

# MicroRNA-409-5p inhibits cell proliferation, and induces G<sub>2</sub>/M phase arrest and apoptosis by targeting DLGAP5 in ovarian cancer cells

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**Abstract.** MicroRNA (miRNA/miR)-409-5p has been reported to be implicated in prostate and breast cancers; however, its functional role in ovarian cancer (OC) remains unclear. Therefore the aim of the present study was to investigate the clinical significance and biological function of miR-409-5p in OC. Here, reverse transcription-quantitative PCR analysis was performed to detect miR-409-5p expression in OC tissues and cell lines. The association between miR-409-5p expression and the clinicopathological characteristics of patients with OC was assessed using the Fisher's exact test. Furthermore, the Cell Counting Kit-8 assay was performed to assess cell proliferation. Cell cycle distribution and apoptosis were evaluated via flow cytometric analysis, and the target gene of miR-409-5p was validated via the dual-luciferase reporter assay. The results demonstrated that miR-409-5p expression was significantly downregulated in OC tissues and cell lines compared with adjacent normal tissues and epithelial cells, respectively. In addition, low miR-409-5p expression was significantly associated with tumor size ( $P=0.044$ ) and the International Federation of Gynecology and Obstetrics staging system ( $P=0.005$ ). Notably, overexpression of miR-409-5p suppressed cell proliferation, and induced G<sub>2</sub>/M phase arrest and apoptosis of OC cells. Mechanistically, discs large-associated

protein 5 (DLGAP5) was identified as a novel target of miR-409-5p, which was negatively regulated by miR-409-5p. DLGAP5 expression was significantly upregulated in OC tissues and cell lines compared with adjacent normal tissues and epithelial cells, respectively. Furthermore, overexpression of DLGAP5 reversed the effects of miR-409-5p on SKOV-3 cell proliferation, and G<sub>2</sub>/M phase and apoptosis. Taken together, these results suggest that miR-409-5p acts as a tumor suppressor in OC by modulating DLGAP5 expression.

## Introduction

Ovarian cancer (OC) is one of the most common malignancies in the female reproductive system and the leading cause of cancer-associated mortality worldwide (1). In 2019, it was estimated that there were ~22,530 new cases and 13,980 mortalities in the United States (2). Despite improvements in the median survival rates following application of the first-line therapy, including cytoreductive surgery and combined chemotherapy or radiotherapy (3,4), the 5-year survival rate of patients with OC remains low (<40%) in most women diagnosed at advanced stages (5). Thus, it is important to understand the molecular pathogenesis of OC for early diagnosis and to develop innovative therapies.

MicroRNAs (miRNAs/miRs) are a class of small non-coding RNA molecules (18-25 nucleotides in length) that participate in several biological processes by negatively regulating gene expression at the post-transcriptional level by binding to the 3'-untranslated regions (UTRs) of mRNAs (6-8). Recent studies have reported that aberrant expression of miRNAs is closely associated with the progression of OC (9,10). Functionally, miR-665 (11) promotes, while miR-506-3p (12) suppresses the proliferation of OC cells by targeting SRC kinase signaling inhibitor 1 and myotubularin-related protein 6, respectively. Notably, aberrantly expressed miRNAs have been reported to act as oncogenes or tumor suppressors (13,14). Among these, miR-409-5p has recently been demonstrated to play a key role in the following tumor cells: miR-409 targets pro-metastatic gene radixin to suppress gastric cancer cell invasion and metastasis (15), while miR-409-5p is upregulated and

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*Abbreviations:* OC, ovarian cancer; DLGAP5, discs large-associated protein 5; UTR, untranslated region; WT, wild-type; MUT, mutant

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promotes cancer development in prostate (16) and breast (17) cancer cells. Notably, bioinformatics analyses have indicated that miR-409-5p is downregulated in OC samples (18,19). However, the functional role of miR-409-5p in OC *in vitro* has not yet been investigated.

Discs large-associated protein 5 (DLGAP5, also known as HURP or KIAA0008), is a member of the DLGAP family, which plays an important role in spindle assembly (20). Recently, DLGAP5 has been suggested to be associated with poor prognosis in non-small cell lung cancer (21,22), anaplastic thyroid carcinoma (23), glioblastoma (24) and pancreatic carcinoma (25). *In vitro* analyses have demonstrated that DLGAP5 knockdown significantly decreases the migratory and invasive abilities of colorectal cancer cells (26), as well as the proliferative ability of hepatocellular carcinoma cells (27,28). Similarly, DLGAP5 was downregulated by NUSAP1 gene silencing, which is associated with cell cycle G<sub>2</sub>/M phase arrest and inhibition of MCF-7 cell proliferation (29). Based on bioinformatics analysis, it was hypothesized that miR-409-5p can regulate DLGAP5 expression. However, the association between miR-409-5p and DLGAP5 in the progression of OC remains unclear.

The present study aimed to investigate miR-409-5p expression and determine its association with the clinicopathological characteristics of patients with OC. In addition, the role of miR-409-5p on OC cell functions was investigated, including proliferation, cell cycle progression and apoptosis. Furthermore, the functional mechanism of miR-409-5p in regulating the proliferation of OC cells was determined.

## Materials and methods

**Collection of tissue samples.** A total of 39 paired OC tissues and adjacent normal tissues (at least 4 cm from the tumor edge) were collected from patients (age range, 25–68 years; mean age, 45.6±6.5 years) via resection at Mindong Hospital Affiliated to Fujian Medical University (Fujian, China) between October 2017 and September 2018. Tissues samples were immediately snap-frozen and stored at -80°C until subsequent experimentation. Patient characteristics, including age, tumor size and histological grade are listed in Table I. All patients were staged according to the International Federation of Gynecology and Obstetrics (FIGO) staging system (30) and graded based on the histological grade (31). The exclusion criteria included patients which had received chemotherapy, radiotherapy, hormone therapy or other antitumor therapies. The inclusion criteria are patients diagnosed with NSCLC and confirmed not to receive any antitumor therapies. The present study was approved by the Institutional Ethics Committee of Mindong Hospital Affiliated to Fujian Medical University (Fujian, China; approval no. MHFM-39A) and performed in accordance with the Declaration of Helsinki. Written informed consent was provided by all patients prior to the study start.

