miR-210-3p regulates cell growth and affects cisplatin sensitivity in human ovarian cancer cells via targeting *E2F3*

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Abstract. The potential role of microRNA (miR)-210-3p in carcinogenesis and the cisplatin sensitivity of ovarian cancer were evaluated in the present study. The relative expression levels of miR-210-3p in cisplatin-sensitive SKOV-3 cells and cisplatin-resistant SKOV-3/DDP cells were determined using reverse transcription-quantitative polymerase chain reaction analysis. miR-210-3p mimics and inhibitors were transfected into SKOV-3/DDP cells. Cell Counting Kit-8, scratch and Transwell invasion assays and flow cytometry were conducted to evaluate the role of miR-210-3p in ovarian cancer cells. A luciferase reporter assay was used to verify the association between miR-210-3p and E2F transcription factor 3 (*E2F3*). Drug sensitivity was evaluated by treating the cells with cisplatin. The expression level of miR-210-3p was lower in SKOV-3/DDP cells than in SKOV-3 cells. Compared with the untransfected control, SKOV-3 cells transfected with miR-210-3p exhibited a significantly higher survival rate. The overexpression of miR-210-3p inhibited SKOV-3/DDP cell proliferation, migration and invasion, and promoted cell apoptosis. By contrast, the inhibition of miR-210-3p promoted cell migration and invasion. The luciferase reporter assay confirmed that *E2F3* was a direct target gene of miR-210-3p. Cisplatin treatment resulted in a sharp decrease in the survival rate of SKOV-3/DDP cells transfected with the miR-210-3p mimics. The decrease in cell survival rate caused by the overexpression of miR-210-3p was rescued by the overexpression of *E2F3* in SKOV-3/DDP cells. Taken together, these results suggest that miR-210-3p may act as a tumor suppressor in ovarian cancer cells and affect the sensitivity of cells to cisplatin by directly targeting *E2F3*. This indicates its potential use as a therapeutic target for improving drug resistance in ovarian cancer.

Introduction

Ovarian cancer is one of the most common types of cancer in women and the most life-threatening gynecologic tumor, which led to ~151,900 cases of mortality worldwide in 2012 (1,2). There are no obvious clinical symptoms in the early stages of this disease. Although the international promotion of treatment protocols consisting of surgery and neoadjuvant chemotherapy have significantly improved the survival rate of patients with ovarian cancer, the 5-year-survival rate remains <45% (3). Drug resistance is responsible for the failure of platinum-based chemotherapy, wherein patients initially respond to these agents but relapse ~6 months following their initial chemotherapy (4). Therefore, there is an urgent need to understand the multiple mechanisms underlying the progression of ovarian cancer and chemotherapy drug resistance to develop effective targeted therapies.

As a type of gene regulatory factor, microRNAs (miRNAs) are endogenous, non-coding RNAs with a length of ~22 nucleotides that are widely involved in physiological processes, including cell growth and replication (5). miRNAs specifically bind to the 3'-untranslated region (UTR) of the target mRNAs (6). In 2006, Chan and Loscalzo (7) found that miRNAs are involved in ovarian cancer oncogenesis, and numerous subsequent studies have demonstrated the importance of miRNAs in cell development, invasion, apoptosis and drug sensitivity in ovarian cancer. For example, miR-7 exhibited specific methylation in resistant cell lines and was associated with poor prognosis in patients with ovarian cancer (8); miR-509-3p sensitized ovarian cancer cells to cisplatin treatment by targeting anti-apoptotic genes, including *MCL1*, B-cell lymphoma 2 (*BCL2*) and *BCL2L2* (9); the overexpression of miR-630 promoted SKOV3 cell proliferation and migration (10); and the overexpression of miR-18b was associated with the metastasis of ovarian cancer cells via phosphatase and tensin homolog (PTEN) (11). In addition, the E2F transcription factor 3 (*E2F3*) has been shown to act as a crucial protein in the cell cycle process and is a known target of miR-210-3p. Studies have shown that *E2F3* is downregulated at the protein level upon induction of the expression of miR-210 in human ovarian cancer (12).

The present study aimed to characterize the expression level of miR-210-3p between a cisplatin-resistant human

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ovarian cancer cell line (SKOV-3/DDP) and a cisplatin-sensitive cell line (SKOV-3) to evaluate the regulatory mechanisms of miR-210-3p and its target gene, *E2F3*, in the carcinogenesis and cisplatin sensitivity of SKOV-3/DDP cells.

