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MicroRNA-18a suppresses ovarian carcinoma progression by targeting CBX7 and regulating ERK/MAPK signaling pathway and epithelial-to-mesenchymal transition

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Abstract. – OBJECTIVE: Ovarian carcinoma (OC) is one prevalent fatal malignancy in gynecology. Currently, there is an imperative need to better investigate the pathogenesis of OC. Accumulating evidence has indicated that microRNAs (miRNAs) play pivotal roles in OC occurrence and development. In this study, we mainly investigated the potential roles of miR-18a in OC progression.

PATIENTS AND METHODS: We first examined miR-18a expressions in OC tissue samples and cell lines using quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Moreover, OC patients involved in current study were assigned into two groups based on the mean miR-18a expression level. Kaplan-Meier analysis was carried out to assess the overall survival rate of miR-18a in OC patients. Next, we investigated whether miR-18a could regulate OC cell proliferation abilities by using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assays. Next, transwell assay was used to detect the effects of miR-18a on cell invasion and migration. We further performed Luciferase reporter assays by cotransfecting with miR-18a mimics and the Luciferase reporter vector containing CBX7 3'UTR-WT or MUT. We then performed immunohistochemistry (IHC) assays to determine the expression of CBX7 in OC tissues.

RESULTS: QRT-PCR results indicated that miR-18a expressions were notably decreased in OC related cell lines and tissues. Moreover, the low miR-18a expression was related to the malignant phenotype and poor prognosis of OC patients. Overexpression of miR-18a in OC cells could prominently suppress the proliferation, migration and invasion abilities via modulating ERK/MAPK pathway and epithelial-to-mesenchymal transition (EMT). Furthermore, CBX7 was confirmed as a functional target of miR-18a, indicating that miR-18a exerted the suppressive functions in OC cells partially via the regulation of CBX7. Additionally, restoration of miR-18a remarkably reduced the OC tumor growth in vivo.

CONCLUSIONS: Taken together, our study rationally suggested that miR-18a may serve as an effective diagnostic and therapeutic biomarker for OC.

Key Words:
MiR-18a, Ovarian cancer, CBX7, ERK/MAPK, Epithelial-to-mesenchymal transition.

Introduction

Ovarian carcinoma (OC), as one prevalent gynecological malignant tumor around the world, has a high incidence and is one severe tumor which affects the reproductive tracts of females[1]. Due to the lack of effective early screening methods, OC patients are often detected in the late stages and have a poor prognosis[2]. Additionally, distant metastases and recurrence continue to be the leading threat to advanced OC patients[3,4]. The widely used treatment methods, such as surgery, chemotherapy, and radiotherapy, were mostly adopted for OC treatment[5]. However, due to the lack of reliable diagnostic biomarker, the diagnosis and therapeutic approaches for OC patients remain challenging[6]. Therefore, further research on the molecular mechanism implicated in OC development is essential for effective OC treatments.

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MicroRNAs are promising molecular biomarkers for the diagnosis and prognosis of cancers[7] and can regulate multiple key cellular processes via targeting the 3'-UTRs of target genes, thereby leading to translational inhibition or mRNA degradation[8]. MiRNAs exerted vital functions in the development of many cancers by acting as either tumor suppressor or oncogene[9,10]. Growing attention has been paid to the functions of miRNAs in various types of malignancies. In particular, Ahmad et al[11] found that miR-135a inhibited breast cancer cell proliferation via regulating ELK1 and ELK3; Bai et al[12] reported that miR-20a-5p promoted cell growth via targeting RUNX3 in triple-negative breast cancer; Chen et al[13] revealed that miR-148a suppressed non-small cell lung cancer cell migration and invasion via targeting Wnt1. All of these reports showed the direct correlation between the aberrant miRNA expressions and tumor progression. However, the functional relevance of miR-18a in OC remains elusive.

Chromobox homolog 7 (CBX7) is a chromobox family protein which belongs to polycomb repressive complex 1 (PRC1)[14]. Increasing evidence[15] has showed the function of CBX7 for tumor treatment. However, the functions of CBX7 in cancer development remain controversial. CBX7 acts as either oncogene or tumor suppressor depending on the cellular environment and tumor types. Ni et al[16] indicated that CBX7 suppressed pancreatic carcinoma cell proliferation, invasion, and migration via the inhibition of PTEN/Akt signaling. On the contrary, Zhang et al[17] found oncogenic roles of CBX7 in gastric cancer. These results indicated that the specific functions of CBX7 in tumor progression may be different between cancers. In this research, we focused on the functions and underlying molecular mechanisms of CBX7 in OC development.

Material and Methods

Tissue Samples

OC tissue samples and the matched para-carcinoma tissues were obtained from 50 OC patients who underwent surgery at the Qilu Hospital of Shandong University from July 2015 to August 2017. Informed consent was provided by all patients before surgery. No patients had received any treatment prior to tissue collection. All tissue specimens were frozen in liquid nitrogen immediately and then stored at –80°C for further assays. The current study was approved by the Ethics Committee of Qilu Hospital of Shandong University. This investigation was conducted in accordance with the Declaration of Helsinki.

Cell Culture

OC cell lines (SKOV3, OVCAR3, HO8910, and A2780) and the normal immortalized human ovarian surface epithelial cells IOSE29 were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Carlsbad, CA, USA), including 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) at 37°C and 5% CO_2.

