miR-638 suppresses proliferation by negatively regulating high mobility group A1 in ovarian cancer cells

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Abstract. Ovarian cancer is one of the most common gynecological diseases with high mortality rates. Previous studies have shown that microRNA (miR)-638 is associated with tumorigenesis. The present study aimed to assess the role and underlying mechanisms of miR-638 in ovarian cancer. miR-638 expression was detected in ovarian cancer tissues and miR-638 was overexpressed or knocked down in ovarian cancer OVCAR-3 and Caov-3 cells. The clinical results revealed that miR-638 expression was downregulated in ovarian cancer tissues compared with in adjacent normal tissues. miR-638 expression was also found to be relatively low in OVCAR-3 cells whilst being relatively high in Caov-3 cells among the five ovarian cancer cell lines tested. miR-638 overexpression inhibited cell viability, arrested the cell cycle at the G_1 phase and promoted apoptosis in OVCAR-3 cells. By contrast, miR-638 knockdown increased Caov-3 cell viability, facilitated cell cycle progression and inhibited apoptosis. miR-638 reduced the expression of high mobility group A1 (HMGA1) by directly targeting its 3' untranslated region. HMGA1 overexpression reversed the inhibition of proliferation induced by miR-638 overexpression in OVCAR-3 cells. These results suggest that miR-638 may serve to be a suppressor of ovarian cancer by regulating HMGA1, which may provide a potential therapeutic target for ovarian cancer.

Introduction

Ovarian cancer is the second most common gynecological malignancy after uterine corpus cancer and it is the eighth most common cause of cancer-associated death globally, with a 5-year survival rate <45% (1,2). However, mortality from ovarian cancer is the highest among all gynecological malignancies (1). Due to the lack of early-stage symptoms and reliable diagnostic methods, it remains difficult to detect the occurrence of ovarian cancer at the early stage (3). Frequently, when the patient exhibits typical symptoms of ovarian cancer, metastasis has already occurred, such that >70% patients are diagnosed at the late stage (4). The main therapeutic method for ovarian cancer includes surgery and adjuvant treatment for chemotherapy, but these treatment strategies can also be combined with radiotherapy and biological therapy (5). Although ovarian cancer can be completely curable after initial surgery and chemotherapy, the majority of patients with advanced disease tend to suffer tumor recurrence, where the 10-year survival rate for patients with ovarian cancer at stages III and IV is <30% (6). Therefore, identification of additional early detection markers that are also sensitive is crucial for the early diagnosis and treatment of ovarian cancer.

MicroRNAs (miRNAs or miRs) are non-coding RNA molecules that are 17-27 nucleotides in length and serve a regulatory role in various life processes (7). Numerous studies have shown that miR-638 serves as a suppressive factor in various malignant tumors, including gastric, breast and cervical cancer, where its expression is reduced (8-10). However, in other malignant tumors, such as esophageal squamous cell carcinoma and melanoma, it is abundantly expressed and functions as a promoter of malignant physiology (11,12). The role of miR-638 in ovarian cancer cells remain poorly understood. Bafilomycin A1 treatment was able to inhibit the proliferation of the ovarian cancer cell line HO-8910, which was accompanied by miR-638 upregulation (13), suggesting that increased miR-638 levels may inhibit the proliferation of ovarian cancer cells.

High mobility group A1 (HMGA1) is a chromosomal binding protein that is involved in various cell processes by regulating gene transcription (14). Recent evidence suggested that HMGA1 protein is expressed at high levels during embryonic development, which is then reduced to markedly low levels or not detected after aging (15). In 1983, the abnormal expression of HMGA1 was first identified in aggressive cervical cancer cells, following which the role of HMGA1 in malignant cancer became gradually elucidated (16), including

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in breast, gastric and thyroid cancer (17-20). Furthermore, HMGA1 is also known to be highly expressed in ovarian cancer tissues (21), where downregulation of HMGA1 can inhibit the proliferation of ovarian cancer cells *in vitro* and tumor formation *in vivo* (22). Therefore, it was hypothesized that miR-638 may be involved in the regulation of malignancy in ovarian cancer cells by regulating HMGA1.

The present study aimed to explore the effects of miR-638 on ovarian cancer cell proliferation, cell cycle and apoptosis, in addition to investigating the possible underlying mechanism. The results may facilitate the early detection and treatment of ovarian cancer, thereby helping to reduce ovarian cancer-related mortality.

Materials and methods

Clinical ovarian cancer sample collection. A total of 30 paired ovarian cancer tissues and adjacent normal tissues (2 cm from ovarian cancer tissues) were collected from female patients with ovarian cancer (mean age, 41.5±4.9 years) following excision of the tumor. The cancer tissues were collected in the Second Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China) between February 2018 and February 2019 with informed consent signed by the patients. All procedures were performed in accordance with the principles outlined in the Declaration of Helsinki. All the protocols were approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University.

Cell culture. The ovarian cancer cell lines A2780, ES-2, OVCAR-3 and Caov-3 were purchased from Procell Life Science & Technology Co., Ltd. SKOV-3 and 293T cells were obtained from Shanghai Zhongqiaoxinzhou Biotechnology Co., Ltd. A2780, Caov-3 and 293T cells were cultured in DMEM (HyClone; Cytiva) supplemented with 10% FBS (Biological Industries). ES-2 cells were cultured in McCoy's 5A medium (Procell Life Science & Technology Co., Ltd.) supplemented with 10% FBS. OVCAR-3 cells were cultured in RPMI-1640 medium (HyClone; Cytiva) supplemented with 20% FBS and 0.01 mg/ml bovine insulin (Beijing Solarbio Science & Technology Co., Ltd.). SKOV-3 cells were cultured in RPMI-1640 medium supplemented with 15% FBS. All cells were incubated at 37˚C in an incubator with 5% CO_2.

Cell transfection. A total of 100 pmol miR-638 mimics or 100 pmol negative control (NC) mimics were transfected into OVCAR-3 cells, whilst 100 pmol miR-638 inhibitor or 100 pmol NC inhibitor were transfected into Caov-3 cells using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. miR-638 expression and cell viability were detected 24 h after transfection. Apoptosis, cell cycle and protein expression were measured 48 h after transfection.

