

Long non-coding RNA MCM3AP-AS1 drives ovarian cancer progression via the microRNA-143-3p/TAK1 axis

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Abstract. The long non-coding RNA (lncRNA) MCM3AP antisense 1 (MCM3AP-AS1) has previously been shown to be a key regulator of multiple types of cancer; however whether it is important in the context of ovarian cancer (OC) is uncertain. The present study determined that MCM3AP-AS1 expression in samples from patients with OC was significantly increased, and was associated with tumor stage, presence of lymph node metastases and poorer overall survival. The role of this lncRNA was investigated *in vitro*, and it was observed that knockdown of MCM3AP-AS1 impaired OC cell proliferation, migration and colony formation. Similarly, it disrupted tumor growth *in vivo*. The present study further determined that MCM3AP-AS1 was able to directly interact with microRNA (miRNA or miR)-143-3p as a competing endogenous (ce)RNA for this miRNA, thereby regulating the expression of transforming growth factor- β -activated kinase 1 (TAK1), a known target of miR-143-3p in OC. Consistent with this, inhibition of miR-143-3p was sufficient to partially reverse the effects of MCM3AP-AS1-knockdown, which inhibited the proliferation, migration and invasion of OC cells. Together, these results indicate that MCM3AP-AS1 serves as an oncogenic lncRNA in OC by binding to miR-143-3p and thereby promoting TAK1 expression, and suggest that this lncRNA may be a possible target for therapy in OC.

Introduction

Ovarian cancer (OC) is one of most common cancer types in women, with high morbidity and mortality worldwide (1). Despite the therapeutic advances aimed at more effectively treating OC in recent years, overall prognosis remains poor, and patients are often affected by either tumor recurrence or

distant metastasis (2,3). It is thus essential that the molecular mechanisms governing OC are further characterized in order to identify novel diagnostic or therapeutic targets that can be utilized to better understand, prevent and treat this deadly disease.

Long non-coding RNAs (lncRNAs) are RNAs >200 nucleotides in length that largely lack the ability to encode for proteins (4). These lncRNAs play diverse biological roles in regulating essential processes, such as cell proliferation, cell cycle progression, cancer development and cell death (5,6). Numerous lncRNAs are relevant in the context of cancer, and some act as suppressors or promoters of tumor progression in OC, which makes these molecules potential diagnostic and/or therapeutic targets in patients with OC (7,8). MCM3AP anti-sense RNA 1 (MCM3AP-AS1) is an oncogenic lncRNA that was found to promote the progression of non-small cell lung cancer, hepatocellular carcinoma, glioblastoma and papillary thyroid cancer (9-13). Whether this lncRNA is important in the context of OC remains uncertain. The present study assessed the expression, clinical relevance, biological function and molecular mechanisms of MCM3AP-AS1 in OC.

Materials and methods

Clinical samples. The Ethics Committee of the First Hospital of Jilin University (Changchun, China) approved this study, and the participants provided written informed consent. In total, 52 pairs of OC tumors and adjacent non-tumor tissues were collected between March 2013 and March 2014 at the First Hospital of Jilin University. None of the patients had undergone anticancer treatments prior to surgical resection. Two histopathologists independently confirmed all tissue specimen results. All tissue samples were immediately snap-frozen in liquid nitrogen after surgery and subsequently stored at -80°C until use. The patient demographic and clinicopathological features are listed in Table I.

Cell culture and transfection. Two human OC cell lines (SKOV3 and A2780) and a human ovarian surface epithelial cell line HOSEpiCs were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). DMEM (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), 100 mg/ml streptomycin and 100 U/ml penicillin was used for all cell cultures at 37°C in a humidified 5% CO₂ incubator.

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For short hairpin RNA (shRNA) transfection, an shRNA plasmid directly targeting MCM3AP-AS1 (sh-MCM3AP-AS1; 5'-GGGAGUAAGUGAAAGUAAU-3') as well as an appropriate non-targeting plasmid used as a negative control (NC) (sh-NC; 5'-UUCUCCGAACGUGUCACGU-3') were produced by Sangon Biotech Co., Ltd. In addition, Shanghai GenePharma Co., Ltd. provided microRNA (miRNA or miR)-143-3p mimics, NC mimics (miR-NC) and the miR-143-3p inhibitor (miR-143-3p in). SKOV3 cells underwent transient transfection with the abovementioned constructs using Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) based on the provided protocols, and the efficiency of transfection was assessed at 48 h after transfection by reverse transcription-quantitative PCR (RT-qPCR).

RT-qPCR. TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.) was utilized for total RNA extraction from samples, and RNA was then used for RT with TransScript First-Strand cDNA Synthesis SuperMix (TransGen Biotech Co., Ltd.). Subsequently, SYBR Green qPCR SuperMix (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used for quantification of relative gene expression on an ABI Prism 7900 System (Applied Biosystems; Thermo Fisher Scientific, Inc.). Reaction conditions were: 95°C for 1 min, 40 cycles of 95°C for 30 sec and 58°C for 40 sec. The $2^{-\Delta\Delta C_q}$ method was used to compare relative expression following normalization to U6 for miR-143-3p or to GAPDH for MCM3AP-AS1. The primer sequences are presented in Table II.

Cell Counting Kit-8 (CCK-8) assay. Transfected cells were plated in 96-well plates (5×10^3 cells/well) for 24, 48 or 72 h. Subsequently, CCK-8 solution (Dojindo Molecular Technologies, Inc.) was added to these wells for an additional 2-h incubation. The absorbance of each well at 450 nm was then assessed with a microplate reader (Bio-Rad Laboratories, Inc.).

Colony formation assay. SKOV3 cells were seeded into 6-well plates 48 h post-transfection (500 cells/well). After 10 days of growth, the colonies underwent 4% paraformaldehyde fixation, followed by 0.1% crystal violet (Sigma-Aldrich; Merck KGaA) staining.

EdU incorporation assay. For this assay, an EdU kit (Roche Diagnostics) was utilized according to the manufacturer's instructions, and a Zeiss fluorescence photomicroscope (magnification, x200; Carl Zeiss AG) was used for imaging and quantification of 5 random fields per sample.