Cell Transfections

MiR-18a mimics, inhibitors and the negative controls (NC) were obtained from GenePharma Co., Ltd. (Shanghai, China). Then, Lipofectamine® 2000 reagent (Thermo Fisher Scientific, Waltham, MA, USA) was utilized to transfect the miR-18a mimics or inhibitors into OC cells following the manufacturer's protocols. Efficiencies were analyzed by qRT-PCR 48 h after the transfection.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNAs were extracted from OC tissues or cells by TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's recommendations. Then, cDNA was synthesized from the isolated RNA with M-MLV Reverse Transcriptase (Promega, Madison, WI, USA). SYBR® Premix Ex Taq™ II kit (TaKaRa, Shanghai, China) was used to examine the expression levels of genes on ABI 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA, USA). The expression fold change in expression was calculated by the $2^{-\Delta\Delta Ct}$ method. U6 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were endogenous controls for miR-18a and CBX7, respectively. The sequences of the primers were described in Table I.

Immunohistochemistry (IHC)

IHC assays were performed to measure the expressions of CBX7 in OC tissues and adjacent normal tissues. Briefly, 10% formalinfixed and paraffinembedded tissue sections were deparaffinized and rehydrated with xylene and graded alcohols. After pretreatment with citrate buffer in a microwave oven for the endogenous anti-

Table I. Primer sequences for qRT-PCR.

Primer	Sequence
miR-18a forward	5'-GCTGAGCTAAGGTGCATCTAG-3'
miR-18a reverse	5'-TCAACTGGTGTCGTGGAGT-3'
U6 forward	5'-CTCGCTTCGGCAGCACA-3'
U6 reverse	5'-AACGCTTCACGAATTTGCGT-3'
CBX7 forward	5'-GGATGGCCCCCAAAGTACAG-3'
CBX7 reverse	5'-TATACCCCGATGCTCGGTCTC-3'
GAPDH forward	5'-AGGTCGGTGTGAACGGATTTG-3'
GAPDH reverse	5'-TGTAGACCATGTAGTTGAGGTCA-3'

U6: small nuclear RNA, snRNA; CBX7: Chromobox homolog 7; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

gen-retrieval, the endogenous peroxidase activities were repressed by 3% hydrogen peroxide (H_2O_2) in ethanol for 10 min. Then, tissues were incubated with primary CBX7 antibody (1:200, ab91413, Abcam, Cambridge, MA, USA) at 4°C overnight. Next, the slides were incubated with goat anti-rabbit IgG (1:2,000, ab205718, Abcam, Cambridge, MA, USA) labeled by horseradish peroxidase (HRP) for 30 min at room temperature, followed by being stained with DAB as the chromogen and counterstained with hematoxylin. The sections were then photographed with a BX53F microscope (Olympus, Tokyo, Japan). The expressions were calculated following the ratio of positive cells: stained cells/all cells < 25% was considered as negative (-) while the ratio >25% was positive (+)[18,19].

Cell Proliferation Assay

MTT assay was conducted to determine the effects of miR-18a on OC cell viabilities at different time points (0, 24, 48, and 72 h after treatment with miR-18a mimic or inhibitor). First, OC cells treated with miR-18a mimics or inhibitor were plated into a 96-well plate, and after being cultured for indicated times, MTT solution (10 μL; Beyotime, Shanghai, China) was added into the treated cells followed by another 4 h incubation at 37°C. Then, dimethyl sulfoxide (DMSO) solutions were added to dissolve the crystal. Next, a microplate reader (BioTek, Winooski, VT, USA) was used to measure optical density at wavelength of 490 nm.

Transwell Assays

Transwell chamber inserts (8.0 μm pore size; Corning, Corning, NY, USA) with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) present or absent were utilized to examine the functions of miR-18a in OC cell invasion or migration abilities. In brief, treated OC cells were seeded in the upper chambers with serum-free medium whereas medium supplemented with 10% FBS was added to the low chambers as a chemoattractant. Following incubation at 37°C for 48 h, cells remained in the top chambers were carefully wiped away with cotton swabs, while cells that had traversed the membrane were subsequently fixed and stained with formaldehyde (4%) and crystal violet (0.1%), respectively. An inverted microscope (Olympus, Tokyo, Japan) was used to measure and count the invasive and migratory cells in five randomly selected fields.

Western Blot

For protein analysis, OC cells were lysed with iced lysis buffer containing protease and phosphatase inhibitors (Roche, Basel, Switzerland). A bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA) was utilized to determine the protein concentration. Then, proteins were separated with 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, the proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Invitrogen, Carlsbad, CA, USA). The membrane was blocked in Tris-Buffered Saline and Tween-20 (TBST) containing 5%-skim milk for 2 h at room temperature. After that, the membrane was incubated with specific primary antibodies at 4°C overnight. The primary antibodies were as follows: CBX7 (1:2000, ab91413, Abcam, Cambridge, MA, USA), E-cadherin (1:1000, ab40772, Abcam, Cambridge, MA, USA), Vimentin (1:1000, ab45939, Abcam, Cambridge, MA, USA), ERK (1:1000, ab17942, Abcam, Cambridge, MA, USA),

p-ERK (1:2000, ab192591, Abcam, Cambridge, MA, USA) and GAPDH (1:1000, ab9485, Abcam, Cambridge, MA, USA). Subsequently, the membrane was incubated with a HRP-conjugated secondary antibody (1:2000, ab6721, Abcam, Cambridge, MA, USA) at room temperature for 1 h. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. Finally, proteins were detected by enhanced chemiluminescence (ECL; Beyotime, Shanghai, China).

