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MicroRNA-132 reverses cisplatin resistance and metastasis in ovarian cancer by the targeted regulation on Bmi-1

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Abstract. – OBJECTIVE: To explore the role of micro ribonucleic acid-132 (miR-132) in cisplatin (DDP) resistance and metastasis of ovarian cancer and its related mechanisms.

MATERIALS AND METHODS: Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and Western blotting were applied to detect the expression levels of miR-132 and B lymphoma Mo-MLV insertion region 1 homolog (Bmi-1) in maternal SKOV3 cells and cisplatin-resistant SKOV3/DDP cells. SKOV3/DDP cells were transfected with miR-132 mimic and miR-132 mimic negative control (NC). QRT-PCR and Western blotting were used to detect the expression changes in Bmi-1, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was adopted to detect the sensitivity of cells to DDP after transfection with miR-132 mimic. The effect of transfection on the apoptosis was detected via flow cytometry, and that on cell invasion and migration abilities were examined using wound healing assay and transwell assay. Bmi-1 wild-type (wt) and mutant-type (mut) luciferase reporter plasmids were co-transfected with miRNA-132 mimic or miRNA-132 NC, and luciferase activity was analyzed by dual-luciferase reporter system.

RESULTS: QRT-PCR and Western blotting results manifested that the miR-132 expression level in SKOV3/DDP cells was significantly lower than that in SKOV3 cells, while the expression level of Bmi-1 in SKOV3/DDP cells was significantly higher than that in SKOV3 cells. The overexpression of miR-132 could reduce the expression level of Bmi-1 in SKOV3/DDP cells, increase the sensitivity of SKOV3/DDP cells to DDP, and inhibit cell invasion and metastasis. Data detected by the luciferase activity revealed that miR-132 could bind to the three prime untranslated region (3'-UTR) of the Bmi-1 gene and negatively regulate the protein expression.

CONCLUSIONS: MiR-132 may regulate ovarian cancer's sensitivity to DDP and inhibit its invasion and metastasis by targeted regulation on Bmi-1.

Key Words:
MiR-132, Bmi-1, Cisplatin, Ovarian cancer, Drug resistance.

Introduction

Ovarian cancer is a tumor with the highest mortality rate in the female reproductive system[1]. Due to the lack of early clinical symptoms and specific screening indicators, most patients are in the advanced stage when definitely diagnosed. First of all, the optimal cytoreductive surgery followed by paclitaxel and platinum-based combination chemotherapy is the current standard treatment for ovarian cancer[2]. Cisplatin (DDP), as a classic chemotherapeutic drug for ovarian cancer, plays an important role in clinical practice. However, with the deepening of treatment, it will inevitably lead to resistance of tumor cells to DDP[3,4]. Therefore, the 5-year recurrence rate of ovarian cancer in patients is still at a high level. The mechanism of DDP resistance in ovarian cancer cells is not yet clear. Searching for an effective method to inhibit DDP resistance in cancer cells is an urgent problem. In recent years, it has been found that the abnormal expression

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of micro ribonucleic acids (miRNAs) is closely related to the resistance of various tumor cells to DDP[5,6]. The up-regulation or down-regulation of some miRNAs, such as miR-143, miR-20a, and miR-181b, can change the sensitivity of tumor cells to DDP. This phenomenon suggests that the miRNA, as an important regulatory molecule after transcription, may mediate the resistance of tumor cells to DDP[7]. As a member of the miRNA family, miR-132 plays a crucial role in inflammation, angiogenesis, and nerve development[8]. The imbalance of miR-132 has close correlations with the occurrence and development of non-small cell lung cancer, osteosarcoma, breast cancer, liver cancer, prostate cancer, gastric cancer, and pancreatic cancer[9]. Liu et al[10] revealed that the overexpression of miR-132 can improve the sensitivity of cervical cancer cells to radiotherapy by down-regulating B lymphoma Mo-MLV insertion region 1 homolog (Bmi-1). However, the significance of miR-132 in DDP-resistant ovarian cancer has not been reported yet. Therefore, this study aims to explore the expression of miR-132 in DDP-resistant SKOV3/DDP cells, its relationship with drug resistance and its potential mechanism.

Materials and Methods

Cell Culture

SKOV3 cells were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. DDP-resistant SKOV3 cells were induced by DDP concentration gradient, which were named as SKOV3/DDP. Specific induction method: after the SKOV3 cells were firstly passaged, DDP was added and incubated with SKOV3 cells to make the final concentration to 1 nM when the cells adhered to the wall. When the cells covered 85% of the culture dish, the cells were passaged and then incubated at 1 nM on the basis of the last passaged final concentration. After repeated culture and passage of cells, SKOV3/DDP cells that could grow in 100 nM DDP environment were finally obtained. Both SKOV3 and SKOV3/DDP cells were cultured in minimum essential media (MEM; HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 1% penicillin-streptomycin dual antibody solution, and 1% nonessential amino acids, and placed in an incubator with 5% CO_2 at 37°C.