To verify the association between miR-638 and HMGA1, OVCAR-3 cells were co-transfected with 50 pmol miR-638 mimics and 1 μg HMGA1 overexpression plasmid (GenScript), and the detection was performed 24 or 48 h after transfection. NC mimics and empty vector served as the negative controls, respectively. The sequences used were as follows: miR-638 mimics forward, 5'-AGGGAUCGCGGGCGGGUGGCGGCCU-3' and reverse, 5'-GCCGCCACCCGCCCGCGAUCCCUUU-3'; NC mimics forward, 5'-UUCUCCGAACGUGUCACGUTT-3' and reverse, 5'-ACGUGACACGUUCGGAGAATT-3'; miR-638 inhibitor, 5'-AGGCCGCCACCCGCCCGCGAUCCCU-3'; and NC inhibitor, 5'-UUGUACUACACAAAAGUACUG-3'.

Cell counting kit-8 (CCK-8) assay. OVCAR-3 and Caov-3 cells were transferred to 96-well plates ($3x10^3$ cells/well), and cell viability was detected using CCK-8 assay (Sigma-Aldrich; Merck KGaA) at 24, 48 and 72 h after transfection. Briefly, 10 μl CCK-8 solution was added to each well containing 100 μl normal medium, followed by incubation for 1 h at 37˚C with 5% CO_2. The optical density value, representing the viability of the cells was measured using a microplate reader (ELX-800; BioTek Instruments, Inc.) at 450 nm.

Cell cycle assay. The aforementioned OVCAR-3 and Caov-3 cells were first digested with 0.2% trypsin, harvested by centrifugation at 1,000 x g for 5 min at 4˚C and washed with PBS twice 48 h after transfection. Subsequently, Cell Cycle Detection kit (Beyotime Institute of Biotechnology) was used for cell cycle detection according to the manufacturer's protocol. Briefly, the cells ($2x10^4$) were fixed in pre-cooled 70% ethanol at 4˚C for 12 h, stained with 25 μl propidium iodide (PI) solution at 37˚C for 30 min in the dark, analyzed by flow cytometry (NovoCyte; ACEA Bioscience, Inc.; Agilent Technologies, Inc.) and quantified using NovoExpress v1.2.5 (Agilent Technologies, Inc.).

Cell apoptosis. Consistent with the procedure of cell cycle assay, the OVCAR-3 and Caov-3 cells were harvested. Annexin-V/PI kit (Beyotime Institute of Biotechnology) was used for apoptotic detection according to the manufacturer's protocol. Briefly, 200 μl Annexin V-FITC and 10 μl PI were added to resuspend the cells ($2x10^4$). The cells were stained for 15 min in the dark and then placed in an ice bath, followed by analysis using flow cytometry (NovoCyte; ACEA Bioscience, Inc.; Agilent Technologies, Inc.) and quantified using NovoExpress v1.2.5 (Agilent Technologies, Inc.).

TUNEL assay. The transfected OVCAR-3 and Caov-3 cells were first permeabilized by 0.1% Triton X-100. *In Situ* Cell Death Detection kit (Roche Diagnostics) was used for labelling the apoptotic cells according to the manufacturer's protocols. Briefly, when the cell confluency reached 70%, the cells were permeabilized with 200 μl 0.1% Triton X-100 for 15 min at room temperature, and then incubated in TUNEL working solution (Enzyme solution:Label Solution, 1:9) in the dark at 37˚C for 1 h. The cells were counterstained by DAPI solution (Beyotime Institute of Biotechnology) for 5 min at room temperature and then sealed with anti-attenuation sealing reagent (Beijing Solarbio Science & Technology Co., Ltd.). Finally, the apoptotic cells were observed under a fluorescence microscope at x400 magnification (BX53; Olympus Corporation). At least five fields of view were randomly observed under the fluorescence microscope.

Dual-luciferase reporter assay. The putative binding site of miR-638 was predicted by bioinformatics

analysis (https://www.targetscan.org; release 7.2 March 2018). According to the procedure of dual-luciferase reporter assay with certain modifications (23), 293T cells were plated in 12-well plates and incubated in DMEM containing 10% FBS until 70% confluence, followed by incubation in DMEM without FBS for 1 h at 37˚C. The wild-type or mutant 3' untranslated region (UTR) sequences of HMGA1 with suspected miR-638 binding sites was then inserted into the luciferase reporter vector pmiRGLO (Promega Corporation). 293T cells were co-transfected with the constructed reporters (1.5 μg) and miR-638 mimics or NC mimics (75 pmol) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). After 4 h of incubation at 37˚C, the medium was discarded and replaced with normal DMEM for another 48 h. The luciferase activity was detected using the Dual-Luciferase assay kit (Promega Corporation) according to the manufacturer's protocol. The data were presented as Firefly/*Renilla* luciferase activity.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA from OVCAR-3 and Caov-3 cells was extracted with Total RNA Isolating kit (BioTeke Corporation) following the manufacturer's protocols. Complementary DNA was obtained by RT using M-MLV reverse transcriptase kit (Takara Biotechnology Co., Ltd.) and RT Primer (GenScript) according to the manufacturer's protocol. The relative expression level of miR-638 was determined by qPCR amplification using TaKaRa Taq™ HS Perfect Mix (Takara Biotechnology Co., Ltd.) with SYBR Green (BioTeke Corporation). The thermocycling conditions consisted of: Pre-denaturation at 94˚C for 30 sec, followed by 40 cycles at 94˚C for 5 sec and 60˚C for 15 sec. miR-638 expression was normalized against the expression of U6. The relative expression level of miR-638 was converted to fold changes according to the $2^{-\Delta\Delta Cq}$ method (24). The primers used were as follows: miR-638 forward, 5'-AATAGGGATCGCGGGCGG-3' and reverse, 5'-GCAGGGTCCGAGGTATTC-3', and U6 forward, 5'-GCTTCGGCAGCACATATACT-3' and reverse, 5'-GTGCAGGGTCCGAGGTATTC-3'.

