MicroRNA-18a inhibits ovarian cancer growth via directly targeting TRIAP1 and IPMK

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Abstract. The role of microRNA-18a (miRNA/miR-18a) as a tumor suppressor or promoter in a number of different types of cancer has been reported. However, to date, the expression and the effects of miR-18a in epithelial ovarian cancer (EOC) remain elusive. In the present study, the expression of miR-18a in patient EOC tissues and ovarian cancer cell lines was investigated using the reverse transcription-quantitative polymerase chain reaction. Luciferase assays and western blotting were performed to detect the potential direct targets of miR-18a. An A2780cp intraperitoneal mouse model, and Cell Counting Kit 8, flow cytometry and terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling assays, were used to investigate the effect of miR-18a on tumor growth *in vivo* and *in vitro*. The results indicated that the expression of miR-18a was reduced in EOC tissue and in the investigated ovarian cancer cell lines compared with non-malignant (normal) ovarian tissues and the human ovarian epithelium cell line, respectively. Overexpression of miR-18a in the A2780s and A2780cp cell lines significantly induced cell cycle arrest and apoptosis. It was demonstrated that miR-18a directly targets tumor protein p53-regulating inhibitor of apoptosis gene 1 and inositol phosphate multikinase, hence regulating the expression of downstream targets. The A2780cp intraperitoneal mouse model was employed and the results indicated that miR-18a may inhibit A2780cp intraperitoneal tumor growth *in vivo* by inhibiting proliferation and inducing apoptosis. Together, the results of the present study demonstrated that miR-18a has a role as a tumor suppressor by inhibiting proliferation and inducing apoptosis. Assessment of miR-18a expression may provide a novel method for diagnosis and be a therapeutic target for EOC.

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Introduction

Epithelial ovarian cancer (EOC) is the leading cause of mortality from gynecological malignancies. There are >21,290 cases annually in the United States, and 14,180 women can be expected to succumb to the disease in 2015 (1). Due to the lack of effective tools for early detection, the majority of patients with EOC are in an advanced disease stage at diagnosis. The prognosis of EOC is usually poor, with a 5-year survival rate at ~44% (1). Although there have been advancements in surgery and chemotherapy treatment in previous years, the 5-year survival rate for patients with EOC has not been improved significantly in the last two decades (2). Therefore, it is necessary to identify effective biomarkers that can aid diagnosis and therapy for patients with EOC.

MicroRNAs (miRNAs/miRs) are a family of short non-coding RNAs that negatively regulate gene expression at the post-transcriptional level (3). By regulating protein translation, miRNAs have emerged as powerful regulators of a wide range of biological processes. Evidence is emerging for the role of miRNAs in modulating cancer development. One particular class of miRNAs has emerged to target survival signaling pathways or signaling pathways that regulate apoptosis, sensitivity and the cell cycle. This class includes miR-365 and miR-29, which target cyclin D1 and apoptosis regulator Bcl-2 (Bcl-2) in colon cancer (4), and collagen type Iα1 in ovarian cancer (5), respectively. Additionally, miR-19a and miR-195-5p target Bcl-2 in gastric cancer (6).

miR-18a belongs to the miR-17-92 cluster, which includes six miRNAs (miR-17, -20a, -18a, -19a, -19b and -92a) and is located at the chromosome 13q31.1 region (7). Of the six miR-17-92 cluster members, miR-19a and -b have been demonstrated to be key promoters of cancer development and cancer cell proliferation (8,9). Meanwhile, the role of miR-18a in cancer development remains elusive. Several studies have observed that miR-18a is highly expressed in several types of cancer, including nasopharyngeal carcinoma (10), and prostate (11) and gastric (12,13) cancer. The miRNA

promotes cancer development via inducing proliferation and inhibiting apoptosis. Notably, studies have also demonstrated that miR-18a can act as a tumor suppressor in breast (14) and colorectal (15) cancer through inhibiting the expression of hypoxia inducible factor 1α subunit and cell division cycle 42 (14,15). Nam *et al* (16) determined the expression of miR-18a in advanced stages of ovarian cancer and observed that higher miR-18a expression was significantly correlated with a poor prognosis.

To the best of our knowledge, studies have yet to determine the expression and effects of miR-18a in EOC. In the present study, series studies were performed to investigate the effects of miR-18a on ovarian cancer growth and its associated mechanism, and the potential predictive of miR-18a in patients with EOC.

Materials and methods

Collection of tissue samples. Non-malignant (normal) and EOC tissues were obtained with informed patient consent and ethical approval from the Academic Medical Center Institutional Review Board of West China Second University Hospital, Sichuan University (Chengdu, China). The clinical tissues were obtained from the West China Second University Hospital. Experiments described were performed on samples obtained from the Academic Medical Center.

Cell culture and treatment. The ovarian cancer cell lines (A2780cp, A2780s, SKOV3 and CAOV3), the human ovarian epithelium cell line (HOEC) and the HEK293 cells were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (both Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA). All the cells were maintained in a humidified incubator containing 5% CO_2 at 37˚C.