Transfection of MiR-132

MiR-132 mimic and miR-132 mimic negative control (NC) were purchased from GenePharma (Shanghai, China). SKOV3 and SKOV3/DDP cells were transfected with miR-132 mimic or miR-132 mimic NC for 72 h. The transfection was performed using Lipofectamine 2000 (purchased from Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction.

Luciferase Reporter Assay

SKOV3/DDP cells were inoculated into a 12-well plate at 1.5×10^5/well, and when the cell density reached 70%, the corresponding transfection reagents were prepared according to the instructions, including 50 ng wild-type (wt) or mutant-type (mut) reporter plasmids in the three prime untranslated region (3'UTR), miR-132 mimic or miR-132 NC with a final concentration of 20 nM, and Lipofectamine 2000. After the transfection of SKOV3/DDP cells for 48 h, the corresponding luciferase activity was detected using the dual-luciferase reporter gene detection kit (Beyoytime, Shanghai, China).

Real-Time Fluorescence Quantitative Polymerase Chain Reaction (qPCR)

The total messenger RNA (mRNA) of the cells was extracted with the TRIzol assay (Invitrogen, Carlsbad, CA, USA) and reversely transcribed into complementary deoxyribonucleic acids (cDNAs). Reaction conditions of reverse transcription (RT): at 25°C for 10 min, 50°C for 30 min, and 85°C for 5 min. The results were detected *via* the fluorescence qPCR kit. Primer sequences of miR-132: forward: 5'-CCAGCataACAGTCAGCCA-3' and reverse: 5'-TATGGTTTCCGACTCCTCAC-3'. Primer sequences of Bmi-1: forward: 5'-CTGGTTGCCCATTGACAGC-3 and reverse: 5'-CAGAAAATGAATGCGAGCCA-3. Primer sequences of internal reference glyceraldehyde 3-phosphate dehydrogenase (GAPDH): forward: 5'-CATGGCCTCCGTTCCTA-3 and reverse: 5'-GCGGCACGTCAGATCCA-3'. Fluorescence qPCR reaction conditions: at 95°C for 5 min, 95°C for 15 s, and 60°C for 1 min for a total of 40 cycles. The dissolution curve temperature was set to 60-95°C, and 3 repeated wells were set for each sample.

Cell Proliferation Experiment

3-(4,5)-dimethylthiazol(-z-y1)-3,5-diphenyltetrazolium bromide (MTT) assay (Sigma-Aldrich, St. Louis, MO, USA) was used and carried

out according to the instructions of the reagents. After digestion and passage of the cell lines that grew into the logarithmic phase, the cells were inoculated into 96-well plates at 8×10^3/well, and the volume of the complete culture medium was 200 μL. MTT kit was used to detect the proliferation of each group of cells after treatment with DDP at different concentrations for 48 h and the proliferation of SKOV3/DDP cells after successful transfection with miR-132 mimic or miR-132 NC for 24 h, 48 h, 72 h, and 96 h. The absorption value at the wavelength of 450 nm was read, and the cell growth curve was plotted.

Cell Clone Formation Assay

Cells in the logarithmic growth phase were inoculated into a 6-well plate at 500 cells per well. After 24 h of culture, when the cells adhered to the well and spread well, the medium was replaced with a drug-free medium for further culture for 14 d, and the cells were washed with phosphate-buffered saline (PBS) and fixed with 10% formaldehyde, followed by Giemsa staining and photographing. Cell colonies containing more than 50 cells were regarded as 1 clone.

Flow Cytometry

SKOV3/DDP cells in each group at 24 h after transfection were digested into the single cell suspension, washed with PBS and centrifuged for 3 min at 1000 r/min twice. Single cell suspension with a concentration of $1'10^6$ cells/mL was prepared with 1′ Annexin V binding solution, from which 100 μL solution was taken, added with 5 μL compounds of Annexin V and FITC as well as 5 μL Propidium Iodide (PI) solution, cultured at room temperature for 15 min in the dark, and finally diluted with 400 μL 1′ Annexin V binding solution. The apoptosis rate was detected by flow cytometry.

Western Blotting

The lysate was used to extract the total cell protein, which was loaded into sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in the amount of 25 g per well. The sample was subjected to 350 mA electrophoresis, and the gel and activated polyvinylidene difluoride (PVDF; Millipore, Billerica, MA, USA) were placed on a membrane transfer frame under a constant current for 2 h. The PVDF membrane was taken out and sealed with 5% skim milk powder for 1 h. The primary antibody was added for incubation at 4°C for 10 h. Then, the protein was washed with Tris-Buffered Saline with Tween 20 (TBST). After that, the horseradish peroxidase (HRP)-labeled secondary antibody was added for incubation at room temperature for 1 h. The protein was washed with TBST and then developed, and the gray value was analyzed with ImageJ software. Relative expression level of the target protein = target protein/Actin.

