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ORIGINAL ARTICLE

CDCA5 is negatively regulated by miR-326 and boosts ovarian cancer progression

Aihua Gao, Yanrong Hu, Weipei Zhu

Department of Obstetrics and Gynaecology, Second Affiliated Hospital of Soochow University, Suzhou 215004, PR China.

Summary

Purpose: *Ovarian cancer (OC) is the fifth leading cause of cancer death in women and one of the most prevalent malignancies in humans. Therefore, improved methods for OC early detection are urgently needed. Research has demonstrated that microRNAs (miRs) are strongly linked to OC tumorigenesis. The potential regulatory mechanism regarding miR-326 in OC is undefined. The present study was aimed to explore miR-326 expression in OC and its roles in OC progression.*

Methods: *Qualitative (q)RT-PCR was adopted to examine miR-326 expression in OC tissues and cell lines in clinical samples. qRT-PCR and Western blot were applied to detect division cell cycle associated 5 (CDCA5) expression at the messenger RNA (mRNA) and protein levels. CCK-8 and Transwell invasive experiments were utilized to examine cell proliferation and invasion. Cell cycle and apoptosis were analyzed by flow cytometry. Online bioinformatics analysis was employed to predict the target genes of miR-326 and luciferase reporter genes were applied for validation.*

Results: *MiR-326 was remarkably down-regulated in OC tissues and cell lines relative to the corresponding adjacent normal tissues and normal human ovarian surface epithelial cells (HOSE). MiR-326 overexpression markedly restrained the proliferation and invasion of OC cells, whereas miR-326 inhibitors exerted the contrary effect. Additionally, miR-326 up-regulation in OC cells prevented cells from entering S-phase and enhanced apoptosis, a phenomenon that may be due to CDCA5 down-regulation. Furthermore, we proved that CDCA5 was a downstream target of miR-326.*

Conclusions: *MiR-326 represses the proliferation and invasion of OC cells and enhances apoptosis by specifically modulating CDCA5 expression.*

Key words: *apoptosis, CDCA5, cell cycle, miR-326, ovarian cancer*

Introduction

Ovarian cancer (OC) is one of the most prevalent malignancies among women worldwide, with approximately 230,000 new cases each year and approximately 140,000 deaths each year [1]. Despite many advances made in surgical techniques and chemotherapy, the prognosis of patients is still not optimistic due to lack of reliable early-stage diagnosis and treatment methods and the later development of resistance to standard chemotherapies (e.g., platinum-based drugs) [2]. Therefore, it is imperative to probe new therapeutic methods and targets to prevent or reduce drug resistance and thus improve the efficiency of OC treatment.

miRs are tiny endogenously expressed non-coding single-stranded RNAs, approximately 19-25 nucleotides in length, that negatively regulate gene expression by binding to the 3'UTR of target messenger RNAs (mRNAs), resulting in either translation inhibition or mRNA degradation [3]. Previous studies have pointed out the potential role of miRs in cancer and implied that abnormal expression of miRs may be implicated in regulating tumorigen-

Corresponding author: Weipei Zhu, PhD. Department of Obstetrics and Gynaecology, Second Affiliated Hospital of Soochow University, No.1055 Sanxiang Rd, Suzhou 215004, PR China.
Tel: +86-21-38297582, Email: 13906135277@163.com
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esis and progression by modulating cancer-related genes and pathways related to cancer pathogenesis [4]. MiR-326 has been shown in several studies to be linked to embryonic development, immune response, inflammation, tumorigenesis, invasion, metabolism, apoptosis, tumor growth, chemoresistance, and autophagy [5-7]. For instance, miR-326 is markedly down-regulated in gastric cancer tissues and is related to adverse prognosis. Mechanistic research has demonstrated that miR-326 restrains cell proliferation and metastasis by specifically regulating Fascin1 (FSCN1) expression [5]. Furthermore, miR-326 exerts a crucial effect in non-small cell lung cancer (NSCLC) by specifically regulating Cyclin D1 (CCND1) to repress cell proliferation, migration, invasion, and enhance apoptosis [8]. Additionally, miR-326 exerts a tumor-suppressing effect in gynecological malignancies such as breast cancer and endometrial cancer (EC), suggesting that miR-326 may be endowed with the potential to be a novel diagnostic target [9, 10]. Nevertheless, little is still known about the mechanism of miR-326 in OC.

Cell division is a key process in the biological process. Research has revealed that the cell cycle division-associated (CDCA) protein family exerts a vital effect in regulating cell cycle progression. For instance, CDCA1 is implicated in the regulation of nuclear division and microtubule stability [11]. CDCA2 functions by binding to protein phosphatase 1γ (PP1γ) and controlling the DNA damage response during the cell cycle [12,13]. CDCA4 is a cell cycle regulator related to G1/S conversion, and simultaneously it can participate in the regulation of p53 expression [14,15]. In contrast, CDCA5 is initially identified as a substrate to facilitate the synthesis of complexes and as a key regulator of the cohesion and segregation of sister chromatids during the cell cycle [16]. It has been shown that knocking down CDCA5 markedly suppresses cancer cell cycle progression and thus impedes tumor growth [17,18]. Clinical research has also illustrated that CDCA5 expression is remarkably increased in diverse tumor tissues and is linked to unfavorable prognosis [17,19]. Hence, CDCA5 may exert an important specific role in tumor progression and is likely to become a potential molecular prognostic biomarker for cancer therapy. Nonetheless, the mechanism by which CDCA5 is regulated in OC is undefined.