The lentivirus-based miR-18a expression system (lenti-miR-18a) and the negative control (lenti-NC) were purchased from GeneChem Co., Ltd. (Shanghai, China). Cell infection was performed according to the manufacturer's instructions. Cells were infected with lenti-miR-18a using a multiplicity of infection of 10. Puromycin (2 μg/ml) was added for selection at 72 h post-infection. Cell transfection was performed using FuGENE® HD transfection reagent (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instructions.

Briefly, cells were seeded in 6-well plates at a density of 2×10^5 cells/well and cultured for 24 h to reach 70-80% confluence. Plasmids [2 μg; tumor protein p53-regulated inhibitor of apoptosis gene 1 (TRIAP1)-3'untranslated region (UTR), TRIAP1-3'UTR-mutant, IPMK-3'UTR and IPMK-3'UTR-mutant] were diluted in 100 μl DMEM without serum. A total of 5 μl FuGENE HD Transfection Reagent was added to the tubes containing diluted DNA. The suspension was mixed and incubated for 15 min at room temperature, and subsequently added to the 6-well plates.

Cell cycle assay. Cell cycle assays were performed by flow cytometry. Briefly, the cells post-treatment were collected by centrifugation for 3 min at 1,200 x *g* at 4˚C and resuspended in 1 ml cold PBS. Following fixation with 4 ml absolute ethanol, the cells were centrifuged for 3 min at 1,200 x *g* at 4˚C and resuspended in 1 ml PBS. The cells were subsequently stained with 100 μl light sensitive propidium iodide (1 mg/ml; Beyotime Institute of Biotechnology, Beijing, China) and incubated for 5 min at room temperature prior to flow cytometry. A total of 5×10^4 cells from each group were loaded and counted in each phase.

Bioinformatic analysis. The miRWalk database (www .ma.uni-heidelberg.de/apps/zmf/mirwalk) and other programs, including miRanda (http://www.microrna.org/microrna/getGeneForm.do), Sanger miRDB (http://www.mirdb.org/miRDB/) and Targetscan (http://genes.mit.edu/tscan/targetscanS.html), were used variously for target prediction. The online tool miRWalk (version 2.0; www.umm.uni-heidelberg.de/apps/zmf/mirwalk/predictedmirnagene) was used to predict potential target mRNAs of miR-18a. To verify this bioinformatic analysis, the TRIAP1-3'UTR and the IPMK-3'UTR, each of which contains a single miR-18a binding site, were cloned downstream of the luciferase open reading frame. Meanwhile, TRIAP1-3'UTR mutant and IPMK-3'UTR mutant, which contain mutated miR-18a binding sites, were also introduced into the luciferase constructs. The 293 cells were infected with lenti-miR-18a, and puromycin was used to select the cells with stable expression. qPCR analysis confirmed that miR-18a was upregulated in the cells (data not shown). The luciferase constructs (TRIAP1-3'UTR, TRIAP1-3'UTR, IPMK-3'UTR and IPMK-3'UTR mutant) were transfected separately into cells that stably expressed miR-18a. Luciferase activity was determined 4 to 6 h later.

Luciferase assays. The pMiRluc-TRIAP1-3'UTR and pMiRluc-IPMK-3'UTR constructs containing miR-18a binding site were purchased from Fulengen Biotechnology Co. (Guangzhou, China). The luciferase reporter was co-transfected with lenti-NC (negative control) or lenti-miR-18a using FuGENE HD (Roche Diagnostics). Luciferase activity was measured 4 h after by luciferase reporter assay (Promega Corporation, Madison, WI, USA), and the values were normalized with activity of β-galactosidase.

Reverse transcription-qPCR (RT-qPCR). Total RNA was extracted from each experimental group using TRIzol® reagent (Invitrogen Life Technologies; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. RNA concentration was assessed spectrophotometrically at 260 nm (Thermo ND 2000; Thermo Fisher Scientific, Inc. Wilmington, DE, USA). RT was performed using a RT kit (Takara Bio, Inc., Otsu, Japan). PCR was subsequently performed using a Real Time PCR kit (Takara Bio, Inc.) on a Bio-Rad CFX96 thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA), with U6 as an internal control. PCR conditions were as follows: Denaturation at 94˚C for 2 min; 30 cycles of amplification at 94˚C for 30 sec, annealing at 58˚C for 30 sec, and extension at 72˚C for 1 min. This was followed by a terminal elongation step at 72˚C for 10 min. The Cq value of each PCR product was calculated, and the fold-change was analyzed, according to the protocols of previous studies (17). The primers for miR-18a and U6 were purchased from RiboBio Technology

(Guangzhou, China; the sequences were not supplied due to the rules of the company). All experiments were performed in triplicate.

Cell viability detection assay. The Cell Counting Kit-8 (CCK-8) assay (Beyotime Institute of Biotechnology) was performed to detect cell viability according to the manufacturer's protocol. Briefly, 10 μl CCK-8 was added into the cell culture medium and incubated at 37˚C for 4 h. Subsequently, absorbance was measured at 450 nm and each experiment was performed three times.