Wound healing assay. To assess how MCM3AP-AS1 knockdown influenced cell migration, following transfection, cells were added to 12-well plates until becoming fully confluent. Next, a wound was generated in the cell monolayer using a micropipette tip. Cells were then allowed to grow in serum-free medium for 24 h, and were imaged at 0 and 24 h with an inverted microscope (magnification, x100; Leica Microsystems, Inc.).

Transwell invasion assay. For assessment of cell invasion, 24-well plates containing Matrigel-coated chambers (8- μ m pores; Corning Inc.) were used. Transfected cells in

serum-free medium were added to the upper chamber, with normal medium containing 10% FBS being added to the lower chamber for chemoattraction. Following a 24-h incubation, the cells that had undergone invasion were then assessed via 20% methanol fixation and subsequent 0.1% crystal violet staining. The invasive cells in 5 random fields of view were quantified with an inverted microscope (magnification, x200; Leica Microsystems, Inc.).

Luciferase reporter assays. Potential miRNAs interacting with MCM3AP-AS1 were predicted using starBase v2.0 (<http://starbase.sysu.edu.cn/starbase2/>), with miR-143-3p ultimately being selected for further investigation. Next, MCM3AP-AS1 fragments containing the putative miR-143-3p binding sites were produced by Guangzhou RiboBio Co., Ltd. and inserted into the psiCHECK2 vector (Promega Corp.), yielding the wild-type (WT)-MCM3AP-AS1 vector. In addition, a site-directed mutagenesis kit (Tiangen Biotech Co., Ltd.) was used to generate a mutated form of this vector termed mutant (MT)-MCM3AP-AS1. These vectors were then used in luciferase reporter assays, wherein cells were grown in 24-well plates and co-transfected with the WT or MT-MCM3AP-AS1 plasmids alongside appropriate miR-143-3p mimics or controls using Lipofectamine 3000. After 48 h, a Dual-Luciferase Reporter Assay (Promega Corp.) was employed to assess luciferase activity.

Subcellular fractionation. A PARIS Kit (Thermo Fisher Scientific, Inc.) was used to separate the nuclear and cytoplasmic fractions of SKOV3 cells according to the manufacturer's instructions. RT-qPCR was then used to assess U6, GAPDH and MCM3AP-AS1 expression in these fractions, with U6 serving as a nuclear control and GAPDH as a cytoplasmic control.

RNA immunoprecipitation (RIP) assay. After 48 h of transfection with appropriate miRNA mimics or controls, an anti-Argonaute2 (Ago2) antibody (cat. no. MABE253; EMD Millipore) and the Magna RIP RNA-Binding Protein Immunoprecipitation Kit (EMD Millipore) were used to conduct a RIP assay. A NanoDrop spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.) was used to quantify the purified RNA concentration, and the resultant RNA was then used in RT-qPCR to assess the MCM3AP-AS1 and miR-143-3p expression levels.

Animal experiments. The Institutional Animal Care and Use Committee of Jilin University (Changchun, China) approved all the animal experiments conducted in our study. A total of 2×10^6 SKOV3 cells that had been stably transfected using either sh-MCM3AP-AS1 or sh-NC were resuspended in 100 μ l of a 1:1 mixture of serum-free DMEM and Matrigel. These cells were then implanted subcutaneously into 5-week-old female nude BALB/c mice (n=5 mice/group, the Animal Laboratory Center of Jilin University). All mice were bred in standard mouse irradiated food and tap water *ad libitum*, and maintained under conditions of 25°C and 50% humidity with a 12-h light/dark cycle. Tumor growth was assessed using calipers on a weekly basis, with tumor volume (V) being determined as follows: $V = 0.5 \times L \times W^2$, with L and W being the tumor

Table I. Association of MCM3AP-AS1 expression with the clinicopathologic factors of the 52 patients with OC.

Variables	No. of cases	MCM3AP-AS1 expression		P-value
		High, n	Low, n	
Age (years)				0.3953
<60	20	9	11	
≥60	32	19	13	
Sex				0.5735
Male	31	18	13	
Female	21	10	11	
FIGO stage				0.0030
I-II	40	17	23	
III-IV	12	11	1	
Histological grade				0.0641
Well/Moderate	39	18	21	
Poor	13	10	3	
Tumor size (cm)				0.3911
≤3	33	16	17	
>3	19	12	7	
Lymph node metastasis				0.0105
No	38	16	22	
Yes	14	12	2	

OC, ovarian cancer; MCM3AP-AS1, MCM3AP antisense 1.

length and width, respectively. Tumors were allowed to grow for 5 weeks, and the animals were then anesthetized by intraperitoneal injection with 10% chloral hydrate (300 mg/kg), and were sacrificed using cervical dislocation. The xenografts were excised, and weighed, and the fragments were frozen at -80°C for RNA isolation. In addition, a number of tumor samples were used for Ki-67 immunostaining, as described in a previous report (14).

Statistical analysis. Data are represented as means ± SD from at least three replicates and analyzed with SPSS v19.0 (IBM Corp.). Continuous variables were compared via Student's t tests (two-tailed) and one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test for 2 and ≥2 groups, respectively. The association between MCM3AP-AS1 expression and clinicopathological characteristics was assessed with χ^2 test. Correlations were assessed with Pearson's correlation test. Survival curves were plotted using the Kaplan-Meier method and were analyzed using a log-rank test. GraphPad Prism 5 (Graph-Pad Software, Inc.) was used to generate graphs presenting the results. P<0.05 was considered to indicate a statistically significant difference.

Results

Increased MCM3AP-AS1 expression is positively correlated with OC progression. Our study first assessed MCM3AP-AS1 expression in 52 paired OC tissues and adjacent non-tumor control samples, revealing a significant elevation in the

Table II. qPCR primers used for mRNA expression analysis.