Western blotting. Proteins were isolated from the aforementioned OVCAR-3 and Caov-3 cells sing RIPA (Beyotime Institute of Biotechnology) and PMSF solution (Beyotime Institute of Biotechnology). The proteins were quantified using the BCA assay (Beyotime Institute of Biotechnology) and then equal amounts of protein (15-30 μg) were separated by 8-15% SDS-PAGE, transferred onto PVDF membranes and blocked in 5% bovine serum albumin (Biosharp Life Sciences) for 1 h at room temperature. After blocking, the membranes were incubated with primary antibodies against proliferating cell nuclear antigen (PCNA; dilution, 1:1,000; Affinity Biosciences; cat. no. AF0239), caspase-3 (dilution, 1:1,000; Cell Signaling Technology, Inc.; cat. no. 14220), poly(ADP-ribose) polymerase (PARP; dilution, 1:1,000; Cell Signaling Technology, Inc.; cat. no. 9532), cyclin D1 (dilution, 1:500; ABclonal Biotech Co., Ltd.; cat. no. A0310), cyclin B1 (dilution, 1:2,000; ProteinTech Group, Inc.; cat. no. 55004-1-AP), PTEN (dilution, 1:500; ProteinTech Group, Inc.; cat. no. 22034-1-AP), HMGA1 (dilution, 1:1,000; Affinity Biosciences; cat. no. AF5218) or β-actin (dilution, 1:2,000; ProteinTech Group, Inc.; cat. no. 60008-1-Ig) at 4˚C overnight, followed by incubation with the corresponding anti-rabbit or anti-mouse IgG-horseradish peroxidase secondary antibodies (dilution, 1:10,000; ProteinTech Group, Inc.; cat. nos. SA00001-1 and SA00001-2) at 37˚C for 40 min. The protein bands were visualized using enhanced chemiluminescence (7 Sea Pharmatech Co., Ltd.) and quantitatively analyzed using Gel-Pro Analyzer 4.0 (Media Cybernetics, Inc.).

Statistical analysis. Data are presented as the mean ± standard deviation. All experiments were repeated at least three times. Comparisons between ovarian cancer and adjacent normal tissues were performed with paired Student's t-tests. Differences between two independent groups were analyzed using unpaired Student's t-test. For comparison of ≥3 groups, one-way analysis of variance followed by Tukey's post hoc test was performed. Statistical analysis of data was performed using GraphPad Prism 8.0 (GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

miR-638 is expressed in ovarian cancer tissues and cell lines. The expression levels of miR-638 in clinical ovarian cancer samples were first measured. miR-638 levels were found to be significantly lower in ovarian cancer samples compared with those in adjacent normal tissues (Fig. 1A). The expression levels of miR-638 in different ovarian cancer cell lines were subsequently measured by RT-qPCR. Caov-3 cells had the highest miR-638 expression level, followed by SKOV3, A2780 and ES-2 cells (Fig. 1B). OVCAR-3 cells exhibited the lowest miR-638 expression level (Fig. 1B). Therefore, OVCAR-3 cells were used for miR-638 overexpression and Caov-3 cells were used for miR-638-knockdown in subsequent experimentation. After transfection with miR-638 mimics, the miR-638 levels in OVCAR-3 cells were significantly increased compared with those in parental or cells transfected with NC mimics (Fig. 1C). After the Caov-3 cells were transfected with the miR-638 inhibitor, miR-638 expression was significantly decreased compared with that in cells transfected with NC inhibitor (Fig. 1D). These results suggested that miR-638 overexpression in OVCAR-3 cells and miR-638 knockdown in Caov-3 cells were successful.

Effect of miR-638 on cell viability, apoptosis and cell cycle in ovarian cancer cells. Cell viability was measured at 24, 48 and 72 h after transfection by CCK-8 assay. The viability of OVCAR-3 cells transfected with miR-638 mimics was significantly decreased compared with that in cells that were subjected to NC mimics transfection. By contrast, miR-638 inhibitor transfection significantly promoted Caov-3 cell viability compared with that in cells transfected with the NC inhibitor (Fig. 2A). In addition, the expression of PCNA, an indicator of cell proliferation (25), was found to be significantly downregulated in OVCAR-3 cells transfected with miR-638 mimics compared with that in cells transfected with NC mimics (Fig. 2B). By contrast, PCNA expression was significantly upregulated after miR-638 inhibitor transfection in Caov-3 cells compared with that in cells transfected

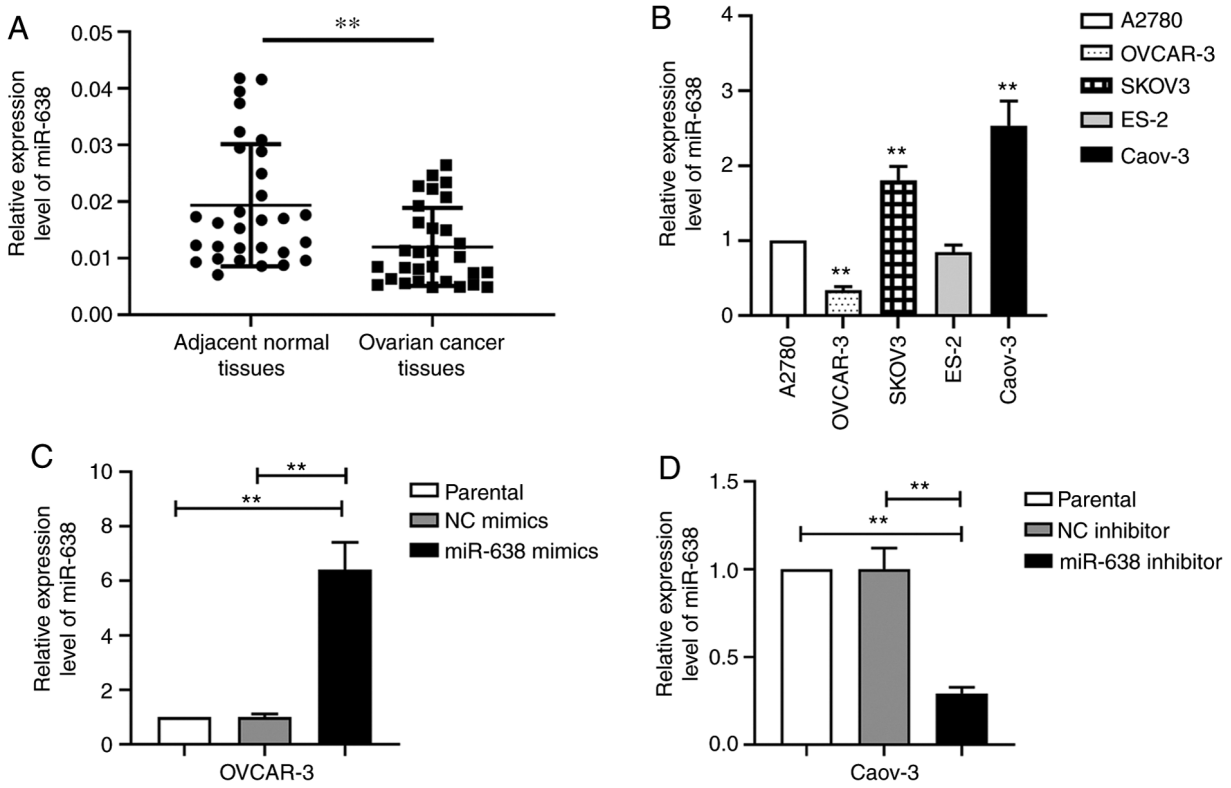

Figure 1. miR-638 expression in ovarian cancer tissues and cell lines. (A) Expression of miR-638 in ovarian cancer and adjacent normal tissues. **P<0.01. (B) Relative expression of miR-638 in the five different ovarian cancer cell lines. **P<0.01 vs. A2780 cells. (C) Expression of miR-638 in OVCAR-3 cells after transfection with miR-638 mimics. (D) Expression of miR-638 in Caov-3 cells after transfection with miR-638 inhibitor. Data are presented as the mean ± SD, n=3. **P<0.01. NC, negative control; miR, microRNA.

