

miR-183 modulated cell proliferation and apoptosis in ovarian cancer through the TGF- β /Smad4 signaling pathway

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Abstract. An increasing body of evidence has revealed that the aberrant expression of microRNAs (miRNAs/miRs) is involved in the development and progression of ovarian cancer (OC). miR-183 has been demonstrated to act as a tumor suppressor and oncogene in various types of human cancers. However, the biological role of miR-183 in OC still remains unclear. The aim of the present study was to investigate the role of miR-183 and evaluate its underlying mechanism in OC. In the present study, miR-183 was observed to be upregulated in OC tissues and cell lines as determined by reverse transcription-quantitative polymerase chain reaction. The effects of miR-183 on OC were further investigated via western blotting, MTT, wound healing, Transwell and immunofluorescence analyses. Downregulation of miR-183 markedly inhibited cell proliferation, migration and invasion, and promoted apoptosis in OC cells. Furthermore, it was initially confirmed that mothers against decapentaplegic homolog 4 (Smad4) was identified as an efficient target of miR-183 by luciferase activity assay. Finally, the results revealed that miR-183 directly regulated biological function via the transforming growth factor (TGF)- β /Smad4 signaling pathway in OC cells. In conclusion, the results of the present study suggested that miR-183 exerted tumor-promoting roles in OC, at least partially by regulating Smad4 via the TGF- β /Smad4 signaling pathway. Therefore, miR-183 may serve as a potential target for the diagnosis and prognosis of OC.

Introduction

Ovarian cancer (OC) is the most lethal malignant gynecological cancer that constitutes 4% of all cancers diagnosed in women worldwide (1,2). The majority of patients are diagnosed at an

advanced stage and the 5-year overall survival rate of patients with OC is <40% (3-6). Unfortunately, despite the current standard treatment of combining surgery with chemotherapy being efficient, the majority of patients ultimately develop recurrence (7-9). Therefore, clinical biomarkers and more efficient therapeutic targets are urgently required to treat OC as well as the elucidation of the molecular pathogenesis of OC.

MicroRNAs (miRNAs/miRs) are endogenous non-coding, single-stranded small regulatory RNA molecules (21-23 nucleotides) (10). A body of evidence has suggested that miRNA is an important regulatory factor of gene expression and miRNA dysregulation is frequently associated with biological processes, including cell proliferation (11), differentiation (12), angiogenesis (13,14), apoptosis (15) and the adhesion of tumor cells, including OC (16-19). miR-183 is located on chromosome 7q32 and has been reported to be overexpressed in the different types of tumors, including prostate cancer and breast cancer (20-22). In addition, a previous study has also reported that miR-183 was implicated in the regulatory mechanisms of tumor invasion and metastasis (23). However, the potential role of miR-183 in the pathogenesis of OC remains unclear. Based on these results, it was hypothesized that the aberrant expression of miR-183 may be an important mediator of cell growth, invasion and apoptosis.

Transforming growth factor- β (TGF- β) is a secreted homodimeric protein and the TGF- β signaling pathway serves pivotal roles in a variety of biological processes. It's essential biological and pathological activities are mediated by mothers against decapentaplegic homolog (Smad) signaling pathways (24). Smad4 is a central-mediator that serves complex and contradictory roles, and cooperates with other transcription factors to regulate the TGF- β signaling pathway during tumorigenesis (25). Accumulating data has reported that Smad4 is involved in various cellular responses and that dysregulation of Smad4 is closely associated with a variety of human cancers, including prostate, pancreatic, colorectal and thyroid cancer (26-29).

The aim of the present study was to investigate the association between miR-183 and the development and progression of OC. It was observed that miR-183 was markedly upregulated in the OC clinical samples and cell lines. In addition, functional assays revealed that downregulation of miR-183 could inhibit cell proliferation and invasion, and induce apoptosis in OC. In addition, it was demonstrated that Smad4 is a direct target

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of miR-183 via a luciferase activity assay. Taken together, the results of the present study suggested that miR-183 may serve an efficient regulatory role and thus, may be a novel strategy and prognostic marker for the diagnosis and prognosis of OC.

Materials and methods

Tissue samples and cell lines. Tissues were obtained from 30 female patients, who were histopathologically and clinically diagnosed at Zhongnan Hospital of Wuhan University (Hubei, China) between January 2016 and December 2017 and their average age was 57±12 years. The present study was approved by the Ethics Committee of Zhongnan Hospital of Wuhan University and every patient provided written informed consent. The tissues were collected prior to chemotherapy and radiotherapy, and all fresh specimens were stored at -80°C until the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay.

Human OC cell lines (SKOV-3 and OVCAR3) and normal human ovarian surface epithelial (HOSE) cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were grown in RPMI-1640 containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and then maintained at 37°C in a humidified incubator with 5% CO₂.

Cell transfection. miR-183 mimics, miR-183 inhibitors, negative control (NC) and the luciferase reporter plasmid were designed and synthesized by Invitrogen; Thermo Fisher Scientific, Inc. The sequences were as follows: miR-183 mimics forward, 5'-UGAGGUAGUAAGUUG UAUUGUU-3' and reverse, 5'-CAAUACAACUUAUA CCUCAUU-3'; and miR-183 inhibitors, 5'-TATGGCACT GGTAGAATTCAC-3'; NC forward, 5'-CAGUACUUU UGUGUAGUACAA-3' and reverse, 5'-UUGUACUACACA AAAGUACUG-3'. The SKOV3 and OVCAR3 cells were transfected with miR-183 mimics, miR-183 inhibitors or NC using Lipofectamine® 2000™ (Invitrogen; Thermo Fisher Scientific, Inc.) at a final concentration of 50 nM according to the manufacturer's protocol. Subsequently, cells were cultured with fresh medium containing 10% FBS for 48 h prior to further experiments.

RT-qPCR. Total RNA was extracted from 5.0×10³ cells and tissues using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and cDNA was synthesized using the SYBR® PrimeScript™ RT-PCR kit (Takara Biotechnology Co., Ltd., Dalian, China) at 37°C for 30 min according to the manufacturer's instructions. RT-qPCR was performed using the SYBR Premix ExTaq (Takara Bio, Inc., Otsu, Japan). U6 small RNA was used as an endogenous control. The thermocycling conditions for qPCR were as follows: 95°C for 5 min followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec. Fold changes were quantified using the 2^{-ΔΔC_q} method (30). Primers were as follows: miR183 forward, 5'-CGCGGTATGGCACTG GTAGA-3' and reverse, 5'-AGTGCAGGGTCCGAGGTA TTC-3'; TGF-β forward, 5'-GAAGTGGATCCACGAGCCC AAG-3' and reverse, 5'-GCTGCACTTGCAGGAGCGCAC-3'; Smad4 forward, 5'-GTCGAAACAACGCTATTGACT-3' and reverse, 5'-CAGAGAATTGTCGCCGTACAT-3'; β-actin

forward, 5'-TGACGGGGTACCCACACTGTGCCCCAT CTA-3' and reverse, 5'-CTAGAAGCATTTGCGGTGGAC GATGGAGGG-3'.