Materials and methods

Cell lines, culture conditions and miRNA transfection. The 293T human embryonic kidney cell line was purchased from the Chinese Academy of Sciences (Beijing, China) and was cultured in Dulbecco's modified Eagle's medium (DMEM; 10-013-CVR; Corning Incorporated, Corning, NY, USA) for conducting a luciferase reporter assay. The SKOV-3/DDP cell line was selected for the present study following referral to previous studies (13). The SKOV-3 cisplatin-sensitive human ovarian cancer cell line and the SKOV-3/DDP cisplatin-resistant cell line were obtained from Huiying Biological Technology Co., Ltd., (Shanghai, China). The SKOV-3 and SKOV-3/DDP cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; HyClone; GE Healthcare Life Sciences, Logan, UT, USA), penicillin (100 U/ml), and streptomycin (100 µg/ml) in an incubator with 5% CO₂, at 37°C.

The SKOV-3/DDP cells in logarithmic phase were diluted to a density of 1.0×10^5 /ml, plated in six-well plates and incubated at 37°C in a humidified atmosphere of 5% CO₂ for 36 h. The SKOV-3/DDP cells were transfected with 50 nM of the miRNA mimics, mimic negative control (NC), miRNA inhibitor and inhibitor NC using Lipofectamine® 2000 (11668019; Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. For the cell proliferation, apoptosis, wound healing and migration assays, the cells were divided into the following mimic and inhibitor groups: i) Mimic groups: Mimic NC subgroup, transfected with the mimics control sequence; miR-210-3p mimic experimental subgroup, transfected with miR-210-3p mimics; ii) inhibitor groups: Inhibitor NC subgroup, transfected with the inhibitor control sequence; miR-210-3p inhibitor experimental subgroup, transfected with the miR-210-3p inhibitors sequence.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) in accordance with the manufacturer's protocol. The concentration and purity of the extracted RNA samples were detected with an ultraviolet spectrophotometer at a wavelength of 260 nm. Following this, ~1 µg of RNA and 3 µM primer F+R were transcribed to cDNA using Thermoscript RT kits (Takara Bio, Inc., Otsu, Japan). RT-qPCR analysis was performed using a SYBR-Green PCR master mix kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) with ~1 µg cDNA on an ABI 7500 Real-Time PCR system; U6 small nuclear RNA was used as an internal control. The specific primer sequences are shown in Table I. PCR amplification was conducted using the following cycling conditions: Initial denaturation at 95°C for 5 min; 40 cycles of denaturation at 95°C for 10 sec, annealing at 60°C for 30 sec, and extension at 72°C for 1 min; followed by a final extension at 72°C for 10 min. Three independent experiments were performed to detect the relative gene expression

level. The relative expression was quantified using the $2^{-\Delta\Delta C_q}$ method (14).

Cell proliferation assay. The cells ($\sim 1 \times 10^3$) in the logarithmic growth phase were plated per well, in 96-well culture plates. Cell proliferation was examined using a conventional Cell counting kit-8 (CCK-8) assay. CCK-8 solution (Beyotime Institute of Biotechnology, Shanghai, China) was used in accordance with the manufacturer's protocol. The plates were incubated in the dark for 3 h, and the absorbance value at 450 nm wavelength was recorded.

Cell apoptosis assay. The effect of miR-210-3p on apoptosis was detected through flow cytometry using the Annexin V-fluorescein isothiocyanate (FITC) cell apoptosis kit (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The SKOV-3/DDP cells transfected with miR-210-3p mimics, miR-210-3p inhibitor and corresponding NC controls were cultured in serum-free Roswell Park Memorial Institute 1640 medium (RPMI-1640; 10-040-CVR; Corning Incorporated) at 37°C in a humidified atmosphere of 5% CO₂ for 36 h. All cells were collected and washed three times with phosphate-buffered saline (PBS; pH 7.4) and suspended in the staining buffer provided in the kit. The cells were mixed with 5 µl of Annexin V-FITC and propidium iodide (PI) and were subjected to FACScan flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA) following incubation for 10 min at room temperature. The cells were pipetted into a test tube and 300 µl of PBS was added to each tube. PI (~1 µl; 50 µg/ml) was added to each sample and cell apoptosis was measured within 30 min. Cells that were Annexin V-positive and PI-negative were considered apoptotic.

Wound healing assay. Following transfection, a scratch was produced in the monolayer cultures of cells using sterile 200-µl pipette tips. The detached cells were removed by washing twice with PBS and the plates were incubated in a medium without FBS. Images were captured immediately (0 h) and after 24 h. Images of five random fields were captured under an inverted microscope, and the numbers of migratory cells were counted. Each experiment was repeated at least three times.