In this work, qRT-PCR was applied to detect miR-326 expression in clinical specimens and OC cell lines. Subsequently, we detected the effects of miR-326 on proliferation, invasion and apoptosis of OC cells by gain-of-function or loss-of-function experiments. Thereafter, the potential mechanism of miR-326 in OC progression was explored using bioinformatics analysis and luciferase reporter gene experiments. Ultimately, a compensation experiment was applied to confirm the effect of the miR-326/CDCA5 axis on the development of OC carcinogenesis.

Methods

Clinical specimen collection and cell line culture

Nine pairs of primary OC tissues and matched tumor-adjacent normal tissues preserved from June 2017 to April 2019 were collected at the Second Affiliated Hospital of Soochow University. This work was endorsed by the Ethics Committee of the Hospital. All subjects provided written informed consent.

Human OC cell lines ES-2, SKOV-3, A2780, OCVAR5 and HOSE were procured from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, HyClone, USA) containing 10% fetal bovine serum (FBS) (HyClone, USA) and 100 mL penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) in incubator at 37°C, with 5% CO_2 and 95 % humidity.

Transfection

CDCA5 overexpression plasmid (CDCA5) and its control (pcDNA), small interference RNA (siRNA) targeting CDCA5 (si-CDCA5) and its negative control (si-NC), miR-326 mimic (miR-326) and its negative control (miR-NC), miR-326 inhibitor (anti-miR-326) and its control (NC) were purchased from GenePharma Co., Ltd. (Shanghai, China). OC cells were transfected with plasmids, siRNAs, miRs or miR inhibitors using Lipofectamine® 2000 (Invitrogen, Carlsbad, CA, USA).

RNA isolation and qRT-PCR

The total RNA in OC tissues and cell lines was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). To detect miR-326 expression, qRT-PCR was conducted in a Bio-Rad CFX96 RT-PCR system (Bio-Rad, Hercules, CA, USA) using Taqman probes (Invitrogen, Carlsbad, CA, USA) under the guidance of the instructions. U6 served as an internal reference. For the detection of CDCA5 mRNA expression, the RNA was reverse-transcribed using PrimeScript RT Master Mix (Perfect Real Time, TaKaRa, Dalian, China). qRT-PCR was conducted on a Bio-Rad CFX96 RT-PCR system using SYBR Premix ExTaq II (TliRNaseH Plus) (TaKaRa, Dalian, China). GAPDH worked as an internal reference. Data were processed using the $2^{-\Delta\Delta CT}$ method.

MTT

The MTT experiment was conducted for drug sensitivity detection using Cell Proliferation Kit I (Roche, Mannheim, Germany) according to the manufacturer's instructions. Concisely, 1.0×10^4 cells were seeded into 96-well plates in 100 µL DMEM with reagents. An equal volume of DMSO was used as control. The absorbance of Formazan products was measured at 570 nm and the reference wavelength was 690 nm.

Cell invasion analysis

The upper surface of the filter was coated with Matrigel, 600 μL of solution was supplemented to the bottom of the compartment, and serum-free medium containing tumor cells was supplemented to the top. After 24 h of incubation, serum-free medium was aspirated, adherent cells on the upper surface were scraped off, cells were fixed with 100% methanol, and crystal violet staining was carried out. Cells invading Matrigel in 3 random fields of view were counted.

Cell cycle analysis

Cells were collected and trypsinized, rinsed in cold phosphate-buffered saline (PBS), and fixed in 80% cold ethanol in PBS. Prior to staining, cells were precipitated in a frozen centrifuge and resuspended in cold PBS. A final concentration of bovine pancreatic ribonuclease (Sigma-Aldrich) was supplemented at 2 mg/mL, and the cells were incubated at 37°C for 30 min, followed by incubation with 20 mg/mL propyl iodide ingot (Sigma-Aldrich) for 20 min. The cell cycle was measured using flow cytometry (BD Biosciences, San Jose, CA, USA).

Dual-luciferase reporter gene experiment

The 3'UTR sequence and the mutated 3'UTR sequence of the CDCA5 gene were cloned into the pGL3.0 plasmid to construct the dual-luciferase reporter vectors (the wild-type CDCA5-3'UTR (CDCA5-wt) and the mutant CDCA5 -3'UTR (CDCA5-mut). The vector was cotransfected into OC cells with miR-326 mimics or inhibitors, and fluorescence intensity was detected after 48 h of incubation using the dual-luciferase assay kit (Promega, Madison, WI, USA).