Dual-Luciferase Reporter Assay

The wild type (WT) CBX7 3′-UTR or the mutant (Mut) sequence of CBX7 was inserted into the pGL3 vectors (Promega, Madison, WI, USA). For Luciferase assays, OC cells were cotransfected with wild-type or mutant CBX7-3'UTR and miR-18a mimics by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocols. Luciferase activity was determined by the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) after transfection for 48 h.

Xenograft Tumor Formation Assay

Xenograft experiments were carried out to investigate the functions of miR-18a restoration in the tumorigenicity of OVCAR3 cells *in vivo*. Female BALB/c nude mice (4-6-week-old) were randomly assigned into two groups. OVCAR3 cells were stably transfected with lentiviral miR-18a (lenti-miR-18a) or the negative lentiviral miR-control (lenti-control) and injected subcutaneously into the flanks of the mice in different groups. Once Xenograft tumors had been established in the mock-treated mice, tumor volumes were examined every 3 days according to the following formula: tumor volumes (mm^3) = length×width^2/2.

Statistical Analysis

Each experiment was conducted at least 3 times. Statistical analysis was performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Differences between two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). Kaplan-Meier method and log-rank test were applied to estimate the overall survival rates (OS) and compare the survival curves, respectively. $p<0.05$ was regarded as statistically significant difference.

Results

MiR-18a was Downregulated in OC Tissues and Associated With the Poor Prognosis

To confirm the clinical significance of miR-18a in OC, we first examined the expression of miR-18a in OC tissue samples and adjacent normal tissue samples using qRT-PCR. Results indicated that miR-18a was prominently downregulated in OC tissue samples in comparison to the normal tissues (Figure 1A). Moreover, OC patients involved in the current study were assigned into

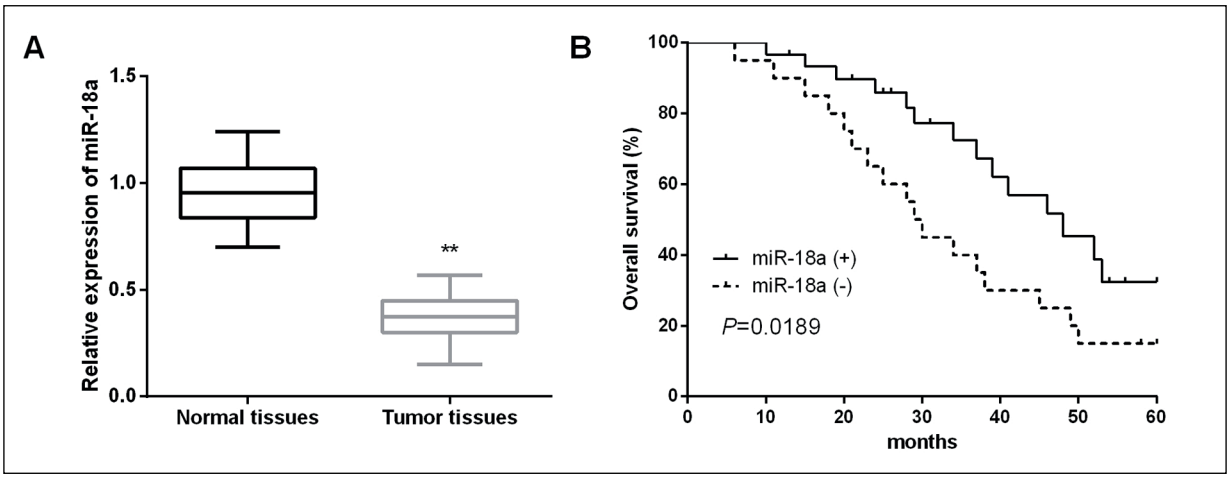

Figure 1. MiR-18a was decreased in OC and associated with poor prognosis. **A,** MiR-18a expressions in OC tissues were detected by qRT-PCR. **B,** Kaplan-Meier analysis of OC patients with different miR-18a expressions. $^{**}p<0.01$.

two groups based on the mean miR-18a expression level. Kaplan-Meier analysis was carried out to assess the prognostic functions of miR-18a in OC patients. The results presented that OC patients with low miR-18a expressions have evident shorter OS than patients with high miR-18a expression (Figure 1B). We also determined the functional values of miR-18a in the clinicopathological characteristics of OC patients. Findings demonstrated that lower miR-125b-5p expression was significantly related to TNM stage, lymph-node metastasis, FIGO stage, and distant metastasis of OC (Table II).

