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Original Article

Long non-coding RNA MCM3AP-AS1 inhibits cell viability and promotes apoptosis in ovarian cancer cells by targeting miR-28-5p

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Abstract: Background: Ovarian cancer (OC) is one of the most malignant cancers in women. Increasing numbers of studies have shown that microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) play important roles in OC progression and prognosis. Purpose: The present study aimed to characterize the functions and primary molecular mechanisms of lncRNA-MCM3AP-AS1 in OC. Materials and methods: Quantitative real-time reverse transcription (qRT-PCR) was performed to measure MCM3AP-AS1 and microRNA-28-5p (miR-28-5p) expression in OC tissues and adjacent normal tissues, respectively. A receiver operating characteristic (ROC) curve was used to analyze the prognosis of OC. Luciferase reporter gene assay was carried out to identify regulation of MCM3AP-AS1 on miR-28-5p transcription activity. Methylthiazoletetrazolium (MTT) assay was used to detect the effect of MCM3AP-AS1 on the viability ability of OC cells. Flow cytometry analysis was used to measure the effect of MCM3AP-AS1 on the apoptosis of OC cells. Results: Both MCM3AP-AS1 and miR-28-5p expression were down-regulated in OC tissues compared to adjacent normal tissues. MCM3AP-AS1 and miR-28-5p expression was significantly associated with lymphatic metastasis and TNM stage. The area under the ROC curve values for MCM3AP-AS1 and miR-28-5p were 0.714 and 0.863, respectively. A positive correlation between MCM3AP-AS1 and miR-28-5p expression was also observed. Moreover, MCM3AP-AS1 directly and positively regulated miR-28-5p transcription. MCM3AP-AS1 suppressed viability and promoted apoptosis of OC cells via targeting miR-28-5p in OC. Conclusions: MCM3AP-AS1 could be a potential therapeutic target for the therapy of OC through miR-28-5p.

Keywords: Long noncoding RNA, MCM3AP-AS1, miR-28-5p, ovarian cancer, viability, apoptosis

Introduction

Among gynecologic cancers, ovarian cancer (OC) has a high rate of mortality and will continue to represent a severe threat to women's health in the future [1]. Currently, standard clinical therapies for this condition by combining with radiotherapy, surgery and chemotherapy could improve the overall survival of OC patients [2]. However, because of a lack of early symptoms and effective biomarker screening, many patients with OC have been diagnosed at the late-stage [3]. Therefore, discovery of functional and molecular mechanisms of OC remains extremely urgent.

Long non-coding RNAs (lcnRNAs), characterized by more than 200 nucleotides, and microRNAs (miRNAs), characterized by less than 22 nucleotides, are the two most important members of non-coding RNAs (ncRNAs), which lack of proteins coding capacity [4]. Previously evidence has demonstrated that lncNRAs and miRANs participate in multiple psychological processes, such as cell viability, tissue differentiation, and metabolic regulation [4, 5]. Dysregulation of lncRNAs and miRNAs may lead to a series of dysfunction and disease, including various human tumors [5]. Recently, emerging evidence has suggested that there may be a cross-modulation between lncRNAs and miRNAs [6, 7]. LncRNAs may serve as a competing endogenous RNA (ceRNA) or as a RNA sponge in regulating expression and function of miRNAs [8-10]. In addition, miRNAs are also report-

Table 1. Primer sequences for qRT-PCR analysis

Gene	Primer sequences
GAPDH	Forward: 5'-TGTTCGTCATGGGTGTGAAC-3'
	Reverse: 5'-ATGGCATGGACTGTGGTCAT-3'
MCM3AP-AS1	Forward: 5'-CACTCTGTGCTCCTCTTCCA-3'
	Reverse: 5'-CGTTGCTGTGAGGATGTCTG-3'
U6	Forward: 5'-CTCGCTTCGGCAGCACA-3'
	Reverse: 5'-AACGCTTCACGAATTTGCGT-3'
miR-28-5p	Forward: 5'-AAGGAGCUCACAGUCUAUUGAG-3'
	Reverse: 5'-AAAGGAGCUCACAGUCUUAUUG-3'

ed to be involved in gene expression regulation via binding to the 3'-untranslated regions (3'-UTR) of targeted genes [11]. Interaction of lncRNAs and miRNAs could affect the initiation and progression of multiple cancers by directly or indirectly regulating the expression of tumor-related genes [12].

The MCM3AP gene encodes a MCM3 associated-protein, reported as chromatin-bound acetyltransferase, which could inhibit DNA replication [13]. LncRNA-MCM3AP-AS1 is the antisense RNA to the protein coding gene MCM3AP and might be involved in the regulation of splicing, editing, transport, degradation of expressed mRNA. A recent study suggested that MCM3AP-AS1 was upregulated in human non-small-cell lung carcinoma (NSCLC) [14] and lung adenocarcinoma (LUAD) [15]. However, the function and mechanism of lncRNA-MCM3AP-AS1 in OC remain unknown, and its research is only at the beginning stages. LncRNAs could typically regulate gene expression by interacting with miRNAs or expressed mRNA. Research increasingly has suggested that miRNAs play important roles in the development of human cancers [16, 17]. Numerous miRNAs, such as miR-34c-5p, miR-200 family, miR-125b and miR-28-5p, have been associated with the regulation of OC progression [18-20]. The lncRNAs and their targeted or interacting miRNAs can be predicted by consulting bioinformatics databases, such as miRanda [21], miRTarBase [22] and TargetScan [23].

In the present study, the expression level of lncRNA-MCM3AP-AS1 was examined in 42 primary OC tissues and cell lines. Cell viability and apoptosis abilities were evaluated by interfering (inhibiting or increasing) with lncRNA-MCM3AP-AS1 expressions *in vitro*. These studies reveal that expression of miRNA-28-5p is down-regulated and positively correlated with lncRNA-MCM3AP-AS1. Thus, there is a co-effect of MCM3AP-AS1 and miR-28-5p on the viability and apoptosis of OC cells *in vitro*.