**Cell culture.** The OC cell lines, SKOV-3 and OVCAR3, and the ovarian epithelial cell line, IOSE80, were purchased from the American Type Culture Collection. Cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (both purchased from Gibco; Thermo Fisher Scientific, Inc.) at 37°C with 5% CO<sub>2</sub>.

**Cell transfection.** Prior to transfection, SKOV-3 and OVCAR3 cells were seeded into 24-well plates at a density of 2x10<sup>5</sup> cells/well and cultured until they reached 90% confluence. Subsequently, miR-409-5p mimics (1.5 μl; 5'-UAAUAGUAAAGGAGGGAAGCAG-3') or miR-negative control (NC; 20 pmol/μl; 5'-ACUCUAUCUGCACGCUGA CUU-3') (Shanghai GenePharma Co., Ltd.) were mixed with 50 μl Opti-MEM medium (Thermo Fisher Scientific, Inc.), which was further mixed with 1 μl Lipofectamine<sup>®</sup> 3000 transfection reagent (Thermo Fisher Scientific, Inc.) for 10 min at room temperature. OC cells were transfected for 48 h at room temperature. For rescue experiments, SKOV-3 cells in the miR-409-5p mimics or miR-NC groups were transfected with 0.5 μl pcDNA3.1-DLGAP5 or empty pcDNA3.1 plasmid (Sangon Biotech Co., Ltd.) at 37°C for 48 h. All transfections were performed for 48 h, followed by subsequent experimentation.

**Reverse transcription-quantitative PCR (RT-qPCR).** Total RNA was isolated from tissue samples or cell lines using TRIzol<sup>®</sup> reagent (Thermo Fisher Scientific, Inc.) and single-stranded cDNA was synthesized using the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) or the M-MLV cDNA synthesis kit (Promega Corporation). The temperature protocol for RT was as follows: 95°C for 100 sec, 60°C for 60 sec and 65°C for 120 sec. qPCR was subsequently performed using TaqMan MicroRNA assay kits or SYBR Premix Ex Taq II (all purchased from Applied Biosystems; Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used: Initial denaturation at 95°C for 15 min followed by 40 cycles of denaturation at 94°C for 15 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec. The following primer sequences were used for qPCR: miR-409-5p forward, 5'-AAG CAAGGTTACCCGCTTTG-3' and reverse, 5'-AGTCGGGTG TCGGTGCAA-3'; U6 forward, 5'-CTCGCTTCGGCAGCA CA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'; DLGAP5 forward, 5'-TTGTGAGGGTTCCTGCTTCG-3' and reverse, 5'-TTCCTGTGTCGACTGGCAA-3'; and GAPDH forward, 5'-GGTGAAGGTCGGAGTCAACG-3' and reverse, 5'-GCATCGCCCCACTTGATTTT-3'. Relative expression levels of miR-409-5p and DLGAP5 were calculated using the 2<sup>-ΔΔC<sub>q</sub></sup> method (32), and normalized to U6 and GAPDH, respectively. All experiments were performed in triplicate.

**Cell Counting Kit-8 (CCK-8) assay.** Transfected SKOV-3 and OVCAR3 cells were seeded into 96-well plates at a density of 3,000 cells/well and the CCK-8 assay (Dojindo Molecular Technologies, Inc.) was performed to assess cell proliferation. At 0, 24, 48 and 72 h, cells were incubated with 10 μl CCK-8 solution for 1 h and cell proliferation was subsequently analyzed at a wavelength of 450 nm, using a microplate reader (SpectraMax M2; Molecular Devices, LLC). Each sample was performed in triplicate.

**Flow cytometry.** Transfected SKOV-3 and OVCAR3 cells were harvested and digested using 0.25% trypsin. Subsequently, single cell suspension containing 1x10<sup>6</sup> cells was prepared by discarding the digestion solution and performing centrifugation at 300 x g for 10 min at 4°C. For

Table I. Association between miR-409-5p expression and the clinicopathological characteristics of patients with ovarian cancer (n=39).

Characteristic	Patients, n	miR-409-5p expression		P-value <sup>a</sup>
		Low (n=25)	High (n=14)	
Age, years				0.721
<55	29	18	11	
≥55	10	7	3	
Tumor size, cm				0.044 <sup>b</sup>
<3	25	13	12	
≥3	14	12	2	
FIGO stage				0.005 <sup>c</sup>
Early (I-IIA)	32	24	8	
Advanced (IIB-IV)	7	1	6	
Histological grade				0.287
Low/moderate	27	19	8	
High	12	6	6	
Lymph node metastasis				0.218
Negative	31	18	13	
Positive	8	7	1	
Menopause				>0.999
Pre-	16	10	6	
Post-	23	15	8	
Differentiation				>0.999
Well/moderate	25	16	9	
Poor	14	9	5	
Depth of invasion				0.445
T1-T3	30	18	12	
T4	9	7	2	

<sup>a</sup> $\chi^2$  test, <sup>b</sup>P<0.05; <sup>c</sup>P<0.01. miR, microRNA; FIGO, International Federation of Gynecology and Obstetrics.

analysis of cell cycle distribution, cells were resuspended in 100  $\mu$ l RNase A (Sigma-Aldrich; Merck KGaA) for 30 min at 37°C, fixed with pre-cooled 70% ethanol overnight at 4°C and stained with 50  $\mu$ g of RNase-containing propidium iodide (PI; Sigma-Aldrich; Merck KGaA) solution for 30 min at 4°C in the dark. For cell cycle distribution analysis, the percentage of cells in the G<sub>0</sub>/G<sub>1</sub>, S and G<sub>2</sub>/M phases were determined using a flow cytometer (BD Biosciences).