Target gene	Primer (5'-3')
U6	F-TCCGATCGTGAAGCGTTC R-GTGCAGGGTCCGAGGT
miR-143-3p	F-GTGAGATGAAGCACTGTAGC R-GTGCAGGGTCCGAGGT
MCM3AP-AS1	F-GCTGCTAATGGCAACACTGA R-AGGTGCTGTCTGGTGGAGAT
TAK1	F-TCTGGATGTCCCTGAGATCGT R-GCTCACCTGACCAGGTTCTG
GAPDH	F-AAGGTGAAGGTCGGAGTCAA R-AATGAAGGGGTCATTGATGG

F, forward; R, reverse; TAK1, transforming growth factor- β -activated kinase 1; MCM3AP-AS1, MCM3AP antisense 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; mRNA, messenger RNA; PCR, polymerase chain reaction.

expression of this lncRNA in tumor samples (Fig. 1A). Consistent with this, when OC cell lines (SKOV3 and A2780) were examined, a significant increase compared with that in the human ovarian surface epithelial cell line HOSEpiC was observed (P<0.001 for SKOV3; P<0.01 for A2780; Fig. 1B). As SKOV3 cells expressed the highest levels of MCM3AP-AS1, these cells were used for all downstream experiments

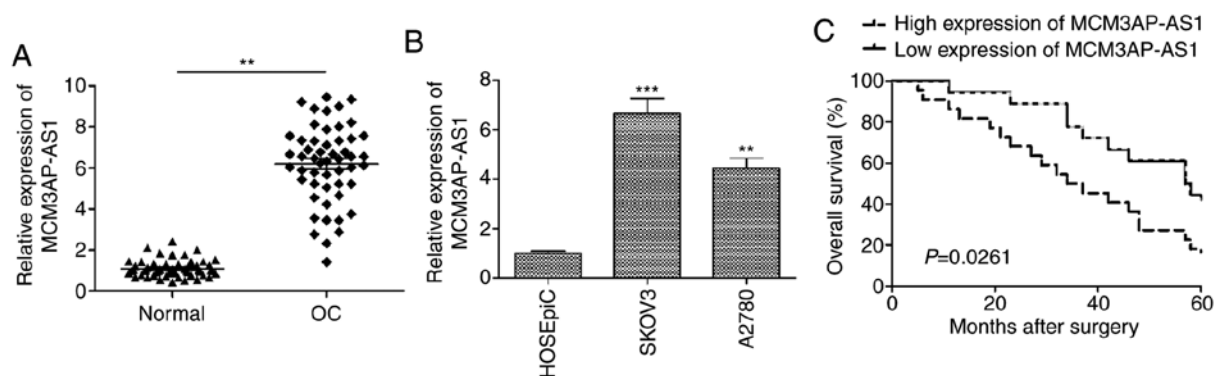


Figure 1. Relative expression of MCM3AP-AS1 in OC tissues and cell lines and overall survival curves analysis. (A) Expression levels of MCM3AP-AS1 in OC tissues and adjacent normal lung tissues were assessed by RT-qPCR. ** $P < 0.01$. (B) Expression level of MCM3AP-AS1 was determined in two OC cell lines (SKOV3 and A2780) and a human ovarian surface epithelial cell line HOSEpiC. ** $P < 0.01$, *** $P < 0.001$, compared with the HOSEpiC cells. (C) Association between overall survival time and differential MCM3AP-AS1 expression in OC was analyzed by a Kaplan-Meier survival curve. All experiments were performed in triplicate and are expressed as mean \pm SD. MCM3AP-AS1, MCM3AP antisense 1; OC, ovarian cancer.