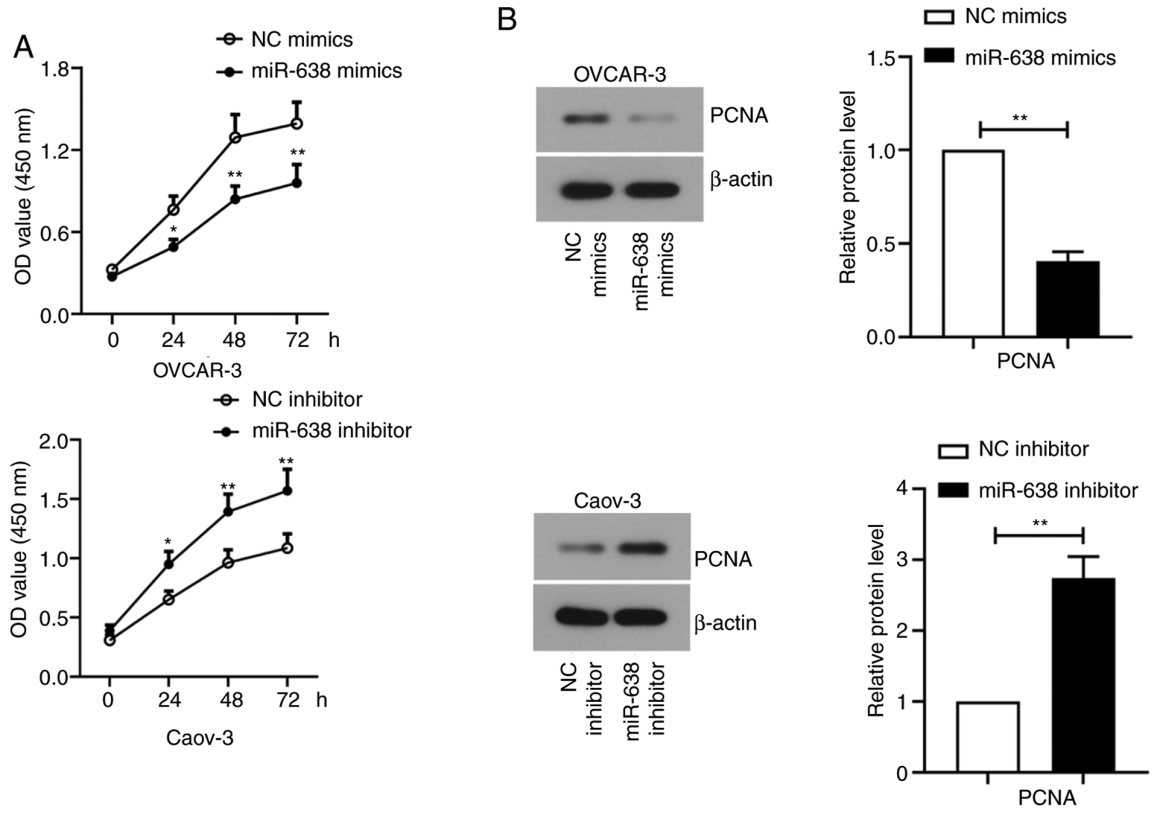

Figure 2. Effects of miR-638 on the viability in OVCAR-3 and Caov-3 cells. (A) Cell Counting Kit-8 assay was used to determine the effects of miR-638 mimics or miR-638 inhibitor on ovarian cancer cell line viability. *P<0.05 and **P<0.01 vs. NC mimics or NC inhibitor. (B) Expression of PCNA in OVCAR-3 and Caov-3 cells. Data are presented as the means ± SD, n=3. **P<0.01. NC, negative control; miR, microRNA; PCNA, proliferating cell nuclear antigen; OD, optical density.