Wound Healing Assay

The cells were inoculated to a 6-well plate and cultured under suitable conditions. When the fusion degree of the cells reached 90%-100%, scratches were made slowly and evenly at the bottom of the 6-well plate with a 10 μL spearhead perpendicular to the plate, and then the floating cells were washed off with PBS. Subsequently, the cells were added with the serum-free culture medium and cultured in an incubator. At 0 h and 48 h after scratching and culture, the migration distance of the scratch area of cells was observed under a microscope, and different fields of view were randomly selected for photographing.

Transwell Migration and Invasion Experiments

The cells after different treatments were collected, the serum-free medium was added into the chamber where 1×10^5 cells were placed. The chamber was placed in a well plate containing 10% fetal bovine serum medium as induction. Cell mobility (without Matrigel) was detected at 24 h after the culture, cell invasion (with Matrigel paved in the chamber) was examined at 48 h after the culture, and the cells in the upper layer of the chamber were brushed off while those in the lower layer were subjected to photographing and observation through crystal violet staining after the culture. The number of cells was calculated to compare the invasion and migration abilities of each group.

Statistical Analysis

Analysis and processing of experimental data were conducted using Statistical Product and Service Solutions 19.0 software (IBM, Armonk, NY, USA). Measurement data were expressed as mean ± standard deviation ($\bar{x}\pm s$), and the intergroup comparison was performed using the *t*-test or one-way analysis of variance. $\alpha=0.05$ was taken as the test level.

Results

Higher Resistance of SKOV3/DDP Cells to DDP Than SKOV3 Cells and the Expressions of MiR-132 and Bmi-1 in Ovarian Cancer Cells

To investigate the DDP resistance of SKOV3/DDP cells, MTT assay was adopted to detect the inhibition of DDP in gradient concentration on maternal SKOV3 cells and SKOV3/DDP cells. The results showed that the half maximal inhibitory concentration (IC_{50}) of DDP to SKOV3 was 4.5 μM, and that to SKOV3/DDP cells was 64 μM. The drug resistance of SKOV3/DDP cells was 14 times that of SKOV3 cells, showing a statistically significant difference ($p<0.05$) (Figure 1A). After the resistance of SKOV3/DDP to DDP was proved, the expression level of miR-132 in SKOV3/DDP cells and SKOV3 cells was detected *via* RT-PCR. As shown in Figure 1B, the results demonstrated that the expression of miR-132 in SKOV3/DDP cells was significantly lower than that in SKOV3 cells, and the expression level in SKOV3/DDP cells was about 1/4 of that in SKOV3 cells, displaying a statistically significant difference ($p<0.05$). We also found that the expression level of Bmi-1 in SKOV3/DDP was significantly higher than that in SKOV3 cells (Figure 1C, 1D).

Up-Regulating the MiR-132 Changed DDP Sensitivity of SKOV3/DDP Cells and Inhibited Their Proliferation

It was found in preliminary research that the miR-132 expression in drug-resistant cells was significantly down-regulated compared with that in maternal cells. To explore whether the down-regulation of expression resulted in resistance of SKOV3 cells to DDP, cell transfection was utilized to up-regulate the miR-132 expression in SKOV3/DDP cells, and the changes in cell sensitivity to DDP were observed. QRT-PCR confirmed that the miR-132 expression level in SKOV3/DDP cells transfected with miR-132 mimic could be significantly up-regulated compared with that in cells transfected with miR-132 NC ($p<0.05$). According to subsequent MTT results (Figure 2B), the overexpression of miR-