Western blot

Cells were lysed in RIPA lysis buffer (Sigma, St. Louis, MO, USA). Protein lysates were prepared and protein concentrations were measured using the bicinchoninic acid (BCA) protein assay kit (Beijing, China). Equal amounts of protein specimens were loaded onto 10% SDS-PAGE, transferred to PVDF membranes, and blocked with 5% skim milk. The membrane was incubated overnight with anti-CDCA5 (1:1000, ab192237, ABCAM, Cambridge, UK) and anti-GAPDH (1:2000, ab181602, ABCAM, Cambridge, UK), rinsed and incubated with horseradish peroxidase-conjugated antibody (Sigma-Aldrich) for 1.5 h. Following the protocol, the blotted protein was examined using an improved chemiluminescence (ECL) system.

Flow cytometry

Cells were transfected for 24 h and then cultured in serum-free medium for 24 h. Cells were collected and the buffer was added to resuspend the cells, the cell density was modulated to 1-5×10^6 cells/mL. 0.1mL of cell suspension was taken and tested after Annexin V-FITC/propidium iodide (PI) double staining under the manufacturer's instructions. The data were processed using Flow Jo software.

Statistics

The data were represented as the mean±standard deviation (SD) of three independent experiments, and were analyzed by SPSS 18.0 software. A t-test was adopted for comparisons between two groups and one-way ANOVA was exploited for comparisons among multiple groups. P<0.05 signified statistical significance.

Results

MiR-326 expression was remarkably down-regulated in OC tissues and cell lines

To elaborate on the role of miR-326 in OC, miR-498 expression in OC tissues and cell lines was assessed by qRT-PCR. As exhibited in Figure 1A, miR-326 expression was remarkably reduced in OC tissues relative to the normal group. Furthermore, miR-326 expression was remarkably reduced in OC cell lines as opposed to HOSE cells (Figure 1B). Briefly, miR-326 was aberrantly downregulated in OC tissues and cell lines.

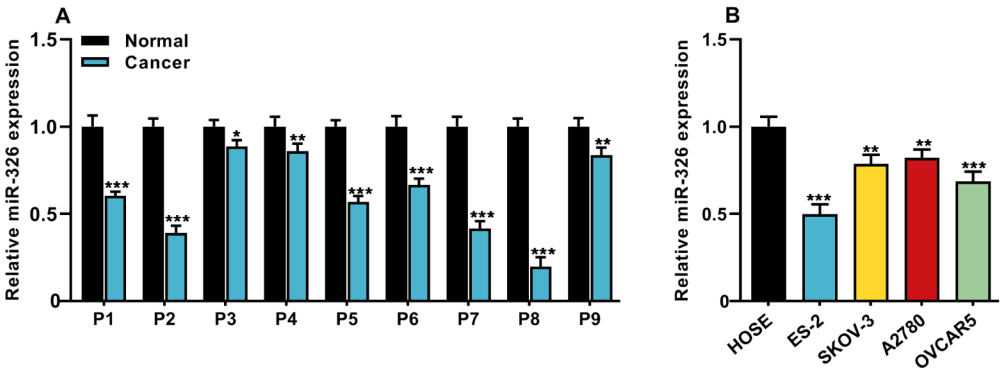

Figure 1. Significantly down-regulated expression of miR-326 in OC tissues and cell lines. **A:** The relative expression of miR-326 in nine groups of OC tissues and matched tumor-adjacent normal tissues were detected by qRT-PCR. **B:** qRT-PCR analyzed miR-326 expression in HOSE cells and OC cell lines (ES-2, SKOV-3, A2780 and OCVAR5) (*p<0.05, **p<0.01, ***p<0.001).

MiR-326 restrained the proliferation and invasion of OC cells in vitro and enhanced apoptosis

To decipher the mechanistic role of miR-326 in ovarian carcinogenesis, miR-326 mimics (miR-326) or its blank control (miR-NC) was transfected into ES-2 cell lines for miR-326 overexpression experiments. The efficiency of miR-326 transfection was verified using qRT-PCR, and the results revealed that miR-326 mimics markedly increased miR-326 expression in the ES-2 cell line (Figure 2A). To evaluate the potential role of miR-326 in OC tumorigenesis, the MTT method was applied to determine whether miR-326 affected cell proliferation in OC. The results demonstrated that the proliferation of ES-2 cells was remarkably impeded after transfection with miR-326 mimics (Figure 2B). Additionally, invasion experiments exhibited that miR-326 mimics remarkably repressed the invasion of ES-2 cells (Figure 2C). Through flow cytometry detection, it was discovered that miR-326 overexpression markedly reduced the percentage of S phase and remarkably increased the percentage of G1 phase (Figure 2D). Meanwhile, miR-326 overexpression markedly enhanced the apoptosis of ES-2 cells (Figure 2E). Overall, the findings implied that miR-326 reduced the tumorigenicity of OC cells *in vitro*.