Western blot analysis. Total protein was extracted from cells using 3 ml of lysis buffer comprised of well-mixed solution containing 7 mol/l urea, 2 mol/l thiourea, 5 ml/l isocratic pH gradient buffer (pH 3–10), 65 mmol/l dithiothreitol, 40 g/l 3-(3-cholamidopropyl) dimethylammonio]-1-propanesulfo- nate, 5 mg/l protease inhibitor and 10 ml/l trypsin inhibitor, and homogenized on ice. The protein concentration was measured using the BCA method and total protein (20 μg) was separated by 10% SDS-PAGE. Proteins were then transferred to poly- vinylidene difluoride membranes and blocked with 5% skim milk in TBST solution containing 5% bovine serum albumin (Gibco; Thermo Fisher Scientific, Inc.) and 0.1% Tween-20, at room temperature for 1 h. The membranes were probed with primary antibodies at room temperature overnight. The specific primary antibodies were as follows: Smad4 (cat. no. ab40759; 1:1,000 dilution; Abcam, Cambridge, MA, USA), TGF-β (cat. no. ab64715; 1:1,000 dilution; Abcam), p21 (cat. no. ab109520; 1:1,000 dilution; Abcam), p27 (cat. no. ab32034; 1:1,000 dilution; Abcam), Cyclin D1 (cat. no. ab16663; 1:1,000 dilution; Abcam) and GAPDH (cat. no. ab9485; 1:1,000 dilution; Abcam). Then the membranes were incubated with the horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody (cat. no. ab6721; 1:2,000; Abcam) for 1 h at room temperature. GAPDH was used as the loading control. Protein bands were visualized with the Amersham Enhanced Chemiluminescence system (Amersham; GE Healthcare, Chicago, IL, USA) and the results were measured using ImageJ software 1.48 (National Institutes of Health, Bethesda, MD, USA).

Measurement of cell viability by MTT. Cell proliferation was assessed using an MTT assay. Briefly, cells were seeded into 96-well plates at 5.0×10³ cells/well in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and incubated at 37°C in 5% CO₂. Cell proliferation was measured using an MTT assay kit (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and dimethyl sulfoxide used to dissolve the purple formazan. The optical density of cells was determined at 490 nm using a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).

Migration and invasion assays. Cell migration was assessed using a 24-well Transwell chamber with a non-coated membrane in the top chamber and 5×10⁴ cells were plated in RPMI-1640 medium in the top chamber of inserts. For the Transwell invasion assay, 5×10⁴ cells were plated in the top chamber of inserts that were coated with Matrigel (BD Biosciences, San Jose, CA, USA). Subsequently, the lower chambers were filled with RPMI-1640 medium supplemented with 10% FBS. Following incubation for 24 h, the non-migrating and non-invading cells on the upper membrane surface were removed using a cotton swab. The cells in the lower membrane surface were washed with PBS three times, fixed with 4% paraformaldehyde at room temperature for 30 min and stained with 0.5% crystal violet for 4 h at room temperature. The cells were counted under a

light microscope in five random fields (magnification, x200). Each experiment was performed in triplicate.

Wound healing assay. A wound healing assay was used to evaluate the migratory ability of the transfected cells. A total of 5×10^4 cells were seeded in 6-well plates until the cells grew to 100% confluence. The scratch wound was created in the surface of the plates using a pipette tip. The cells were washed with PBS and replaced with DMEM. Cells were then observed at 0, 24 and 48 h under a microscope.

Colony formation assay. Following transfection for 48 h, SKOV3 and OVCAR3 cells were seeded in 6-well plate with complete growth medium and incubated for 2 weeks at 37°C in a humidified incubator with 5% CO₂. Following this incubation period, the cells were washed with PBS, fixed with 4% para-formaldehyde at room temperature for 30 min and stained with 0.5% crystal violet for 4 h at room temperature. Cell colonies were counted and photographed using a light microscope. The experiment was repeated in triplicate.

Flow cytometry analysis. A total of 5×10^4 cells were seeded in 6-well plates, cultured for 48 h and then cells were collected. Then the cells were fixed with precooled 70% ethanol for 30 min at room temperature, and collected following centrifugation at 12,000 x g for 5 min at room temperature. Next, the cells were resuspended in PBS containing 50 mg/ml propidium iodide and 50 mg/ml RNaseA (cat. no. 40711ES10; Shanghai Yeasen Biotechnology, Co., Ltd., Shanghai, China) for 30 min. Then cells were incubated for 1 h at 37°C in the dark, and analyzed using flow cytometry (FACSCalibur; BD Biosciences) and analyzed using FlowJo 10.06 software (FlowJo LLC, Ashland, OR, USA). The experiment was repeated three times.

Immunofluorescence analyses. A total of 1×10^5 cells were plated onto coverslips in 6-well plates and transfected with miR-183 mimics or miR-183 inhibitors. Following transfection for 48 h, the coverslips were fixed with 4% formaldehyde for 24 h at room temperature and stained with the primary antibodies overnight at 4°C after blocking cells with 3% bovine serum albumin for 20 min at room temperature (Gibco; Thermo Fisher Scientific, Inc.). The specific primary antibodies were as follows: Smad4 (cat. no. ab40759; 1:1,000 dilution) and TGF- β (cat. no. ab64715; 1:1,000 dilution; both Abcam). Then the coverslips were incubated with the HRP-conjugated anti-rabbit secondary antibody (cat. no. ab205718; 1:2,000; Abcam) for 1 h at room temperature in the dark. Coverslips were counterstained with 4',6-diamidino-2-phenylindole (Molecular Probes; Thermo Fisher Scientific, Inc.) for 20 min at room temperature for the visualization of nuclei. The results were observed using a fluorescence microscope (Leica Microsystems GmbH, Wetzlar, Germany) and analyzed using ImageJ software version 1.48 (National Institutes of Health).

Luciferase reporter assays. A search for putative targets of mi-183 was performed with TargetScan Human 7.2 (www.targetscan.org/vert_72/) and miRanda software (www.microrna.org/). Cells (1×10^5) were seeded in 96-well plates and grown in RPMI-1640 containing 10% FBS at 37°C in a

humidified atmosphere containing 5% CO₂. Following 24 h, psiCheck-2 with the 3'-untranslated region (UTR) of Smad4 was cotransfected with miR-183 mimics wild-type; (WT)], or mutated miR-183 mutant; (MUT). mimics using Lipofectamine 3000™ (Invitrogen; Thermo Fisher Scientific, Inc.). Following transfection for 48 h, luciferase activity was detected via a luciferase assay using a Dual Luciferase Reporter Assay kit (Promega Corporation, Madison, WI, USA). Normalized luciferase activity was reported as luciferase activity/*Renilla* luciferase activity. Three independent experiments were performed.

Statistical analysis. All data in the study were assessed using SPSS 18.0 statistical software (SPSS, Inc., Chicago, IL, USA). Comparisons between groups for statistical significance were performed with Student's t-test and multiple group comparisons were conducted via one-way analysis of variance with Tukey's post hoc test. Data are expressed as the mean \pm standard deviation. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

miR-183 is upregulated in OC tissues and cell lines. To investigate whether miR-183 is associated with the progression of OC, the present study determined the expression levels of miR-183 in OC tissues and cell lines by RT-qPCR. The results revealed that the expression of miR-183 was increased in the OC tissues when compared with the normal tissues (Fig. 1A). In addition, SKOV3 and OVCAR3 cells were also investigated and the results indicated that the miR-183 expression levels were markedly higher in OC cell lines than in the HOSE cell line (Fig. 1B). The present study also assessed the levels of Smad4 in cell lines using RT-qPCR, western blotting and an immunofluorescence assay. The data implied that Smad4 expression in the OC cell lines was markedly lower when compared with HOSE cells (Fig. 1C-E). The colony formation and Transwell assays were conducted to assess cell proliferation, migration and invasion abilities, the number of colonies formed, and the number of migrating and invading cells in each group (Fig. 2A-C). These results indicated that all of these measures were significantly increased in OC cell lines when compared with HOSE cells.