Invasion assay. Following transfection, the cells were divided and washed twice with PBS. The cells (5×10^5) were seeded in the upper chamber of a Transwell insert (12-µm pores) covered with Matrigel (0.7 mg/ml; Collaborative Research, Inc., TX, USA). The lower chamber was filled with RPMI medium (400 ml). Following incubation for 24 h, the non-migrated cells in the upper chamber were gently removed and the migrated cells present on the lower surface of the insert were stained with formaldehyde. Images were captured under an optical microscope following staining with crystal violet.

Luciferase reporter assay. To verify whether miR-210-3p directly targets and inhibits *E2F3*, the entire 3'-UTR of *E2F3* was cloned downstream of the luciferase gene, into the multiple cloning site of the pMIR-REPORT (Promega Corporation, Madison, WI, USA) vector, to construct the pmiR-*E2F3*-wild-type (WT) plasmid. TargetScan (<http://www.targetscan.org/>)

Table I. Primer sequences for RT-qPCR analysis.

Primer name	Sequence
hsa-miR-210-3p-RT	5'-GTCGTATCCAGTGCCTGTCGTGGAGTCGGCAATTGCACTGGATACGACTCAGCCG-3'
hsa-miR-210-3p-F-qRT	5'-AGGCTGTGCGTGTGACA-3'
hsa-miR-210-3p-R-qRT	5'-AGTGCCTGTCGTGGAGTCG-3'
U6-F	5'-CGATACAGAGAAGATTAGCATGGC-3'
U6-R	5'-AACGCTTCACGAATTTGCGT-3'

F, forward; R, reverse; miR, microRNA; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

targetscan.org/vert_71/) was used to predict the binding sites of miR-210-3p on *E2F3*, and to introduce sequence mutations to construct the pmiR-*E2F3*-mutant (MUT) vectors. pRL-TK, the *Renilla* luciferase expression vector (Promega Corporation), was used as an internal reference. The 293T cells were transfected with 50 nM miR-210-3p mimics or NC, with the luciferase reporter vector using Lipofectamine® 2000. The transfected cells were cultured at 37°C in a humidified atmosphere of 5% CO₂ for 24 h. The activity of luciferase was determined according to the Dual Luciferase Reporter Gene Assay system (Promega Corporation).

Western blot analysis. The SKOV-3 and SKOV-3/DDP cells (~1x10⁵) with cisplatin (Meilun Dalian, Liaoning Sheng, China) at concentrations of 0.5, 1, 2, 5, 10, 20 and 40 mg/l in 12-well culture plates were then cultured at 37°C in a humidified atmosphere of 5% CO₂ for 48 h. Total cell lysate was prepared by RIPA lysis (Beyotime Institute of Biotechnology). The concentration of protein was determined using the Bicinchoninic Acid Protein Assay kit (Shanghai Solarbio Bioscience & Technology Co., Ltd., Shanghai, China). The proteins were separated on a 10% polyacrylamide gel (Shanghai Solarbio Bioscience & Technology Co., Ltd.) by electrophoresis and the separated proteins were transferred onto polyvinylidene fluoride membranes. The membranes were blocked with 5% bovine serum albumin (FBS; Gibco; Thermo Fisher Scientific, Inc.) for 1 h at room temperature. The proteins were incubated with primary antibody against *E2F3* (rabbit anti-human, 1:1,000, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and a rabbit anti-human primary antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH, mouse anti-human, 1:1,000, Shanghai Solarbio Bioscience & Technology Co., Ltd.) overnight at 4°C. Following the addition of horseradish peroxidase-labeled secondary antibody (1:5,000, Shanghai Solarbio Bioscience & Technology Co., Ltd.) and incubation for 1 h at room temperature, the protein signals were detected with chemiluminescence. The Gel Doc EZ Imager (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to capture the protein image. ImageJ software version 1.8.0 (National Institutes of Health, Bethesda, MD, USA) was used to determine the gray value of the target band.

