis-related proteins, and can reduce the apoptotic rate in the patients via regulating miR-23c to target NLRP3.[24] However, it remains unclear whether MALAT1 can regulate the cell biological functions of patients with EOC through targeting miR-145-5p. Therefore, in this study, MALAT1 and miR-145-5p expression in the tissues and serum of the patients was first analyzed, and the clinical values of the two genes in the disease were observed. In our study, serum MALAT1 expression was remarkably higher while miR-145-5p expression was remarkably lower in the study group. According to the ROC curves, the AUCs of the two for diagnosing EOC were 0.897 and 0.878, respectively, which indicates that the two have high diagnostic efficiency for the disease. Subsequently, Pearson correlation analysis showed that serum MALAT1 expression was negatively correlated with miR-145-5p expression in the patients. Based on the Cox regression analysis, high MALAT1 expression, tumor size, degree of differentiation, case staging, and lymph node metastasis were the independent risk factors affecting the

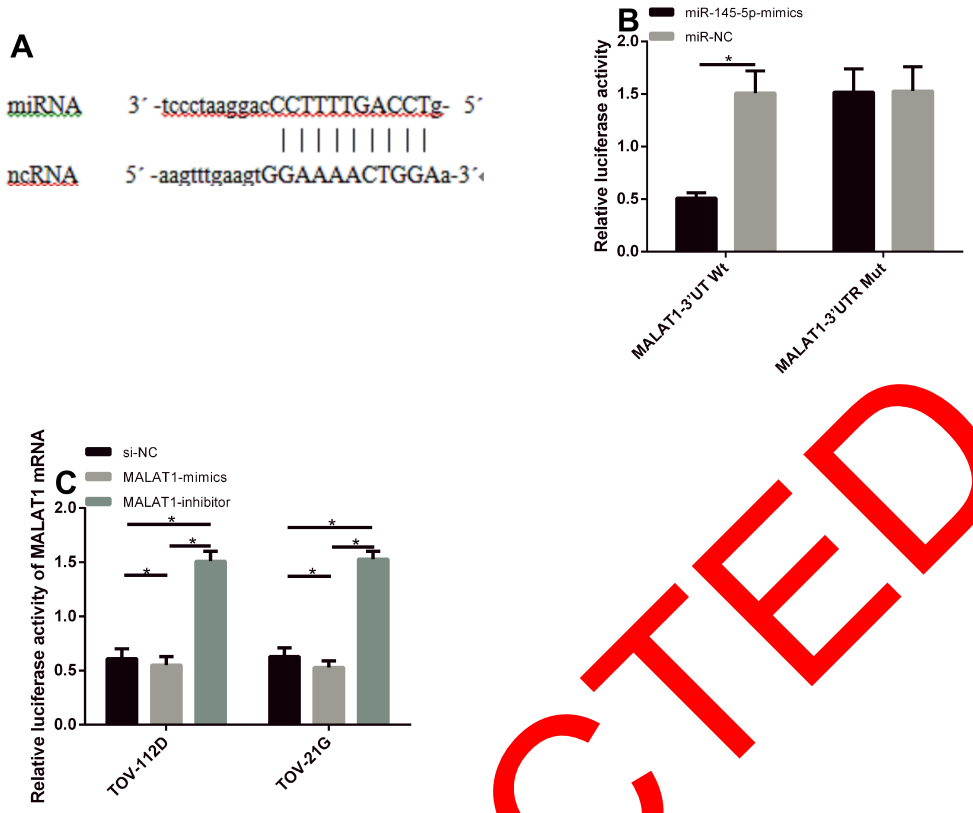

Figure 6 Gene identification of MALAT1. (**A**) The binding site between MALAT1 and miR-145-5p. (**B**) Results of dual luciferase reporter gene assay. (**C**) miR-145-5p expression in TOV-112D and TOV-21G cells after transfection.
Note: *Indicates P<0.05.

prognosis of the patients. According to Yang M and others, poor differentiation and positive lymph node metastasis are also the independent risk factors,[25] which is similar to findings of this study. Additionally, the EOC patients were also followed up for 5 years, and the results showed that their 5-year OSR was 65.2%. The 5-year OSR of patients with high MALAT1 expression was remarkably lower than that of those with low expression. This also suggests that serum MALAT1 is an independent prognostic factor that affects the 5-year OSR of EOC patients.

We speculate that MALAT1 and miR-145-5p were involved in the development and progression of EOC, so we further verified this conjecture through experiments of cell biology. According to previous studies, MALAT1 upregulates in tumor tissues and ovarian cancer cell lines, so inhibiting its expression can reduce the cells' proliferation, invasion, and metastasis and promote their apoptosis.[26] Many miRNAs downregulate in EOC. For instance, miR-145 expression reduces in the cancer tissues and cell lines of the disease, so overexpressing this miR can remarkably inhibit the proliferation, migration, and invasion of EOC cells.[27] In our study, MALAT1 and miR-145-5p expression in human normal ovarian epithelial cells and EOC cell lines was detected. MALAT1 expression was remarkably higher while miR-145-5p expression was remarkably lower in EOC cell lines. Subsequently, we selected the cell lines with significant differences for transfection, and observed their biological functions. After the transfection with MALAT1-inhibitor and miR-145-5p-mimics plasmids, TOV-112D and TOV-21G cells that were transfected with MALAT1 had remarkably lower proliferation and invasion and a remarkably higher apoptotic rate. Those transfected with miR-145-5p had remarkably lower proliferation and invasion and higher apoptosis. We also observed the effects of the two genes on apoptosis-related proteins, and found that inhibiting MALAT1 or overexpressing miR-145-5p could increase Caspase-3 and Bax levels and reduce Bcl-2 levels. These findings suggest that MALAT1 and miR-145-5p can become potential therapeutic targets for EOC. Then, for exploring the correlation between the two, the rescue experiment was performed. The cells in the MALAT1-inhibitor + miR-145-5p-inhibitor group were not different from those in the si-NC group with respect to their proliferation, invasion, and apoptosis, but they had remarkably