MiR-18a Overexpression Significantly Inhibited OC Cell Proliferation

Based on these results, we assumed that miR-18a exerted an inhibitory effect on OC progression. Firstly, we investigated whether miR-18a could regulate OC cell proliferation abilities by MTT assays. Results showed that, compared to the normal human ovarian surface epithelial cells IOSE29, the miR-18a expressions in all OC cells were remarkably reduced (Figure 2A). Then, OVCAR3 and SKOV3 cells were selected for further functional assays. Briefly, miR-18a mimics or inhibitor were transfected into OVCAR3 and SKOV3 cells, respectively. Then, qRT-PCR analysis confirmed that miR-18a was successfully over-expressed or inhibited in OVCAR3 and SKOV3 cells (Figure 2B, 2C). Furthermore, results of MTT assays indicated that miR-18a overexpression notably inhibited OVCAR3 cell proliferation (Figure 2D). On the other hand, the inhibition of miR-18a in SKOV3 cells evidently enhanced the proliferation capacities (Figure 2E).

MiR-18a Upregulation in OC Cells Impaired the Invasion and Migration Abilities

We investigated whether miR-18a overexpression influenced OC cell invasion and migration by performing transwell assay. We found that the migration and invasion capacities of OVCAR3 cells were significantly inhibited by miR-18a mimics (Figure 3A, 3B). In contrast, the migration and invasion capacities of SKOV3

Table II. Correlation of miR-18a expression with the clinicopathological characteristics of the ovarian cancer patients.

Clinicopathological features	Cases (n = 50)	miR-18a[b] expression		p-value
		High (n = 20)	Low (n = 30)	
Age (years)				0.5359
> 60	27	10	16	
≤ 60	27	10	14	
Family history of cancer				0.4366
Yes	27	8	17	
No	27	12	13	
Tumor size (cm)				0.3921
≥ 5.0	29	9	16	
< 5.0	25	11	14	
TNM stage				0.0015*
I-II	24	16	7	
III	30	4	23	
Lymph-node metastasis				0.0021*
Yes	28	3	20	
No	26	17	10	
Pausimenia				
Yes	30	11	15	0.4656
No	24	9	15	
FIGO stage				0.0018*
I-II	23	15	7	
III-IV	31	5	23	
Distant metastasis				
0.0024*				
Yes	30	4	22	
No	24	16	8	

TNM: tumor-node-metastasis; FIGO: International Federation of Gynecology and Obstetrics; [b]The mean expression level of miR-18a was used as the cutoff; *Statistically significant.

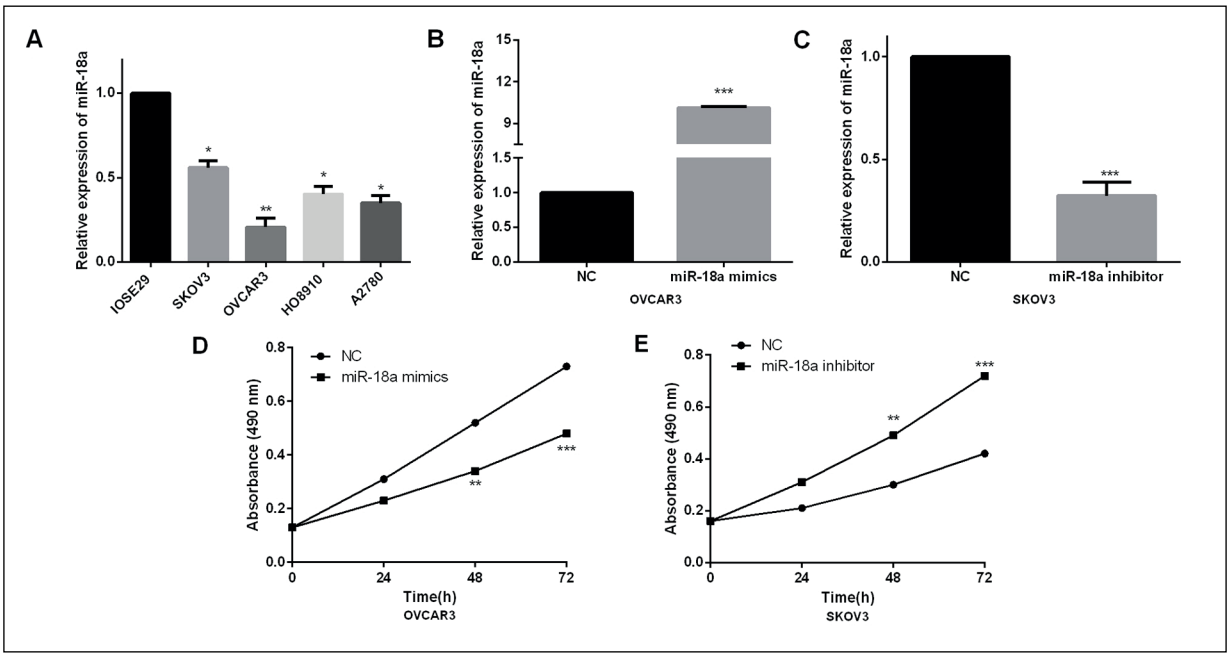

Figure 2. MiR-18a restoration prominently repressed OC cell proliferation. **A,** QRT-PCR analysis was performed to measure miR-18a expressions in OC cells. **B,** MiR-18a overexpression in OVCAR3 cells were determined by qRT-PCR. **C,** MiR-18a inhibition in SKOV3 was confirmed by qRT-PCR. **D, E,** MTT assays were carried out to detect the effects of miR-18a on the proliferation ability of OC cells. **p*<0.05, ***p*<0.01, ****p*<0.001.

cells were significantly enhanced by miR-18a inhibitor (Figure 3C, 3D). Therefore, these findings demonstrated that miR-18a was involved in the regulation of OC cell invasion and migration and exerted anti-tumor functions in OC progression.