Cisplatin sensitivity analysis. The viability of the SKOV-3 and SKOV-3/DDP cells treated with cisplatin at concentrations of 0.5, 1, 2, 5, 10, 20 and 40 mg/l for 48 h was evaluated. The *E2F3*

sequence was inserted into a pReceiver vector (GeneCopoeia, Inc., Rockville, MD, USA) to construct the pReceiver *E2F3* vector. The cells in the experiment were assigned to the five following groups: SKOV-3/DDP, NC (SKOV-3/DDP with empty vector), miR-210-3p mimics (SKOV-3/DDP with miR-210-3p mimics), *E2F3* (SKOV-3/DDP with pReceiver *E2F3* vector), and miR-210-3p mimic + *E2F3* (SKOV-3/DDP with miR-210-3p mimics and pReceiver *E2F3* vector). All cells were exposed to cisplatin and the absorbance value was detected using the CCK-8 assay. The results are expressed as the cell survival rate, which was determined according to optical density (OD) as follows: Percentage survival=(mean OD value of treated group/mean OD value of control group) x100%.

Statistical analysis. The software SPSS 21.0 (IBM Corp., Armonk, NY, USA) was used to analyze statistical significance. All assays were independently performed three times and measurement data are presented as the mean ± standard deviation. A two-sided Student's t-test was used to analyze the differences between the two groups. The significance of differences between groups were evaluated with one-way analysis of variance followed by Student-Newman-Keuls post hoc tests. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of miR-210-3p is significantly decreased in SKOV-3/DDP cells. RT-qPCR analysis was used to assess the expression levels of miR-210-3p in SKOV-3/DDP and SKOV-3 cells, which revealed that the expression of miR-210-3p was significantly lower in the SKOV-3/DDP cells than in the SKOV-3 cells (Fig. 1A). Following treatment with cisplatin, the viability of the SKOV-3/DDP cells was significantly higher compared with that of the SKOV-3 cells (Fig. 1B). These results suggest that the downregulated expression of miR-210-3p may affect the growth of ovarian cancer cells and their resistance to cisplatin.

Overexpression of miR-210-3p represses the ability of cell proliferation. To assess the influence of miR-210-3p on the proliferation of SKOV-3/DDP cells, miR-210-3p mimics or NC were transfected into SKOV-3/DDP cells. The expression level of miR-210-3p was significantly increased in the cells transfected with miR-210-3p mimics (Fig. 2A). The CCK-8 analysis

Figure 1. Expression of miR-210-3p. (A) Relative expression levels of miR-210-3p of the SKOV-3 human ovarian cancer cell line and SKOV-3/DDP cisplatin-resistant cell line. (B) Cell proliferation ability of the SKOV-3 human ovarian cancer cell line and SKOV-3/DDP cisplatin-resistant cell line. The viability of SKOV-3 and SKOV-3/DDP cells treated with cisplatin at concentrations of 0.5, 1, 2, 5, 10, 20 and 40 mg/l for 48 h was evaluated. * $P < 0.05$ compared with SKOV-3 cells; ** $P < 0.01$ compared with SKOV-3 cells ($n = 3$). miR, microRNA.

revealed that the ability of cells to proliferate was significantly lower in the SKOV-3/DDP cells than in the NC group of cells (Fig. 2B).

Overexpression of miR-210-3p promotes cell apoptosis. To clarify the influence of miR-210-3p on cell apoptosis, Annexin V/PI staining was performed to detect apoptotic cells. The proportion of apoptotic cells was significantly higher in the cells transfected with the miR-210-3p mimics than for those transfected with the mimics control sequence, suggesting that the overexpression of miR-210-3p promoted cell apoptosis (Fig. 3A and B).

miR-210-3p suppresses the migration of SKOV-3/DDP cells. To examine the influence of miR-210-3p on cell metastasis, a wound healing assay was conducted for the SKOV-3/DDP cells. The results of the Transwell assay suggested that the number of cells passing through the membrane in the miR-210-3p mimic group was significantly decreased compared with that in the corresponding control ($P < 0.01$; Fig. 4A and B). The expression level of miR-210-3p was significantly decreased in the cells transfected with miR-210-3p inhibitors (Fig. 4C). The number of cells passing through the membrane in the miR-210-3p inhibitor group was significantly increased compared with

Figure 2. Overexpression of miR-210-3p inhibits SKOV-3/DDP cell proliferation. (A) Expression levels of miR-210-3p in SKOV-3/DDP cells following transfection with the miR-210-3p mimics. (B) OD450 of SKOV-3/DDP cells following transfection with the miR-210-3p mimics. ** $P < 0.01$ compared with NC ($n = 3$). miR, microRNA; NC, negative control cells transfected with the mimics control sequence; OD, optical density.

that in the corresponding control ($P < 0.01$; Fig. 4D and E). These results clearly indicated that the ectopic expression of miR-210-3p inhibited cell migration.

miR-210-3p inhibits cell invasion in SKOV-3/DDP cells. The tumor invasion assay revealed that the number of invasive cells among the SKOV-3/DDP cells transfected with miR-210-3p mimics was significantly decreased compared that in the corresponding control ($P < 0.01$; Fig. 5A and B), whereas the miR-210-3p inhibitor promoted cell invasion compared with that in the corresponding control ($P < 0.01$; Fig. 5C and D). These data suggest that the ectopic expression of miR-210-3p inhibited cell metastasis and invasion.