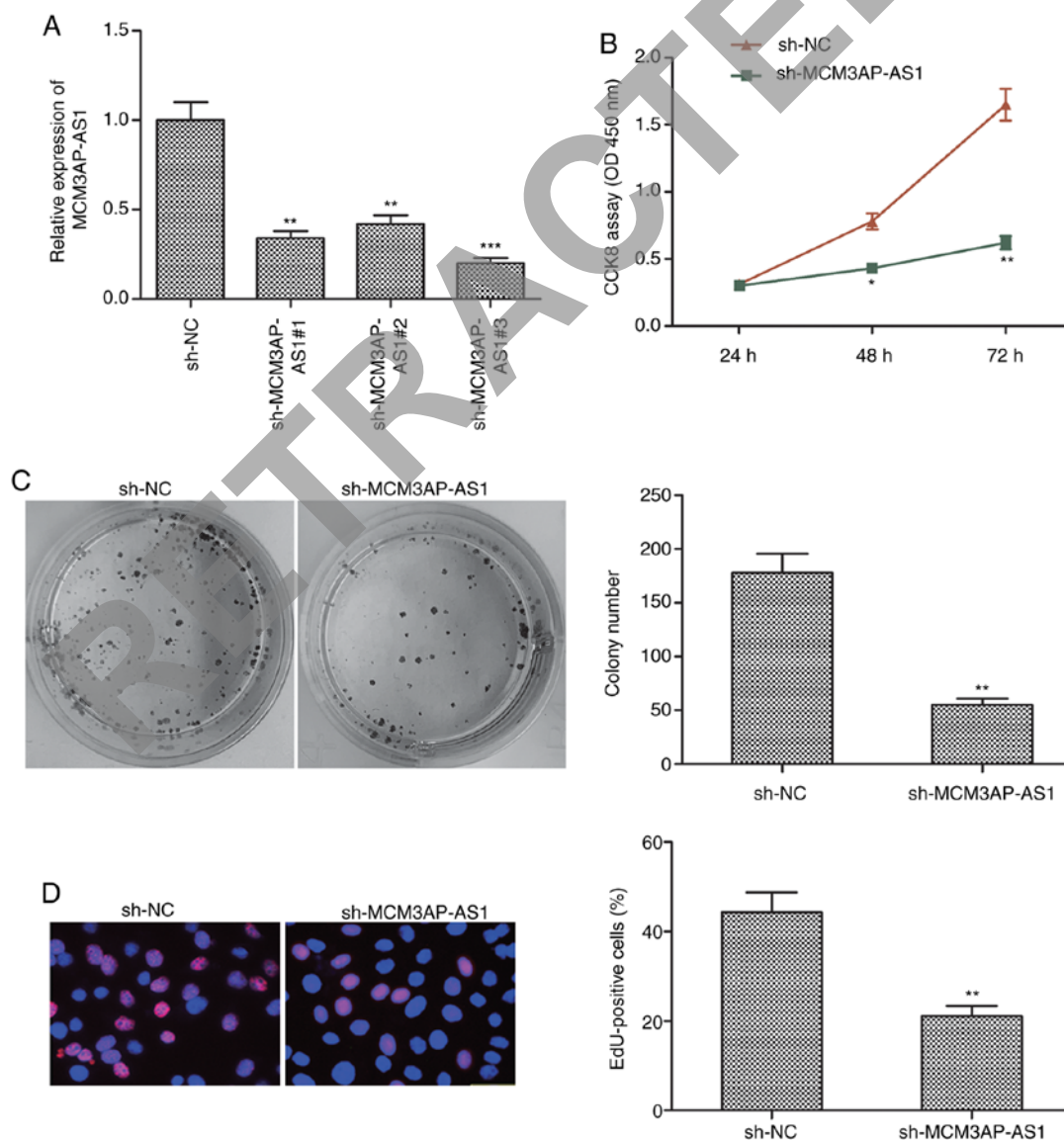


Figure 2. Knockdown of MCM3AP-AS1 inhibits cell proliferation of OC cells. (A) The expression level of MCM3AP-AS1 was determined in SKOV3 cells transfected with three shRNAs against MCM3AP-AS1 (sh-MCM3AP-AS1#1, sh-MCM3AP-AS1#2 and sh-MCM3AP-AS1#3) and non-targeting plasmid (sh-NC). (B) Cell proliferation was determined in SKOV3 cells transfected with sh-MCM3AP-AS1 and sh-NC using the CCK-8 assay. (C) Cell colony formation was assessed in SKOV3 cells transfected with sh-MCM3AP-AS1 and sh-NC. Magnification, $\times 10$. (D) EdU immunofluorescence staining assay was performed in SKOV3 cells transfected with sh-MCM3AP-AS1 and sh-NC. Magnification, $\times 100$. All experiments were performed in triplicate and are expressed as mean \pm SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the sh-NC group. MCM3AP-AS1, MCM3AP antisense 1; OC, ovarian cancer.

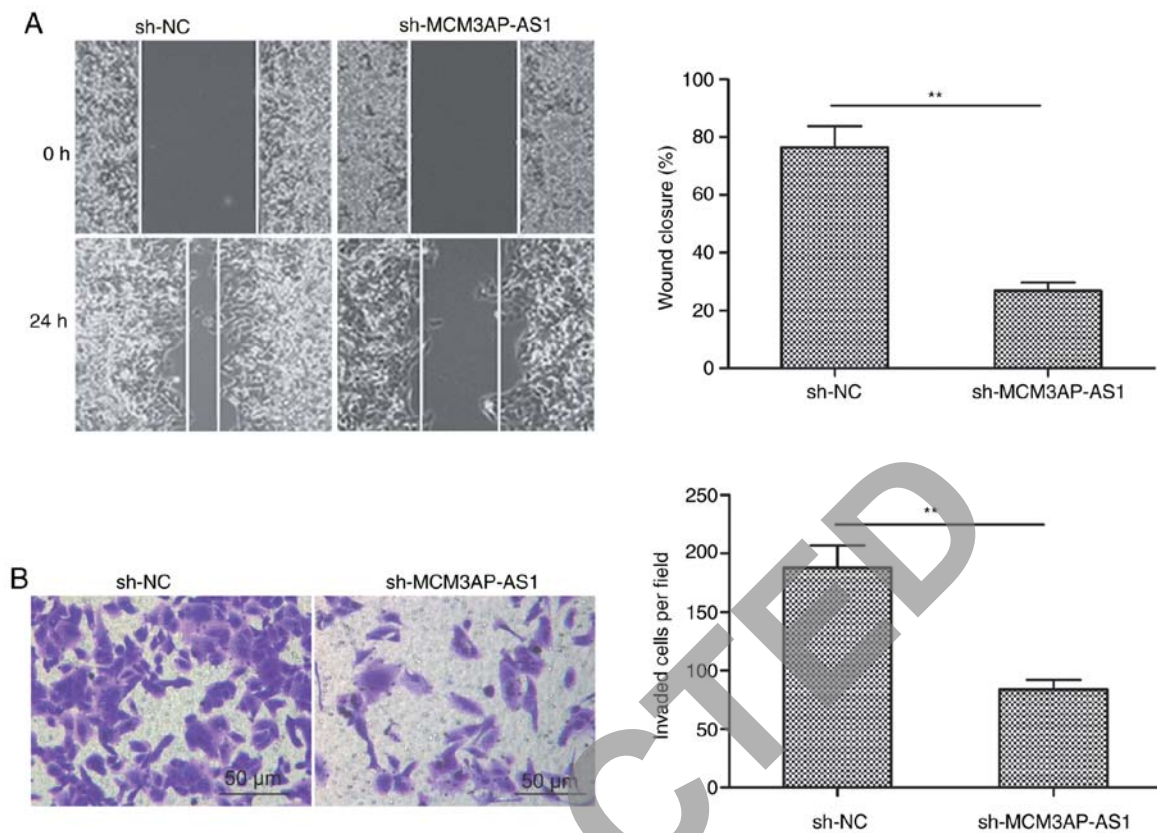


Figure 3. Knockdown of MCM3AP-AS1 inhibits cell migration and invasion of OC cells. (A) Cell migration was determined in SKOV3 cells transfected with sh-MCM3AP-AS1 and sh-NC using a wound healing assay. (B) Cell invasion was examined in SKOV3 cells transfected with sh-MCM3AP-AS1 and sh-NC using the Transwell invasion assay. All experiments were performed in triplicate and are expressed as mean \pm SD. ** $P < 0.01$. MCM3AP-AS1, MCM3AP antisense 1; OC, ovarian cancer.

(Fig. 1B). To assess how MCM3AP-AS1 expression is associated with clinicopathological features in patients with OC, the median MCM3AP-AS1 expression level was used to separate patients into MCM3AP-AS1-high and -low expression groups. As shown in Table I, MCM3AP-AS1 expression was positively correlated with advanced International Federation of Gynecology and Obstetrics (FIGO) staging ($P = 0.0030$) and lymph node metastasis (0.0105) (Table I). In addition, Kaplan-Meier survival analysis revealed that MCM3AP-AS1-high patients had a significantly poorer overall survival than that of MCM3AP-AS1-low patients ($P = 0.0261$; Fig. 1C). These findings suggest a possible key role for MCM3AP-AS1 in OC progression.