Material and methods

Clinical specimens

In this study, the OC and adjacent noncancerous tissue samples were collected from Zhejiang Cancer Hospital between April 2013 and September 2015. Informed consent was obtained from each patient, and this study obtained approval from the Ethics Committee at Zhejiang Cancer Hospital.

Cell lines

Human ovarian surface epithelium (HOSE) and OC cell lines (A2780, TOV-21G, A1847, TOV-112D, SKOV-3 and OVCAR-3) were obtained from the Chinese Academy of Sciences (Shanghai, China). All cells were maintained in DMEM medium (Gibco) with 10% fetal bovine serum (FBS, Gibco), and 100 U/ml penicillin at 37°C in an incubator with 5% CO_2.

Vector construction and transduction

The MCM3AP-AS1 full-length cDNA was amplified by PCR by using PrimerSTAR Max DNA Polymerase Mix (Takara) from OVCAR-3 cells. The PCR products were inserted into pcDNA3.0 vector (Invitrogen). According to previous studies [24, 25], the DNA fragments for MCM3AP-AS1 shRNA were synthesized, cloned into human U6 promoter plasmid (pU6), and then sub-cloned into a lentiviral vector. The constructed plasmids and packaging vectors were co-transfected into HEK293T cells. And then the lentivirus was obtained by ultra-centrifugation, concentration, and validation. A total of 5×10^4 OVCAR-3 and A2780 cells were transduced with MCM3AP-AS1 expressing vector, MCM3AP-AS1 shRNA vector and empty vector by using 8 μg/mL polybrene (Sigma). And the stable cells were filtered by using G418 (Life Technologies, 0.8 mg/mL).

Oligonucleotide transfection

Mock, miR-28-5p mimics and miR-28-5p inhibitors were synthesized from GenePharma

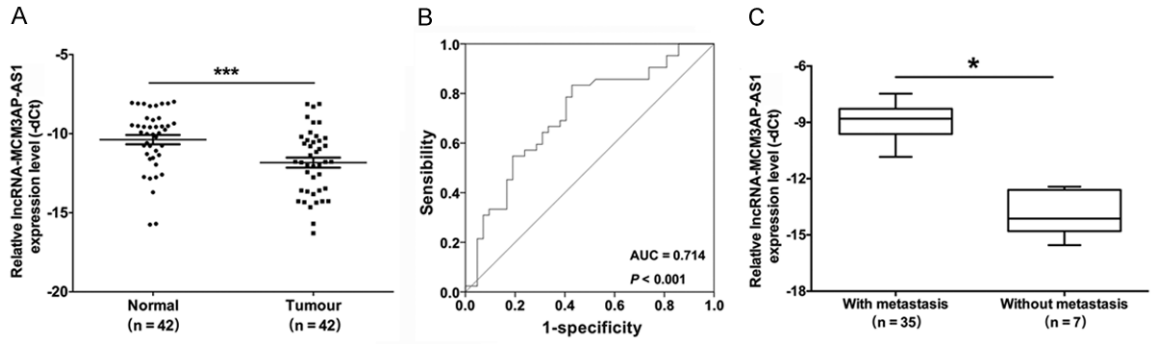

Figure 1. MCM3AP-AS1 is down-regulated in OC tissues. A. The expression level of MCM3AP-AS1 was detected by qRT-PCR in 42 pairs of OC tissues and paired adjacent normal tissues (***P < 0.001). B. The receiver operating characteristic curve (ROC) was used to analyze the cut-off score of MCM3AP-AS1. C. MCM3AP-AS1 expression was examined by qRT-PCR assay in non-metastatic and metastatic tissues (*P < 0.05).

(Shanghai, China). OVCAR-3 and A2780 cells (5×10^4 cells/well) were seeded in 6-well plates and transfected with mock, miR-28-5p mimics and miR-28-5p inhibitors for 48 hours by using Lipofectamine 2000 Reagent (Invitrogen, CA, USA), respectively.

Quantitative real-time reverse transcription (qRT-PCR) assay

Total RNAs of the OC tissues and treated OC cells were extracted by using TRIzol reagent (Takara, Japan). 2 μg of RNAs were used as template to reverse transcript into cDNA by using BestarTM qPCR RT kit (DBI Bioscience, China). The SYBR-Green PCR Master Mix (Takara) was used to analyze the expression levels of MCM3AP-AS1 and miR-28-5p. The qRT-PCR system are shown in Table S1. The primer sequences in this study is shown in **Table 1**. Relative expression levels were analyzed using $2^{-\Delta\Delta Ct}$ method [26].

miRNA target prediction

Analysis of MCM3AP-AS1 predicted-miR-28-5p targets was performed by using TargetScan (http://targetscan.org/).

Dual luciferase reporter assay

The binding site of MCM3AP-AS1 including MCM3AP-AS1-Wild and MCM3AP-AS1-Mut were constructed into pGL3 promoter vector (Realgene, Nanjing, China) to generate reporter plasmids. Briefly, cells (5×10^4 cells/well) were seeded into 24-well plates and transfected with corresponding reporter plasmids by using Lipofectamine 2000 Reagent (Invitrogen, CA, USA). After 48 hours, the reporter gene activity was analyzed by using Dual-Luciferase Reporter Assay System (Promega).

Cell viability assay

The treated OVCAR-3 and A2780 cells (3000 cells/well) were cultured in 96-well plates and then 20 mL MTT solution (5 mg/ml, Sigma, Cat No. M2128) was added at 1, 2, 3, 4 and 5 days. After incubation for 4 hours, 200 mL dimethyl sulfoxide (DMSO) was added. After mixing, a microplate reader was used to assess the corresponding absorbance at 490 nm.