For apoptosis analysis, SKOV-3 and OVCAR3 cells were resuspended in 500  $\mu$ l 1X binding buffer (Sigma-Aldrich; Merck KGaA), fixed with pre-cooled 70% ethanol overnight at 4°C and stained with 1  $\mu$ l Annexin V-FITC (Sigma-Aldrich; Merck KGaA) for 15 min at 4°C in the dark, followed by incubation with 5  $\mu$ l PI for 15 min at 4°C in the dark. Early and late apoptotic cells were subsequently analyzed using a flow cytometer (BD Biosciences).

**Dual-luciferase reporter assay.** TargetScan7.1 (<http://www.targetscan.org>) was used to predict the binding sites between miR-409-5p and DLGAP5. The DLGAP5 3'-UTRs containing wild-type (WT) or mutant (MUT) miR-409-5p binding sites were synthesized and cloned into pmirGLO Dual-Luciferase

vectors (Promega Corporation), yielding DLGAP5-WT and DLGAP5-MUT, respectively. Subsequently, 1.5x10<sup>5</sup> SKOV-3 or OVCAR3 cells were seeded into 12-well plates and co-transfected with 30 nM miR-409-5p mimics or miR-NC and 300 ng DLGAP5-WT or DLGAP5-MUT for 48 h at 37°C using Lipofectamine<sup>®</sup> 3000 (Thermo Fisher Scientific, Inc.). Following incubation, luciferase activities were detected using the Dual-Luciferase Reporter Assay kit (Promega Corporation). Firefly luciferase activity was normalized to *Renilla* luciferase activity.

**Western blotting.** The SKOV-3 and OVCAR3 cells were lysed using RIPA lysis buffer (Beyotime Institute of Biotechnology), and the lysates were incubated for 30 min on ice and centrifuged at 300 x g for 10 min at 4°C. Protein samples were quantified using a BCA protein assay (Beyotime Institute of Biotechnology). Equal amounts of protein (30  $\mu$ g per lane) were separated via 12% SDS-PAGE, transferred onto PVDF membranes and blocked with 5% skimmed milk in TBS containing 0.1% Tween-20 for 2 h at room temperature. The membranes were incubated with primary antibodies against DLGAP5 (1:1,000; cat. no. ab70744), CDK1 (1:500;

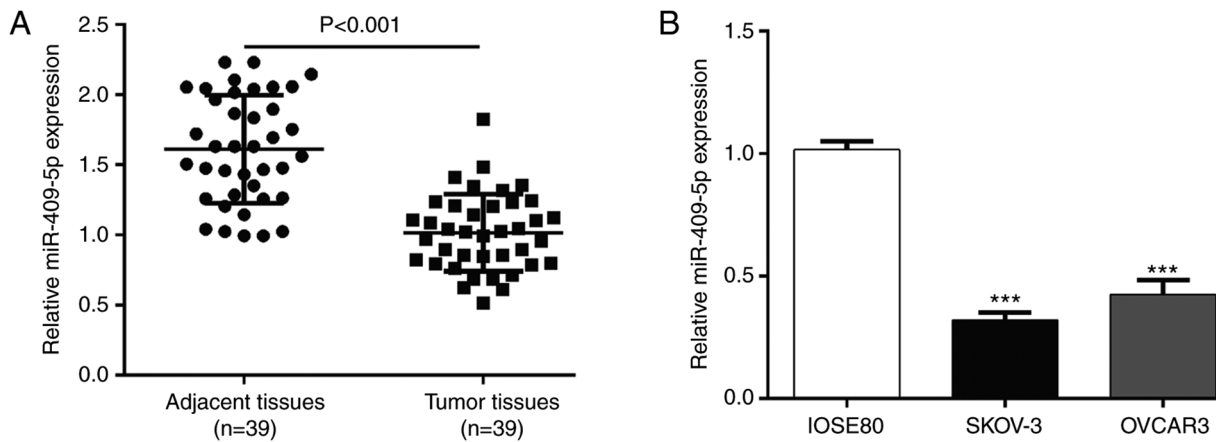


Figure 1. miR-409-5p expression is significantly downregulated in ovarian cancer. (A) RT-qPCR analysis was performed to detect miR-409-5p expression in 39 tumor tissues and matched adjacent normal tissues. Wilcoxon signed rank test was used to compare the differences between tumor tissues and adjacent normal tissues. (B) RT-qPCR analysis was performed to detect miR-409-5p expression in ovarian cancer cell lines (SKOV-3 and OVCAR3) and normal ovarian IOSE80 epithelial cells. Data are presented as the mean  $\pm$  standard deviation (n=3). \*\*\*P<0.001 vs. IOSE80 cells. miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR.

cat. no. ab131450), Cyclin B1 (1:100; cat. no. ab215436), Bad (1:1,000; cat. no. ab90435), Bcl-2 (1:500; cat. no. ab196495) and GAPDH (1:1,000; cat. no. ab245355) (all purchased from Abcam) overnight at 4°C. The membranes were washed three times with TBST and incubated with HRP-conjugated secondary antibody (1:5,000; cat. no. ab97051; Abcam) at room temperature for 2 h. Protein bands were visualized using enhanced chemiluminescence reagents (Thermo Fisher Scientific, Inc.) and quantified using ImageLab\_v3.0 software (Bio-Rad Laboratories, Inc.).