Effects of miR-183 on OC cell proliferation. The present induced overexpression of miR-183 and anti-miR-183 via transfection with lentivirus in SKOV3 and OVCAR3 cells to explore the biological functions of miR-183 in OC. The success of transfection was validated by fluorescence microscopy and RT-qPCR (Fig. 3A and B). The MTT and colony formation assays were conducted to investigate the effects of miR-183 on cell proliferation. The results suggested that overexpression of miR-183 markedly increased the growth rate of SKOV3 and OVCAR3 cells (Fig. 3C). Increased and decreased colony formation was observed in the miR-183 mimics and miR-183 inhibitors groups, respectively, when compared with the control group (Fig. 3D). These results indicated that miR-183 may be involved in the regulation of OC cell growth.

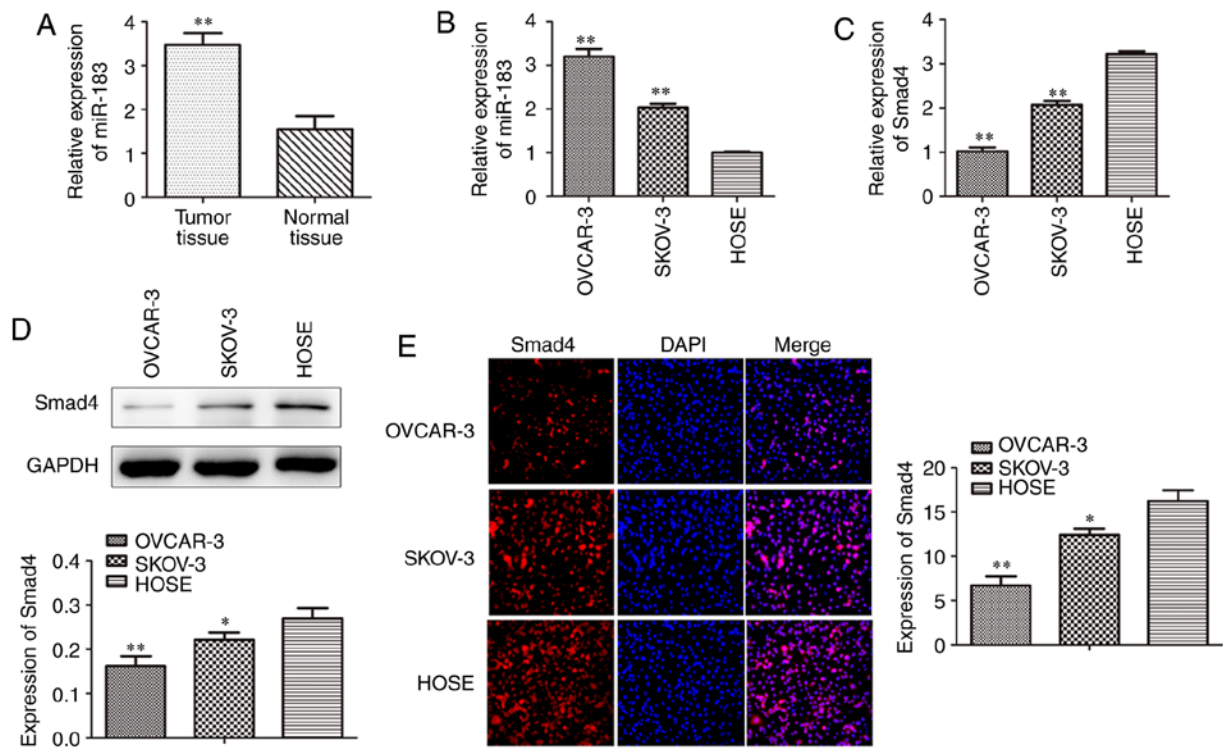


Figure 1. miR-183 was upregulated in OC tissues and cell lines. (A) miR-183 expression in OC tissues and paired normal tissues was examined by RT-qPCR. **P<0.01 vs. normal tissues group. (B) miR-183 and (C) Smad4 expressions in OC cell lines and a human epithelial cell line were examined by RT-qPCR. (D) Western blotting and (E) immunofluorescence analysis were used to detect Smad4 expression (magnification, x200). The results are expressed as the mean \pm standard deviation of three independent experiments and each was performed in triplicate. *P<0.05 and **P<0.01 vs. HOSE group. miR, microRNA; OC, ovarian cancer; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; DAPI, 4',6-diamidino-2-phenylindole; Smad4, mothers against decapentaplegic homolog 4.

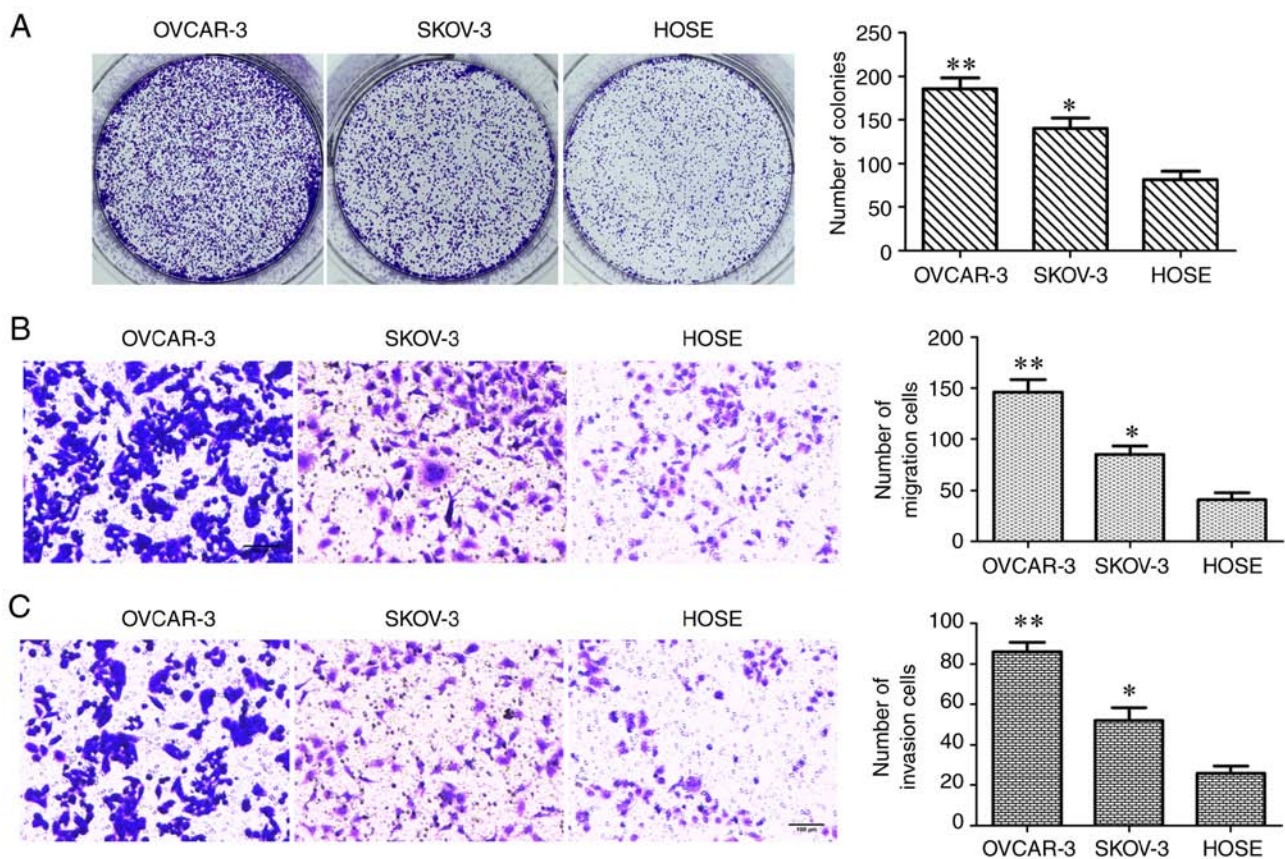


Figure 2. Cell proliferation, migration and invasion abilities. The proliferation of cells was determined by (A) a colony formation assay. (B and C) Transwell assays were also conducted to analyze cell (B) migration and (C) invasion (magnification, x200). *P<0.05 and **P<0.01 vs. HOSE cells.