Flow cytometry analysis

The treated OVCAR-3 and A2780 cells (6×10^7 cells) at the logarithmic phase were suspended with 100 μl 1 × binding buffer and double stained with Annexin V-FITC/PE for 15 minutes in the dark before detection. The apoptotic cells were detected by flow cytometry (BD Biosciences) by using a FACS Calibur Flow Cytometer (BD Biosciences, San Jose, CA, USA), and analyzed with the ModFit LT 2.0 software.

Statistical analysis

The data was estimated by SPSS (version 23.0, Inc., Chicago, IL, USA). The results were analyzed by using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. A receiver operating characteristic (ROC) curve was established to evaluate its diagnostic value. All data are shown as mean ± standard deviation (SD). P < 0.05 indicates that there was a statistically significant difference in all experiments.

Table 2. Correlation analysis between MCM3AP-AS1 expression and clinicopathological characteristics of patients with ovarian cancer

Characteristics	No. of patients	Mean ± SD	*P* value
Total no. of patients	42		
Age (year)			
> 60	20 (47.6%)	11.12 ± 1.02	0.156
≤ 60	22 (52.4%)	12.01 ± 1.35	
Invasion			
T0-T2	31 (73.8%)	10.18 ± 1.07	0.206
T3-T4	11 (26.2%)	12.79 ± 1.23	
Lymphatic metastasis			
N0	35 (83.3%)	9.14 ± 0.87	0.026*
N1-N3	7 (16.7%)	13.79 ± 1.01	
Distal metastasis			
M0	38 (90.5%)	10.87 ± 1.20	0.673
M1	4 (9.5%)	12.47 ± 1.34	
TNM stage			
0 & I & II	35 (83.3%)	9.14 ± 0.87	0.026*
III & IV	7 (16.7%)	13.79 ± 1.01	

*P < 0.05, T: Tumor; N: Node; M: metastasis; TNM stage: Pathologic tumor, node, metastasis stage.

Results

MCM3AP-AS1 is down-regulated in OC tissues

Forty-two OC tissues were randomly sorted and paired adjacent normal tissues Zhejiang Cancer Hospital. The expression level of MCM3AP-AS1 was assessed by qRT-PCR assay. The results certified that the expression level of MCM3AP-AS1 was observably down-regulated in OC tissues relative to adjacent normal tissues (P < 0.0001, **Figure 1A**). An ROC curve was used to evaluate the sensitivity and specificity and analyze the incidence of OC. The results prove that the area under the ROC curve value was 0.714 (*P* < 0.001, **Figure 1B**). As shown in **Table 2**, MCM3AP-AS1 expression was significantly associated with lymphatic metastasis and TNM stage (*P* = 0.026) of OC tissues (**Figure 1C**).

MCM3AP-AS1 suppresses cell viability and promotes apoptosis

The expression level of MCM3AP-AS1 in human ovarian surface epithelium (HOSE) and OC cell lines (A2780, TOV-21G, A1847, TOV-112D, SKOV-3 and OVCAR-3) was also examined. MCM3AP-AS1 was dramatically decreased in all OC cells related to HOSE cells. Among six OC cells, the expression level of MCM3AP-AS1 was lowest in OVCAR-3 cells and highest in A2780 cells (**Figure 2A**). Therefore, OVCAR-3 and A27-80 cells were selected as the target cells for the following studies.

To verify the effects of MCM3AP-AS1 on the viability and apoptosis of OC cells, OVCAR-3 cells were transfected with control, and lenti-MCM3AP-AS1, and A2780 cells were transfected with control and lenti-shMCM3AP-AS1, respectively. The expression level of MCM3AP-AS1 was analyzed by qRT-PCR assay. The results indicated the successful overexpression of MCM3AP-AS1 in OVCAR-3 cells by transfecting with lenti-MCM3AP-AS1 (*P* < 0.0001, **Figure 2B**) and reduced expression of MCM3AP-AS1 in A2780 cells was achieved by silencing MCM3AP-AS1 expression (*P* < 0.0001, **Figure 2C**).

The impact of MCM3AP-AS1 expression on the growth of OC cell was further validated by using an MTT assay. Overexpression of MCM3AP-AS1 significantly inhibited the proliferative ability of OVCAR-3 cells (*P* < 0.0001, **Figure 2D**). Similarly, silencing of MCM3AP-AS1 by siRNAs significantly increased the viability ability of A2780 cells (*P* < 0.0001, **Figure 2E**).

Additionally, flow cytometry analysis was adopted to assess the effect of MCM3AP-AS1 on the apoptosis ability of OC cells. The results indicate that the apoptosis rate of OVCAR-3 cells transfected with blank, control and lenti-MCM3AP-AS1 was 9.67%, 10.67%, and 55.16%, respectively, suggesting that MCM3AP-AS1 overexpression significantly promoted the apoptosis capacity of OVCAR-3 cells (*P* < 0.0001, **Figure 2F**). Similarly, the average percentage of apoptosis of A2780 cells transfected with blank, control and lenti-shMCM3AP-AS1 was 17.06% (*P* < 0.0001), 16.86%, and 6.99%, suggesting that silencing of MCM3AP-AS1 by siRNAs significantly inhibited the apoptosis capacity of A2780 cells (**Figure 2G**).