E2F3 is the target gene of miR-210-3p. TargetScan was used to predict the potential miR-210-3p targets. *E2F3* was selected for further examination as it contained a conserved binding site for miR-210-3p (Fig. 6A). The results of the dual luciferase reporter assay (Fig. 6B) indicated that the dual luciferase activity of pmiR-*E2F3*-WT was significantly lower in the miR-210-3p mimics group than in the control group ($P < 0.05$), whereas that of pmiR-*E2F3*-MUT was not significantly decreased ($P > 0.05$). The levels of *E2F3* in the SKOV-3/DDP and SKOV-3 cells were significantly decreased with the overexpression of miR-210-3p (Fig. 6C). Therefore, miR-210-3p may directly target *E2F3* and negatively regulate its expression.

Figure 3. Overexpression of miR-210-3p induces apoptosis of SKOV-3/DDP cells. (A) Representative images showing Annexin V/PI staining results in SKOV-3/DDP cells following different treatments. (B) Detection of apoptosis following Annexin V/PI staining. ** $P < 0.01$ ($n = 3$). miR, microRNA; NC, negative control cells transfected with the mimics control sequence; PI, propidium iodide; FITC, fluorescein isothiocyanate.

Figure 4. miR-210-3p inhibits the migration of SKOV-3/DDP cells. (A) Representative images of the overexpression of miR-210-3p showing cell migration results in SKOV-3/DDP cells following different treatments. (B) Recovery rates of cell migration. (C) Expression levels of miR-210-3p in SKOV-3/DDP cells following transfection with the miR-210-3p inhibitors. (D) Representative images of the inhibited expression of miR-210-3p, showing cell migration of SKOV-3/DDP cells following different treatments. (E) Recovery rates of cell migration. Magnification, $\times 100$. ** $P < 0.01$ ($n = 3$). miR, microRNA; NC, negative control cells transfected with the mimic or inhibitor control sequence.

Figure 5. miR-210-3p inhibits the invasion of SKOV-3/DDP cells. (A) Representative images of the overexpression of miR-210-3p showing cell invasion results in SKOV-3/DDP cells with different treatments. (B) Numbers of invasive cells in the mimic experiment. (C) Representative images of the inhibition of expression of miR-210-3p showing cell invasion results in SKOV-3/DDP cells with different treatments. (D) Numbers of invasive cells in the inhibitors experiment. Magnification, x100. ** $P < 0.01$ ($n = 3$). miR, microRNA; NC, negative control cells transfected with the mimic or inhibitor control sequence.

Figure 6. miR-210-3p directly targets *E2F3* in 293T cells. (A) Binding site of miR-210-3p at the 3'-UTR of *E2F3* and mutated 3'-UTR of *E2F3*. (B) Western blotting of the expression of *E2F3* in SKOV-3 and SKOV-3/DDP cells. (C) Relative luciferase activity. * $P < 0.05$ ($n = 3$). miR, microRNA; NC, negative control cells transfected with the mimics control sequence; 3'-UTR, 3'-untranslated region; WT, wild-type; MUT, mutant; *E2F3*, E2F transcription factor 3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Overexpression of miR-210-3p affects the sensitivity of SKOV-3/DDP cells to cisplatin by targeting E2F3. A CCK-8 assay was performed to assess the viability of cells following

treatment with different concentrations of cisplatin. In comparison with the SKOV-3/DDP group, the IC_{50} value of cisplatin in the miR-210-3p mimics group was significantly decreased. The analysis of cell survival rate suggested that, with different concentrations of cisplatin, the cell survival rates in the SKOV-3/DDP and miR-210-3p mimics groups were higher compared with those in the SKOV-3 group ($P < 0.05$). The cell survival rates of the NC and miR-210-3p mimics + *E2F3* groups did not differ significantly ($P > 0.05$) from that of the SKOV-3/DDP group, whereas that of the miR-210-3p mimics group was significantly decreased ($P < 0.05$; Fig. 7). In conclusion, transfection with the miR-210-3p mimic significantly reduced the effect of cisplatin on the SKOV-3/DDP cells. The overexpression of *E2F3* rescued the miR-210-3p mimics-induced sensitivity to cisplatin.