Western blotting. Following treatment, cells were lysed on ice for 20 min with radioimmunoprecipitation assay buffer (Beyotime Institute of Biotechnology). A total of 20 μg protein was separated by SDS-PAGE on a 8-12% gel and electronically transferred onto a polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). Following blocking at 4˚C overnight with 5% no-fat milk in TBT Tween 20, the membranes were incubated with the recommended dilutions of primary antibodies against TRIAP1 (dilution, 1:800; cat. no. LS-C346398-50; LifeSpan Biosciences, Inc., Seattle, WA, USA), IPMK (dilution, 1:500; cat. no. TA337886; OriGene Technologies, Inc., Beijing, China), AMP-activated protein kinase (AMPK) (dilution, 1:1,000; cat. no. 2603), phosphorylated (p)-AMPK (dilution: 1:800; cat. no. 2535) p53 (dilution, 1:2,000; cat. no. 2524), cleaved caspase 3 (CC3) (dilution, 1:500; cat. no. 9661), Akt (dilution, 1:1,000; cat. no. 13038), p-Akt (dilution, 1:1,000; cat. no. 4060), cyclin D1 (dilution, 1:800; cat. no. 2978; all Cell Signaling Technology, Inc., Danvers, MA, USA) and glyceraldehyde-3-phosphate dehydrogenase (dilution, 1:5,000; cat. no. SC47724; Santa Cruz Biotechnology, Inc. Dallas, TX, USA) for 1 h at 37˚C. The membranes were subsequently incubated with the following secondary antibodies: Horseradish peroxidase (HRP)-conjugated anti-rabbit Immunoglobulin G (IgG; dilution, 1:10,000; cat. no. ZB2301); and the HRP-conjugated anti-mouse IgG antibody (dilution, 1:10,000; cat. no. ZB2305) both from OriGene Technologies, Inc. at room temperature for 2 h. Peroxidase-labeled bands were visualized using an enhanced chemiluminescence kit (EMD Millipore), according to the manufacturer's protocol.

Terminal deoxynucleotidyl transferase-deoxyuridine triphosphate nick end labeling (TUNEL) assay. TUNEL assay was performed using a DeadEnd™ Fluorometric TUNEL system (Promega Corporation) to detect apoptotic cells in A2780cp and A2780s cells and A2780cp tumor tissues, according to the manufacturer's instructions. Cell nuclei with green fluorescent staining were defined as TUNEL-positive and visualized using a fluorescence microscope (DTX500; Nikon Corporation, Tokyo, Japan). For quantification of the TUNEL-positive cells, the number of green fluorescent cells was counted in randomly selected fields (magnification, x200). The cell nuclei were subsequently counter-stained with 4',6-diamidino-2-phenylindole (Beyotime Institute of Biotechnology).

Animal study. To establish the A2780cp intraperitoneal cancer model, A2780cp cells ($8x10^6$) infected with lenti-miR-18a and lenti-NC (negative control), were injected into the intraperitoneal space of BALB/c nude female mice (6-8 weeks old; ~20 g). A total of 6 mice per group were used. The mice were obtained from the Animal Center of Sichuan University and housed at 26˚C, with a 12-h light/dark cycle and *ad libitum* access to food and water. At 30 days after tumor cell injection, the mice were anesthetized using diethyl ether (100 mg/kg; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and sacrificed. The dissected tumors were weighed. The tumors and viscera were examined grossly and microscopically stained with hematoxylin and eosin. Animal studies were performed with approval from with the Institutional Animal Care and Treatment Committee of Sichuan University.

Immunostaining of proliferating cell nuclear antigen (PCNA). Expression of PCNA was analyzed using mouse anti-human PCNA antibody (cat. no. I0710; Santa Cruz Biotechnology, Inc.). Paraffin-embedded tumor sections (3-5 μm) were mounted on 3-aminopropyl triethoxysilane-coated glass slides. Sections were deparaffinized in xylene, treated with a graded series of alcohol [100, 95 and 80% ethanol/double-distilled H_2O (v/v)] and rehydrated in PBS (pH 7.4). Antigen retrieval was performed by heating for 3 min in a pressure cooker with 0.1 mol/l citrate buffer (pH 6.0). Endogenous peroxide was blocked with 3% H_2O_2 for 10 min. Following PBS washes, slides were blocked with 5% normal goat serum (Beyotime Institute of Biotechnology) in PBS for 15 min at room temperature and incubated with primary anti-PCNA antibody (1:200) in blocking solution overnight at 4˚C. All slides were subsequently incubated with biotin-conjugated goat anti-mouse or goat anti-rat secondary antibody (both dilution 1:200; cat. nos. SP9001 and SP9002, respectively; OriGene Technologies, Inc.) for 15 min at 37˚C and with streptavidin-biotin complex at 37˚C for 15 min. The immunoreaction was visualized using diaminobenzidine peroxide solution and the nuclei were counterstained with hematoxylin. All specimens were observed using Olympus BX600 microscopes and SPOT Fiex camera (both Olympus Corporation, Tokyo, Japan). The total number of cells and the number of PCNA-positive cells were counted, and the percentage of PCNA-positive cells was calculated.