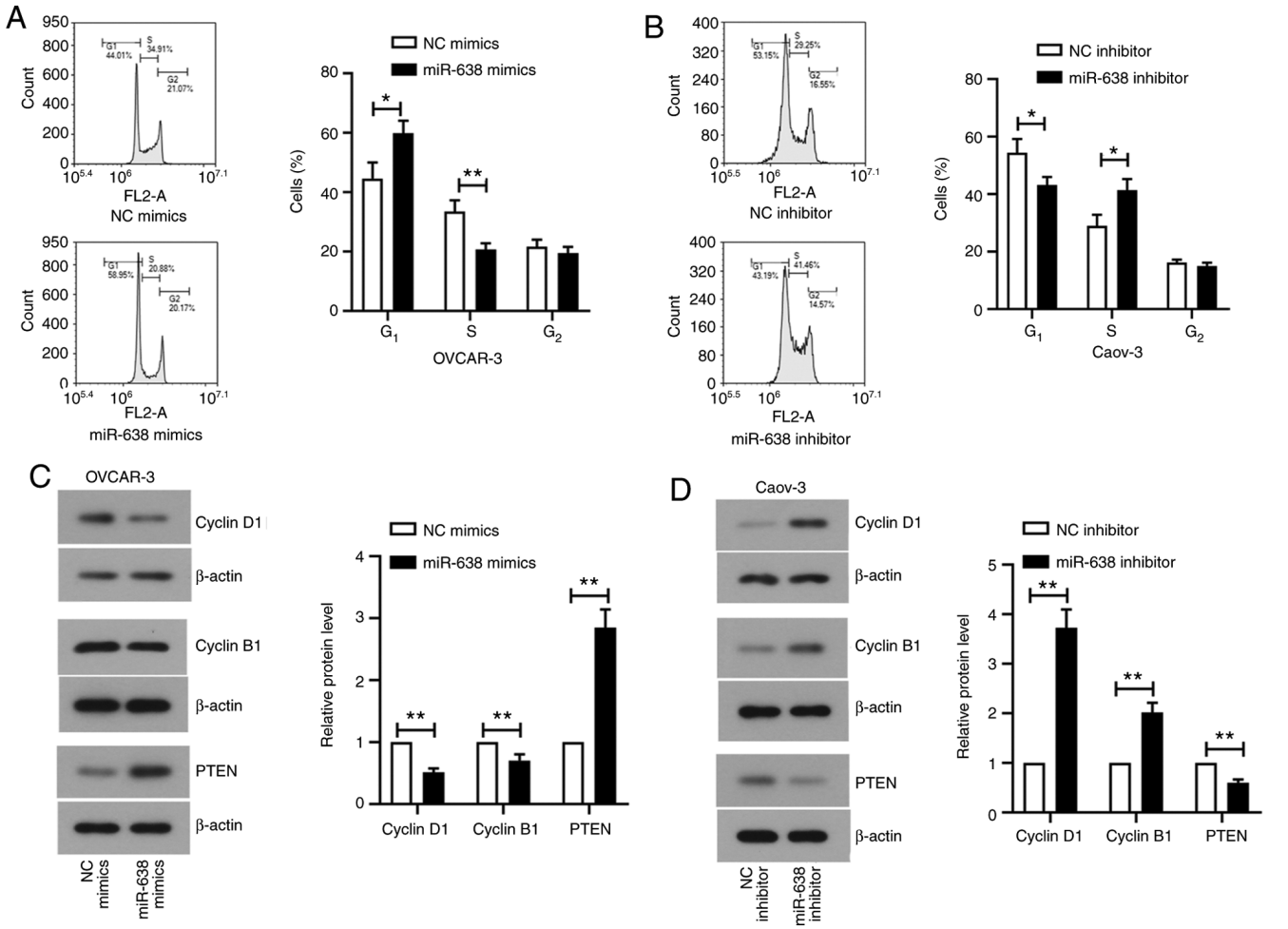

Figure 3. Effects of miR-638 on cell cycle progression in OVCAR-3 and Caov-3 cells. Flow cytometry was used to determine the effect of (A) miR-638 mimics in OVCAR-3 cell cycle progression and the effect of (B) miR-638 inhibitor in Caov-3 cell cycle progression. Protein expression levels of cyclin D1, cyclin B1 and PTEN in (C) OVCAR-3 and (D) Caov-3 cells. Data are presented as means ± SD, n=3. *P<0.05 and **P<0.01. NC, negative control; miR, microRNA.

with NC inhibitor (Fig. 2B). To confirm whether miR-638 was involved in cell cycle regulation, cell cycle status was assessed after transfection by flow cytometry. There was a significant increase in the number of cells at the G_1 phase and a significant decrease in the number of cells at the S phase in miR-638 mimics-transfected OVCAR-3 cells (Fig. 3A). Opposite trends were observed in Caov-3 cells transfected with miR-638 inhibitor (Fig. 3B). miR-638 overexpression was found to significantly suppress cyclins D1 and B1 expression whilst significantly increasing PTEN expression in OVCAR-3 cells compared with that in cells transfected with mimics NC (Fig. 3C). By contrast, opposite results were observed in Caov-3 cells transfected with the miR-638 inhibitor (Fig. 3D).

TUNEL staining and flow cytometry assay were next used to measure apoptosis after transfection. The number of TUNEL-positive OVCAR-3 cells was significantly increased after transfection with miR-638 mimics compared with that in cells transfected with mimics NC (Fig. 4A), whilst this number was significantly decreased after transfection of Caov-3 cells with miR-638 inhibitor compared with that in cells transfected with inhibitor NC (Fig. 4B). Flow cytometry assay also subsequently showed that the percentage of apoptotic cells was significantly increased in OVCAR-3 cells after miR-638 mimics transfection but significantly decreased in Caov-3 cells after miR-638 inhibitor transfection compared with those in their respective NCs (Fig. 4C and D). In addition, the expression levels of apoptotic markers cleaved-caspase-3 and cleaved-PARP, were found to be significantly increased with miR-638 overexpression in OVCAR-3 cells and significantly decreased with miR-638 knockdown in Caov-3 cells compared with the levels exhibited by their corresponding NCs (Fig. 4E and F). These data suggest that miR-638 is involved in proliferation, cell cycle arrest and apoptosis in ovarian cancer cells.

HMGA1 is the direct target of miR-638. The putative binding site of miR-638 was predicted by bioinformatics analysis. The results showed that there was a potential association between miR-638 and HMGA1 (Fig. 5A). To verify this prediction, luciferase reporter assay was performed in 293T cells. Transfection with miR-638 mimics significantly inhibited the luciferase activity of the wild-type reporter gene containing HMGA1-3'UTR compared with that in cells co-transfected with the NC mimics (Fig. 5B), whilst miR-638 mimics did not affect the luciferase activity of the mutated reporter gene containing HMGA1-3'UTR (Fig. 5B). These results suggested that miR-638 could specifically bind to HMGA1 mRNA. To