Discussion

In the present study, the role of miR-210-3p in the carcinogenesis and cisplatin sensitivity of ovarian cancer cells was investigated. The expression of miR-210-3p was significantly lower in cisplatin-resistant SKOV3/DDP cells than in chemosensitive SKOV3 cells. Transfection of the SKOV3/DDP cells with miR-210-3p mimics resulted in the inhibition of cell proliferation, migration and invasion, and the induction of cell apoptosis. *E2F3* was verified as the direct target of miR-210-3p. The transfection of SKOV3/DDP cells with miR-210-3p mimics also increased the sensitivity of cells to cisplatin. However, the overexpression of *E2F3* attenuated this effect of the overexpression of miR-210-3p.

Several studies have suggested the important role of miR-210 in cancer. However, there are contradictory results

Figure 7. Effects of miR-210-3p and *E2F3* on the sensitivity of SKOV-3/DDP cells to cisplatin. SKOV-3/DDP, NC, miR-210-3p mimic, *E2F3*, and miR-210-3p mimic + *E2F3* groups refer to SKOV-3/DDP cells transfected with no sequence, empty vector, miR-210-3p mimics, pReceiver *E2F3* vector, and both miR-210-3p mimic and pReceiver *E2F3* vectors, respectively. * $P<0.05$ and ** $P<0.01$ compared with SKOV-3/DDP, NC, and miR-210-3p mimic + *E2F3* ($n=3$). miR, microRNA; NC, negative control; *E2F3*, E2F transcription factor 3.

with respect to the role of miR-210 as an oncogene or gene suppressor. miR-210 is upregulated in head and neck cancer, pancreatic tumors (15), glioma (16), non-small cell lung cancer (17) and prostate cancer (18). By contrast, the expression of miR-210 is downregulated in esophageal squamous cell carcinoma (19), bladder cancer (20), angiosarcoma (21) and renal cell carcinoma (22). The function of miR-210 in ovarian cancer remains to be fully elucidated. Under hypoxic conditions, miR-210 is upregulated in epithelial ovarian cancer tissues and ovarian cancer cell lines (23). miR-210 is located on chromosome 11p15.5, on which allelic loss is observed in ovarian cancer (24,25). Giannakakis *et al* (12), reported that miR-210 gene copy number was deleted in 64% ovarian cancer samples and was associated with the expression levels of mature miR-210. The level of miR-210 is also reduced in primary ovarian cancer cells compared with that in effusions (26). These results are consistent with the observations of the present study. Therefore, miR-210-3p is not only a simple tumor-stimulating miRNA in cancer but may have a dual role as an oncogene and a tumor suppressor. The results of the present study revealed that the overexpression of miR-210-3p suppressed cell multiplication, migration and invasion abilities, and promoted cell apoptosis in cisplatin-resistant SKOV3/DDP cells. Therefore, miR-210-3p mainly functions as a tumor suppressor gene in SKOV3/DDP cells and may prevent the progression of ovarian cancer.

The protein *E2F3* is a key molecule involved in cell cycle progression. *E2F3a* acts as an oncogene in ovarian cancer (27). The *E2F3* isoforms (*E2F3a* and *E2F3b*) are overexpressed in ovarian cancer tissues, compared with normal tissues; of these, the expression of *E2F3a* is known to be associated with tumor stage (28). The present study verified that miR-210-3p directly targeted the 3'-UTR region of *E2F3* and it was hypothesized

that downregulation of the expression of miR-210-3p may affect certain signaling pathways in ovarian cancer. miR-210-3p suppressed SKOV3/DDP cell multiplication via *E2F3* through cell cycle arrest, as observed in esophageal squamous cell carcinoma.

miRNAs have been investigated in multiple tumor types for their roles in drug resistance. For example, the deregulation of miR-340-5p and miR-128 led to an increase in cisplatin resistance in osteosarcoma cells and glioma cells, respectively (29,30). The serum level of miR-210 was associated with the sensitivity to cisplatin-based chemotherapy in non-small cell lung cancer (31). Zhao *et al* (32), reported on the association between the expression of miR-9 and increased response of cancer cells to cisplatin treatment. In the present study, the overexpression of miR-210-3p reduced the suppressive effect of cisplatin on resistant cells, however, the overexpression of *E2F3* eliminated the miR-210-3p-induced resistance to cisplatin. Taken together, the present study highlights the potential role of miR-210-3p as a novel therapeutic target for ovarian cancer. miR-210-3p affects the sensitivity of cisplatin-resistant cells to cisplatin by targeting *E2F3* in ovarian cancer. Further investigations are warranted to evaluate the specific regulation network of miR-210-3p and its target gene in ovarian cancer and develop effective drugs for clinical application.