Knockdown of MCM3AP-AS1 disrupts OC cell proliferation. To knockdown this lncRNA in OC cells, a shRNA specific for MCM3AP-AS1 was transfected into SKOV3 cells, leading to a significant decrease in MCM3AP-AS1 expression, as expected following targeted shRNA transfection when compared to the sh-NC group (Fig. 2A). After knockdown of this lncRNA, CCK-8 assay was used to measure cell proliferation, revealing that the proliferative activity of SKOV3 cells was significantly decreased after MCM3AP-AS1 knockdown when compared to the sh-NC group ($P < 0.01$ at 72 h) (Fig. 2B). Colony formation assay yielded comparable results (Fig. 2C). EdU assay was able to further confirm a marked decrease in SKOV3 cell proliferation following sh-MCM3AP-AS1 transfection in contrast to sh-NC transfection (Fig. 2D). These results

confirmed that a reduction in MCM3AP-AS1 expression was able to impair OC proliferation.

Knockdown of MCM3AP-AS1 disrupts OC cell migration and invasion. Wound healing and Transwell invasion assays were next used to assess how MCM3AP-AS1 knockdown influences the ability of SKOV3 cells to undergo migration and invasion, respectively. The results revealed that reduced MCM3AP-AS1 expression significantly impaired the cell migration (Fig. 3A) and invasion (Fig. 3B) activities when compared to the sh-NC group ($P < 0.01$).

MCM3AP-AS1 directly targets miR-143-3p in OC cells. Our study next used starBase 2.0 in order to predict miRNAs that have the potential to directly bind MCM3AP-AS1 based on their complementarity. This predictive analysis identified miR-143-3p as one such putative MCM3AP-AS1-binding miRNA (Fig. 4A). This was confirmed via luciferase reporter assays, which revealed a significant decrease in WT but not in MT MCM3AP-AS1 luciferase reporter activity upon miR-143-3p overexpression in SKOV3 cells ($P < 0.01$; Fig. 4B). Our study next assessed the expression of MCM3AP-AS1 in the nuclear and cytoplasmic fractions of SKOV3 cells to evaluate its potential to serve as a ceRNA for miR-143-3p, and the results indicated that it was primarily located within the cytoplasm (Fig. 4C). RIP assay additionally confirmed that MCM3AP-AS1 and miR-143-3p were more abundant in Ago2 pellets than in IgG pellets prepared