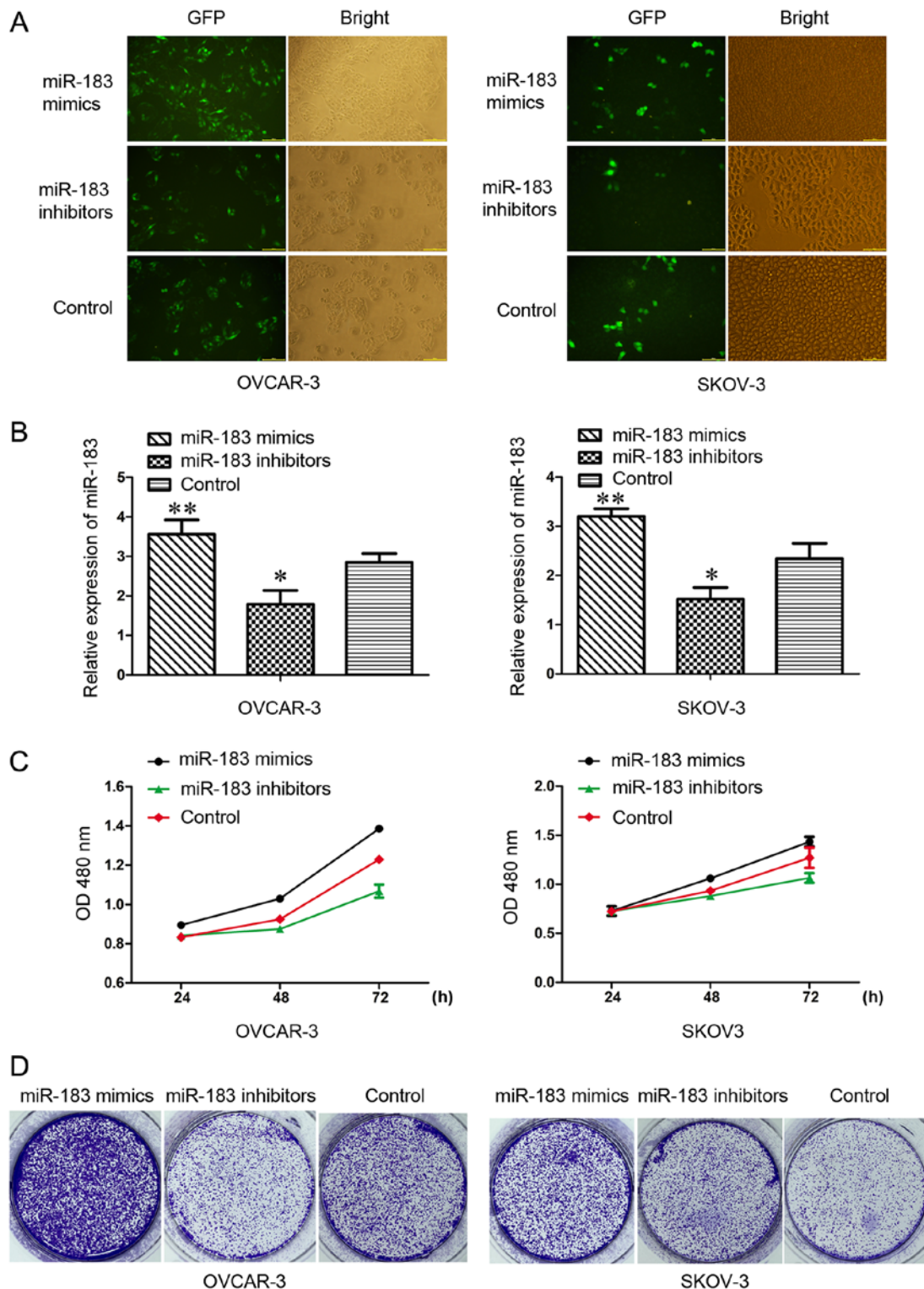


Figure 3. Effect of miR-183 on OC cell proliferation. (A and B) Transfection efficiency was determined by (A) fluorescence microscopy following transduction with recombinant lentivirus and (B) reverse transcription-quantitative polymerase chain reaction (magnification, x200). (C) The proliferation of SKOV3 and OVCAR3 cells was assessed by MTT assay. (D) Crystal violet-stained colonies from the colony formation assay for each group. Data are presented as the mean \pm standard deviation. * $P < 0.05$ and ** $P < 0.01$ vs. control. miR, microRNA; OC, ovarian cancer; GFP, green fluorescent protein; OD, optical density.

miR-183 mediates the cell cycle and apoptosis in OC cells. To determine whether miR-183 is able to influence the cell cycle, the present study performed a flow cytometry assay and observed that the downregulation of miR-183 could markedly arrest a greater number of cells in the S phase when compared with the control group, whereas the cells

in the G1 phase were decreased. Furthermore, the number of cells in the S phase were reduced when transfected with miR-183 mimics when compared with the control group ($P < 0.05$; Fig. 4A) and the number of cells in the G1 phase was increased when transfected with miR-183 mimics when compared with the control group ($P < 0.01$). The