Statistical analysis. All data were analyzed using one-way analysis of variance. Statistical analyses were performed using SPSS Statistics version 19.0 (IBM SPSS, Armonk, NY, USA). Values are expressed as mean ± standard error. $P<0.05$ was considered to indicate a statistically significant difference.

Results

miR-18a is downregulated in malignant ovarian tissues and ovarian cancer cells. To determine the expression level of miR-18a in ovarian malignant and normal tissues, malignant ovarian and non-malignant (normal) tissues were collected and used for miR-18a detection by qPCR. As shown in Fig. 1A, miR-18a expression in ovarian malignant tissues was significantly downregulated compared with the normal ovarian tissues ($P<0.001$). Furthermore, the expression of miR-18a in several ovarian cancer cell lines and the normal ovarian cell line was determined. The results indicated that miR-18a expression was significantly lower in the ovarian cancer cell lines (A2780cp, A2780s, SKOV3 and CAOV3) compared with the non-malignant HOEC cell line (all $P<0.01$; Fig. 1B).

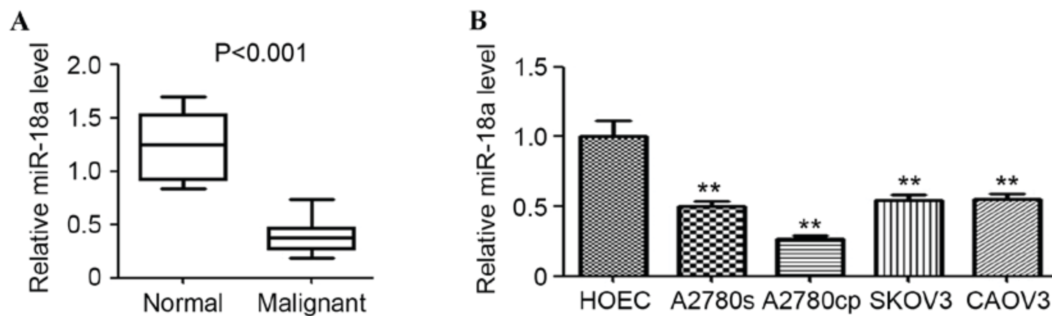

Figure 1. Expression of miR-18a in malignant ovarian tissues and ovarian cancer cell lines. (A) Box plots show the relative expression levels of miR-18a in 12 pairs of non-malignant (normal) and malignant ovarian tissues as determined by qPCR. Each box shows the median and the interquartile range. (B) qPCR analysis of miR-18a expression in human ovarian cancer cells (A2780s, A2780cp, SKOV3 and CAOV3) compared with HOECs. Data are depicted as mean ± standard error. **P<0.01. HOEC, human ovarian epithelial cells; miR/miRNA, microRNA; qPCR, quantitative polymerase chain reaction.

miR-18a significantly inhibits proliferation and induces apoptosis of ovarian cancer cells in vitro. A lentivirus-based miR-18a expression system was established to overexpress miR-18a in two ovarian cancer cell lines (A2780cp and A2780s) to investigate the potential involvement of miR-18a in inhibiting the development of ovarian cancer. As shown in Fig. 2A and B, qPCR confirmed that miR-18a expression was significantly upregulated when miR-18a was overexpressed in A2780cp and A2780s cells compared with the NC (P<0.01). Analysis of cell viability by CCK-8 assay indicated that overexpression of miR-18a significantly inhibited cell growth in the ovarian cancer cell lines (A2780s and A2780cp) compared with the NC (P<0.01; Fig. 2C).

Flow cytometric analysis of the cell cycle indicated a significant reduction in the number of cells in the S-phase and a significant increase in the G0/G1 phase when miR-18a was overexpressed in the A2780cp and A2780s cells compared with the NC (P<0.01; Fig. 2D and E). However, no significant differences were observed in the G2/M phase between the two groups (Fig. 2D and E). Analysis of cell apoptosis by TUNEL assay indicated significant increases in the number of apoptotic cells when miR-18a was overexpressed in the A2780cp and A2780s cells (P<0.01; Fig. 2F and G).

miR-18a directly targets TRIAP1 and IPMK. In order to investigate the direct targets of miR-18a involved in cancer cell proliferation and apoptosis in human ovarian cells, a large number of potential target proteins in a database library were screened to identify potential microRNA binding seed sequences within the 3'-UTR. Bioinformatic analysis (Targetscan micro, RNA.org, microRNASeq) identified TRIAP1 and IPMK as candidate targets (Fig. 3A and B).

Luciferase expression in the cells overexpressing miR-18a and also expressing the TRIAP1-3'UTR construct was significantly reduced. The same finding was observed for the cells overexpressing miR-18a and also expressing the IPMK-3'UTR construct (P<0.01; Fig. 3C and D). No significant reduction in luciferase expression of cells transfected with miR binding site mutant plasmids was observed (Fig. 3C and D).