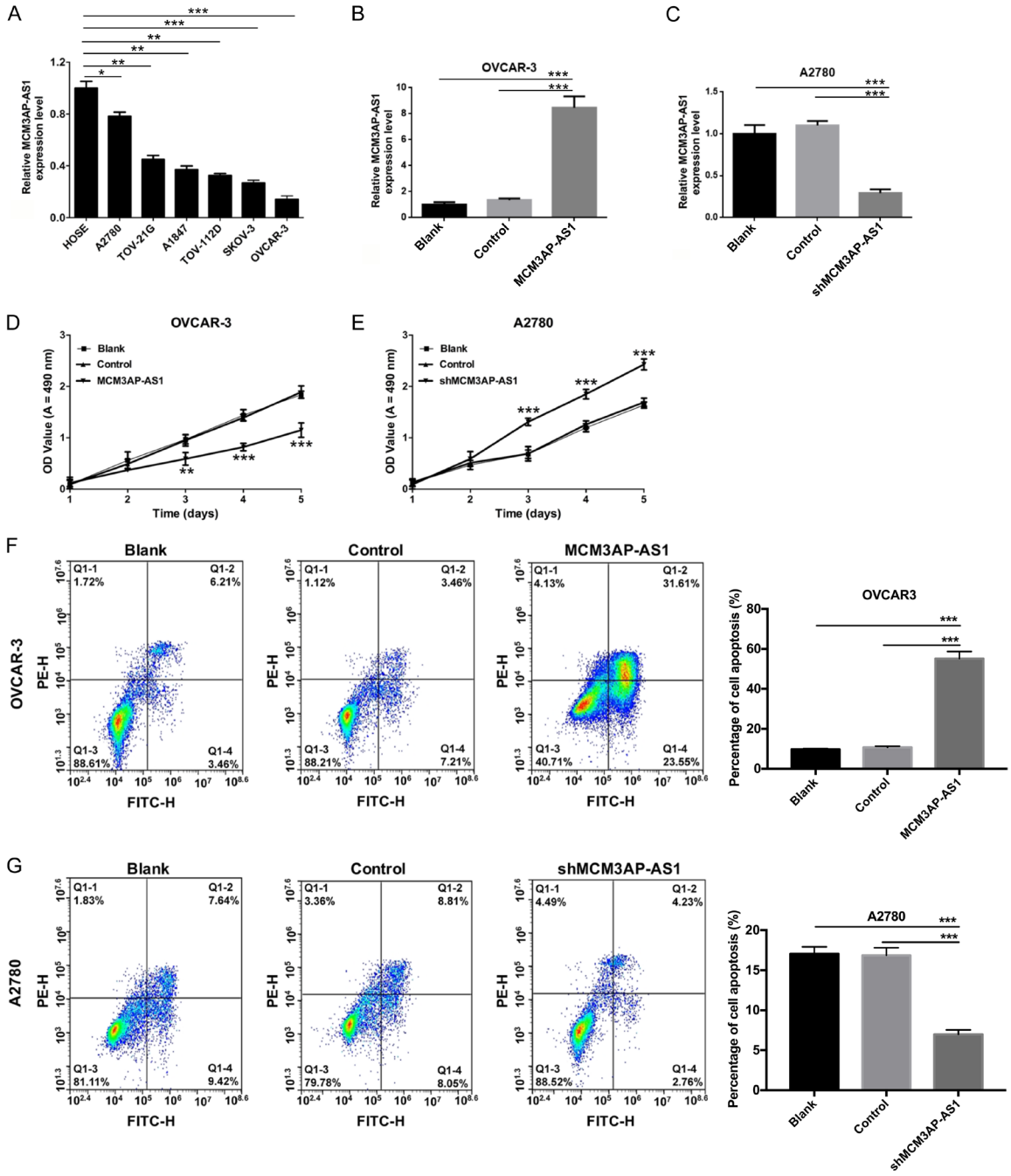

Figure 2. MCM3AP-AS1 suppresses the viability and promotes the apoptosis of OC cells. A. qRT-PCR was used to analyze the expression level of MCM3AP-AS1 in human ovarian surface epithelium (HOSE) and OC cell lines (A2780, TOV-21G, A1847, TOV-112D, SKOV-3 and OVCAR-3, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). B. The expression level of MCM3AP-AS1 was measured by qRT-PCR in OVCAR-3 cells transfected with control and Leti-MCM3AP-AS1 ($***P < 0.001$). C. The expression level of MCM3AP-AS1 was measured by qRT-PCR in A2780 cells transfected with control and shMCM3AP-AS1 ($***P < 0.001$). D. MTT assay was performed to detect the viability of OVCAR-3 cells transfected with control and MCM3AP-AS1 ($**P < 0.01$, $***P < 0.001$). E. MTT assay was performed to detect the viability ability of A2780 cells transfected with control and shMCM3AP-AS1 ($***P < 0.001$). F. OVCAR-3 cells were transfected with control and MCM3AP-AS1, and subsequently, apoptotic cell death was measured by flow cytometry analysis using Annexin V-FITC and PE staining. The relative proportions of early apoptosis are shown in the right lower quadrant. The relative proportions of late apoptosis are shown in the right upper quadrant ($***P < 0.001$). G. Cell apoptosis was detected by Annexin V-FITC and PE staining in A2780 cells transfected with control and shMCM3AP-AS1 ($***P < 0.001$).