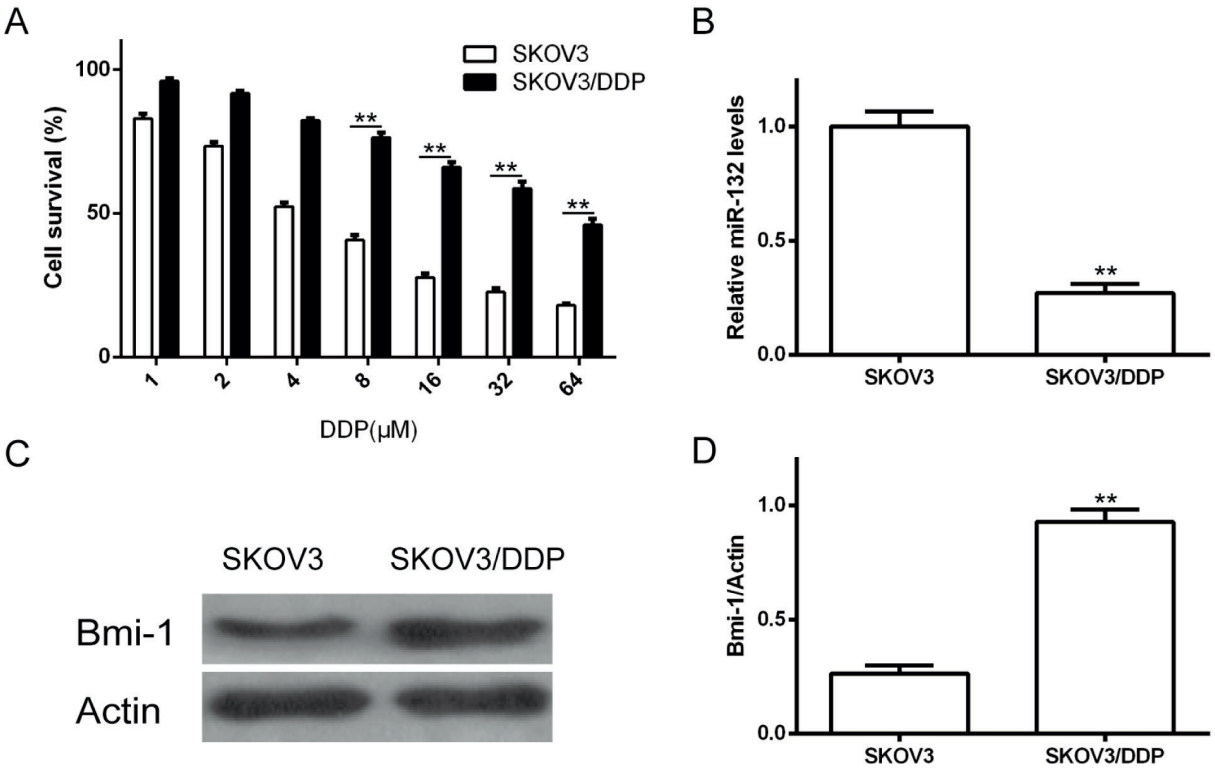

Figure 1. Identification of miR-132 and Bmi-1 expression in cisplatin (DDP)-resistant SKOV3/DDP and SKOV3 cells. ***A,*** Cell proliferation curves of SKOV3/DDP and SKOV3 cells were determined by MTT assay after treated with indicated concentrations of cisplatin for 48 h. ***B-D,*** The expression level of miR-132 and Bmi-1 was detected in SKOV3/DDP and SKOV3 cells. Data shown are representative of 3 independent experiments. Data are presented as means ± SD (n = 3). **$p<0.01$ *vs.* control group.