**Statistical analysis.** Statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, Inc.). All experiments were performed in triplicate and data are presented as the mean  $\pm$  standard deviation. An unpaired Student's t-test was used to compare differences between two groups, while one-way ANOVA followed by Dunnett's test or Tukey's post hoc test were used to compare differences between multiple groups. Wilcoxon signed rank test was used to compare miR-409-5p/DLGAP5 expression levels in tumor tissues and adjacent normal tissues. Patients were divided into high (n=14) and low expression groups (n=5), according to the median miR-409-5p expression value (1.021). Fisher's exact test was used to assess the association between miR-409-5p expression and the clinicopathological characteristics of patients with OC. P<0.05 was considered to indicate a statistically significant difference.

## Results

**miR-409-5p expression is significantly downregulated in OC.** To determine the potential role of miR-409-5p in the progression of OC, RT-qPCR analysis was performed to detect its expression in 39 histologically diagnosed OC tumor tissues and matched adjacent normal tissues. The results demonstrated that miR-409-5p expression was significantly lower in OC tumor tissues compared with adjacent normal tissues (P<0.001; Fig. 1A). Similarly, miR-409-5p expression was significantly lower in the OC cell lines (SKOV-3 and

OVCAR3) compared with normal ovarian epithelial cells (Fig. 1B).

Next, the association between miR-409-5p expression and the clinicopathological characteristics of patients with OC was analyzed. As presented in Table I, miR-409-5p expression was significantly associated with tumor size (P=0.044) and FIGO stage (P=0.005).

**Overexpression of miR-409-5p inhibits the proliferation of OC cells.** Gain-of-function assays were performed to determine the biological function of miR-409-5p in OC cells *in vitro*. RT-qPCR analysis demonstrated that transfection with miR-409-5p mimics significantly upregulated miR-409-5p expression in both SKOV-3 and OVCAR3 cells compared the miR-NC group (Fig. 2A). The results of the CCK-8 assay indicated that transfection with miR-409-5p mimics significantly attenuated the proliferation of SKOV-3 and OVCAR3 cells (Fig. 2B). As presented in Fig. 2C, the percentage of SKOV-3 (57.8 $\pm$ 0.6 vs. 63.5 $\pm$ 0.9) and OVCAR3 (41.4 $\pm$ 1.3 vs. 46.3 $\pm$ 0.8) cells in the G<sub>0</sub>/G<sub>1</sub> phase and SKOV-3 cells in the S phase (16.7 $\pm$ 1.2 vs. 20.1 $\pm$ 1.6) significantly decreased following transfection with miR-409-5p mimics compared with the miR-NC group. Overexpression of miR-409-5p significantly increased the percentage of SKOV-3 cells in the G<sub>2</sub>/M phase from 16.4 $\pm$ 0.8 to 25.6 $\pm$ 0.8 and OVCAR3 cells from 17.7 $\pm$ 1.1 to 22.0 $\pm$ 0.6. In addition, overexpression of miR-409-5p mimics significantly elevated early and late apoptosis in both SKOV-3 and OVCAR3 cells (Fig. 2D). Taken together, these results suggest that miR-409-5p negatively regulates OC cell proliferation by affecting cell cycle progression and apoptosis.

**DLGAP5 is a direct target of miR-409-5p.** TargetScan software was used to identify the direct targets of miR-409-5p. Among the predicted targets, DLGAP5, a cell cycle-related gene (20), was identified as a potential target of miR-409-5p, and DLGAP5 harbored a putative miR-409-5p binding site in its 3'-UTR (Fig. 3A). The dual-luciferase reporter assay was performed in OC cells by constructing DLGAP5 WT or MUT reporter plasmids. The results demonstrated that