CBX7 Was a Target of MiR-18a in OC Cells

To further investigate the mechanisms of miR-18a in regulating the biofunctions of OC cells, we explored the candidate target of miR-18a by target analysis. The results showed that CBX7 3'UTR contained target sequences for miR-18a (Figure 4A). To confirm the association between CBX7 and miR-18a in OC cells, we further performed Luciferase reporter assays by cotransfecting with miR-18a mimics or containing CBX7 3'UTR-WT or MUT. We found that the transfection of miR-18a mimics significantly reduced CBX7 3'UTR-WT Luciferase activities, while it had no significant inhibition effect on the CBX7 3'UTR-MUT Luciferase activities in OC cells (Figure 4B). In addition, results showed that the CBX7 expression were notably inhibited by miR-18a mimics in OVCAR3 cells while miR-18a inhibitor significantly promoted the CBX7 expression in SKOV3 cells (Figure 4C, 4D). Taken together, CBX7 was verified to be a functional target for miR-18a in OC cells.

MiR-18a Regulated ERK/MAPK Signaling Pathway and EMT in OC Cells

We then investigated the prognostic value of CBX7 in OC patients. IHC assays revealed that CBX7 mainly localized at the nucleus and significantly higher in OC tissues (Figure 5A, 5B). Moreover, Kaplan-Meier analysis indicated that OC patients with higher CBX7 expression presented poorer OS than patients with lower CBX7 expressions (Figure 5C). Subsequently, to further investigate the potential molecular mechanism underlying OC tumorigenesis, ERK/MAPK signaling pathway and EMT were chosen as research targets. Western blot analysis demonstrated that p-ERK expressions were markedly inhibited by miR-18a overexpression in OVCAR3 cells whereas notably enhanced by miR-18a inhibition in SKOV3 cells. However, the ERK expressions were not altered by miR-18a overexpression or inhibition (Figure 5D). In the meanwhile, the functions of miR-18a in OC cell EMT were also investigated. The expression of EMT-related markers was measured by Western blots. The findings indicated that the E-cadherin expres-