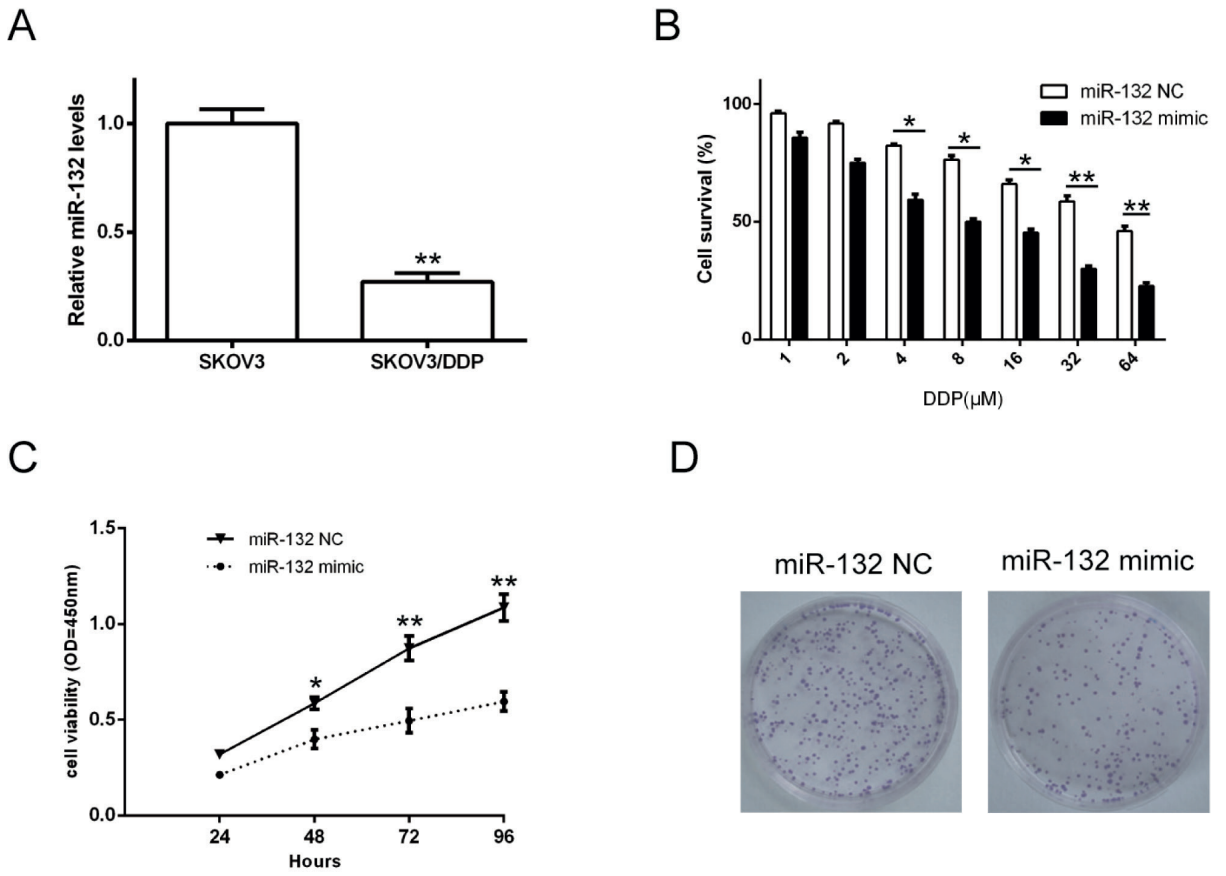

Figure 2. Overexpression of miR-132 reversed cisplatin resistance of SKOV3/DDP cells. ***A,*** Expression of miR-132 in SKOV3/DDP cells transfected with miR-132 mimic or miR-132 NC. ***B,*** SKOV3/DDP cells were transfected with miR-132 mimic or miR-132 NC, and the effects on proliferation were determined by MTT assay after treated with indicated concentrations of cisplatin for 48 h. ***C,*** MTT assay and Colony-forming assay were performed to determine the proliferation of SKOV3/DDP cells transfected with miR-132 mimic compared to negative control. Data are presented as means ± SD (n = 3). *p<0.05, **p<0.01 *vs.* control group.

132 could significantly improve the sensitivity of SKOV3/DDP cells to DDP in miR-132 mimic group compared with that in miR-132 NC group, with IC_{50} values of 8 μM and 64 μM, respectively. At the same time, the proliferation experiment showed that compared with miR-132 NC group, miR-132 mimic group had weaker proliferation ability at 24 h, 48 h, and 72 h, indicating that the overexpression of miR-132 overexpression could inhibit the proliferation of SKOV3/DDP cells in ovarian cancer, and the difference was statistically significant (p<0. 05). The clone formation experiment also revealed that the number of proliferative cells in the miR-132 mimic group was significantly smaller than that in the miR-132 NC group after 14 days (Figure 2C and 2D). To sum up, up-regulating miR-132 could inhibit the proliferation of SKOV3/DDP cells and also increased its sensitivity to DDP.

Up-Regulating the MiR-132 Expression Promoted SKOV3/DDP Cell Apoptosis

To explore the mechanism of the overexpression of miR-132 in inhibiting SKOV3/DDP cell proliferation, the effect of up-regulation of miR-132 on cell apoptosis was examined. Apoptosis was detected by flow cytometry, which revealed that the apoptosis rate in the miR-132 mimic group was 16%, which was significantly higher than that in the miR-132 NC group (6%) (Figure 3A, 3B). Western blotting was further applied to explore the molecular pathways involved in inducing apoptosis. The results showed that the overexpression of miR-132 mimic could markedly increase the expression levels of B-cell lymphoma 2 (Bcl-2)-associated X protein (BAX), cleaved caspase-3 and cleaved poly ADP-ribose polymerase (PARP), and down-regulate the expression level of Bcl-2. The above results indicate