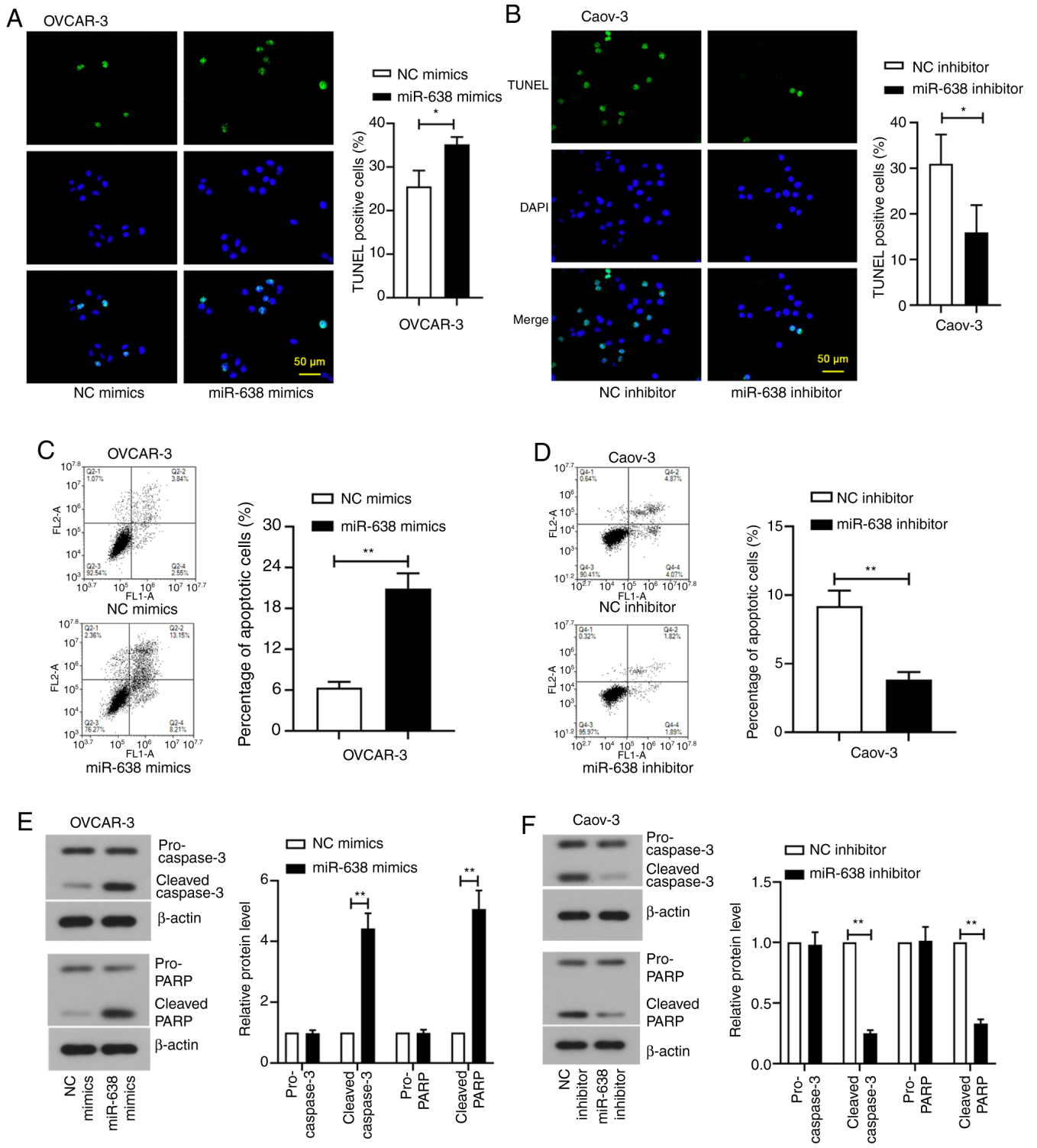

Figure 4. Effects of miR-638 on apoptosis in OVCAR-3 and Caov-3 cells. TUNEL assay was used to determine the effect of (A) miR-638 mimics on OVCAR-3 cell apoptosis and the effect of (B) miR-638 inhibitor on Caov-3 cell apoptosis. Flow cytometry was used to determine the effect of (C) miR-638 mimics on OVCAR-3 cell apoptosis and the effect of (D) miR-638 inhibitor on Caov-3 cell apoptosis. Protein expressions of cleaved caspase-3 and cleaved PARP in (E) OVCAR-3 and (F) Caov-3 cells. Data are presented as means ± SD, n=3. *P<0.05 and **P<0.01, respectively. NC, negative control; miR, microRNA; PARP, poly(ADP-ribose) polymerase.

further verify the targeting association between miR-638 and HMGA1, HMGA1 protein expression was detected by western blotting. HMGA1 protein expression was significantly reduced in OVCAR-3 cells transfected with miR-638 mimics and notably increased in Caov-3 cells transfected with miR-638 inhibitor compared with those in their corresponding NC (Fig. 5C and D). These results suggest that HMGA1 is the direct target of miR-638 in ovarian cancer cells.

HMGA1 overexpression reverses miR-638 mimics-induced inhibition of proliferation and cell cycle arrest. To verify whether miR-638 functions as a suppressor gene by regulating

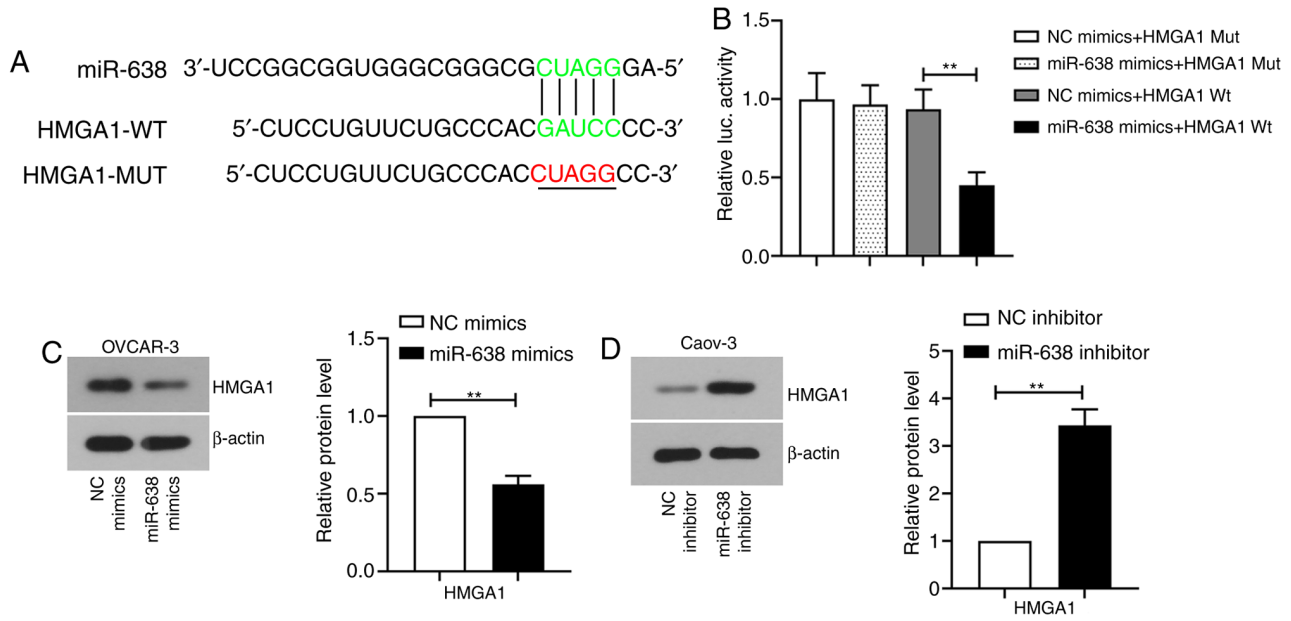

Figure 5. HMGA1 is a target of miR-638. (A) miR-638 and its putative binding sequence in HMGA1 3'-UTR. (B) Luciferase reporter assay of the interaction between the 3'UTR of HMGA1 and miR-638 in OVCAR-3 cells. Western blotting for HMGA1 expression after transfection in (C) OVCAR-3 and (D) Caov-3 cells. Data are presented as means ± SD, n=3. **P<0.01. NC, negative control; miR, microRNA; HMGA1, high mobility group A1; 3'UTR, 3'untranslated region; MUT, mutant; WT, wild-type; Luc., luciferase.