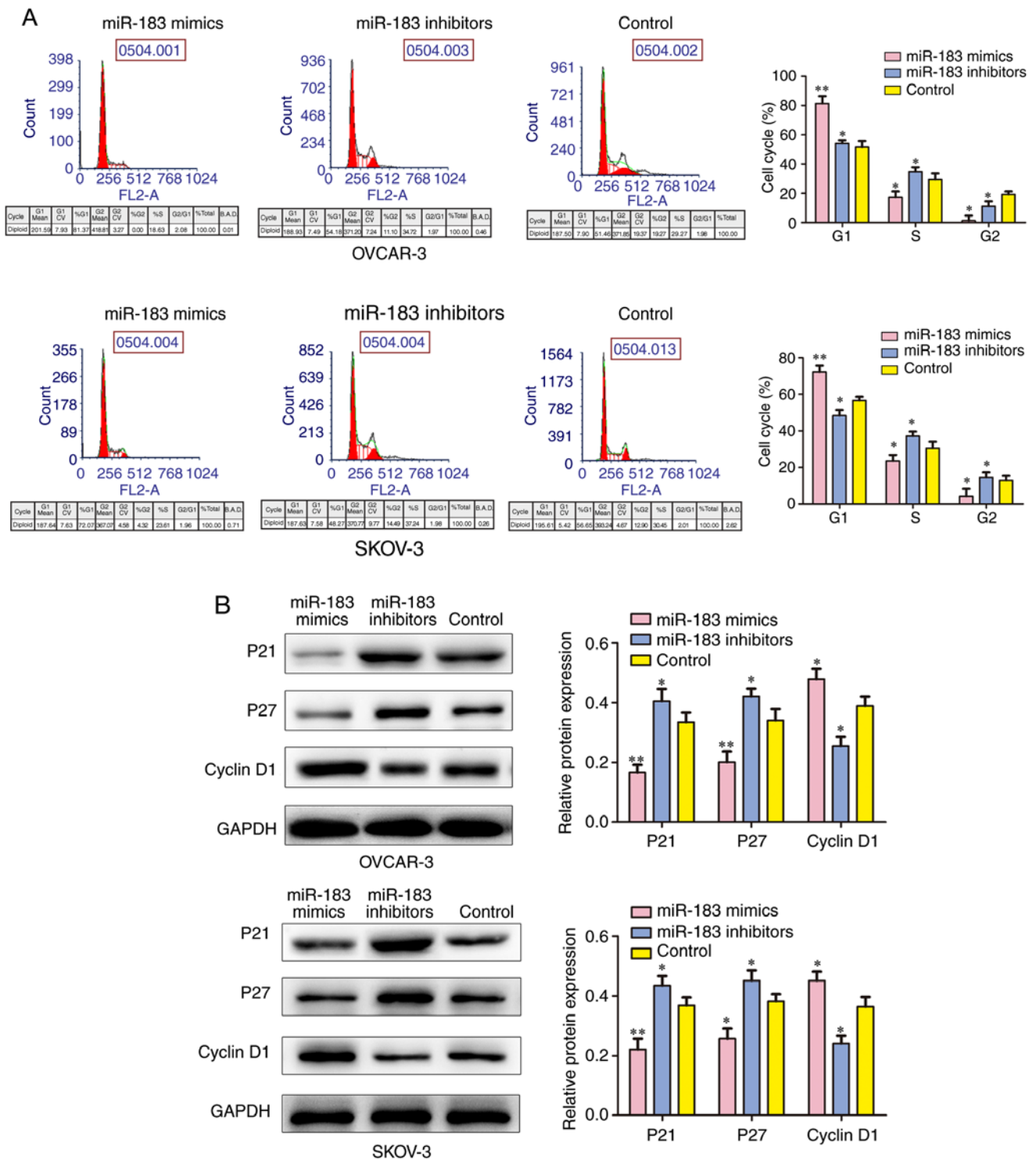


Figure 4. Overexpression of miR-183 regulates the cell cycle. (A) Histograms presenting the percentages of OC cells in the G1, S and G2 phases following miR-183 transfection. (B) Protein expression of cell cycle-associated markers were detected by western blot assay. Data are presented as the mean \pm standard deviation. * $P < 0.05$ and ** $P < 0.01$ vs. control. miR, microRNA; OC, ovarian cancer.

western blotting assay was conducted to detect several cell cycle-associated factors. The expressions of p21 and p27 protein were revealed to be reduced by miR-183 mimics when compared with the control group. By contrast, the expression of Cyclin D1 was reduced in the miR-183 inhibitors group when compared with the control group (Fig. 4B). In order to further investigate the influence of miR-183 expression on cell apoptosis, the percentage of apoptotic

cells was measured by flow cytometry. The results indicated that the percentage of apoptosis was significantly increased in the cells transfected with miR-183 inhibitors. In addition, overexpression of miR-183 markedly decreased the number of apoptotic cells when compared with the control group ($P < 0.01$; Fig. 5A). A western blot assay was employed to detect the protein expression of apoptosis-associated factors. The results demonstrated that downregulation

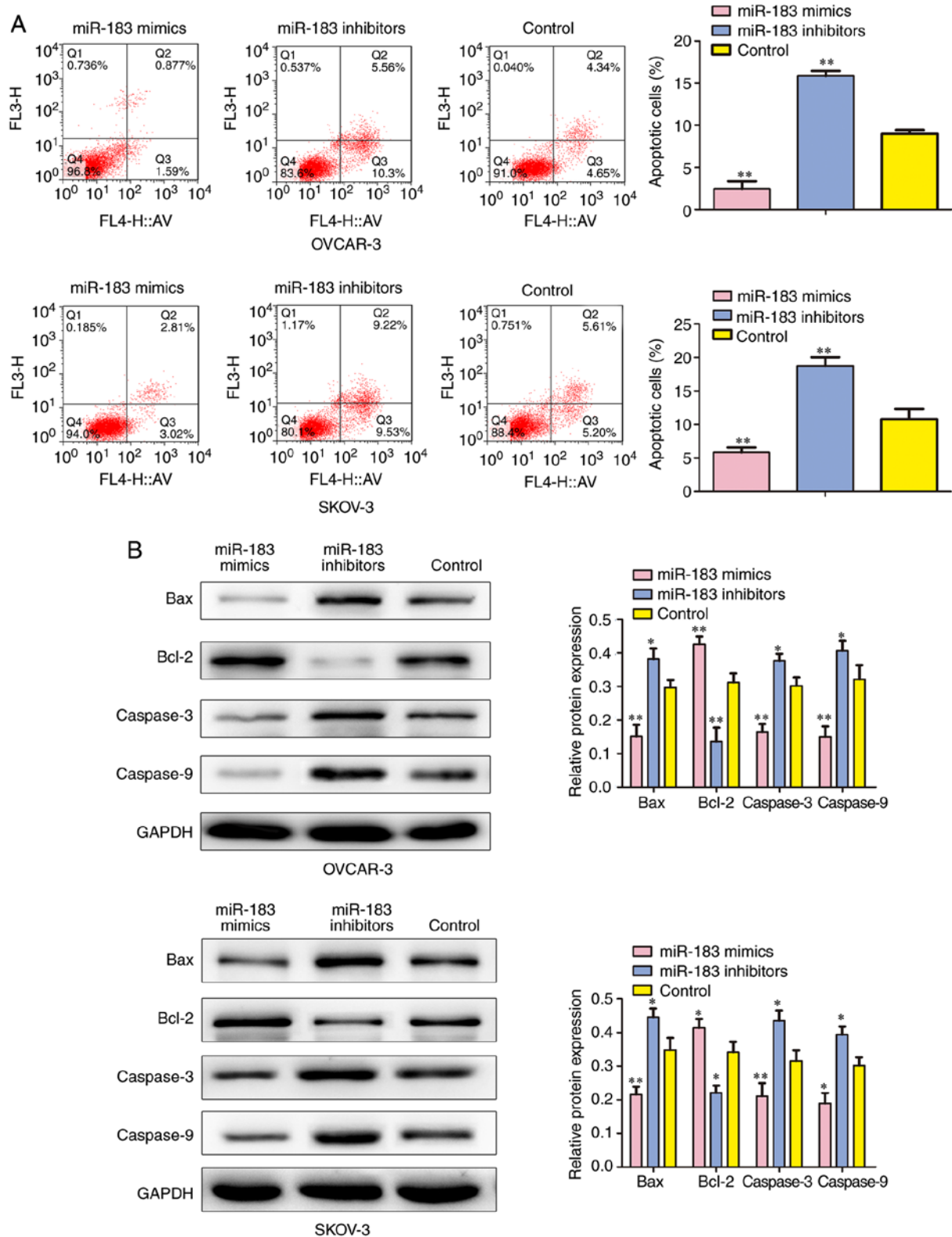


Figure 5. Effect of miR-183 on cell apoptosis. (A) SKOV3 and OVCAR3 cell apoptosis was examined by flow cytometry analysis. (B) Expression of apoptosis-associated markers were detected by western blot assay. Data are presented as the mean \pm standard error of the mean of three independent experiments. * P <0.05 and ** P <0.01 vs. control. miR, microRNA; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein.