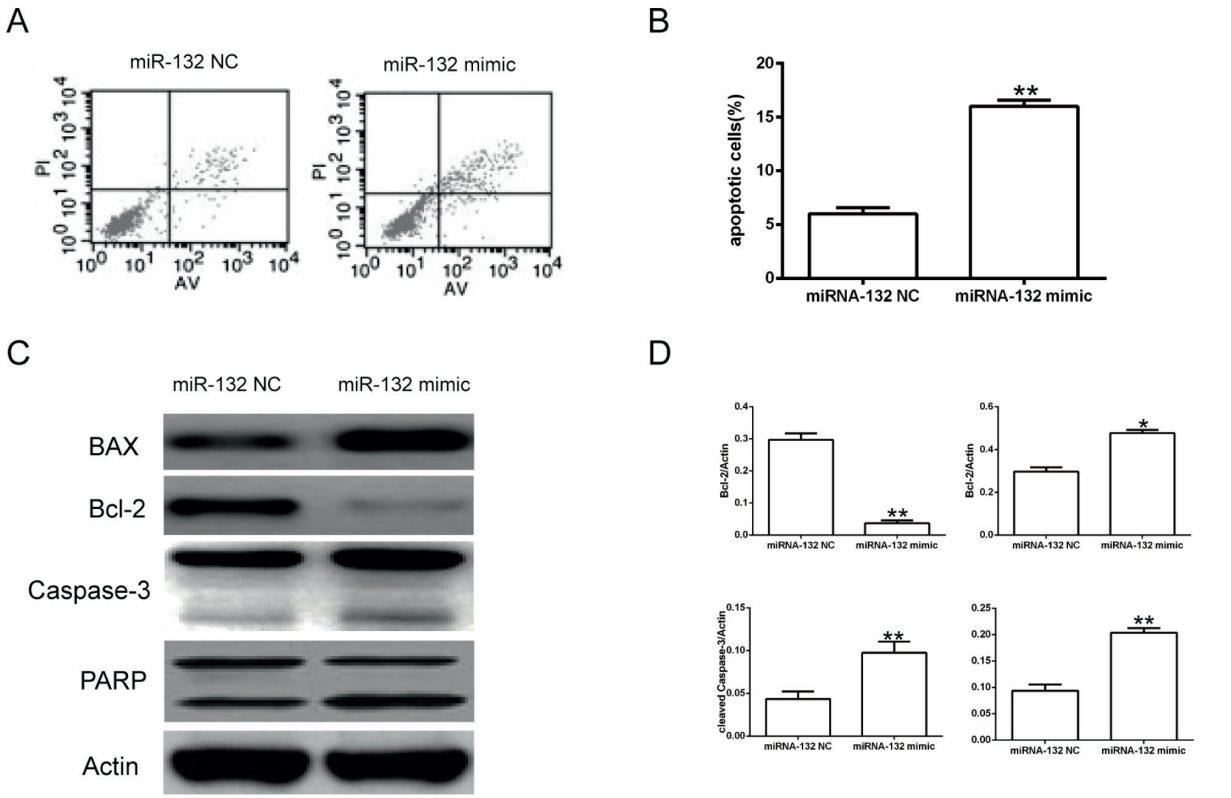

Figure 3. Overexpression of miR-132 induced apoptosis in SKOV3/DDP cells. ***A-B,*** Representative data of FACS and statistical graph analyses of cell apoptosis in SKOV3/DDP cells transfected with miR-132 mimic or miR-132 NC. ***C-D,*** The proteins levels of BAX, Bcl-2, caspase-3, and PARP were determined by Western blotting after SKOV3/DDP cells transfected with miR-132 mimic or miR-132 NC for 48 h. Data are presented as means ± SD (n=3). **p*<0.05, ***p*<0.01 *vs.* control group.

that the overexpression of miR-132 can promote cell apoptosis by up-regulating pro-apoptotic protein Bax, inhibiting the expression of anti-apoptotic protein Bcl-2, initiating the mitochondrial apoptosis pathway and activating expressions of downstream cleaved caspase-3 and cleaved PARP (Figure 3C, 3D).

Up-Regulating the MiR-132 Expression Suppressed SKOV3/DDP Cell Migration and Invasion

Invasion and metastasis are important reasons for the progression and recurrence of malignant tumors. However, many studies have shown that tumor cells often have enhanced invasion and metastasis abilities while acquiring drug resistance characteristics. Therefore, the influences of miR-132 on SKOV3/DDP cell migration and invasion were further investigated using the wound healing assay and transwell chamber assay. It was found that the migration ability of SKOV3/DDP cells in the miR-132 mimic group was lower than that in the miR-132 NC group after overexpressing miR-132, which was consistent with the wound healing assay results. Transwell chamber experiments on the migration and invasion revealed that the migration and invasion ability of SKOV3/DDP cells decreased significantly after the overexpression of miR-132, and the difference was statistically significant ($p<0.05$, Figure 4). In conclusion, the up-regulation of the miR-132 expression could markedly inhibit the migration and invasion of SKOV3/DDP cells.

MiR-132 Targeted Bmi-1 and Regulated its Expression in SKOV3/DDP Cells

Liu et al[10] denoted that miR-132 can target and down-regulate the expression level of Bmi-1 in cervical cancer cells, thus promoting the sensitivity of cervical cancer cells to radiotherapy. Hence, we wondered whether miR-132 could also target the expression of Bmi-1 in SKOV3/DDP cells, thus playing a related biological role. To verify this conception, the expression of Bmi-

1 in SKOV3 cells and SKOV3/DDP cells was detected by RT-PCR and Western blotting. Western blotting results manifested that the Bmi-1 expression in SKOV3/DDP cells was evidently up-regulated compared with that in its maternal cells (Figure 5A). Then, the expression level of the Bmi-1 mRNA after the overexpression of miR-132 in SKOV3/DDP cells was examined. The results confirmed that the expression level of the Bmi-1 mRNA was remarkably down-regulated compared with that in the miR-132 NC group after the overexpression of miR-132, displaying a statistically significant difference ($p<0.05$). Consistent with RT-PCR results, Western blotting results demonstrated that the protein level of Bmi-1 transfected with miR-132 mimic was decreased significantly, with a statistically significant difference ($p<0.05$) (Figure 5B). To further verify whether miR-132 targeted Bmi-1 in SKOV3/DDP cells, resulting in degradation of the Bmi-1 mRNA and further lowering the Bmi-1 protein level, the dual-luciferase reporter gene assay was performed to verify whether miR-132 bound to the 3'-UTR of the Bmi-1 mRNA, thus