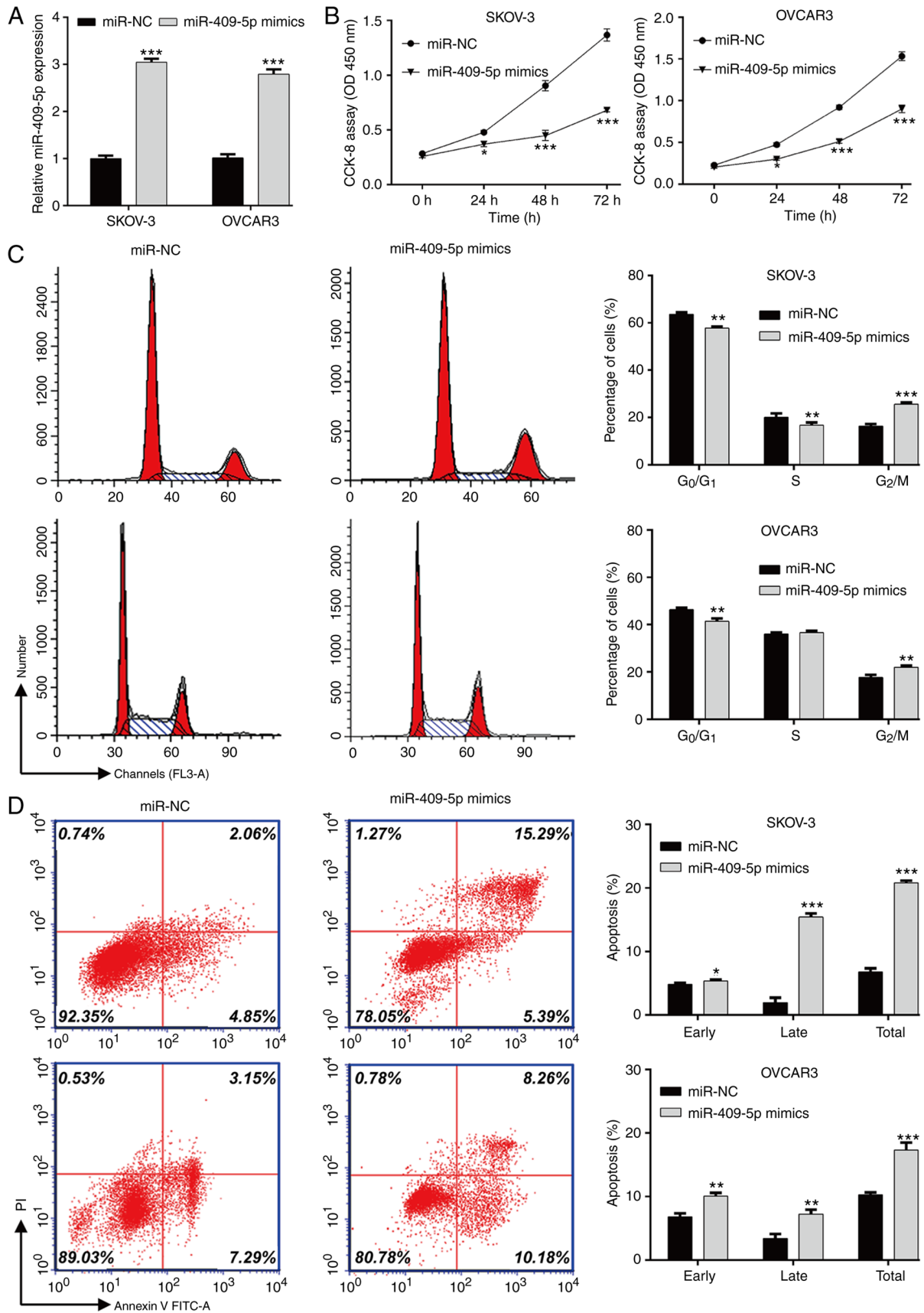
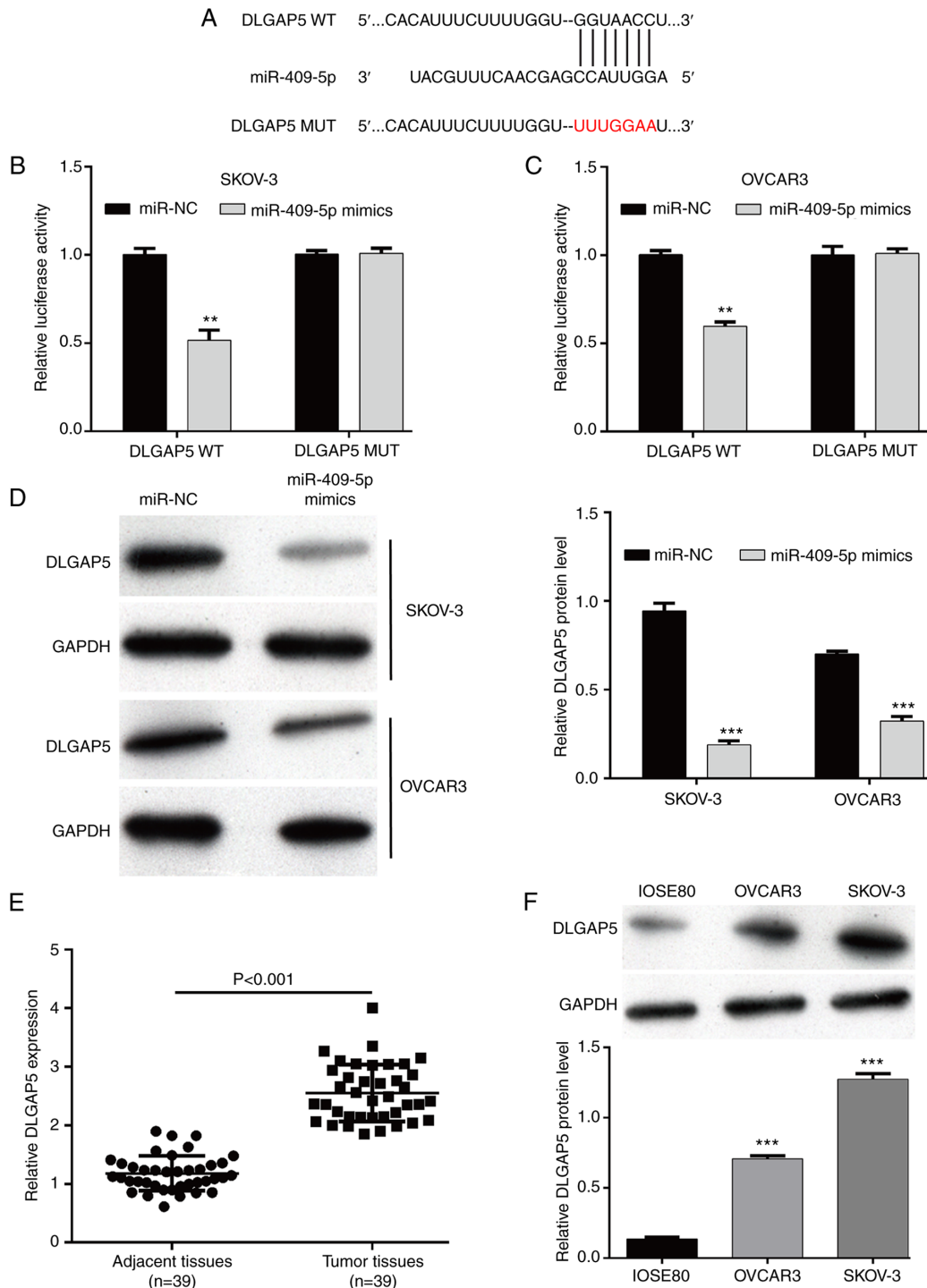


Figure 2. Overexpression of miR-409-5p inhibits cell proliferation, and induces G<sub>2</sub>/M phase arrest and apoptosis in ovarian cancer cells. (A) Reverse transcription-quantitative PCR analysis was performed to detect miR-409-5p expression in SKOV-3 and OVCAR3 cells transfected with miR-409-5p mimics or miR-NC. (B) The CCK-8 assay was performed to assess the proliferation of SKOV-3 and OVCAR3 cells transfected with miR-409-5p mimics or miR-NC. (C) The percentage of transfected cells in the G<sub>0</sub>/G<sub>1</sub>, S and G<sub>2</sub>/M phases were analyzed via flow cytometry with PI staining. (D) Early and late apoptotic cells were determined in transfected SKOV-3 and OVCAR3 cells via flow cytometry with Annexin V/PI staining. Data are presented as the mean ± standard deviation (n=3). \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001 vs. the miR-NC group. miR, microRNA; NC, negative control; CCK-8, Cell Counting Kit-8; PI, propidium iodide; OD, optical density.