of miR-183 markedly suppressed the expression of B-cell lymphoma 2 (Bcl-2), and induced Bcl-2-associated X protein (Bax), Caspase-3 and Caspase-9 levels (Fig. 5B). Therefore, these results demonstrated that miR-183 could induce cell cycle progression and suppress the apoptosis of OC cells.

miR-183 promotes cell migration and invasion. To explore the role of miR-183 in the migration and invasion of OC cells, wound healing and Transwell invasion assays were performed to investigate the migration and invasion abilities of SKOV3 and OVCAR3 cells following transfection with miR-183 mimics

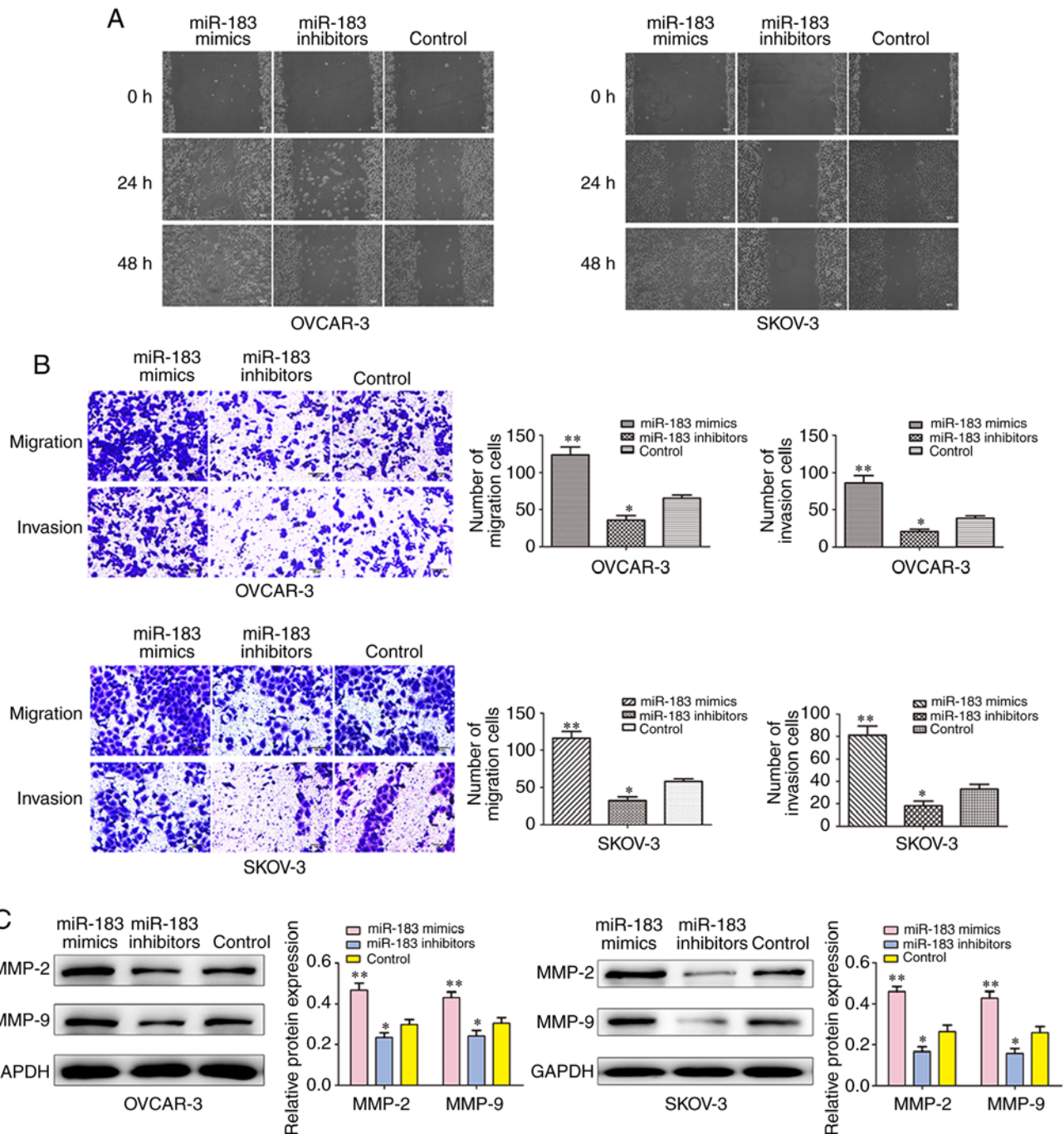


Figure 6. miR-183 enhances cell migration and invasion in OC. (A and B) Cell migration and invasion abilities were determined using a (A) wound healing assay and (B) Transwell assay (magnification, x200). (C) The relative protein expression was detected by western blot assay. Data are presented as the mean \pm standard deviation. * $P < 0.05$ and ** $P < 0.01$ vs. control. miR, microRNA; MMP, matrix metalloproteinase; OC, ovarian cancer.

and inhibitors. The wound healing assay revealed that the cells in the control group migrated less so than the miR-183 mimics group but more so than the miR-183 inhibitor group (Fig. 6A). As shown in Fig. 6B, the cells that were transfected with the miR-183 mimics had promoted migratory and invasive abilities when compared with the cells in the control group. The results of the Transwell assay indicated that overexpression of miR-183 lead to significant enhancements in cell migration. In addition, western blot analysis was performed to determine whether the protein levels of invasion-associated markers were also affected by miR-183. Overexpression of miR-183 significantly increased the expressions of matrix metalloproteinase

(MMP)-2 and MMP-9 (Fig. 6C). These results indicated that the downregulated expression of miR-183 inhibited the cell migration and invasion of OC cells.

miR-183 directly regulates biological function via the TGF- β /Smad4 signaling pathway in OC cells. To determine the underlying mechanism of miR-183 in OC, the present searched for the potential targets of miR-183 by using prediction programs including TargetScan and miRanda. As shown in Fig. 7A, Smad4 3'-UTR was predicted to be a potential target of miR-183. To determine whether Smad4 is a direct target of miR-183, luciferase reporter constructs were combined with