In conclusion, the overexpression of miR-210-3p repressed the cell proliferation, apoptosis, migration and invasion of cells, and the inhibition of miR-210-3p promoted cell migration and invasion. The overexpression of miR-210-3p decreased the sensitivity of SKOV-3/DDP cells to cisplatin treatment via *E2F3*. miR-210-3p may serve a tumor suppressor role in ovarian cancer cells and be a potentially valuable therapeutic target for improving cisplatin resistance in ovarian cancer cells.

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Availability of data materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

HZ made substantial contributions to conception and design this study. YJ, JW, SX, FG and LY performed all the experiments, and carried out all the analysis and interpretation of data. YJ and JW were involved in drafting the manuscript and revised it critically for important intellectual content. LY gave final approval of the version to be published. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work is appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. *CA Cancer J Clin* 65: 87-108, 2015.
- Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 136: E359-E386, 2015.
- Jemal A, Siegel R, Xu J and Ward E: Cancer statistics, 2010. *CA Cancer J Clin* 60: 277-300, 2010.
- Davis A, Tinker AV and Friedlander M: 'Platinum resistant' ovarian cancer: What is it, who to treat and how to measure benefit? *Gynecol Oncol* 133: 624-631, 2014.
- Wang J and Sen S: MicroRNA functional network in pancreatic cancer: From biology to biomarkers of disease. *J Biosci* 36: 481-491, 2011.
- Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 116: 281-297, 2004.
- Chan SY and Loscalzo J: MicroRNA-210: A unique and pleiotropic hypoxamir. *Cell Cycle* 9: 1072-1083, 2010.
- Vera O, Jimenez J, Pernia O, Rodriguez-Antolin C, Rodriguez C, Sanchez Cabo F, Soto J, Rosas R, Lopez-Magallon S, Esteban Rodriguez I, *et al*: DNA methylation of miR-7 is a mechanism involved in platinum response through MAFK overexpression in cancer cells. *Theranostics* 7: 4118-4134, 2017.
- Chen W, Du J, Li X, Su J, Huang Y, Ding N, Zhang M and Jiang S: miR-509-3p promotes cisplatin-induced apoptosis in ovarian cancer cells through the regulation of anti-apoptotic genes. *Pharmacogenomics* 18: 1671-1682, 2017.
- Zhang S, Zhang JY, Lu LJ, Wang CH and Wang LH: MiR-630 promotes epithelial ovarian cancer proliferation and invasion via targeting KLF6. *Eur Rev Med Pharmacol Sci* 21: 4542-4547, 2017.
- Han X, Zhang Y, Wang D, Fu X, Li M and Wang A: Upregulation of microRNA-18b induces phosphatase and tensin homolog to accelerate the migration and invasion abilities of ovarian cancer. *Oncol Lett* 14: 5631-5637, 2017.
- Giannakakis A, Sandaltzopoulos R, Greshock J, Liang S, Huang J, Hasegawa K, Li C, O'Brien-Jenkins A, Katsaros D, Weber BL, *et al*: miR-210 links hypoxia with cell cycle regulation and is deleted in human epithelial ovarian cancer. *Cancer Biol Ther* 7: 255-264, 2008.
- Xu Y, Wang C, Su J, Xie Q, Ma L, Zeng L, Yu Y, Liu S, Li S, Li Z and Sun L: Tolerance to endoplasmic reticulum stress mediates cisplatin resistance in human ovarian cancer cells by maintaining endoplasmic reticulum and mitochondrial homeostasis. *Oncol Rep* 34: 3051-3060, 2015.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
- Huang X, Ding L, Bennewith KL, Tong RT, Welford SM, Ang KK, Story M, Le QT and Giaccia AJ: Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumor initiation. *Mol Cell* 35: 856-867, 2009.
- Malzkorn B, Wolter M, Liesenberg F, Grzendowski M, Stühler K, Meyer HE and Reifenberger G: Identification and functional characterization of microRNAs involved in the malignant progression of gliomas. *Brain Pathol* 20: 539-550, 2010.
- Puissegur MP, Mazure NM, Bertero T, Pradelli L, Grosso S, Robbe-Sermesant K, Maurin T, Lebrigand K, Cardinaud B, Hofman V, *et al*: miR-210 is overexpressed in late stages of lung cancer and mediates mitochondrial alterations associated with modulation of HIF-1 activity. *Cell Death Differ* 18: 465-478, 2011.
- Ren D, Yang Q, Dai Y, Guo W, Du H, Song L and Peng X: Oncogenic miR-210-3p promotes prostate cancer cell EMT and bone metastasis via NF- κ B signaling pathway. *Mol Cancer* 16: 117, 2017.
- Tsuchiya S, Fujiwara T, Sato F, Shimada Y, Tanaka E, Sakai Y, Shimizu K and Tsujimoto G: MicroRNA-210 regulates cancer cell proliferation through targeting fibroblast growth factor receptor-like 1 (FGFR1). *J Biol Chem* 286: 420-428, 2011.
- Yang X, Shi L, Yi C, Yang Y, Chang L and Song D: MiR-210-3p inhibits the tumor growth and metastasis of bladder cancer via targeting fibroblast growth factor receptor-like 1. *Am J Cancer Res* 7: 1738-1753, 2017.
- Nakashima S, Jinnin M, Kanemaru H, Kajihara I, Igata T, Okamoto S, Tazaki Y, Harada M, Masuguchi S, Fukushima S, *et al*: The role of miR-210, E2F3 and ephrin A3 in angiosarcoma cell proliferation. *Eur J Dermatol* 27: 464-471, 2017.
- Yoshino H, Yonemori M, Miyamoto K, Tatarano S, Kofuji S, Nohata N, Nakagawa M and Enokida H: microRNA-210-3p depletion by CRISPR/Cas9 promoted tumorigenesis through revival of TWIST1 in renal cell carcinoma. *Oncotarget* 8: 20881-20894, 2017.
- Li L, Huang K, You Y, Fu X, Hu L, Song L and Meng Y: Hypoxia-induced miR-210 in epithelial ovarian cancer enhances cancer cell viability via promoting proliferation and inhibiting apoptosis. *Int J Oncol* 44: 2111-2120, 2014.
- Vandamme B, Lissens W, Amfo K, De Sutter P, Bourgain C, Vámos E and De Grève J: Deletion of chromosome 11p13-11p15.5 sequences in invasive human ovarian cancer is a subclonal progression factor. *Cancer Res* 52: 6646-6652, 1992.
- Viel A, Giannini F, Tumiotto L, Sopracordevole F, Visentin MC and Boiocchi M: Chromosomal localisation of two putative 11p oncosuppressor genes involved in human ovarian tumours. *Br J Cancer* 66: 1030-1036, 1992.
- Vaksman O, Stavnes HT, Kaern J, Trope CG, Davidson B and Reich R: miRNA profiling along tumour progression in ovarian carcinoma. *J Cell Mol Med* 15: 1593-1602, 2011.
- Reimer D, Hubalek M, Riedle S, Skvortsov S, Erdel M, Concin N, Fiegl H, Müller-Holzner E, Marth C, Illmensee K, *et al*: E2F3a is critically involved in epidermal growth factor receptor-directed proliferation in ovarian cancer. *Cancer Res* 70: 4613-4623, 2010.