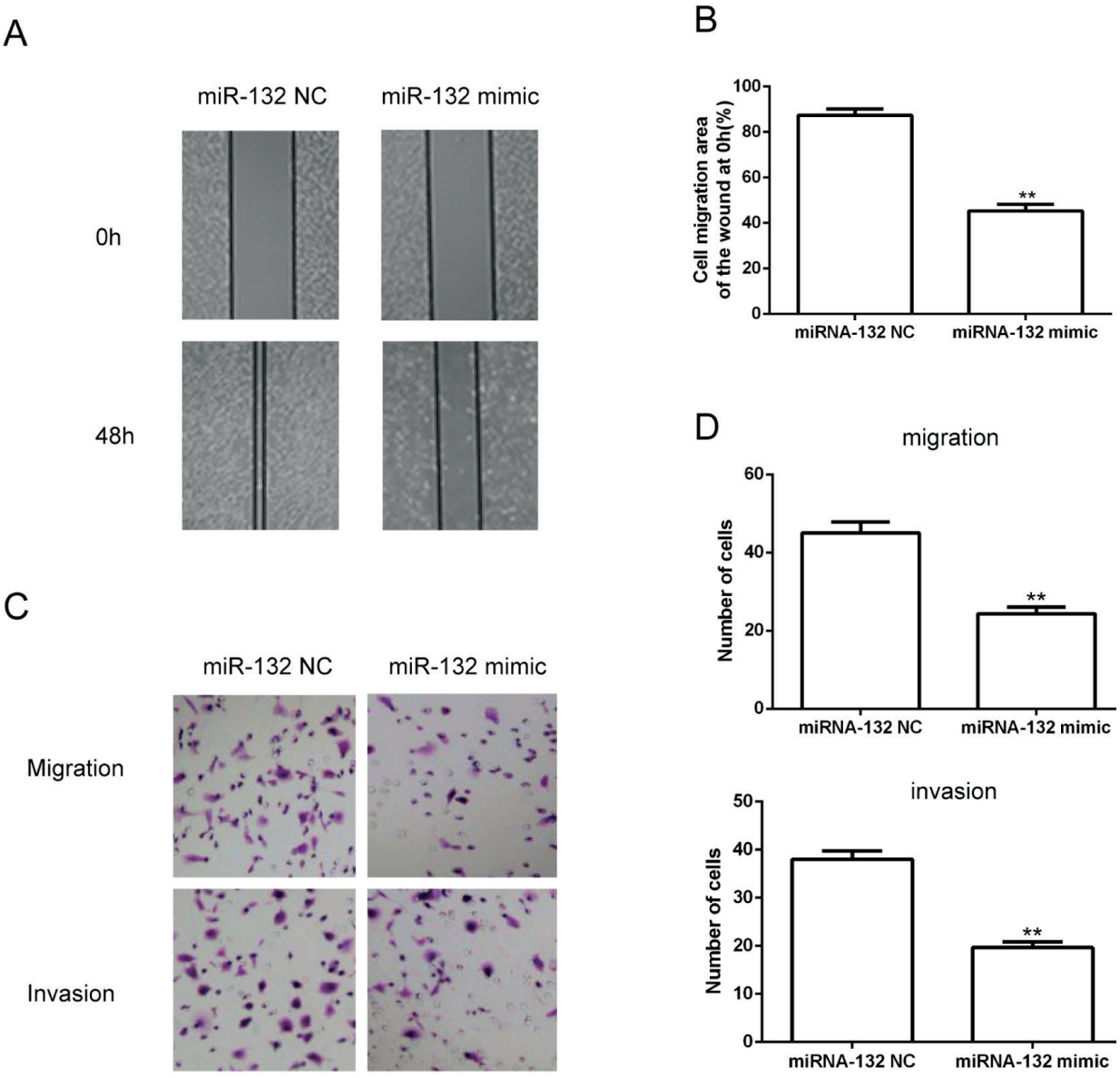

Figure 4. miR-132 can affect SKOV3/DDP invasion ability. ***A, B,*** SKOV3/DDP cells were transfected with miR-132 mimic or miR-132 NC for 48 h, and the effects on cell migration were determined with cell scratch test. ***C, D,*** The effects on cell migration and invasion were determined with cell transwell test. Data are presented as means ± SD (n = 3). **$p<0.01$ *vs.* control group.