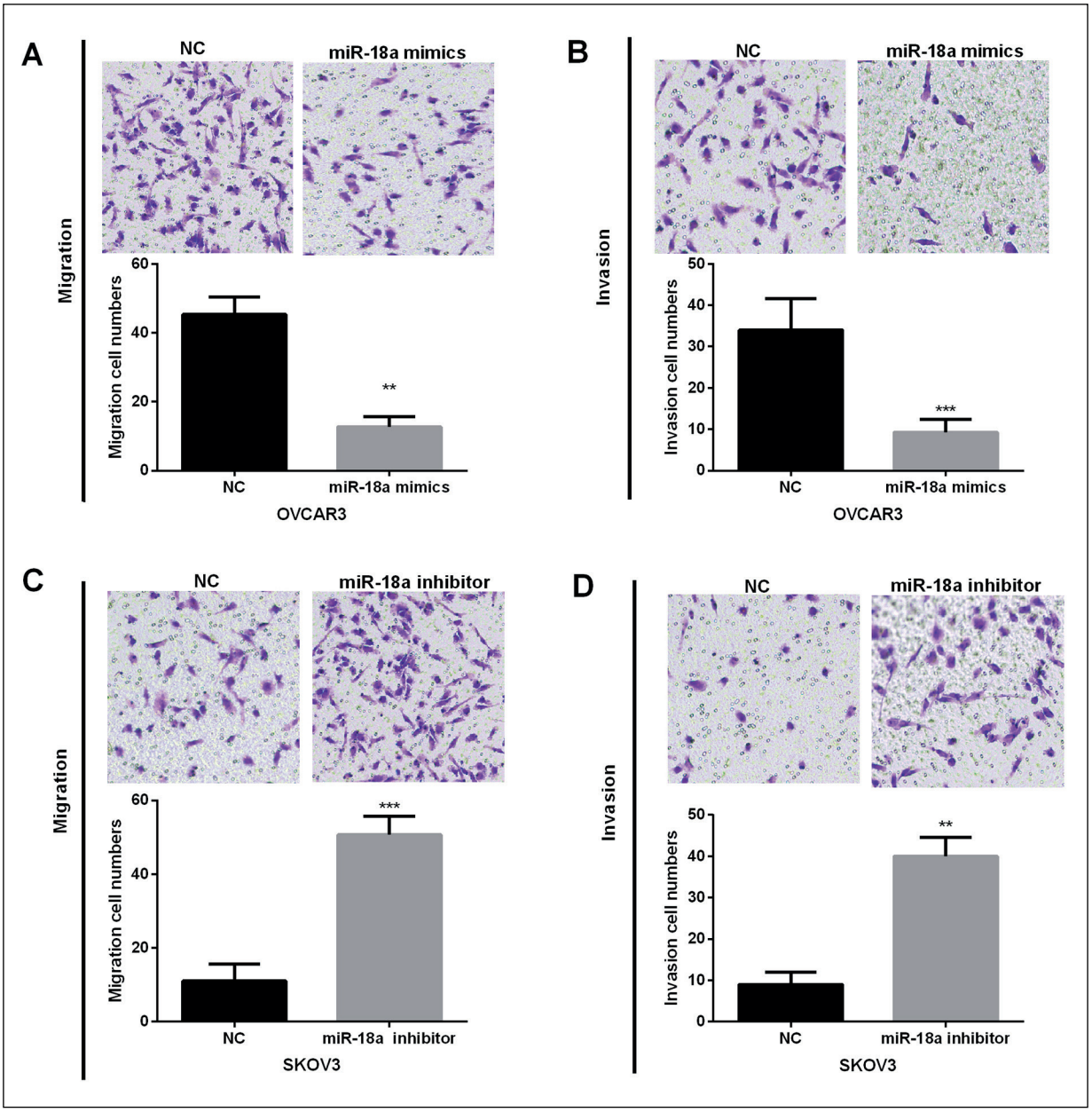

Figure 3. MiR-18a upregulation suppressed OC cell invasion and migration. **A, B,** Transwell assays were carried out to detect the invasion and migration capacities of miR-18a-overexpressed OVCAR3 cells. **C, D,** Invasion and migration capacities of miR-18a-suppressed SKOV3 cells were observed by transwell assays. Magnification 200×. **p<0.01, ***p<0.001.

sions in OVCAR3 cells were markedly increased while N-cadherin and vimentin expressions were significantly suppressed by miR-18a upregulation; on the contrary, miR-18a knockdown notably repressed the E-cadherin expressions and enhanced the vimentin and N-cadherin expressions in SKOV3 cells (Figure 5D). Collectively, these findings revealed that miR-18a functioned as a tumor suppressor in OC progression via regulation of ERK/MAPK and EMT pathway.

MiR-18a Overexpression Inhibited OC Xenograft Growth

The sizes of the xenograft ovarian tumors were detected to elucidate the functions of miR-18a in tumor growth *in vivo*. Results showed that miR-18a overexpression markedly slowed down the growth rate and the sizes of tumors originating from miR-18a-overexpressed OVCAR3 cells were prominently smaller than those of the negative controls (Figure 6A, 6B). These data

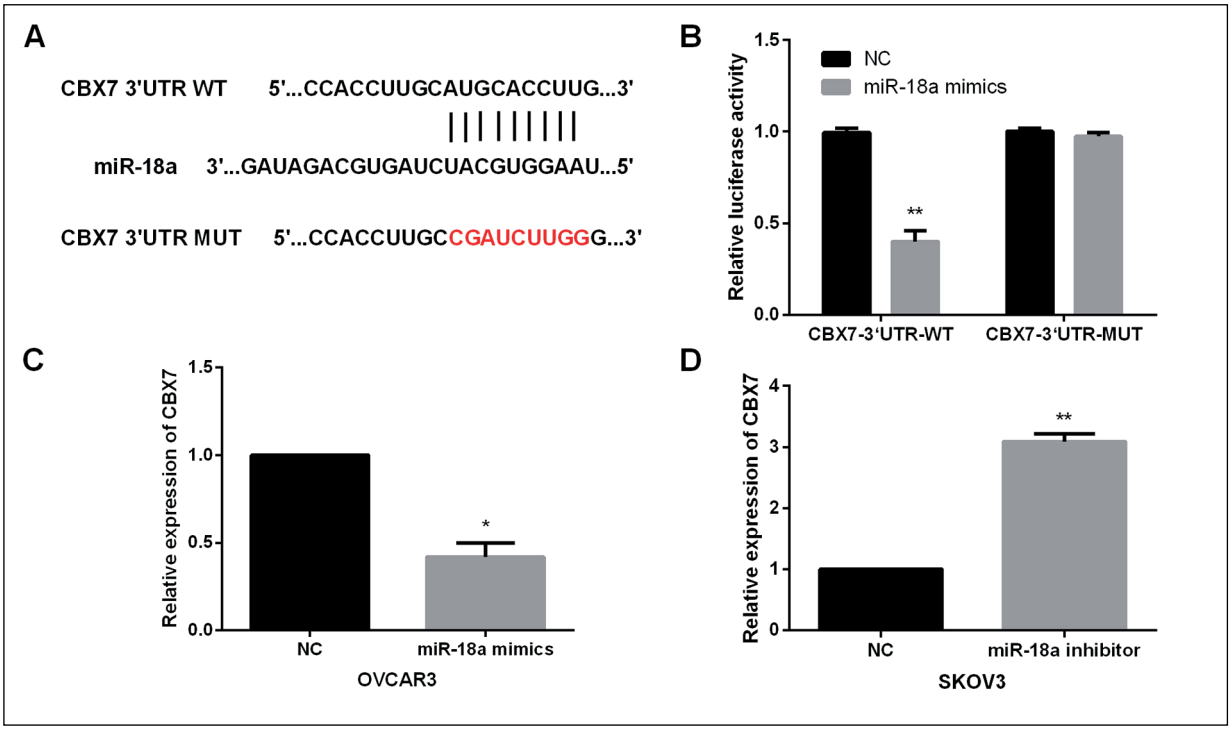

Figure 4. CBX7 was a functional target for miR-18a in OC cells. **A,** Predicted miR18a target sequences in the 3'UTR of CBX7. **B,** Luciferase activities in OC cells were determined after cotransfection with wide-type or mutant-type CBX7 3'-UTR and miR-18a mimics. **C, D,** Effects of miR-18a on CBX7 expressions in OC cells. *p<0.05, **p<0.01.

provided evidence to support the conclusion that the miR-18a overexpression weakened ovarian tumor malignancy *in vivo*.