28. Reimer D, Hubalek M, Kiefel H, Riedle S, Skvortsov S, Erdel M, Hofstetter G, Concin N, Fiegl H, Müller-Holzner E, *et al*: Regulation of transcription factor E2F3a and its clinical relevance in ovarian cancer. *Oncogene* 30: 4038-4049, 2011.
29. Song L, Duan P, Gan Y, Li P, Zhao C, Xu J, Zhang Z and Zhou Q: MicroRNA-340-5p modulates cisplatin resistance by targeting LPAATbeta in osteosarcoma. *Braz J Med Biol Res* 50: e6359, 2017.
30. Yi DY, Su Q, Zhang FC, Fu P, Zhang Q, Cen YC, Zhao HY and Xiang W: Effect of microRNA-128 on cisplatin resistance of glioma SHG-44 cells by targeting JAG1. *J Cell Biochem* 119: 3162-3173, 2018.
31. Li ZH, Zhang H, Yang ZG, Wen GQ, Cui YB and Shao GG: Prognostic significance of serum microRNA-210 levels in nonsmall-cell lung cancer. *J Int Med Res* 41: 1437-1444, 2013.
32. Zhao HM, Wei W, Sun YH, Gao JH, Wang Q and Zheng JH: MicroRNA-9 promotes tumorigenesis and mediates sensitivity to cisplatin in primary epithelial ovarian cancer cells. *Tumour Biol* 36: 6867-6873, 2015.