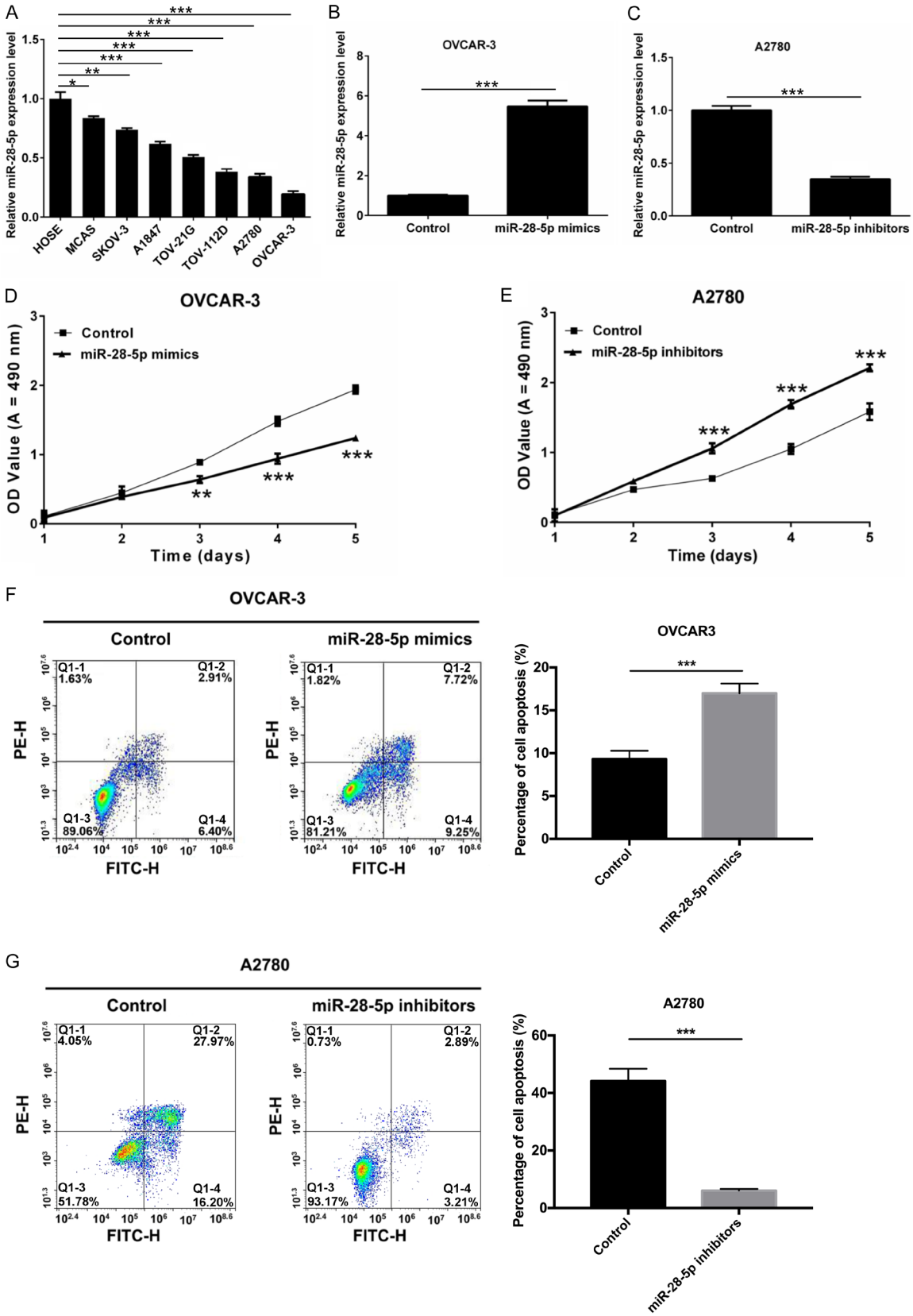

Figure 3. miR-28-5p suppresses the viability and promotes the apoptosis of OC cells. A. qRT-PCR was used to analyze the expression level of miR-28-5p in human ovarian surface epithelium (HOSE) and OC cell lines (A2780, TOV-21G, A1847, TOV-112D, SKOV-3 and OVCAR-3, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). B. The expression level

of miR-28-5p was measured by qRT-PCR in OVCAR-3 cells transfected with control and miR-28-5p mimics (***P < 0.001). C. The expression level of miR-28-5p was measured by qRT-PCR in A2780 cells transfected with control and miR-28-5p inhibitors (***P < 0.001). D. MTT assay was performed to detect the viability ability of OVCAR-3 cells transfected with control and miR-28-5p mimics (**P < 0.01, ***P < 0.001). E. MTT assay was performed to detect the viability ability of A2780 cells transfected with control and miR-28-5p inhibitors (***P < 0.001). F. OVCAR-3 cells were transfected with control and miR-28-5p mimics, and subsequently, apoptotic cell death was measured by flow cytometry analysis with Annexin V-FITC and PE staining. The relative proportions of early apoptosis are shown in the right lower quadrant; The relative proportions of late apoptosis are shown in the right upper quadrant (***P < 0.001). G. Cell apoptosis was detected by Annexin V-FITC and PE staining in A2780 cells transfected with control and miR-28-5p inhibitors (***P < 0.001).

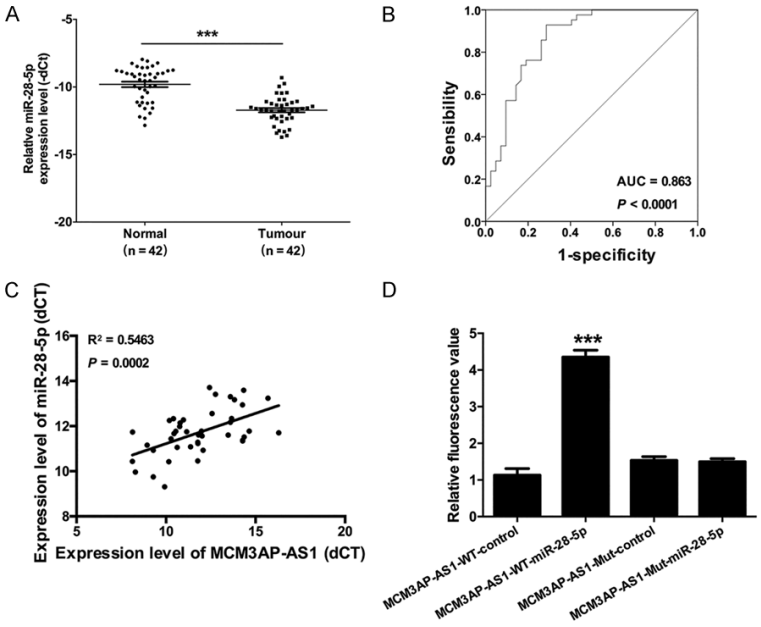

Figure 4. MCM3AP-AS1 positively regulates miR-28-5p transcription. A. The expression level of miR-28-5p was detected by qRT-PCR in 42 pairs of OC tissues and paired adjacent normal tissues (***P < 0.001). B. The receiver operating characteristic curve (ROC) was used to analyze the cut-off score of miR-28-5p. C. Correlation between MCM3AP-AS1 and miR-28-5p expression was analyzed (R2 = 0.5463, P = 0.0002). D. The relative fluorescence value was detected by the luciferase reporter gene assay in OVCAR-3 cells co-transfected with wild type or mutant MCM3AP-AS1 and control or miR-28-5p, respectively (***P < 0.001).