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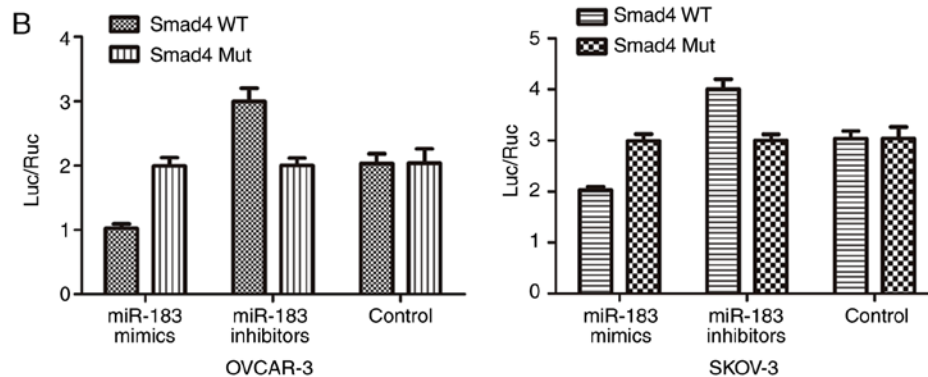
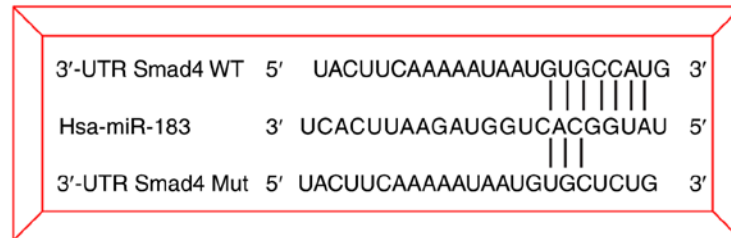
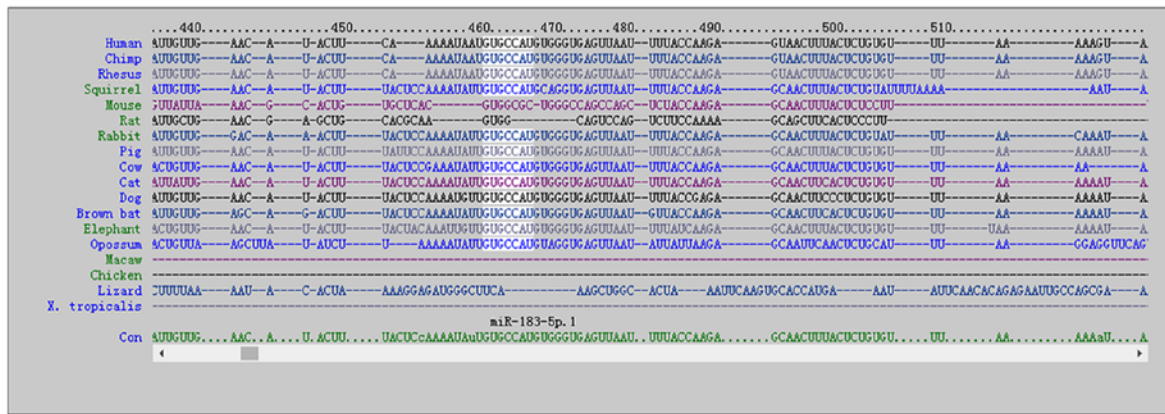


Figure 7. SMAD4 is a direct target gene of miR-183. (A) The predicted binding sites of miR-183 on SMAD4 mRNA are presented. (B) The luciferase activity was measured following 48 h. Data are the presented as the mean \pm standard deviation of three separate experiments. Smad4, mothers against decapentaplegic homolog 4; miR, microRNA; WT, wild-type; Mut, mutant; 3'UTR, 3'-untranslated region; Luc/Ruc, luciferase activity/*Renilla* luciferase activity.

the 3'-UTR of Smad4 mRNA containing the miR-183 binding sites and the mutant. The results of the luciferase reporter assay indicated that the luciferase activity in the Smad4 3'-UTR WT group that was transfected with miR-183 mimics, was markedly decreased when compared with the control group (Fig. 7B). However, this effect was abolished among the three groups cotransfected with the Smad4-Mut vector. These results were further supported via assessment of the protein levels of SMAD4 in the OC cell lines treated with miR-183 mimics and miR-183 inhibitors (Fig. 8B).

Additionally, the expressions of TGF- β and SMAD4 were detected by RT-qPCR, western blotting and immunofluorescence analyses following transfection. The results demonstrated that the mRNA and protein expressions of TGF- β and SMAD4 were significantly suppressed in cells that were transfected with miR-183 mimics when compared with the control group, and cells transfected with miR-183 inhibitors had significantly upregulated TGF- β and SMAD4 expressions in SKOV3 and OVCAR3 cells (Figs. 8 and 9). All of these results demonstrated that SMAD4 may be a direct target of miR-183 and suggest that miR-183 may participate

in the regulation of cell biological function, at least in part through the TGF- β /Smad4 signaling pathway in OC.

Discussion

An increasing body of evidence has suggested that miRNAs are important regulators in different cellular processes (31) and are frequently dysregulated in various types of cancer, including OC (32). miR-183 was overexpressed and acted as an oncogene in several types of cancers, including gastric, bladder and colon cancers (33-35). Ren *et al* (36) identified that miR-183 was markedly overexpressed in OSCC. In addition, Li *et al* (33) reported that miR-183 was markedly upregulated in gastric cancer. This may suggest that miR-183 is involved in the pathogenesis of OC and that it may be a biomarker for the diagnosis and treatment of OC. Therefore, the present study was conducted to further investigate the functional role of miR-183 in OC. The results revealed that miR-183 was upregulated in OC tissues and cell lines. Furthermore, SKOV3 and OVCAR3 were used as an *in vitro* model to examine the functional impact of miR-183 inhibition or overexpression on cell behavior.