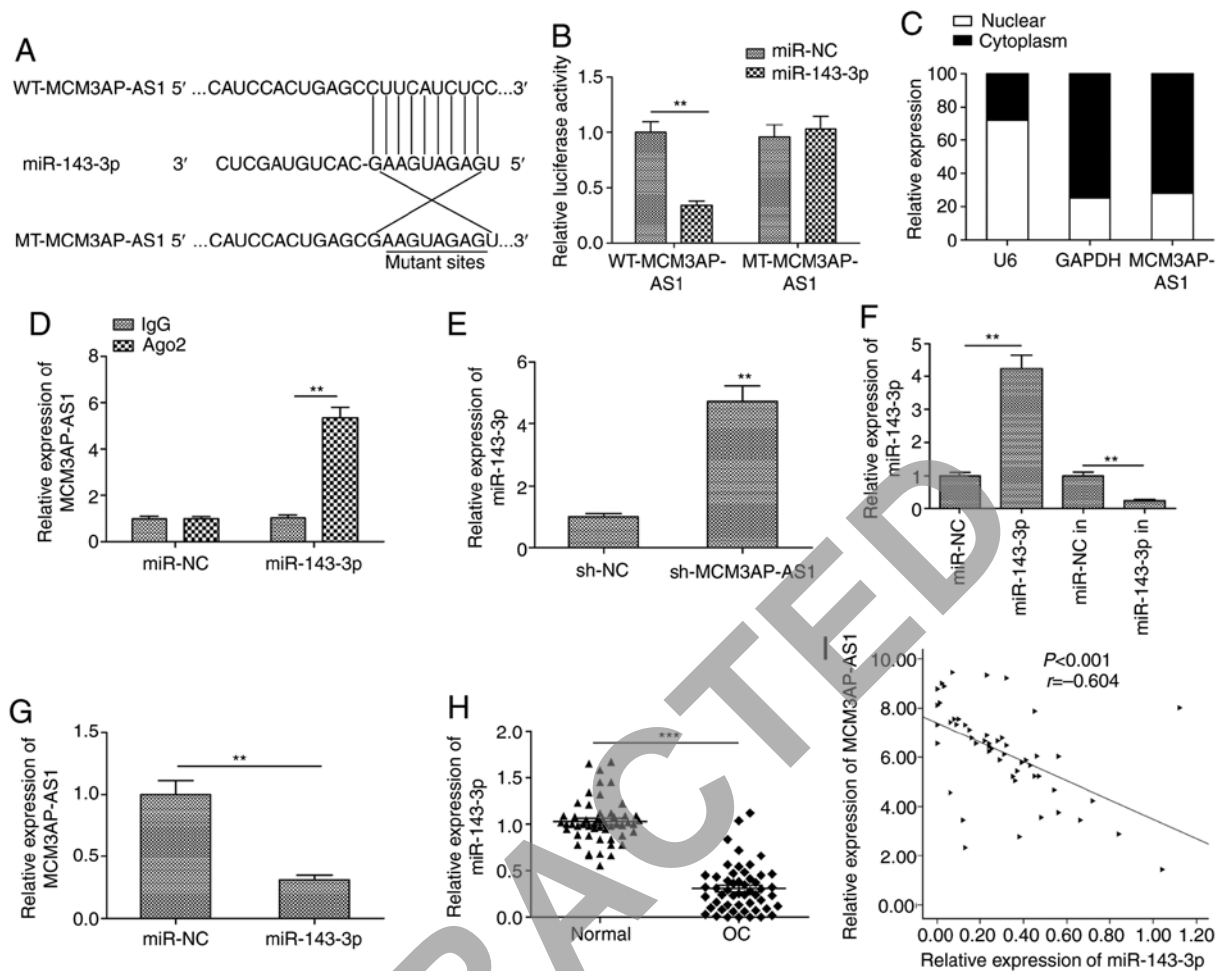


Figure 4. miR-143-3p serves as a direct target of MCM3AP-AS1 in OC cells. (A) The predicted binding site and mutant sites between MCM3AP-AS1 and miR-143-3p was explored by Starbase 2.0. (B) Dual-luciferase reporter assay revealed that miR-143-3p mimics negatively regulated the luciferase activity of MCM3AP-AS1-WT, rather than MCM3AP-AS1-MT. WT, wild-type; MT, mutant type. ** $P < 0.01$, compared with the miR-NC. (C) Relative expression of MCM3AP-AS1 in the cell cytoplasm or nucleus in SKOV3 cells was examined by RT-qPCR. U6 was used as the nuclear control and GAPDH was used as the cytoplasmic control. (D) The association between MCM3AP-AS1 and miR-143-3p was determined by RIP assay. ** $P < 0.01$, compared with the IgG group. (E) miR-143-3p expression was increased in SKOV3 cells transfected with sh-MCM3AP-AS1 when compared with sh-NC. ** $P < 0.01$, compared with the sh-NC. (F) The expression of miR-143-3p was detected in SKOV3 cells transfected with miR-143-3p mimic (miR-143-3p) or miR-143-3p inhibitor (in). ** $P < 0.01$. (G) MCM3AP-AS1 expression was decreased in SKOV3 cells transfected with miR-143-3p mimics. ** $P < 0.01$, compared with miR-NC. (H) Expression of miR-143-3p was examined in OC tissues and adjacent normal tissues. *** $P < 0.01$. (I) Correlation between MCM3AP-AS1 expression and miR-143-3p expression in OC tissues was analyzed by Pearson's correlation analysis. All experiments were performed in triplicate and are expressed as mean \pm SD. MCM3AP-AS1, MCM3AP antisense 1; OC, ovarian cancer.

from SKOV3 cells (Fig. 4D). In addition, knockdown of MCM3AP-AS1 expression led to a significant increase in the miR-143-3p level in SKOV3 cells when compared to the sh-NC group ($P < 0.01$; Fig. 4E). We also detect the expression of miR-143-3p in SKOV3 cells transfected with miR-143-3p mimic (miR-143-3p) or miR-143-3p inhibitor (in), and found that transfection with the miR-143-3p mimic significantly increased miR-143-3p expression ($P < 0.01$; Fig. 4F), while transfection with the inhibitor significantly decreased miR-143-3p expression in SKOV3 cells when compared with the relevant NC group ($P < 0.01$; Fig. 4F). When miR-143-3p was overexpressed, this was associated with a significant decrease in MCM3AP-AS1 expression ($P < 0.01$; Fig. 4G). Moreover, reduced miR-143-3p expression levels were further observed in OC tissues (Fig. 4H), and this expression in tissue samples was negatively correlated with that of MCM3AP-AS1 (Fig. 4I), consistent with a role for miR-143-3p as an MCM3AP-AS1 target in OC cells.

Inhibition of miR-143-3p is a mechanism by which MCM3AP-AS1 influences OC progression. Our study next assessed whether inhibition of miR-143-3p activity might be a mechanism by which MCM3AP-AS1 affects OC cells. For that purpose, rescue experiments were conducted in SKOV3 cells co-transfected with sh-MCM3AP-AS1 and miR-143-3p inhibitors. MCM3AP-AS1 downregulation led to an increase in miR-143-3p expression that was partially reversed by miR-143-3p inhibition (Fig. 5A). It was also found that, when miR-143-3p expression was inhibited in the rescue experiments, the ability of MCM3AP-AS1 downregulation to impair the proliferation, migration and invasion of SKOV3 cells was markedly attenuated (Fig. 5B-F).

Transforming growth factor- β -activated kinase 1 (TAK1) is known to be a miR-143-3p target gene in the context of OC (15). Therefore, our study sought to assess whether MCM3AP-AS1 might regulate TAK1 expression in OC cells. Indeed, when MCM3AP-AS1 expression was