miR-28-5p inhibits viability and promotes apoptosis of OC cells

After bioinformatics prediction and previous screening of several miRNA candidates in cell lines, a focus was made on miR-28-5p, whose expression level was significantly down-regulated in human ovarian surface epithelium (HOSE) and OC cell lines (A2780, TOV-21G, A1847, TOV-112D, SKOV-3 and OVCAR-3, **Figure 3A**). Furthermore, to investigate the effects of miR-28-5p on OC viability and apoptosis, OVCAR-3 cells were transfected with control and miR-28-5p mimics, and A2780 cells were transfected with control and miR-28-5p inhibitors. The results indicate the success of construction of the over-expression or silencing of miR-28-5p *in vitro* (**Figure 3B** and **3C**). Over-expression of miR-28-5p significantly inhibited the proliferative ability of OVCAR-3 cells (P < 0.0001, **Figure 3D**). Similarly, miR-28-5p inhibitors significantly increased the viability of A2780 cells (P < 0.0001, **Figure 3E**). The average percentage of apoptosis of OVCAR-3 cells transfected with control and miR-28-5p mimics was 9.31%, and 16.97% (P < 0.0001), respectively, suggesting that miR-28-5p significantly promoted the apoptosis of OVCAR-3 cells (**Figure 3F**). Similarly, the average percentage of apoptosis of A2780 cells transfected with control and miR-28-5p inhibitors was 16.58% (P < 0.0001), and 6.37%, suggesting that miR-28-5p inhibitors significantly inhibited the apoptosis of A2780 cells (**Figure 3G**).

MCM3AP-AS1 is positively correlated with miR-28-5p transcription

To further evaluate miR-28-5p expression in OC, qRT-PCR was used to measure the miR-28-5p expression in OC tissues (n = 42) and paired adjacent normal tissues (n = 42). The results proved that miR-28-5p was significantly decreased in OC tissues relative to paired adjacent normal tissues (**Figure 4A**). Furthermore, miR-28-5p was significantly associated with lymphatic metastasis and TNM stage (P = 0.048) based on clinicopathological analyses in OC tissues (**Table 3**). The incidence of OC using ROC curve analysis of miR-28-5p between 42 OC patients and paired adjacent normal tissues was subsequently analyzed. The area

Table 3. Correlation analysis between miR-28-5p expression and clinicopathological characteristics of patients with ovarian cancer

Characteristics	No. of patients	Mean ± SD	*P* value
Total no. of patients	42		
Age (year)			
> 60	20 (47.6%)	10.87 ± 1.36	0.513
≤ 60	22 (52.4%)	12.35 ± 1.74	
Invasion			
T0-T2	31 (73.8%)	10.84 ± 1.52	0.627
T3-T4	11 (26.2%)	12.13 ± 0.95	
Lymphatic metastasis			
N0	35 (83.3%)	9.29 ± 0.93	0.048*
N1-N3	7 (16.7%)	13.66 ± 1.03	
Distal metastasis			
M0	38 (90.5%)	10.37 ± 1.16	0.564
M1	4 (9.5%)	12.48 ± 0.95	
TNM stage			
0 & I & II	35 (83.3%)	9.29 ± 0.93	0.048*
III & IV	7 (16.7%)	13.66 ± 1.03	

*P < 0.05, T: Tumor; N: Node; M: metastasis; TNM stage: Pathologic tumor, node, metastasis stage.

under the ROC curves was 0.863 ($P < 0.001$, **Figure 4B**). Subsequently, the expression relationship between MCM3AP-AS1 and miR-28-5p was also analyzed. The results indicated a positive correlation between MCM3AP-AS1 and miR-28-5p expression ($R^2 = 0.5463$, $P = 0.002$, **Figure 4C**). To determine whether MCM3AP-AS1 interacts with miR-28-5p transcriptional regulation in OC cells, we speculated that MCM3AP-AS1 might directly regulate miR-28-5p expression. Wild-type MCM3AP-AS1 or mutant MCM3AP-AS1 was cloned into the pGL3-basic luciferase reporter vector. Luciferase reporter gene assays confirmed that MCM3AP-AS1 enhanced the fluorescence activity (**Figure 4D**).

MCM3AP-AS1 suppresses proliferative ability and induces apoptosis of OC cells via miR-28-5p