Discussion

Due to unclear early symptoms of OC and lacking effective screening approaches, OC is frequently diagnosed at late stages with high relapse rates and poor prognosis[20,21]. Therefore, developing novel biomarkers for early detections of OC has become a hot spot in tumor investigation[22]. However, the inherent deficiencies of current diagnostic strategies for OC treatment limit their clinical applications. Hence, there is an imperative need to identify minimally invasive and highly accurate biomarkers for earlier detection of OC. With the advancement of molecular biology, molecular targeted therapy has become a new treatment for malignant tumors[23]. Recently, numerous miRNAs have been proven to predict OC metastasis and prognosis, serving as molecular biomarkers and therapeutic agents for OC treatment. For example, Zheng et al[24] indicated that miR-101 suppressed OC cell proliferation and invasion via downregulating SOCS-2. Li et al[25] proposed that miR-221 overexpression promoted OC cell proliferation via regulating the apoptotic protease activating factor-1. In the present research, we investigated the functional effects and mechanism of miR-18a in OC progression.

MiR-18a appears to act as a cancer suppressor or an oncogene in various tumors, playing pivotal roles in carcinogenesis[26]. MiR-18a is a research hotspot in recent years. Fan et al[27] proposed that miR-18a upregulation enhanced triple negative cancer cell autophagy via repressing mTOR signaling pathway; Humphreys et al[28] reported that miR-18a played a tumor suppressive role in colorectal cancer and inhibited CDC42 expression; Chen et al[29] observed that miR-18a promoted nasopharyngeal carcinoma proliferation and metastases via downregulating DICER1. In the current study, we found that miR-18a expressions were notably decreased in OC and the low miR-18a expression was related to the malignant features and poorer prognosis of OC patients. Moreover, functional assays showed that miR-18a overexpression markedly suppressed OC cell proliferation, invasion and migration abilities. We also found that miR-18a upregulation significantly inhibited