MiR-326 inhibition facilitated cell proliferation and invasion, but restrained apoptosis in vitro

MiR-326 inhibitor (anti-miR-326) was transfected into the A2780 cell line. The data of qRT-PCR experiment manifested that miR-326 inhibitor remarkably decreased miR-326 expression in A2780 cells (Figure 3A). We then analyzed the inhibitory effects of miR-326 expression on A2780 cell proliferation, invasion, cell cycle progression and apoptosis. The results illustrated that inhibition of miR-326 remarkably facilitated cell proliferation (Figure 3B) and invasion (Figure 3C). Simultaneously, inhibition of miR-326 markedly increased the percentage of G1 phase and decreased the

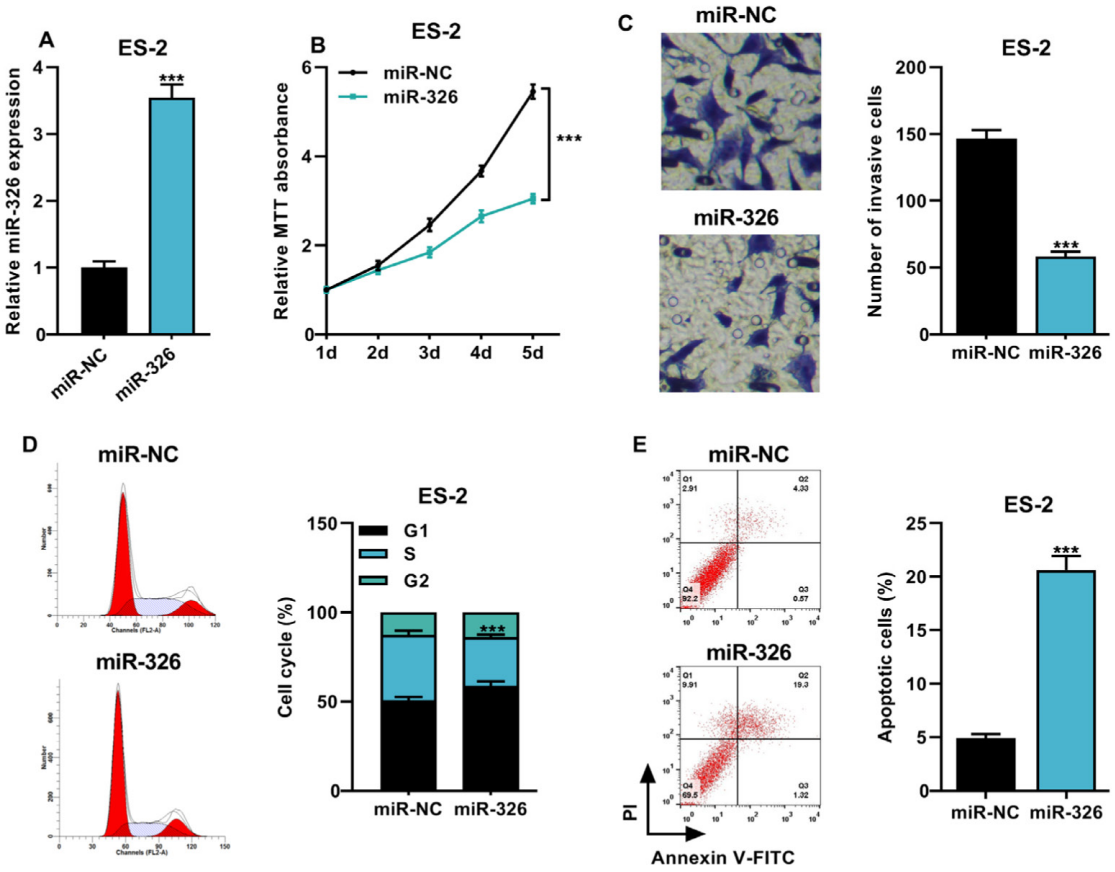

Figure 2. MiR-326 repressed proliferation and invasion of OC cells *in vitro* and enhanced OC cell apoptosis. **A:** MiR-NC or miR-326 mimics were transfected with ES-2 cells for 24 h. qRT-PCR was adopted to determine the efficiency of miR-326 overexpression in ES-2 cells. **B:** MTT was used to determine the effect of miR-NC or miR-326 mimics on ES-2 cell proliferation. **C:** Invasion assays were performed to determine cell invasion after transfecting ES-2 cells with miR-NC or miR-326 mimics. **D:** Flow cytometry was exploited to detect the distribution of cell cycle after miR-NC or miR-326 mimics transfected into ES-2 cells. **E:** Flow cytometry was used to detect the apoptosis of ES-2 cells after miR-NC or miR-326 mimics were transfected (*p<0.05, **p<0.01, ***p<0.001).

percentage of S phase in A2780 cells (Figure 3D). Furthermore, miR-326 inhibition remarkably suppressed the apoptosis of ES-2 cells (Figure 3E).