**Figure 3.** DLGAP5 is a direct target of miR-409-5p. (A) TargetScan software revealed a potential target site for miR-409-5p in the 3'-UTR of human DLGAP5 mRNA. To disrupt the interaction between miR-409-5p and DLGAP5 mRNA, the target site was mutated (highlighted in red). (B and C) The dual-luciferase reporter assay was performed in SKOV-3 and OVCAR3 cells following co-transfection with miR-409-5p mimics or miR-NC and WT or MUT DLGAP5 3'-UTR constructs. Data are presented as the mean  $\pm$  standard deviation ( $n=3$ ). (D) Western blot analysis was performed to detect DLGAP5 protein expression in SKOV-3 and OVCAR3 cells following transfection with miR-409-5p mimics or miR-NC. (E) Reverse transcription-quantitative PCR analysis was performed to detect DLGAP5 mRNA expression in 39 paired tumor tissues and matched adjacent normal tissues. Wilcoxon signed rank test was used to compare the differences between tumor tissues and adjacent normal tissues. (F) Western blot analysis was performed to detect DLGAP5 protein expression in ovarian cancer cell lines (SKOV-3 and OVCAR3) and normal ovarian IOSE80 epithelial cells.  $^{**}P<0.01$  vs. the miR-NC group;  $^{***}P<0.001$  vs. the miR-NC group or IOSE80 cells. DLGAP5, discs large-associated protein 5; miR, microRNA; UTR, untranslated region; NC, negative control; WT, wild-type; MUT, mutant.

luciferase activity decreased in the DLGAP5 WT groups in SKOV-3 (Fig. 3B) and OVCAR3 (Fig. 3C) cells following transfection with miR-409-5p. Furthermore, DLGAP5 protein expression significantly decreased following overexpression

of miR-409-5p in both SKOV-3 and OVCAR3 cells (Fig. 3D). Notably, DLGAP5 mRNA expression was significantly upregulated in OC tumor tissues compared with adjacent normal tissues (Fig. 3E). Consistently, DLGAP5 protein