Western blotting was subsequently employed to determine the expression of TRIAP1 and IPMK proteins, and their downstream targets. Overexpression of miR-18a in A2780cp and A2780s cells markedly inhibited TRIAP1 and IPMK expression (Fig. 3E and F). The expression of cell cycle-associated proteins p-Akt, p-AMPK and cyclin D1 was also downregulated by miR-18a, whereas no significant reduction of Akt and AMPK expression was observed. Furthermore, miR-18a also significantly induced the expression of the apoptosis-associated protein p53, p21 and cleaved caspase 3 in the A2780cp and A2780s cells (Fig. 3E). Collectively, the results indicated that miR-18a directly targets TRIAP1 and IPMK, and hence regulates the expression of the downstream targets.

miR-18a inhibits ovarian tumor growth in vivo. To further elucidate the effects of miR-18a on ovarian tumor growth *in vivo*, the A2780cp intraperitoneal mouse model was employed. The tumor was collected (Fig. 4A) and the weight was measured 30 days after the A2780cp injection. The mean tumor weight was 1.05±0.11 and 0.37±0.04 g in the NC and miR-18a group (P<0.01; Fig. 4B). Furthermore, the proliferative and apoptotic activity of the A2780cp tumors were determined by PCNA staining and TUNEL assay. When compared with the negative controls, lower numbers of PCNA-positive cells and higher numbers of apoptotic cells were found in the miR-18a group (P<0.01; Fig. 4C and D). Together, the results in the present study indicated that miR-18a may inhibit A2780cp intraperitoneal tumor growth *in vivo* by inhibiting proliferation and inducing apoptosis.

Discussion

An initial analysis of the expression and effects of miR-18a in EOC indicated that the expression of miR-18a was lower in EOC tissue and all ovarian cancer cell lines investigated compared with non-malignant (normal) ovarian tissues and the HOEC cell line. Thus, we hypothesize that miR-18a may act as a tumor suppressor in the development of EOC. The lentivirus-based miR-18a expression system was employed and used to infect ovarian cancer cell lines (A2780s and A2780cp). Overexpression of miR-18a significantly induced cell cycle arrest and apoptosis. Bioinformatic and luciferase reporter gene assay demonstrated that miR-18a directly targeted TRIAP1 and IPMK, and hence regulated the expression of downstream targets. Furthermore, *in vivo* study and