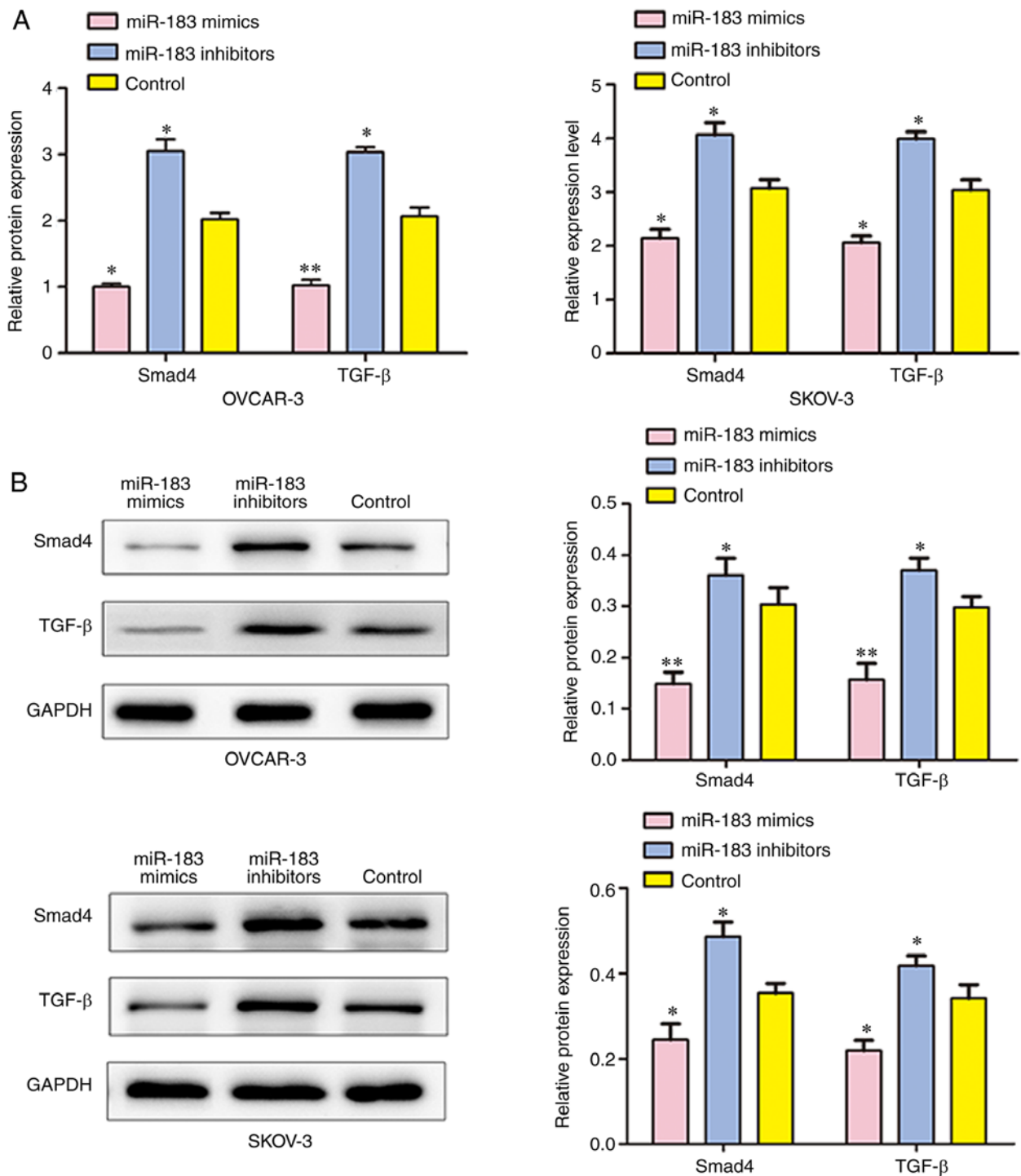


Figure 8. miR-183 regulates biological function via the TGF- β /Smad4 signaling pathway as determined by RT-qPCR and western blotting. (A) The expression of TGF- β and SMAD4 mRNA was measured by RT-qPCR. (B) The protein expression of TGF- β and SMAD4 was detected by western blot analysis. Data are the presented as the mean \pm standard deviation. * $P < 0.05$ and ** $P < 0.01$ vs. control. miR, microRNA; TGF- β , transforming growth factor- β ; Smad4, mothers against decapentaplegic homolog 4; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

The results revealed that downregulation of miR-183 suppressed proliferation, migration and invasion, and promoted cell apoptosis in SKOV3 and OVCAR3 cells, while overexpression of miR-183 enhanced cell growth, migration and invasion. Cell cycle analysis demonstrated that downregulation of miR-183 could markedly arrest more cells in the S phase when compared with the control group. Furthermore, when compared with the control group, the number of cells

in the S phase was reduced when cells were transfected with miR-183 mimics. In addition, a luciferase reporter assay was performed and the results revealed that SMAD4 was a novel target gene of miR-183. These results were further supported by the protein expression analyses of SMAD4 in the OC cell lines treated with miR-183 mimics. SMAD4 was demonstrated to be the key mediator of the TGF- β signaling pathway (37,38). In addition, the miR-183 level was inversely

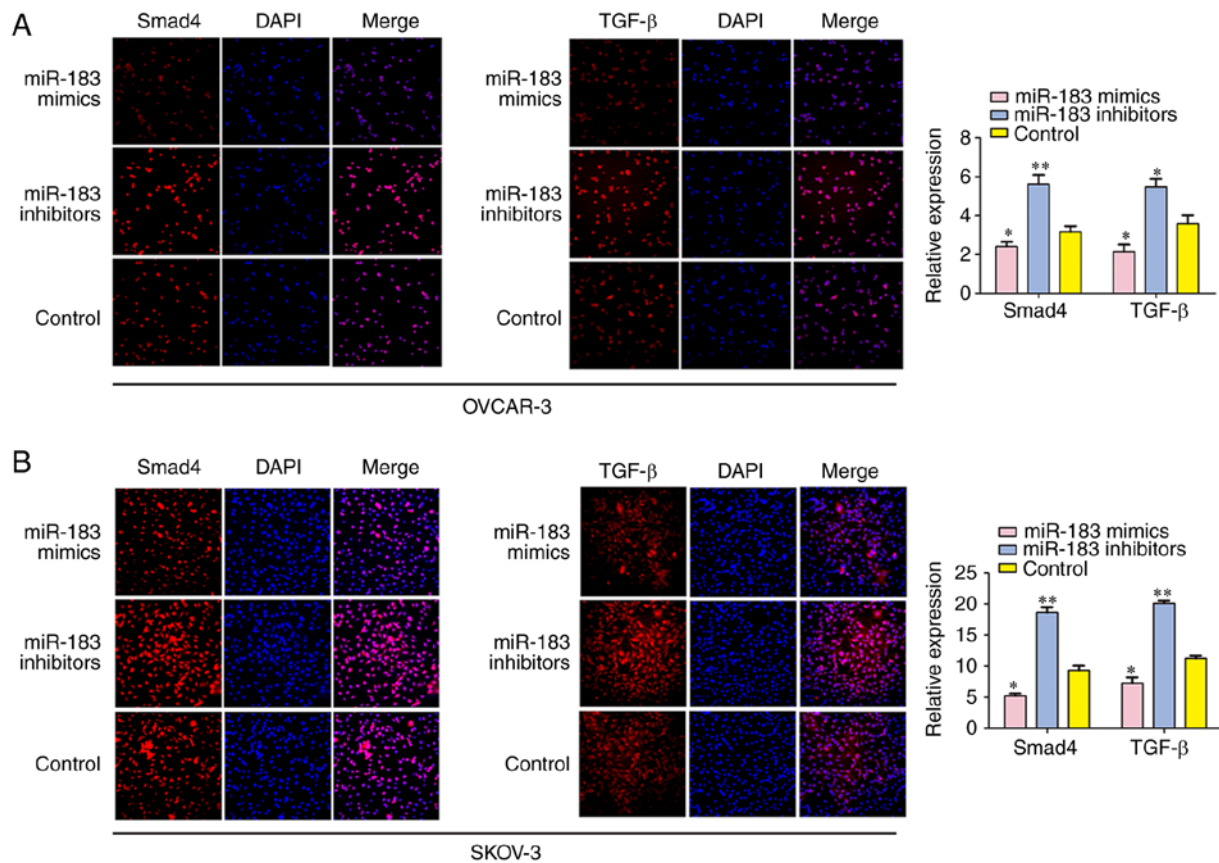


Figure 9. miR-183 regulates biological function via the TGF- β /Smad4 pathway as determined by immunofluorescence assay. (A and B) The protein expression of TGF- β and SMAD4 were detected by immunofluorescence assay in (A) OVCAR-3 and (B) SKOV-3 OC cells (magnification, $\times 200$). Data are the presented as the mean \pm standard deviation. * $P < 0.05$ and ** $P < 0.01$ vs. control. miR, microRNA; TGF- β , transforming growth factor- β ; Smad4, mothers against decapentaplegic homolog 4; DAPI, 4',6-diamidino-2-phenylindole.

correlated with the expression of Smad4 (39). Smad4 was also confirmed to be the pivotal mediator of the TGF- β signaling pathway and recent evidence has suggested that SMAD4 has dual roles with tumor-suppressive and tumor-promoting effects in different types of cancers (40-46). Accordingly, the present results revealed that miR-183 overexpression significantly downregulated Smad4 and activation of the TGF- β /Smad4 signaling pathway.

In conclusion, to the best of our knowledge, the present study for the first time, demonstrated that miR-183 was increased in OC tissues and cells. Notably, the results revealed that miR-183 serves an oncogene-like function by regulating the TGF- β /Smad4 signaling pathway, thereby promoting cell proliferation, migration and invasion in OC. Taken together, the present results provide insight into the potential contribution of miR-183 in the progression of OC, and downregulation of miR-183 and activation of Smad4 could be a promising approach for the diagnosis and treatment of OC.

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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

JZ and DJ conceived and designed the study. CZ and BZ performed the experiments. JZ wrote the manuscript. All authors have read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy and integrity of any part of the study are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Zhongnan Hospital of Wuhan University and every patient provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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