HMGA1, OVCAR-3 cells were co-transfected with miR-638 mimics and HMGA1 overexpression plasmid. HMGA1 expression in OVCAR-3 cells was significantly enhanced after transfection with the HMGA1 overexpression plasmid compared with that in cells transfected with the empty vector (Fig. 6A), indicating that HMGA1 was successively transfected into the cells. CCK-8 assay showed that the anti-proliferative effect of miR-638 mimics was significantly reversed by HMGA1 overexpression (Fig. 6B). Flow cytometry analysis of apoptosis and cell cycle distribution showed that miR-638 mimics and HMGA1 co-transfection significantly reduced apoptosis and cell cycle arrest compared with those in cells transfected with miR-638 mimics alone in OVCAR-3 cells (Fig. 6C and D). These results suggested that miR-638 served as a suppressor by targeting HMGA1 in ovarian cancer cells.

Discussion

The present study primarily investigated the effect and potential mechanism of the miR-638/HMGA1 axis on proliferation, cell cycle and apoptosis in ovarian cancer cell lines, including OVCAR-3 and Caov-3 cells. It was found that the expression of miR-638 was relatively low in OVCAR-3 cells and relatively high in Caov-3 cells. In addition, upregulating miR-638 expression significantly inhibited the viability of OVCAR-3 cells, induced cell cycle arrest at the G_1 phase and promoted cell apoptosis, all of which were prevented by miR-638 inhibition in Caov-3 cells. Furthermore, results from the present study showed that miR-638 could downregulate HMGA1 expression by binding to its 3'UTR, where HMGA1 overexpression could reverse the inhibitory effects of miR-638 on ovarian cancer cell physiology. Therefore, these data suggested that miR-638 may serve as a tumor suppressor by targeting HMGA1 expression in ovarian cancer.

Since miRNAs are key factors in modulating tumor occurrence and progression, identifying sensitive miRNAs and understanding their functions may provide new strategies for patients with cancer (26). Previous reports have shown that the levels of multiple miRNAs are altered in human ovarian cancer compared with those of normal tissue (27). Previous studies have reported that the expression levels of miR-603 and miR-31 were decreased (28,29), whilst those of miR-552 and miR-182 were increased in ovarian cancer (30,31). Furthermore, the miR-638 expression was found to be enhanced in ovarian cancer cells after treatment with the antibiotic bafilomycin A1 (13). However, the role of miR-638 in ovarian cancer remains poorly understood, which provided a basis for the present study.

Unchecked cell proliferation is one of the hallmarks of cancer, which may lead to high mortality among patients with ovarian cancer, such that reversing aberrant miRNA expression can effectively inhibit tumor cell proliferation, promote apoptosis and arrest cell cycle progression (32). Zhao *et al* (33) found that miR-638 overexpression could markedly suppress gastric cancer cell proliferation and induce cell cycle arrest at the G_1 phase *in vitro*. By contrast, Cheng *et al* (34) observed that downregulating miR-638 could promote hepatocellular carcinoma cell growth *in vitro* and tumor angiogenesis processes *in vivo*. Consistent with these studies, the present study also found that upregulating miR-638 levels could significantly inhibit ovarian cancer cell viability, induce cell cycle arrest at the G_1 phase and promote cell apoptosis, suggesting that miR-638 is a tumor suppressor in the ovarian cancer pathogenesis.

PCNA is a standard marker of cell proliferation that can be used to effectively evaluate the growth of malignant tumors (35). PCNA dysfunction has been widely used in the diagnosis of various cancer types, including breast (36) and cervical