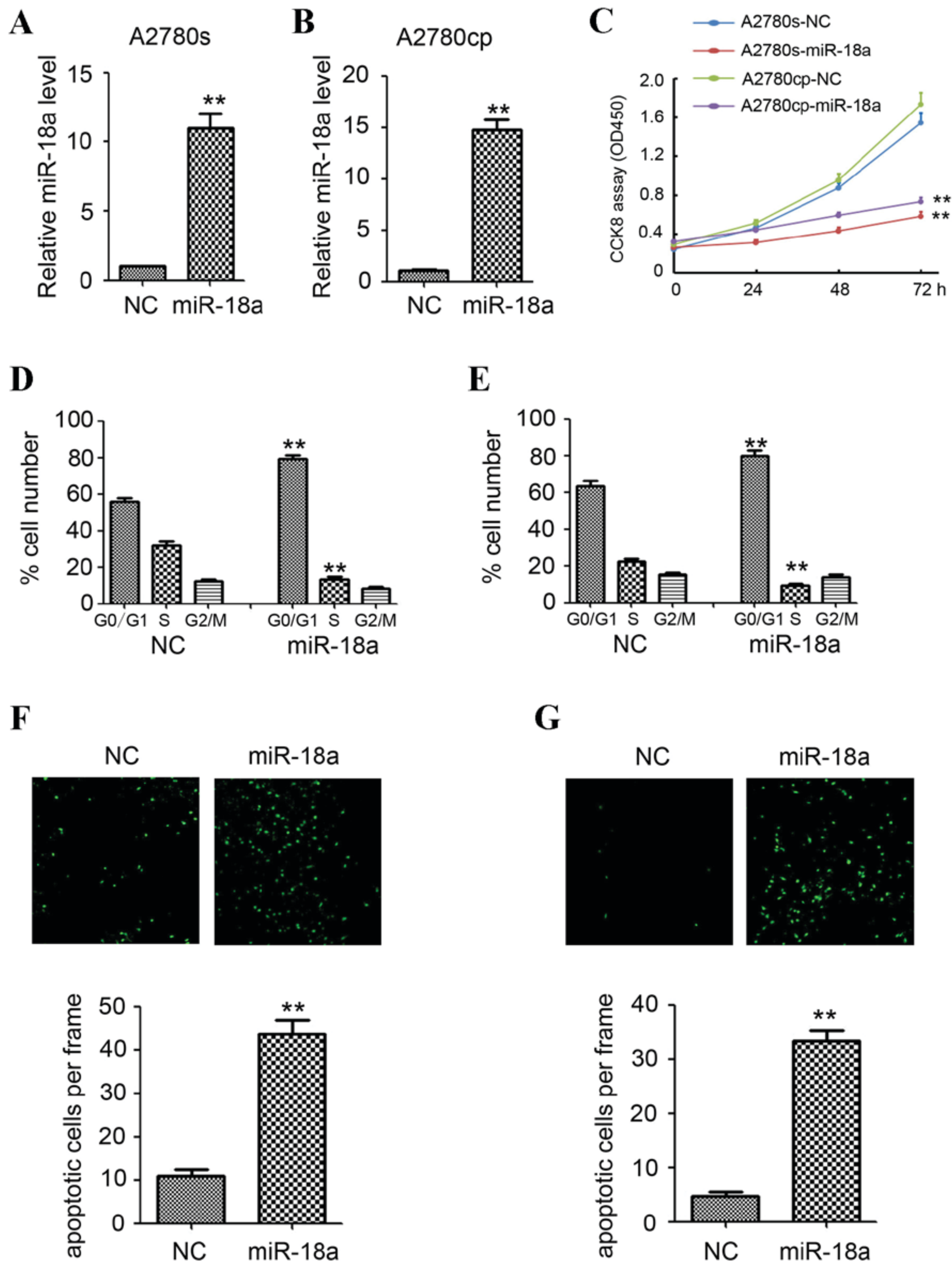

Figure 2. miR-18a inhibits proliferation and induces apoptosis of ovarian cancer cells *in vitro*. qPCR analysis of miR-18a expression in (A) A2780s and (B) A2780cp cells infected with lenti-miR-18a and lenti-miR-NC following selection with puromycin. (C) Cell viability of A2780s and A2780cp cells following infection with lenti-miR-18a or lenti-miR-NC as determined by CCK-8 assay. Flow cytometric analysis of cell cycle distribution in (D) A2780cp and (E) A2780s cells. Apoptotic cells were detected by terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling assay in (F) A2780cp and (G) A2780s cells. Apoptotic cells per frame were analyzed compared with the NC group. Magnification, x200. Data are depicted as mean ± standard error, $^{**}P<0.01$ vs. the NC group. CCK-8, cell counting Kit-8; miR/miRNA, microRNA; OD, optical density; qPCR, quantitative polymerase chain reaction; NC, negative control.

staining indicated that miR-18a may significantly inhibit A2780cp intraperitoneal tumor growth by inhibiting proliferation and inducing apoptosis.

miR-18a is encoded by the miR-17-92a-1 cluster host gene (MIR17HG) gene, which is one of the most frequently deregulated genes in human cancer (7). MIR17HG was originally