miR-326 directly and specifically regulated CDCA5 expression in OC cells

Since the role of miRs in tumor development depends on targeting their crucial target genes, identification of targets of miR-326 is crucial. The miRDB, miRanda and TargetScan databases were utilized to predict the target genes for miR-326. Among them, a potential target binding site for CDCA5 and miR-326 was discovered. Given that CDCA family genes were shown to be linked to prognosis in OC patients, we selected them as target genes for followed-up research [20]. Initially, we tested the relative expression of CDCA5 in nine clinical specimens and found that the CDCA5 mRNA expression in OC patients was remarkably higher as opposed to that in the corresponding adjacent normal group (Figure 4A). Moreover, Pearson's correlation analysis illustrated that miR-326 expression in the specimens was remarkably and negatively related to CDCA5 expression (Figure 4B). Subsequently, to verify that CDCA5 was a direct target of miR-326, pGL3-CDCA5 3'UTR wild-type (CDCA5-wt) and mutant (CDCA5-mut) reporter gene vectors (Figure 4C) were constructed. The dual-luciferase reporter gene experimental results demonstrated that the cotransfection of miR-326 significantly increased the luciferase activity of CDCA5-wt, but exerted no effect on the luciferase activity of CDCA5-mut in ES-2 cells (Figure 4D). Meanwhile, A2780 cells transfected with anti-miR-498 restrained the luciferase activity of CDCA5-wt, but did not affect the CDCA5-mut gene (Figure 4E). To determine whether miR-326 could reduce endogenous CDCA5 expression, we examined the changes in CDCA5 expression after ES-2 transfection with miR-326 mimics and A2780 transfection with miR-326 inhibitors. The data revealed that miR-326 overexpression remarkably

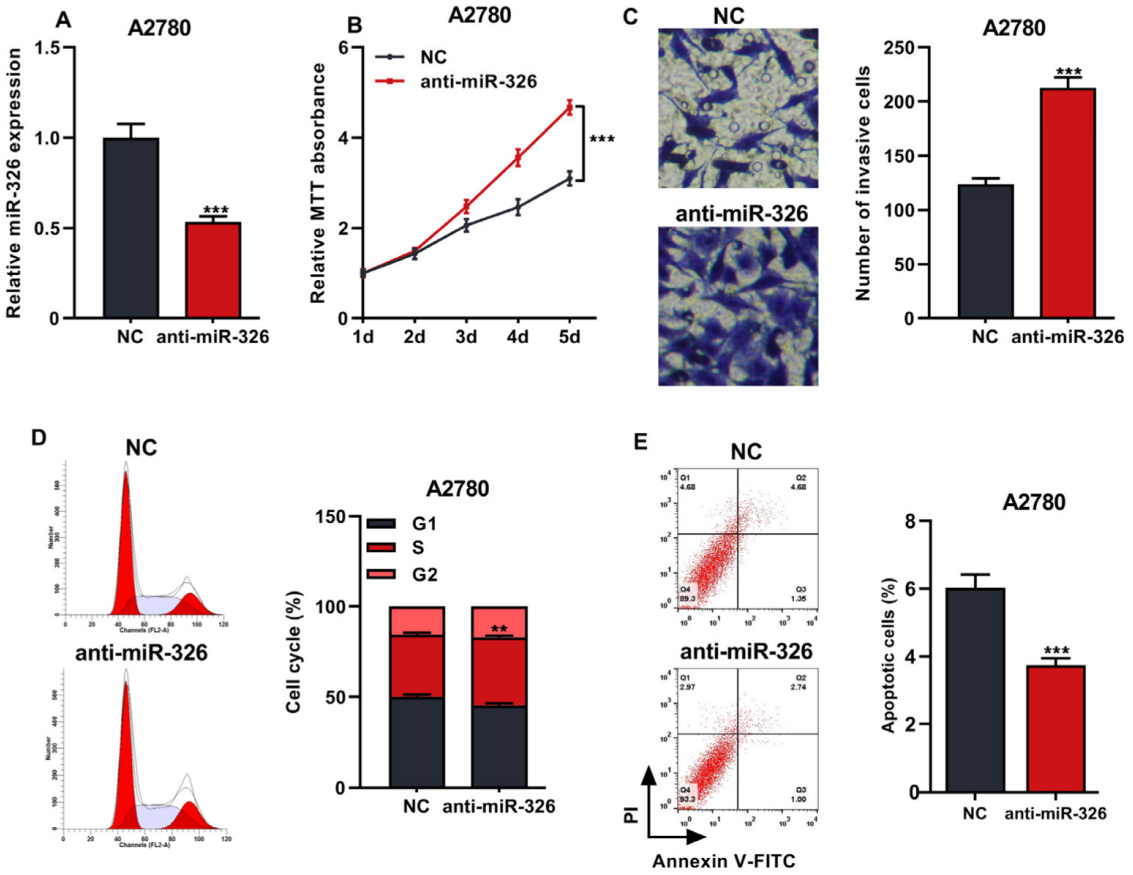

Figure 3. *In vitro* inhibition of miR-326 enhanced cell proliferation and invasion, but suppressed apoptosis. **A:** miR-326 inhibitor (anti-miR-326) and its control (NC) were transfected with A2780 cells for 24 h. qRT-PCR was used to determine miR-326 expression in A2780 cells. **B:** MTT assay was employed to examine the effect of anti-miR-326 or NC on the proliferation of A2780 cells. **C:** Invasion assay was applied to determine cell invasion after transfecting A2780 cells with anti-miR-326 or NC. **D:** Flow cytometry was used to detect cell cycle distribution after transfecting anti-miR-326 or NC into A2780 cells. **E:** Flow cytometry was used to detect the apoptosis of A2780 cells after anti-miR-326 or NC transfection (*p<0.05, **p<0.01, ***p<0.001).

suppressed CDCA5 mRNA expression and protein levels, whereas miR-326 inhibitor enhanced CDCA5 expression (Figures 4F,G). The above results signified that CDCA5 may be a direct target of miR-326 and that its expression was modulated by miR-326.