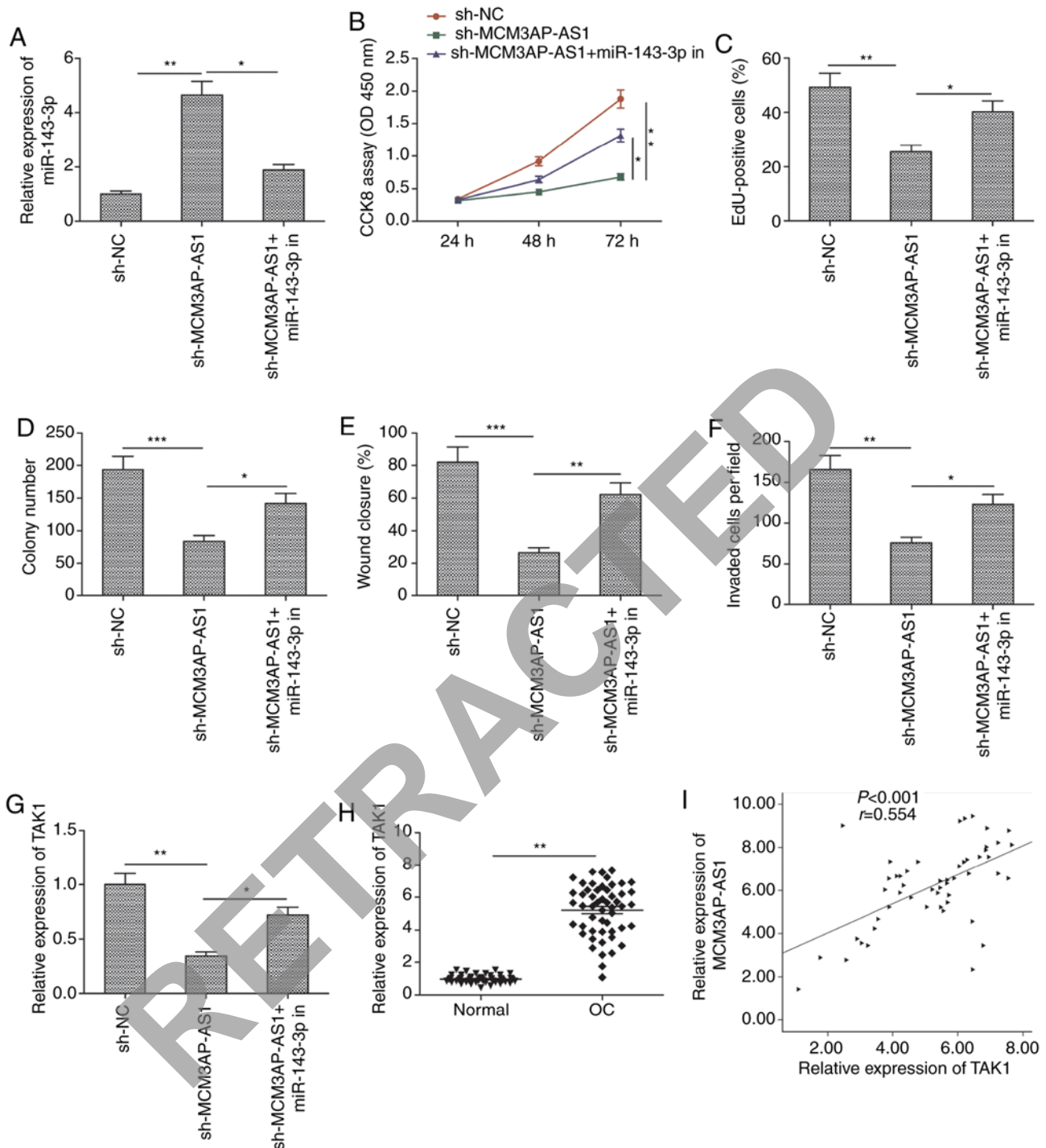


Figure 5. miR-143-3p inhibitor mediates the tumor-suppressive effects of MCM3AP-AS1 knockdown in OC cells. (A) Expression of miR-143-3p was examined in SKOV3 cells transfected with sh-NC, sh-MCM3AP-AS1 and sh-MCM3AP-AS1+miR-143-3p inhibitor (miR-143-3p in). (B and C) Cell proliferation, (D) colony formation, (E and F) migration and invasion were detected in SKOV3 cells transfected with sh-NC, sh-MCM3AP-AS1, and sh-MCM3AP-AS1+miR-143-3p inhibitor (miR-143-3p in). (G) *TAK1* mRNA expression was examined by RT-qPCR in SKOV3 cells transfected with sh-NC, sh-MCM3AP-AS1, and sh-MCM3AP-AS1+miR-143-3p inhibitor (miR-143-3p in). (H) Expression of *TAK1* was examined in OC tissues and adjacent normal tissues. (I) Correlation between MCM3AP-AS1 expression and *TAK1* expression in OC tissues was analyzed by Pearson's correlation analysis. All experiments were performed in triplicate and are expressed as mean \pm SD. * P <0.05, ** P <0.01, *** P <0.001. MCM3AP-AS1, MCM3AP antisense 1; OC, ovarian cancer; TAK1, transforming growth factor- β -activated kinase 1.

knocked down in SKOV3 cells, *TAK1* expression was significantly decreased, whereas this was partially reversed by miR-143-3p inhibition (Fig. 5G). OC tissue samples also exhibited increased *TAK1* expression (Fig. 5H), with this expression being positively correlated with MCM3AP-AS1 expression in OC tissues (Fig. 5I). This suggested that

MCM3AP-AS1, at least in part, exerts its influence on OC via the miR-143-3p/*TAK1* axis.

MCM3AP-AS1 knockdown inhibits tumor growth in vivo. The influence of MCM3AP-AS1 on OC was explored *in vivo* using a xenograft model in which mice were injected with

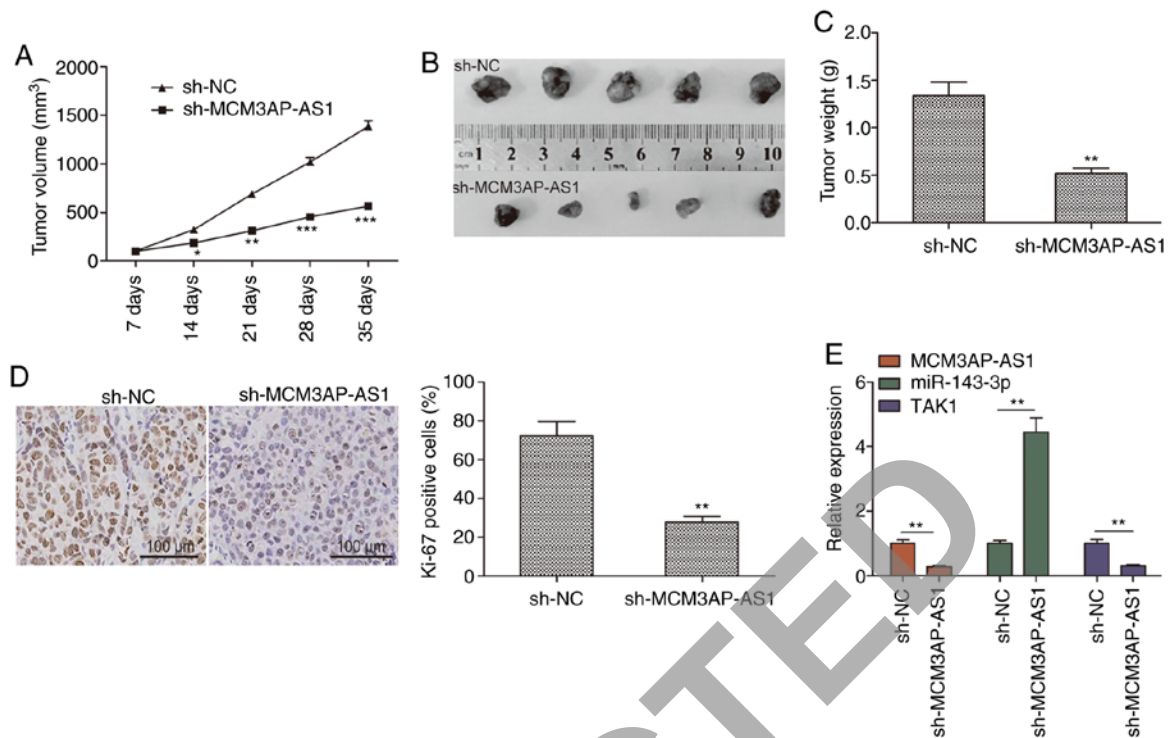


Figure 6. Knockdown of MCM3AP-AS1 inhibits OC tumor growth *in vivo*. (A) Tumor volumes were measured every 7 day until the mice were sacrificed. (B) Tumor images was captured at the end of the experiments. (C) Tumor weight was measured at the end of the experiments. (D) Ki-67 expression was determined in xenograft tumors by IHC. (E) Relative expression of MCM3AP-AS1, miR-143-3p and *TAK1* was determined in xenograft tumors by RT-qPCR. All experiments were performed in triplicate and are expressed as mean \pm SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. MCM3AP-AS1, MCM3AP antisense 1; OC, ovarian cancer; TAK1, transforming growth factor- β -activated kinase 1.