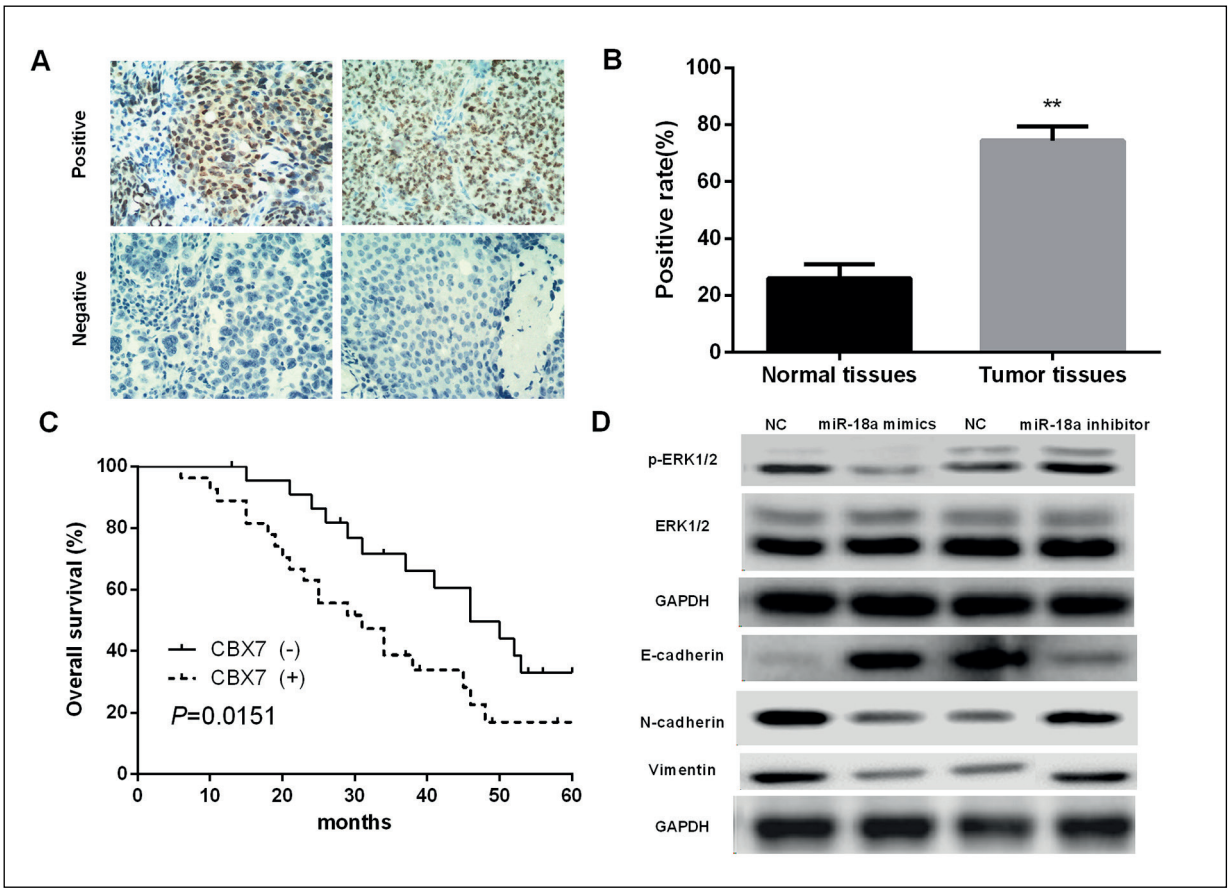

Figure 5. MiR-18a regulated the ERK/MAPK signaling pathway and EMT of OC cells. **A, B,** IHC assay was conducted to determine the CBX7 expressions in OC tissues (magnification 100X). **C,** Kaplan-Meier analysis of OC patients with high and low CBX7 expressions. **D,** Western blot was performed to assess the influence of miR-18a on ERK/MAPK signaling pathway and EMT in OC cells. $^{**}p<0.01$.

OC tumor growth *in vivo*. Consistent with these findings, Liu et al[30] showed that miR-18a inhibited OC growth via directly targeting IPMK and TRIAP1. Based on the existing literature and our experimental results, we speculate that miR-18a may have a function of tumor suppressor gene in OC.

During the development of tumors, high recurrence rates are attributed to abnormal metastases. Epithelial-to-mesenchymal transition (EMT) is

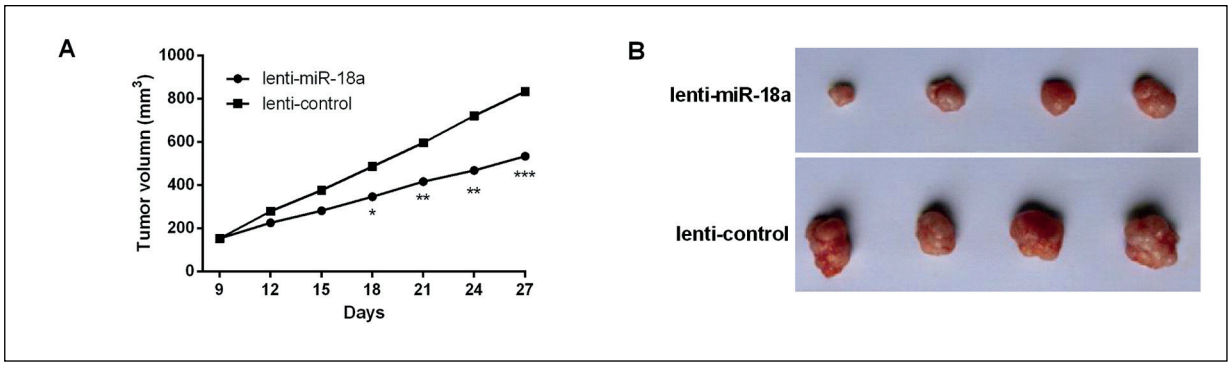

Figure 6. MiR-18a overexpression repressed OC tumor growth *in vivo*. **A,** Schematic representation of OVCAR3 xenograft tumors in the lenti-miR-18a and lenti-control groups. **B,** Tumor growth curves of the groups with different treatments. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

the biological progress where epithelial cells are transformed into mesenchymal cells[31]. In this process, cell adhesion molecules (E-cadherin) were downregulated; on the other hand, the mesenchymal markers (N-cadherin, vimentin) were upregulated[32]. Aceto et al[33] have claimed that the adhesion abilities of cancer cells may inhibit the migration capacities. Thus, EMT was associated with metastasis of tumors. In addition, ERK/MAPK is an important intracellular pathway, which is known for the regulation of cell proliferation, survival, and growth. Here, we investigated the potential molecular mechanism underlying the functions of miR-18a in OC cell proliferation, invasion, and migration through EMT and ERK/MAPK signaling pathways. Findings revealed that the E-cadherin expressions in OVCAR3 cells were markedly increased while p-ERK, N-cadherin, and vimentin expressions were significantly suppressed by miR-18a upregulation. MiR-18a inhibitor could reverse this trend, indicating that low expression of miR-18a reduced ERK/MAPK and EMT signaling pathway in OC cells.

The basic mechanisms by which miRNAs regulate gene expressions are primarily via the modulation of target genes. Thus, it is important to determine the potential targets for miR-18a in OC. In this study, CBX7 was revealed to be a target for miR-18a in OC cells. Several studies have disclosed that CBX7 may have diverse roles in different tumors, including OC. Shinjo et al[34] discovered that CBX7 was correlated with poor prognosis of ovarian clear cell adenocarcinoma. In this investigation, we provided further evidence that CBX7 was up-regulated in OC and indicated poor prognosis of OC patients.

Conclusions

Findings in this investigation showed that miR-18a was down-regulated in OC, which was associated with the aggressive progression and poor prognosis of OC patients. Furthermore, the functional assays indicated that miR-18a overexpression significantly repressed OC cell proliferation, invasion and migration capacities via the regulation of ERK/MAPK pathway and EMT. Additionally, CBX7 was identified as a functional target of miR-18a in OC cells, suggesting that the suppressive functions of miR-18a were partially regulated by CBX7. Furthermore, miR-18a overexpression was observed to notably inhibit tumor growth *in vivo*. The present research may provide the potential theoretical foundation for the diagnosis and treatment of OC. Taken together, our study rationally suggested that miR-18a may serve as an effective diagnostic and therapeutic biomarker for OC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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