CDCA5 restored the effects of miR-326 on OC cell proliferation, invasion and apoptosis

Compensation experiments were conducted to confirm the involvement of the miR-326/CDCA5 axis in the regulation of proliferation, invasion and apoptosis in OC cells. qRT-PCR and Western blot were adopted to examine the transfection efficiency of CDCA5 overexpressing plasmid transfected ES-2 cells. The results indicated that the CDCA5 overexpression plasmid markedly increased CDCA5 mRNA and protein expression in ES-2 cells (Figures 5A,B). Moreover, MTT and invasion experiments uncovered that miR-326 overexpression remarkably repressed the proliferation and invasion of ES-2 cells, but the proliferation and invasion of ES-2 cells were partially restored by CDCA5 overexpression (Figures 5C,D). Flow cytometry was employed to detect the cell cycle and apoptosis of ES-2 cells. The results revealed that miR-326 mimics remarkably enhanced ES-2 cells to reside in G1 phase, while the proportion of cells in S phase decreased and accelerated ES-2 cell apoptosis; conversely, after ES-2 cells were transfected with the CDCA5 overexpression plasmid, the proportion of ES-2 cells in S phase increased and the proportion of apoptosis decreased (Figures 5E,F). In addition, we successfully restrained CDCA5 expression at the mRNA and protein levels by transfecting si-CDCA5 into A2780 cells (Figures 5G,H). The miR-326 inhibitor markedly enhanced the proliferation and invasion of A2780 cells (Figures 5I,J). Furthermore, by down-regulating miR-326 expression, the proportion of A2780 cells in the S-phase increased remarkably, while the proportion in G1 phase decreased and the proportion of apoptosis decreased (Figures 5K,L). When A2780 cells were transfected with si-CDCA5, cell proliferation, invasion, cycle progression, and apoptosis were all partially reversed (Figures 5I-L). The results implied that miR-326 repressed the proliferation and invasion of OC cells and enhanced apoptosis through the miR-326/CDCA5 axis.