According to the positive correlation between MCM3AP-AS1 and miR-28-5p expression, OVCAR-3 cells were transfected with control, MCM3AP-AS1, MCM3AP-AS1 and miR-28-5p control, MCM3AP-AS1 and miR-28-5p mimics, and MCM3AP-AS1 and miR-28-5p inhibitors; A2780 cells were transfected with control, shMCM3AP-AS1, shMCM3AP-AS1 and miR-28-5p control, shMCM3AP-AS1 and miR-28-5p mimics, shMCM3AP-AS1 and miR-28-5p inhibitors. qRT-PCR was used to analyze the expression level of miR-28-5p. Overexpressed MCM3AP-AS1 remarkably increased miR-28-5p expression ($P < 0.001$); miR-28-5p was observably upregulated in OVCAR-3 cells transfected with MCM3AP-AS1 and miR-28-5p mimics compared with MCM3AP-AS1 and miR-28-5p control ($P < 0.001$). The expression level of miR-28-5p was down-regulated in OVCAR-3 cells transfected with MCM3AP-AS1 and miR-28-5p inhibitors compared to MCM3AP-AS1 and miR-28-5p control ($P < 0.001$, **Figure 5A**). Similarly, silencing of MCM3AP-AS1 by siRNAs markedly decreased miR-28-5p expression ($P < 0.001$); miR-28-5p was upregulated in A2780 cells transfected with MCM3AP-AS1 and miR-28-5p mimics compared to MCM3AP-AS1 and miR-28-5p control ($P < 0.001$), and miR-28-5p was markedly down-regulated in A2780 cells transfected with MCM3AP-AS1 and miR-28-5p inhibitors compared to MCM3AP-AS1 and miR-28-5p control ($P < 0.001$, **Figure 5B**).

MCM3AP-AS1 was also found to inhibit the viability of OC cells via miR-28-5p. MTT results indicated that MCM3AP-AS1 suppressed the viability of OVCAR-3 cells via miR-28-5p ($P < 0.05$, $P < 0.01$, $P < 0.001$, **Figure 5C**). MCM3AP-AS1 silencing promoted the viability of A2780 cells via miR-28-5p ($P < 0.05$, $P < 0.01$, $P < 0.001$, **Figure 5D**). In addition, MCM3AP-AS1 promoted the apoptosis of OVCAR-3 cells via miR-28-5p ($P < 0.05$, $P < 0.001$, **Figure 5E**). Silencing of MCM3AP-AS1 inhibited the apoptosis of A2780 cells via miR-28-5p ($P < 0.05$, $P < 0.01$, **Figure 5F**).

Discussion

LncRNAs are non-protein-coding transcripts transcribed by RNA polymerase II [27] that play crucial roles in chromatin structure, such as transcriptional, posttranscriptional, epigenetic, and translational[28-30]. Recent studies have