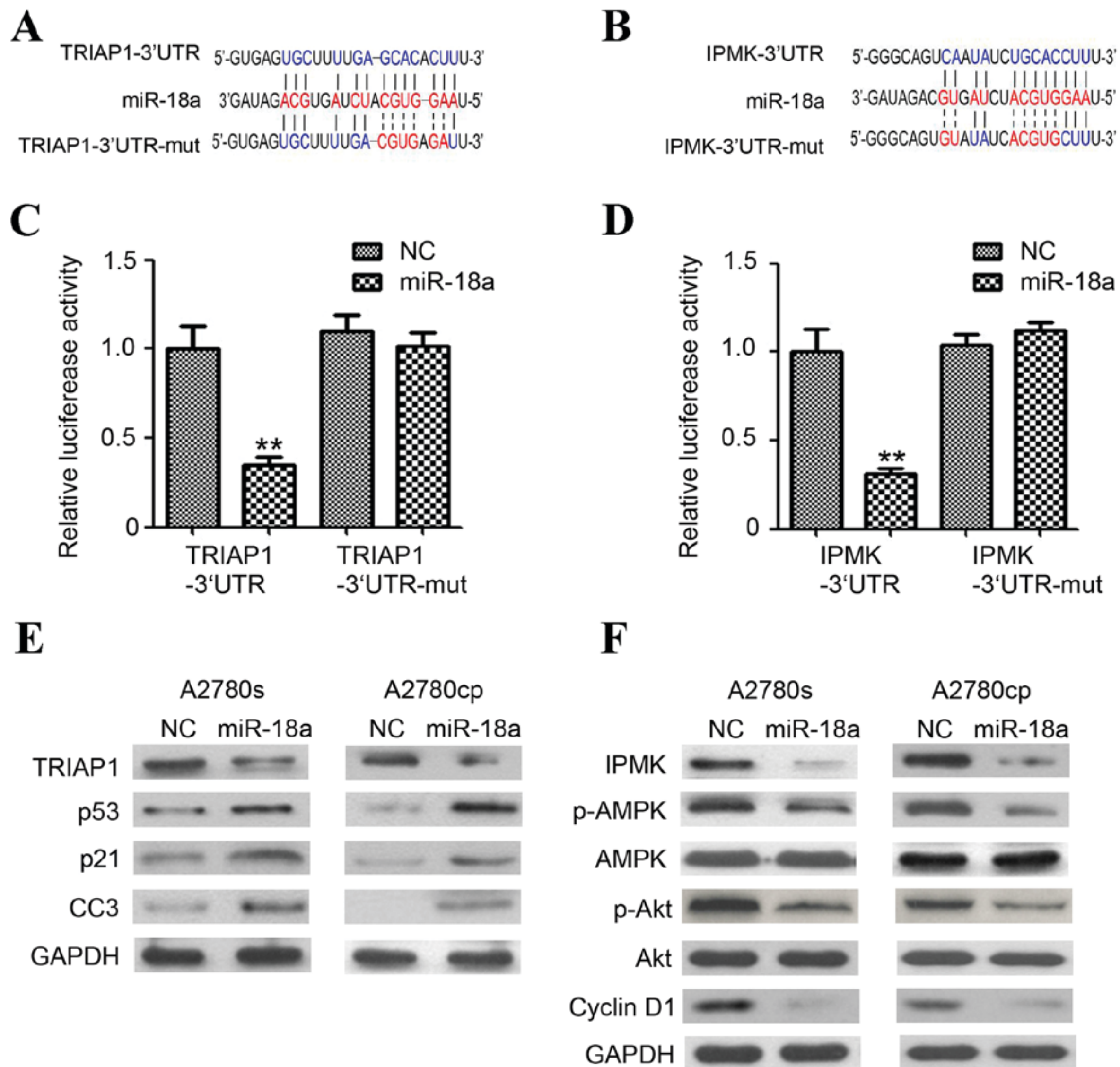

Figure 3. TRIAP1 and IPMK are the targets of miR-18a. (A) Predicted duplex formations between TRIAP1-3'UTR and miR-18a. TRIAP1-3'UTR contains a single predicted miR-18a binding site. (B) Predicted duplex formations between IPMK-3'UTR and miR-18a. IPMK-3'UTR contains one single predicted miR-18a binding site. Sites of target mutagenesis are indicated in red. (C and D) Relative luciferase reporter expression was normalized to NC. Data are depicted as mean ± standard error. **P<0.01 vs. lenti-NC group. (E) Western blot analysis of TRIAP1, p53, p21 and CC3 and GAPDH in A2780s and A780cp cells following infection with lenti-miR-18a and lenti-NC (F) Western blot analysis of IPMK, (p)-adenosine monophosphate (AMP)-activated protein kinase, p-Akt, Akt and cyclin D1 and GAPDH in A2780s and A780cp cells following infection with lenti-miR-18a and lenti-NC. CCS, cleaved caspase 3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IPMK, inositol phosphate multikinase; miR/miRNA, microRNA; NC, negative control; p-AMPK, phosphorylated-adenosine monophosphate-activated protein kinase; TRIAP1, tumor protein p53 (TP53)-regulated inhibitor of apoptosis gene 1; UTR, untranslated region.

identified as a tumor promoter in the heart, thyroid and lungs (18). However, emerging evidence suggests that the loss of MIR17HG may contribute to the development and progression of other types of cancer, thereby implicating a tumor suppressor function (19).

MIR17HG is able to inhibit the proliferation of luminal breast cancer cells by targeting a steroid receptor co-activator (nuclear receptor coactivator 3), cyclin D1 and estrogen receptor 1 (20-23). Previous studies have demonstrated that the tertiary structure of the folded primary-miR-17-92 transcript may contribute to less efficient processing of miR-18a (24,25). The miR-17-92 cluster has been reported to correlate with the poor prognosis of patients with a genetic etiology of ovarian cancer (26). Therefore, miR-18a may serve a homeostatic role in regulating the oncogenic effect of the entire miR-17-92 cluster. The present study demonstrated a lower expression of miR-18a in EOC patient tissues and human ovarian cancer cell lines. Functional study indicated that miR-18a may inhibit A2780s and A2780cp tumor growth *in vitro* and *in vivo* by inhibiting proliferation and inducing apoptosis.

p53 cell survival factor (TRIAP1; p53CSV) contains a p53 binding site within its coding sequence and is upregulated in multiple myeloma (27). TRIAP1 has been demonstrated to suppress apoptosis through interacting with cytoplasmic heat shock protein family A (Hsp70) member 1A and apoptotic peptidase activating factor 1 (18), and inhibiting the expression of p21 (28). Furthermore, TRIAP1 interact with PRELI domain-containing protein 1, mitochondrial (PRELI) to form