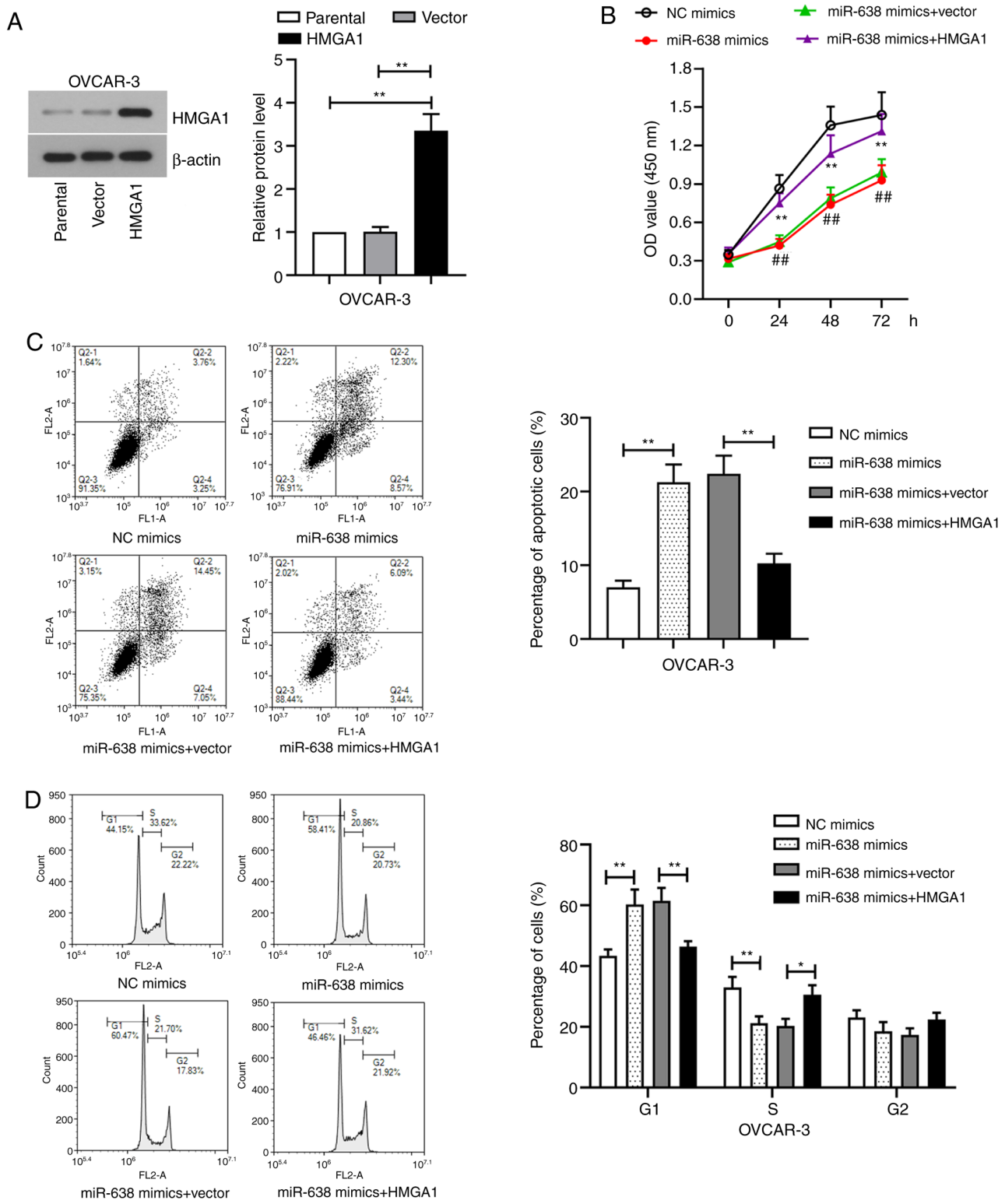

Figure 6. Effects of the miR-638/HMGA1 axis on cell cycle arrest and apoptosis in OVCAR-3 cells. (A) Western blotting was performed to measure HMGA1 expression in OVCAR-3 cells after transfection with the HMGA1-encoding vector. **P<0.01. (B) Cell Counting Kit-8 assay was performed to measure OVCAR-3 cell viability after co-transfection with miR-638 and HMGA1. $^{\#\#}$P<0.01 vs. NC mimics and **P<0.01 vs. miR-638 mimics + vector. Flow cytometry was performed to measure OVCAR-3 (C) cell apoptosis and (D) cell cycle progression after co-transfection with miR-638 and HMGA1. Data are presented as the mean ± SD, n=3. *P<0.05 and **P<0.01. NC, negative control; miR, microRNA; HMGA1, high mobility group A1; OD, optical density.

cancer (37). Consistent with a previous study, results from the present study revealed that the cell viability in OVCAR-3 cells was reduced with miR-638 overexpression, which was accompanied with the decreased expression of PCNA. By contrast, opposite results were observed following miR-638 knockdown in Caov-3 cells. Cyclins D1 and B1 are members of the cyclin family that are associated with cell cycle progression and can also be regulated by miRNA. It has been previously reported

that inhibiting cyclin D1 expression in epithelial ovarian cancer cells by upregulating miR-211 can inhibit the progression of the cell cycle through the G_1 phase (38), whilst decreasing cyclin B1 expression in breast cancer cells by miR-379 transfection can reduce proliferation (39). In the present study, the S-phase cell fraction was decreased in OVCAR-3 cells overexpressing miR-638, but was increased in Caov-3 cells following miR-638 knockdown. In line with the aforementioned data, reduced cyclin D1 and cyclin B1 expression was observed in OVCAR-3 cells transfected with miR-638 mimics, whilst miR-638 inhibitor transfection could reverse this phenomenon in Caov-3 cells. In addition, cyclin D is one of the targets in the PTEN signaling pathway, where PTEN overexpression can prevent the increase in cyclin D1 during cell cycle progression from G_1 to S phase in U-87 and U-251 glioblastoma cells (40). Dysfunction of the suppressor PTEN is a frequent phenomenon observed in numerous types of cancer, such as ovarian cancer (31,41). Cleaved caspase-3 and cleaved PARP are characteristic markers of cell apoptosis (42,43), whereby elevating their expression levels may promote tumor apoptosis. Zhang *et al* (44) reported that miR-148a promoted apoptosis, which was characterized by increased caspase-3 and PARP activation. In another study, Liu *et al* (45) showed that apoptosis, along with cleaved caspase-3 expression were decreased in ovarian granulosa cells with miR-26b knockdown. Findings from the present study suggest that miR-638 overexpression accelerated apoptosis in OVCAR-3 cells by increasing cleaved caspase-3 and cleaved PARP levels. These results indicate that miR-638 upregulation suppressed proliferation, induced cell cycle arrest and promoted apoptosis in ovarian cancer cells.

Bioinformatics prediction indicated that miR-638 may have a potential regulatory effect on HMGA1 by targeting its 3'UTR. HMGA1 is a chromatin factor that is expressed at low levels in normal human tissues but is overexpressed in certain malignant tumors, including cervical, prostate and pancreatic cancer (15). In addition, a previous study has shown that HMGA1 overexpression is also a common feature in ovarian cancer (46). However, the mechanism involved in the regulation of HMGA1 expression has yet to be fully elucidated. Although bioinformatics analysis showed that miR-638 can directly target HMGA1, there is no previous report on the association between miR-638 and HMGA1. Therefore, a dual-luciferase assay was performed to verify the direct binding of miR-638 to HMGA1 3'UTR, and the results confirmed this hypothesis. Wei *et al* (47) found that miR-296 diminished prostate cancer growth and invasion by directly targeting HMGA1. In another study, Chen *et al* (48) found that HMGA1 was a target of the miRNA let-7d-5p, where upregulating let-7d-5p expression could suppress proliferation and facilitate apoptosis in ovarian cancer via the p53 signaling pathway, which was mediated by HMGA1 expression. Based on these aforementioned studies, the present study aimed to clarify whether miR-638/HMGA1 signaling was involved in proliferation, apoptosis and cell cycle progression in ovarian cancer cells. The inhibitory effects of miR-638 overexpression on cell proliferation was reversed by HMGA1 overexpression, suggesting that the growth-suppressive effect of miR-638 in ovarian cancer cells was mediated by HMGA1 repression. However, this conclusion was initially obtained through *in vitro* experiments. Verifying the potentially anti-tumor effects of miR-638 in a xenograft animal model would render results from the present the study more convincing.

In conclusion, the findings of the present study suggest that miR-638 expression is closely associated with ovarian cancer. Upregulating miR-638 can inhibit proliferation, induce cell cycle arrest and promote apoptosis in ovarian cancer cells. Furthermore, the antitumor effects of miR-638 were mediated at least partially by negatively regulating HMGA1 expression in ovarian cancer cells. This finding may help to develop a novel strategy for the prevention and treatment of ovarian cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LM designed the study and drafted the manuscript. YJ contributed to the experiments. WZ and XB analyzed the data. QY was involved in conception and design, and drafting and revision of the manuscript. LM and WZ confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All the protocols were approved by the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China). Informed consent was signed by the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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