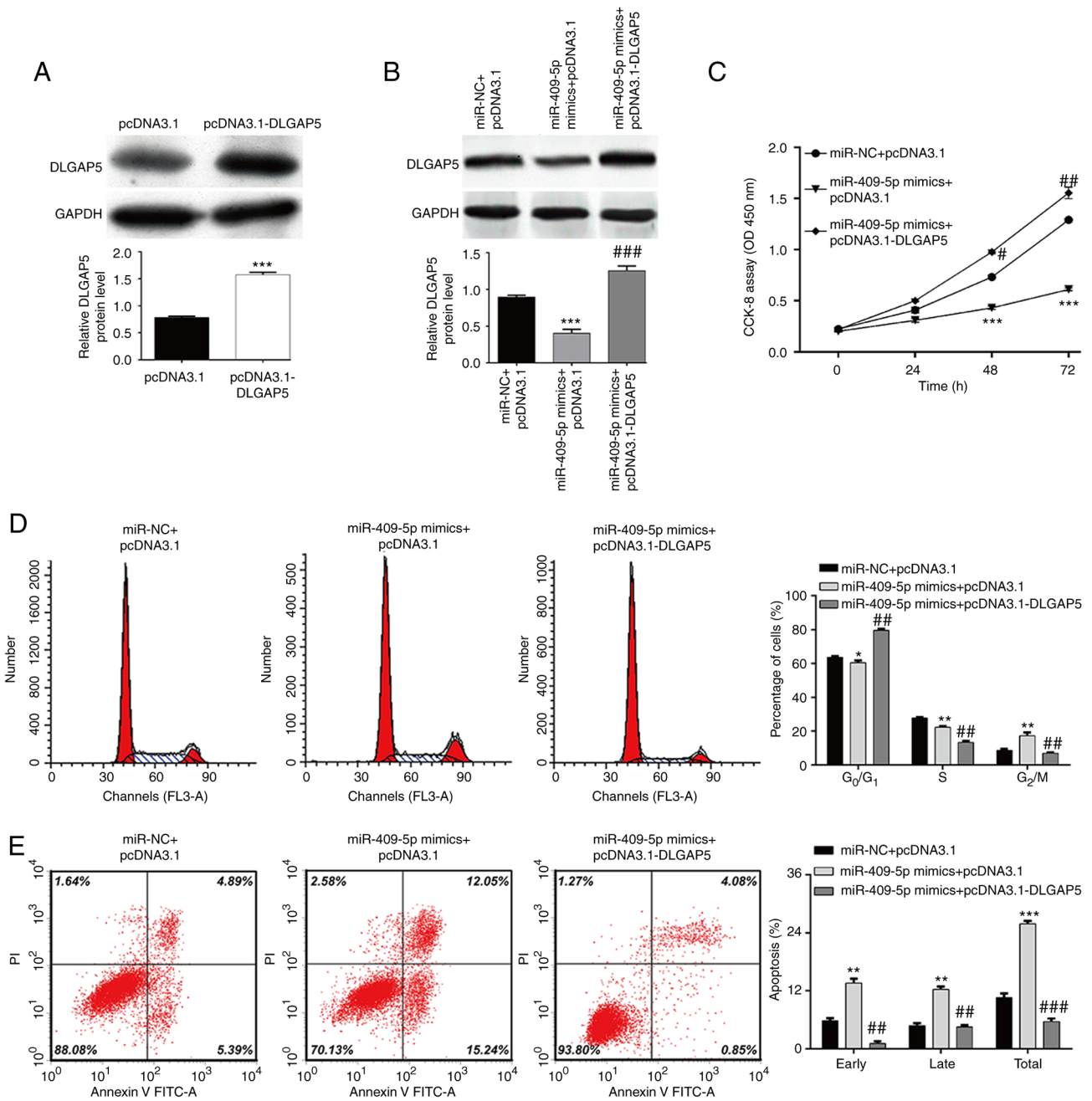


Figure 4. Restoration of DLGAP5 reverses the effects of miR-409-5p on ovarian cancer cell proliferation, cell cycle progression and apoptosis. SKOV-3 cells were transfected with pcDNA3.1-DLGAP5 alone or together with miR-409-5p mimics. (A and B) Western blot analysis was performed to detect the DLGAP5 protein expression in SKOV-3 cells in the different groups. (C) The CCK-8 assay was performed to assess cell proliferation. (D) The percentage of cells in the G<sub>0</sub>/G<sub>1</sub>, S and G<sub>2</sub>/M phases were analyzed in SKOV-3 cells in the different groups via flow cytometry with PI staining. (E) Early and late apoptotic cells were determined in SKOV-3 cells in the different groups via flow cytometry with Annexin V/PI staining. Data are presented as the mean ± standard deviation (n=3). \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 vs. the miR-NC + pcDNA3.1 group; #P<0.05, ##P<0.01, ###P<0.001 vs. the miR-409-5p mimics + pcDNA3.1 group. DLGAP5, discs large-associated protein 5; miR, microRNA; NC, negative control; PI, propidium iodide; CCK-8, Cell Counting Kit-8; OD, optical density.

expression was significantly upregulated in the OC cell lines (OVCAR3 and SKOV-3), compared with normal ovarian IOSE80 epithelial cells (Fig. 3F). Collectively, these results suggest that miR-409-5p negatively regulates DLGAP5 expression by binding to its 3'-UTR.

*Restoration of DLGAP5 abolishes the effects of miR-409-5p on OC cells.* To further investigate whether DLGAP5 expression is associated with miR-409-5p regulating OC cell functions, overexpression of DLGAP5 in SKOV-3 cells was confirmed

following transfection with pcDNA3.1-DLGAP5 (Fig. 4A). Subsequently, co-transfection with miR-409-5p mimics and pcDNA3.1-DLGAP5 was performed in SKOV-3 cells. As presented in Fig. 4B, miR-409-5p-induced downregulation of DLGAP5 protein expression was significantly abolished following co-transfection with miR-409-5p mimics and pcDNA3.1-DLGAP5. The results of the CCK-8 assay demonstrated that restoration of DLGAP5 expression in SKOV-3 cells stably expressing miR-409-5p reversed the inhibitory effect of miR-409-5p on cell proliferation (Fig. 4C). In addition, G<sub>2</sub>/M

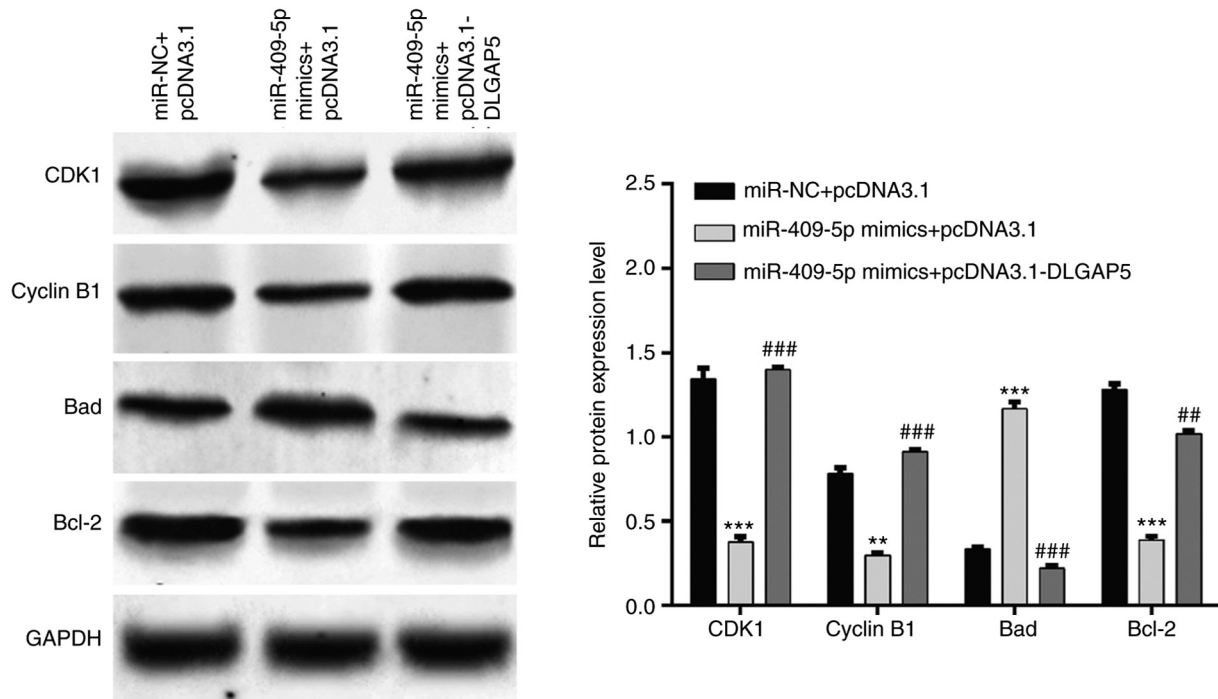


Figure 5. Restoration of DLGAP5 reverses the effects of miR-409-5p on related downstream protein levels. SKOV-3 cells were divided into three groups, miR-NC + pcDNA3.1; miR-409-5p mimics + pcDNA3.1 and miR-409-5p mimics + pcDNA3.1-DLGAP5. Western blot analysis was performed to detect the protein expression levels of CDK1, Cyclin B1, Bad and Bcl-2 in the three groups. Data are presented as the mean  $\pm$  standard deviation (n=3). \*\*P<0.01, \*\*\*P<0.001 vs. the miR-NC + pcDNA3.1 group; \*\*P<0.01, \*\*\*P<0.001 vs. the miR-409-5p mimics + pcDNA3.1 group. DLGAP5, discs large-associated protein 5; miR, microRNA; NC, negative control.