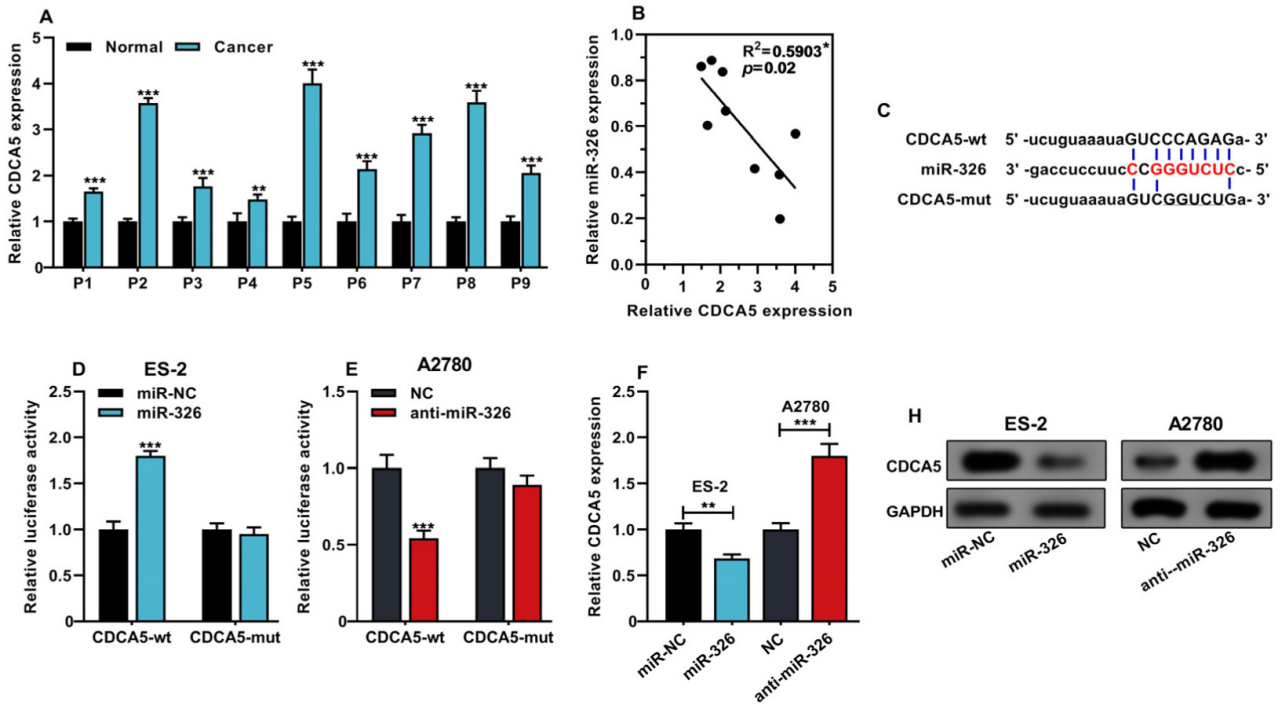

Figure 4. MiR-326 directly and specifically regulated CDCA5 expression in OC cells. **A:** Relative expression levels of CDCA5 in nine groups of ovarian cancer tissues and matched tumor-adjacent normal tissues were measured by qRT-PCR. **B:** Pearson's correlation analysis analyzed the relationship between miR-326 and CDCA5 expression in clinical tissue samples. **C:** MiR-326 target genes were predicted using the online bioinformatics database. **D, E:** Dual-luciferase was used to verify the targeted binding site of miR-326 to CDCA5. **F:** qRT-PCR was applied for detecting mRNA expression of CDCA5 after transfecting ES-2 cells with miR-326 mimics and its control (miR-NC) or transfecting A2780 cells with miR-326 inhibitor (anti-miR-326) and its control (NC). **G:** Western blot was conducted to examine CDCA5 protein expression in ES-2 cells transfected with miR-326 mimics and its control (miR-NC) or A2780 cells transfected with miR-326 inhibitor (anti-miR-326) and its control (NC) (*p<0.05, **p<0.01, ***p<0.001).