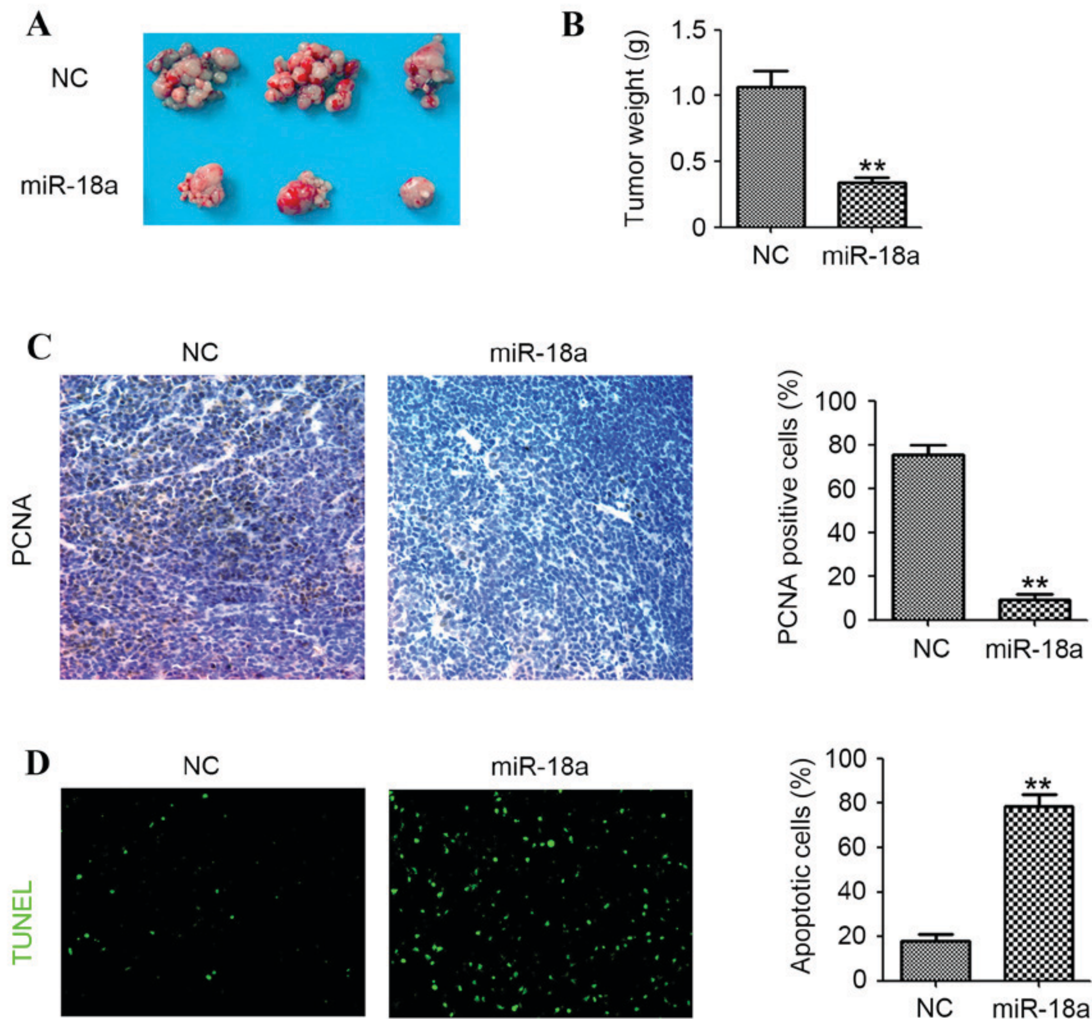

Figure 4. miR-18a inhibits A2780cp intraperitoneal tumor growth in mice. (A) Images of intraperitoneal tumors from mice injected with lenti-miR-18a and lenti-NC A2780cp cells. Following A2780cp cell injection for 30 days, mice were sacrificed and the intraperitoneal tumors were collected, and images were captured. Data are shown as (B) mean tumor weight of mice (n=6). (C) Immunohistochemical staining of PCNA and graph showing the percentage of PCNA-positive nuclei in mice injected with lenti-miR-18a compared with lenti-NC. (D) Detection of apoptosis by terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling assay. Apoptotic cells in each frame were analyzed. Magnification, x200. Data are shown as mean ± standard error **P<0.01 compared with the NC group. miR/miRNA, microRNA; NC, negative control; PCNA, proliferating cell nuclear antigen.

the TRIAP1/PRELI complex, which inhibits apoptosis by promoting cardiolipin accumulation and link p53-mediated cell survival to mitochondrial cardiolipin (29). In the present study, it was demonstrated that TRIAP1 was the direct target of miR-18a by luciferase assay. Additionally, western blotting results indicated that TRIAP1 expression was inhibited by miR-18a. The downstream targets of TRIAP1, including p53, p21 and CC3 also increased following the overexpression of miR-18a. These results provide evidence that miR-18a induces ovarian cancer apoptosis through directly targeting TRIAP1 and hence regulating apoptosis-related protein expression.

IPMK is a key regulatory enzyme in inositol phosphate disposition and is also a member of the inositol phosphokinase 6-kinase family of enzymes, but is not primarily associated with the formation of inositol pyrophosphates. IPMK has been shown to act as an activator of Akt, AMPK and the mechanistic target of rapamycin pathway (30-33). Several studies have suggested that IPMK inhibitors may be beneficial in treating obesity, diabetes mellitus and cell proliferation-associated diseases (31,33,34). In the present study, it was demonstrated that IPMK was the direct target of miR-18a, and the expression of IPMK was significantly inhibited by miR-18a in A2780cp and A2780s ovarian cancer cells. The downstream targets of IMPK, including p-Akt, p-AMPK and cyclin D1, were also regulated by miR-18a. These results demonstrated that miR-18a inhibits ovarian cancer cell proliferation by directly targeting IPMK and hence is able to regulate cell proliferation-associated protein expression.

Collectively, the present study examined the expression of miR-18a in EOC and the potential role of miR-18a in modulating ovarian cancer growth. miR-18a detection by qPCR indicated that miR-18a was reduced in EOC tissues and all ovarian cancer cell lines investigated. Functional study demonstrated the tumor suppressor role of miR-18a in ovarian cancer development. Further study of the mechanism indicated that miR-18a directly targets TRIAP1 and IPMK, hence inducing cell cycle arrest and apoptosis. The present study may provide a novel diagnostic method and be a therapeutic target for EOC.

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