phase arrest (Fig. 4D) and elevated cell apoptosis (Fig. 4E) induced by miR-409-5p overexpression was significantly reversed following restoration of DLGAP5 in SKOV-3 cells. Western blot analysis further demonstrated that miR-409-5p overexpression induced-downregulation of CDK1, Cyclin B1 and Bcl-2 and upregulation of Bad were remarkably attenuated following restoration of DLGAP5 (Fig. 5). Taken together, these results suggest that restoration of DLGAP5 expression can reverse the effects of miR-409-5p on OC cell proliferation, G<sub>2</sub>/M phase arrest and apoptosis.

## Discussion

The results of the present study demonstrated that miR-409-5p expression was significantly downregulated in OC tissues and cell lines, which was associated with tumor size (P=0.0353) and FIGO stage (P=0.0024). Consistent with the results of the present study, downregulated miR-409-3p is associated with TNM stage and lymph node metastasis in human gastric cancer (15). miR-409-3p expression is also significantly downregulated in human bladder cancer (33), lung adenocarcinoma (34), colorectal cancer (35), breast cancer (36,37) and glioma (38). Contrary to the results of the present study, miR-409-3p/5p expression is upregulated in bone metastatic prostate cancer cell lines and human prostate cancer tissues, with higher Gleason scores (16). In addition, miR-409-5p is aberrantly upregulated in both breast cancer tumors and cell lines (17). miR-409-5p functions as an osteogenesis suppressor by targeting Lrp-8 as a positive effect of Wnt signaling (39). The controversial role of miR-409 in different types of cancer may be due to the different tissues and miRNA maturations.

Overexpression assays in the present study demonstrated that overexpression of miR-409-5p inhibited OC cell proliferation, and induced G<sub>2</sub>/M phase arrest and apoptosis. It has been speculated that miR-409 plays an essential role in tumor cell behaviors, including proliferation, cell cycle distribution and apoptosis. For example, miR-409-3p notably suppresses cell proliferation and promotes cell apoptosis in gastric cancer (40), osteosarcoma (41) and papillary thyroid carcinoma (42). The suppressive role of miR-409-3p on tumor cell proliferation has also been observed in breast cancer (36,43), glioma (38), tongue squamous cell carcinoma (44) and osteosarcoma (45). Orthotopic delivery of miR-409-3p/-5p appears to be an attractive therapeutic target for treating bone metastatic prostate cancer by facilitating tumor growth in the murine prostate gland (16). Yu *et al* (17) reported that lentiviral transduction-mediated downregulation of endogenous miR-409-5p expression suppresses MDA-MB-231 and MCF-7 cell proliferation and xenograft development.

Currently, Ras suppressor protein has been identified as a downstream target of miR-409-5p, which is involved in the development of breast cancer (17). The present study identified DLGAP5 as a novel target of miR-409-5p. Notably, overexpression of DLGAP5 abrogated the effects of miR-409-5p on the proliferation of SKOV-3 cells, G<sub>2</sub>/M phase arrest and apoptosis, as well as the regulation of CDK1, Cyclin B1, Bad and Bcl-2 expression levels. The results of the present study are consistent with previous findings, suggesting that silencing of DLGAP5 suppresses cell proliferation, induces G<sub>2</sub>/M phase arrest and apoptosis in hepatocellular carcinoma (27,28) and invasive breast cancer (29). DLGAP5 can promote spindle formation (46) and the cell cycle progression from M to G<sub>0</sub>/G<sub>1</sub> phase, which can be modulated by CDK1/Cyclin B (47). Thus, it is speculated that



miR-409-5p exerts its suppressive effects on OC cell proliferation via G<sub>2</sub>/M arrest and apoptosis by targeting DLGAP5.

Increasing evidence suggest that miRNAs function as important regulators affecting tumor development and progression (48,49). Consequently, antagonizing oncogenic miRNAs or restoration of tumor suppressive miRNAs can represent a reliable tool for improving cancer therapy, which suggests that miRNA-based treatment requires a careful choice of the potential target (50,51).

There are some limitations to the present study. There were only 2 OC cell lines were used, knockdown of miR-409-5p/DLGAP5 experiments are also missing, and *in vivo* experiments should be performed to confirm the results in future research.

In conclusion, the results of the present study demonstrated that overexpression of miR-409-5p downregulated DLGAP5 expression, which suppressed cell proliferation and induced G<sub>2</sub>/M phase arrest and apoptosis. These results suggest that stable overexpression of miR-409-5p may represent a promising approach to improve the treatment of patients with OC.

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### Availability of data and materials

All data generated and/or analyzed during this study are included in this published article.

### Authors' contributions

XJC designed the present study. All the authors confirmed the authenticity of all the raw data. WWL and JL performed the experiments. JFH and ZYC participated in literature research and collected the data. QYS and FY participated in data acquisition and data analysis. YF was involved in drafting the manuscript or revising it critically for important intellectual content. XY participated in data collection and drawing figures and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors have read and approved the final manuscript.

### Ethics approval and consent to participate

The present study was approved by the Institutional Ethics Committee of Mindong Hospital Affiliated to Fujian Medical University (Fujian, China; approval no. MHFM-39A) and performed in accordance with the Declaration of Helsinki. Written informed consent was provided by all patients prior to the study start.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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