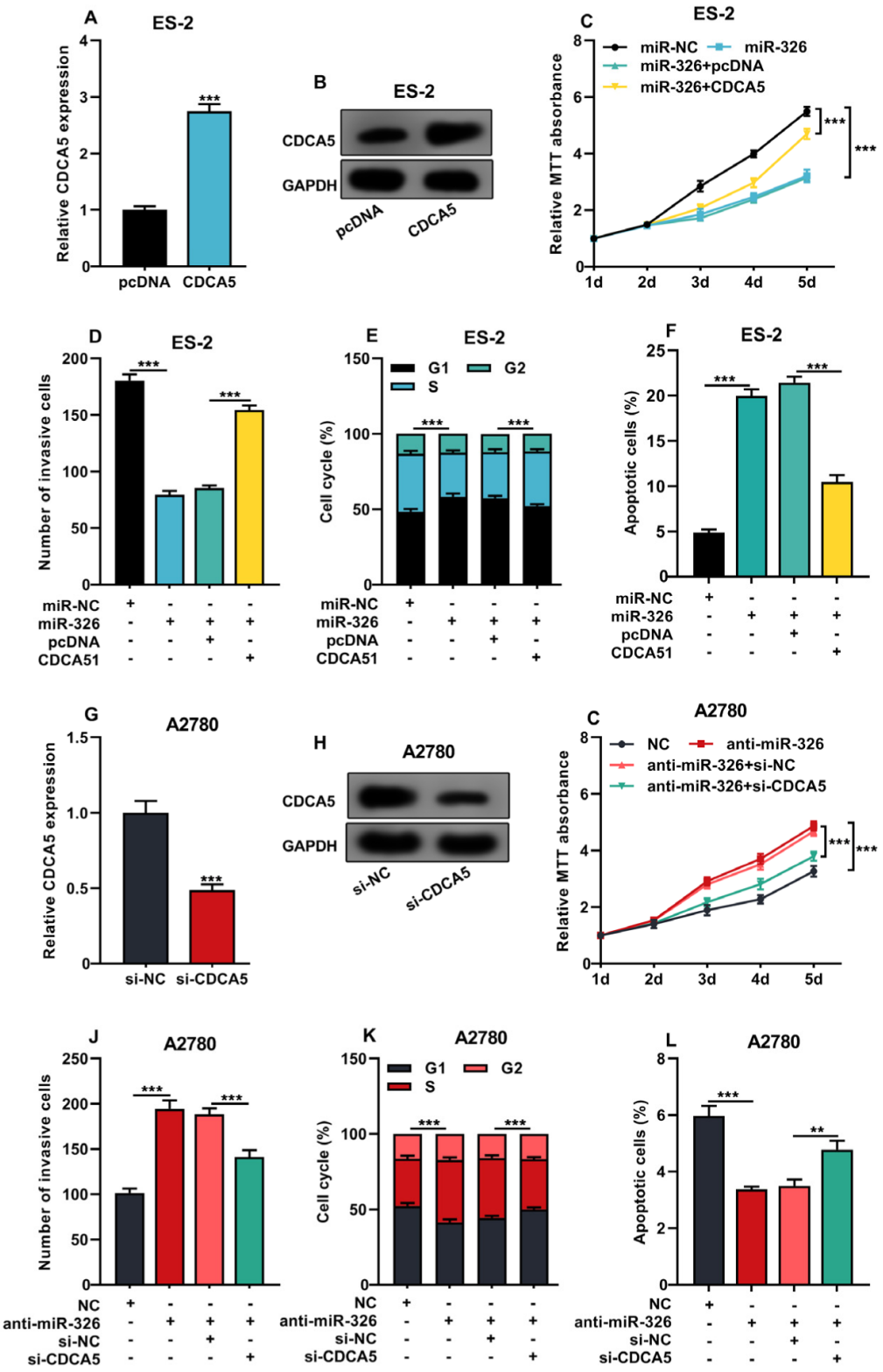

Figure 5. CDCA5 restored the effects of miR-326 on proliferation, invasion and apoptosis of OC cells. **A,B:** qRT-PCR and Western blot were used to detect the expression changes of CDCA4 after ES-2 cells were transfected with CDCA4 overexpression plasmid. **C-F:** ES-2 cells were transfected with miR-NC, miR-326 mimics, miR-326+pcDNA, and miR-326+CDCA5 overexpression plasmids. **C:** MTT was employed to detect cell proliferation after transfection. **D-F:** Invasion assay was used to detect changes in cell invasion after transfection, and flow cytometry was adopted to detect the cell cycle and apoptosis of ES-2 cells after transfection. **J, K:** qRT-PCR and Western blot were used to detect changes in the expression of CDCA4 after transfecting A2780 cells with si-CDCA4. **I-L:** A2780 cells were transfected with NC, anti-miR-326 mimics, anti-miR-326+si-NC, and anti-miR-326+si-CDCA5. MTT detected cell proliferation after transfection. **I:** Invasion assay was utilized to detect changes in cell invasion after transfection. **J-L:** Flow cytometry was employed to detect cell cycle and apoptosis in A2780 cells after transfection (**p<0.01, ***p<0.001).

Discussion

This work experimentally proved that miR-326 was markedly down-regulated in OC cell lines and tumor tissues relative to normal HOSE cells and normal ovarian tissues. Furthermore, we demonstrated that miR-326 impeded the proliferation, invasion and cell cycle processes of OC cells and promoted apoptosis. Besides, CDCA5 was validated to be a direct target of miR-326 in OC. Therefore, we hypothesized that miR-326 was implicated in regulating the development of OC by negatively regulating CDCA5 expression.

Research has revealed that miRs are engaged in the regulation of biological processes including cell proliferation, apoptosis, and metabolism. There is growing evidence that aberrantly expressed miRs are closely linked to diverse malignancies, including OC, and are considered promising biomarkers for clinical applications. Akira Yokoi adopts microarrays to conduct miR expression profiling on 4046 serum samples including 428 OC patients, and constructs a diagnostic model based on ten miR expression to provide a scientific basis for early screening and diagnosis of ovarian cancer (OS) [21]. Besides, research has revealed that miR-99a-5p is significantly enhanced in the serum of OC patients, and the mechanism indicates that exosomal miR-99a-5p from EOC cells facilitates cell invasion by affecting HPMCs through fibronectin and vitronectin upregulation [22]. MiR-326 is validated to exert a crucial effect in cell development, differentiation, metastasis and invasion [23]. For instance, miR-326 is markedly down-regulated in glioblastomas (GBM) cells, and miR-326 overexpression can remarkably suppress cell proliferation and cell migration. In addition, miR-326 expression is restrained by abnormal PI3 kinase signaling [24]. In this work, we similarly observed the remarkable down-regulation of miR-326 expression in OC tissues and cell lines. Additionally, miR-326 significantly enhanced the proliferation and invasion of OC cells. Besides, miR-326 overexpression led to cell cycle arrest at G1 phase in OC cells and resulted in increased apoptosis. Down-regulation of miR-326 expression exerted the opposite effect. The findings suggest that miR-326 is implicated in regulating OC progression.

Notably, miRs regulate their gene expression by targeting complementary regions of bound mRNAs, thereby repressing their translation or regulating their degradation [25]. This means that other genes regulated by miR-326 may also contribute to OC. For instance, miR-326 is down-regulated in gastric cancer, which can restrain GC cell proliferation and induce GC cell G2/M arrest by targeting and regulating NOB1 [26]. In addition, miR-326 targets and regulates the gene network constituted by EGFR, ErbB2, ErbB3, AKT1, AKT2, and AKT3, thereby impeding the ErbB/PI3K pathway and thus exerting an inhibitory effect in breast cancer [27]. In this work, we identified a target binding site for CDCA5 and miR-326 by online bioanalysis. Research has illustrated that CDCA5 is implicated in the progression of diverse human cancers. CDCA5 is up-regulated in tissues of patients with urothelial carcinomas of the upper urinary tract and urinary bladder and is linked to unfavorable prognosis [28]. CDCA5 activates ERK signaling and promotes colorectal cancer cell proliferation via facilitating the phosphorylation of ERK1/2 and c-jun expression [29]. The present research likewise reveals that CDCA5 is markedly upregulated in OC tissues and cells. Dual-luciferase reporter gene experiments confirm that miR-326 can bind to CDCA5 3'-UTR. Furthermore, CDCA5 can reverse the effect of miR-326 on the proliferation, invasion and apoptosis of OC cells.

In conclusion, this work demonstrates that miR-326 is lowly expressed in OC. Besides, miR-326 restrains OC proliferation, migration, and cell cycle progression through specifically modulating CDCA5 and enhancing cell apoptosis, and the findings imply that miR-326 may act as a prospective target for early diagnosis and OC therapy in the future.

Acknowledgements

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Conflict of interests

The authors declare no conflict of interests.

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