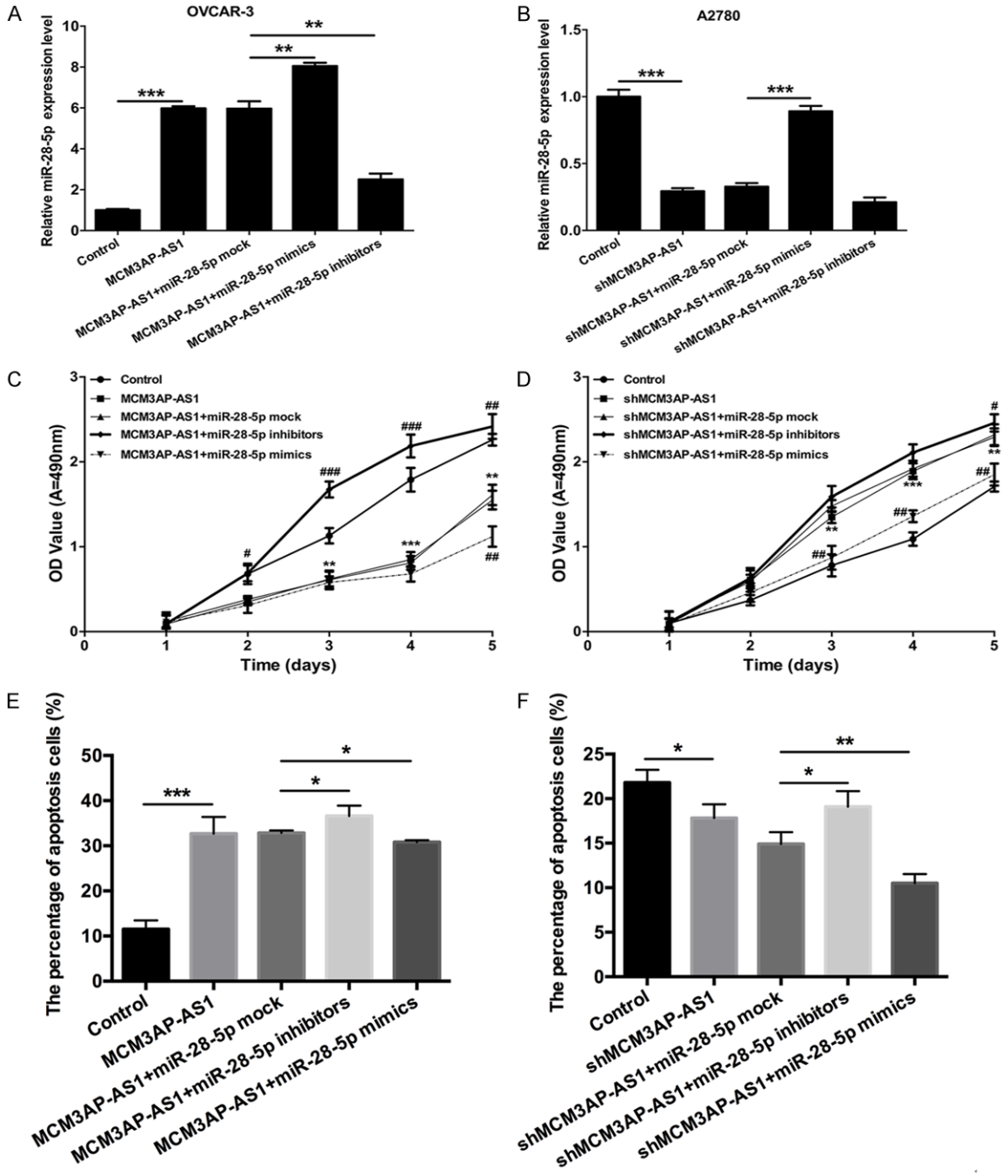

Figure 5. MCM3AP-AS1 suppresses the viability and induces apoptosis of OC cells via miR-28-5p. (A) qRT-PCR was used to analyze the mRNA expression level of miR-28-5p in OVCAR-3 cells transfected with control, MCM3AP-AS1, MCM3AP-AS1 and miR-28-5p control, MCM3AP-AS1 and miR-28-5p mimics, and MCM3AP-AS1 and miR-28-5p inhibitors (***P* < 0.01, ****P* < 0.001). (B) qRT-PCR was used to analyze the mRNA expression level of miR-28-5p in A2780 cells transfected with control, shMCM3AP-AS1, shMCM3AP-AS1 and miR-28-5p control, shMCM3AP-AS1 and miR-28-5p mimics, and shMCM3AP-AS1 and miR-28-5p inhibitors (****P* < 0.001). (C) MTT assay was performed to measure cell viability in the OVCAR-3 cells treated as in (A). ***P* < 0.01, ****P* < 0.001 vs. control group; ##*P* < 0.01, ###*P* < 0.001 vs. miR-28-5p mock group. (D) MTT assay was performed to measure cell viability in the A2780 cells treated as in (B). ***P* < 0.01, ****P* < 0.001 vs. control group; #*P* < 0.05, ##*P* < 0.01 vs. miR-28-5p mock group. (E) The number of apoptotic cells was assessed by flow cytometry in the OVCAR-3 cells treated as in (A); the percentage of apoptotic cells was analyzed in five groups (**P* < 0.05, ****P* < 0.001). (F) The number of apoptotic cells was assessed by flow cytometry in the A2780 cells treated as in (B); the percentage of apoptotic cells was analyzed in five groups (**P* < 0.05, ***P* < 0.01).

indicated that lncRNAs are involved in various biological processes, such as viability [31], carcinogenesis [32], and human diseases [33-35]. For example, recently, studies have indicated that PKC delta VIII and Bcl2 increase the survival rate of OC via lncRNA NEAT1 [36]. Zhou M et al. analyzed the lncRNA expression profiles of OC [37], and Guo QY et al. analyzed and identified the lncRNA-mRNA co-expression patterns, associated with immunity in OC [38]. Previous studies have demonstrated MCM3AP-AS1 was one of the top five most upregulated lncRNAs in NSCLC tumors [14], and MCM3AP-AS1 was one of the identified nineteen lncRNA-miRNA-mRNA regulatory modules in LUAD [15]. MCM3AP-AS1 might be associated with the miR-205-5p-PTEN network in endometrial cancer (EC) [39]. MCM3AP-AS1 has been implicated in apoptosis (BAD) in breast cancer [40]. Previous studies have also shown that MCM3AP-AS1 may be involved in OC [41].

In the present study, the expression level of MCM3AP-AS1 was down-regulated in OC tissues compared to adjacent normal tissues. MCM3AP-AS1 was significantly associated with lymphatic metastasis and TNM stage, and a good prognosis effect of MCM3AP-AS1 was observed in OC. Furthermore, MCM3AP-AS1 suppressed OC cell viability and induced apoptosis. Therefore, MCM3AP-AS1 can inhibit the development of OC.

Studies have shown that miRNAs play important biological functions in tumor progression, including cell viability, inflammation, and metastasis, by targeting mRNAs [42, 43]. Recent theoretical and experimental studies have shown that miRNAs, including miR-223, miR-141, miR-200a, miR-100, miR-199a, and miR-9, are involved in the development of OC [20, 44-46]. Studies have demonstrated the ceRNA activity of lncRNAs, as a miRNA sponge, playing a critical role in pathophysiological conditions and human development [47]. The lncRNA-associated ceRNA network has been examined in gastric cancer [48], breast cancer [49], and glioblastoma multiforme [50]. However, the relationship between MCM3AP-AS1 and miR-28-5p in the development of cancer has not previously been reported.

Several studies on the molecular mechanisms of miRNA have shown that miR-28-5p acted as a tumor suppressor by targeting *RAP1B* in renal cell carcinoma [51] or *IGF-1* via the PI3K/AKT pathway in hepatocellular carcinoma [52]. These results suggest that in OC, MCM3AP-AS1 might be regulator of OC cell viability and apoptosis via miR-28-5p. However, the downstream target gene of miR-28-5p remains unclear and should be explored in OC.

In summary, miR-28-5p was down-regulated in OC tissues. miR-28-5p was significantly associated with distal metastasis and TNM stage, and a good prognosis effect of miR-28-5p for OC was also observed. miR-28-5p suppressed OC cell viability and induced apoptosis. Thus, MCM3AP-AS1 likely inhibits the development of OC. Furthermore, a positive correlation between MCM3AP-AS1 and miR-28-5p was observed in OC tissues. In addition, TargetScan showed a binding site between MCM3AP-AS1 and miR-28-5p, and this result further demonstrated that MCM3AP-AS1 positively regulated miR-28-5p transcription. Furthermore, MCM3AP-AS1 inhibited the progression of cell viability and apoptosis via miR-28-5p in OC.

Conclusions

MCM3AP-AS1 and miR-28-5p might be potential biomarkers and therapeutic targets of OC. However, additional studies are needed to validate this observation in the prognostic and therapeutic effects of MCM3AP-AS1 and miR-28-5p in OC, as well as observations. Moreover, further exploration of the functions and mechanisms of MCM3AP-AS1 and miR-28-5p in OC are needed.

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Disclosure of conflict of interest

None.

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Table S1. Reaction system of quantitative real-time reverse transcription PCR

Reagent	Consumption
SYBR® Premix Ex TaqTM II (2 ×)	5.0 μl
PCR Forward Primer (10 μM)	0.4 μl
PCR Reverse Primer (10 μM)	0.4 μl
ROX Reference Dye (50 ×)	0.2 μl
DNA template	1.0 μl
ddH_2O	3.0 μl
Total	10.0 μl