SKOV3 cells stably expressing either sh-MCM3AP-AS1 or sh-NC. A significant reduction in tumor growth was observed for tumors in which MCM3AP-AS1 had been knocked down relative to control tumors, with a significantly smaller tumor weight at the end of the study (Fig. 6A-C). In addition, Ki-67 staining confirmed a reduction in tumor proliferation in MCM3AP-AS1-knockdown tumors, as evidenced by reduced expression of this proliferative marker (Fig. 6D). RT-qPCR was further used to assess the expression of MCM3AP-AS1, miR-143-3p and *TAK1* in these tumor samples. The results revealed decreased expression of MCM3AP-AS1 and *TAK1* in the sh-MCM3AP-AS1 group, along with a corresponding increase in miR-143-3p expression relative to that of the sh-NC group (Fig. 6E). These results suggested that depleting MCM3AP-AS1 expression can significantly constrain tumor growth *in vivo*.

Discussion

A substantial body of evidence supports a role for long non-coding (lnc)RNAs in the regulation of tumor progression and development, with lncRNAs such as MALAT1, HOXA11-AS and UAC1 having previously been shown to promote ovarian cancer (OC) progression (7,8), while others such as TUG1, BANC1 and H19 having been reported to play a tumor-suppressive role (16,17). Previous studies have demonstrated that MCM3AP-AS1 can promote the progression of several tumor types (9-13). However, the role of MCM3AP antisense 1 (MCM3AP-AS1) in OC remains to be investigated. In this study, a clear upregulation of this lncRNA was observed

in OC tissue samples and cells, with tissue expression being associated with a poorer prognosis. When MCM3AP-AS1 was knocked down, it constrained tumor growth *in vitro* and *in vivo*. MCM3AP-AS1 was able to drive OC progression at least in part by regulating miR-143-3p. Our results clearly demonstrate that MCM3AP-AS1 plays an oncogenic role in OC.

Numerous lncRNAs have been shown to regulate diverse biological processes by serving as ceRNAs capable of binding and sequestering specific target miRNAs (18-20). Using predictive bioinformatics techniques, our study identified miR-143-3p as a miRNA likely to bind MCM3AP-AS1, which was confirmed by dual-luciferase reporter assays. Thus, it was hypothesized that MCM3AP-AS1 may serve as a ceRNA for this target miRNA. To confirm this hypothesis, the subcellular localization of MCM3AP-AS1 was additionally assessed, revealing that it was expressed primarily in the cytoplasm of SKOV3 cells. RIP assays additionally confirmed a direct binding interaction between MCM3AP-AS1 and miR-143-3p, consistent with the role of MCM3AP-AS1 as a miR-143-3p ceRNA that binds and sequesters this miRNA. miR-143-3p has previously been shown to act as a tumor suppressor in several tumor types (21-23). Decreased miR-143-3p expression has been detected previously in OC tissues and cell lines, and overexpression of this miRNA suppresses the growth and metastasis of OC tumors *in vitro* and *in vivo* (15,24). In line with these previous findings, our study observed decreased miR-143-3p levels in OC tissues and cell lines, and found this expression to be negatively correlated with that of MCM3AP-AS1 in OC tissue samples. Importantly, inhibition of miR-143-3p partially abrogated the inhibition

of proliferation and migration observed in SKOV3 cells upon MCM3AP-AS1 knockdown. Together, these findings suggest that MCM3AP-AS1 knockdown inhibits OC growth by regulating miR-143-3p expression.

The role of lncRNAs as ceRNAs allows them to indirectly control the expression of target genes of miRNAs (21-23). Transforming growth factor- β -activated kinase 1 (TAK1) has previously been reported as a miR-143-3p target in OC cells (15). TAK1, a member of the MAP kinase kinase kinase (MAP3K) family, was reported to participate in various cellular processes by regulating several signaling pathways, including the p38MAPK and NF- κ B pathways, which play critical roles in cell survival and proliferation (25,26). TAK1 overexpression can promote the progression of several cancer types, and is considered a key therapeutic target in multiple types of cancer (27,28). In OC tissues, TAK1 has been found to be overexpressed, and its knockdown suppresses the growth and metastasis of OC cells (15,29,30). In line with these findings, our study observed increased TAK1 expression in OC samples, which was positively correlated with that of MCM3AP-AS1. Knockdown of MCM3AP-AS1 was associated with a marked decrease in TAK1 expression in these cells, and this could be partially reversed by inhibiting miR-143-3p. This suggests that, by acting as a miR-143-3p ceRNA, MCM3AP-AS1 is able to regulate TAK1 expression.

In summary, the present study revealed that MCM3AP-AS1 expression was upregulated in OC samples and cell lines, and that the levels are positively associated with advanced FIGO stage, lymph node metastasis and poor overall survival. MCM3AP-AS1 knockdown caused a conspicuous inhibition of proliferation and migratory activity in OC cells *in vitro*, as well as suppression of xenograft tumor growth *in vivo*. MCM3AP-AS1 exerted its oncogenic role in OC by regulating the miR-143-3p/TAK1 axis. Further research is required using additional OC cell lines in order to confirm the biological relevance of these findings, and to validate MCM3AP-AS1 as a potential therapeutic target.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

YW conceived and designed the study. JW and SH performed the experiments and wrote the manuscript. MC reviewed and edited the study.

Ethics approval and consent to participate

The Ethics Committee of the First Hospital of Jilin University (Changchun, China) approved this study, and the

participants provided written informed consent. In addition, The Institutional Animal Care and Use Committee of Jilin University (Changchun, China) approved all the animal experiments conducted in our study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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