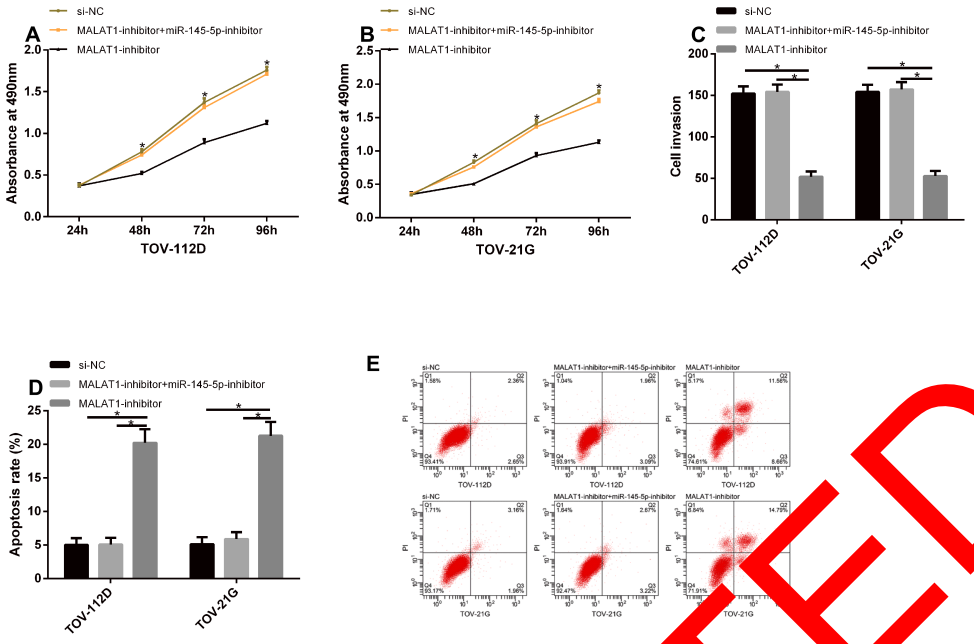

Figure 7 Rescue experiment. (**A** and **B**) The cells in the MALAT1-inhibitor + miR-145-5p-inhibitor group were not different from those in the si-NC group with respect to their proliferation, but they had remarkably increasing proliferation when compared with those in the MALAT1-inhibitor group. (**C**) The cells in the MALAT1-inhibitor + miR-145-5p-inhibitor group were not different from those in the si-NC group with respect to their invasion, but they had remarkably increasing invasion when compared with those in the MALAT1-inhibitor group. (**D**) The cells in the MALAT1-inhibitor + miR-145-5p-inhibitor group were not different from those in the si-NC group with respect to their apoptosis, but they had remarkably reducing apoptosis when compared with those in the MALAT1-inhibitor group. (**E**) Flow cytometry maps.
Note: *Indicates P<0.05.

increasing proliferation and invasion and a remarkably lower apoptotic rate when compared with those in the MALAT1-inhibitor group. This shows a regulatory relationship between the two genes. Therefore, dual luciferase reporter gene assay was conducted for further verifying the relationship. miR-145-5p overexpression remarkably reduced MALAT1-3′UTR Wt luciferase activity, but it had no effect on MALAT1-3′UTR Mut luciferase activity. Moreover, the transfection of MALAT1-mimics could remarkably reduce miR-145-5p expression in TOV-112D and TOV-21G cells, while that of MALAT1-inhibitor could remarkably increase the expression in the cells. These findings demonstrate that MALAT1 has a targeted regulatory relationship with miR-145-5p, i.e. inhibiting MALAT1 could increase miR-145-5p expression, thus inhibiting EOC cells from proliferating and invading and thereby increasing their apoptotic rate.

In this study, we have confirmed that MALAT1 is highly expressed in EOC cells and can mediate their biological functions via regulating miR-145-5p. However, this study still has shortcomings. Firstly, tumor formation in nude mice was not performed in this basic study, and whether MALAT1 expression in cell experiments is consistent with that in experiments in vivo needs further verification. Therefore, we hope to carry out more basic studies, analyze more potential mechanisms of MALAT1 through bioinformatics, and collect samples with more kinds (healthy people, benign ovarian lesions) and types (serum) in future research, so as to verify our research results.

Conclusion

MALAT1 promotes EOC cells' survival through down-regulating miR-145-5p, so it may become a new direction for EOC diagnosis and gene therapy.

Disclosure

The authors report no conflicts of interest for this work. Ye Zhao and Yi-Min Wang are co-corresponding author.

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