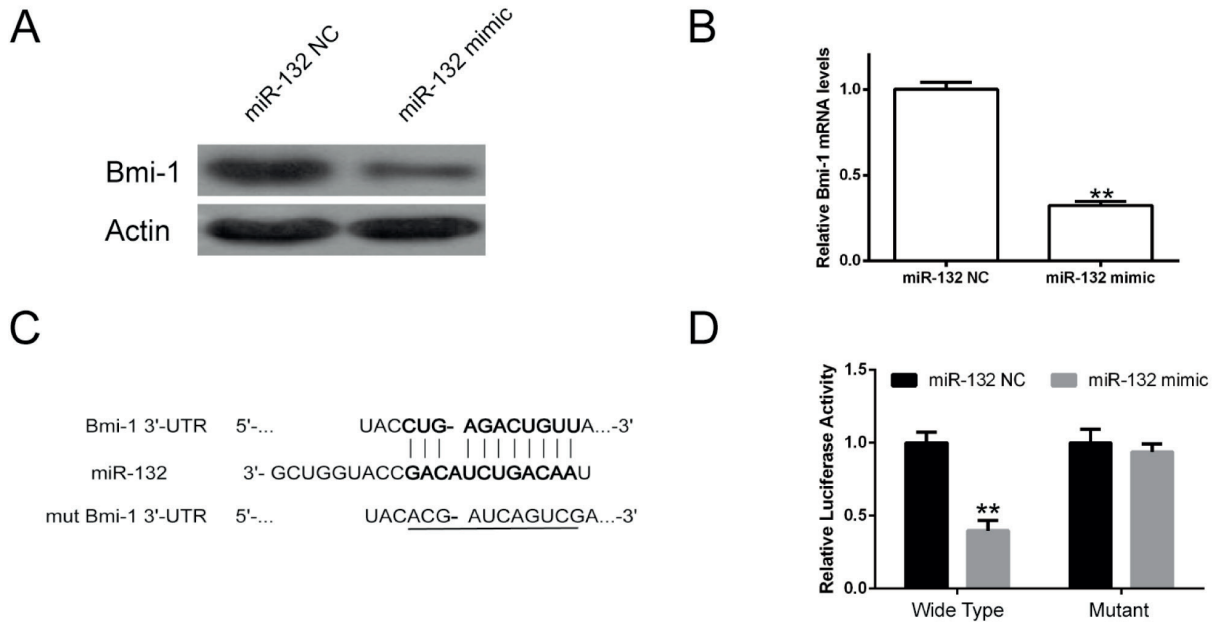

Figure 5. Bmi-1 is a direct target of miR-132 in SKOV3 cells. ***A,*** The levels of Bmi-1 protein were measured by Western blotting in miR-132 overexpressing SKOV3/DDP cells and control cells. ***B,*** The relative Bmi-1 mRNA levels were measured by qRT-PCR in miR-132 overexpressing SKOV3/DDP cells and control cells. ***C,*** The predicted binding sites of miR-132 in the 3'-UTR of Bmi-1. ***D,*** Dual-luciferase reporter assay was used to determine the binding site. mir-132 targeted the wild-type but not the mutant 3′UTR of Bmi-1. Data are presented as means ± SD (n = 3). $^{**}p<0.01$ *vs.* control group.

negatively regulating its expression. The full-length 3'-UTR of Bmi-1 was amplified by PCR and cloned into the downstream of the fluorescein gene in pGL3 vector (Promega, Madison, WI, USA), and this vector was named as wt-3'-UTR. QuickChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) was used to mutate the binding region between Bmi-1 and miR-132. The mutated 3'-UTR was cloned into pGL3 vector and named as mut-3'-UTR. Cells were transfected with miR-132 mimic, miR-132 NC, wt-3'-UTR, and mut-3'-UTR, respectively. At 48 h after transfection, the luciferase value was measured using a dual-luciferase reporting system. The results of dual-luciferase activity detection manifested that compared with that in cells co-transfected with miR-132 NC and mut-3'-UTR, the activity of the luciferase reporter gene in cells co-transfected with wt-3'-UTR and miR-132 mimic markedly decreased ($p<0.05$) (Figure 5D). The above results preliminarily verified that miR-132 could directly target wt-3'-UTR, and miR-132 targeted Bmi-1 and suppressed its expression in SKOV3/DDP cells.

Discussion

Chemotherapy is the main treatment method for ovarian cancer. Combined chemotherapy based on platinum has been proved to be effective. However, DDP resistance tends to occur and chemotherapy fails in the process of using DDP. Therefore, it is very important to study its drug resistance mechanism and try to reverse the drug resistance of ovarian cancer cells.

MiRNAs are a class of non-coding single-stranded RNA molecules with a length of about 22 nucleotides encoded by endogenous genes, which participate in the regulation of gene expression after transcription in animals and plants[11]. Shin et al[12] researches have shown that miRNA can participate in the occurrence and development of tumors and regulate the sensitivity of tumor cells to radiotherapy and chemotherapy. MiR-132 is generally considered to be a miRNA that can inhibit tumor progression. In pancreatic cancer, the promoter of miR-132 is methylated, making its level drop down, while the overexpression of miR-132 can inhibit the invasion level of pancreatic cancer

cells[13]. In breast cancer tissues and cell lines, the expression level of miR-132 is generally decreased. After restoring its expression level, it can inhibit the proliferation, migration, and invasion of breast cancer cells[14]. Throughout the existing studies, no investigation has been found on the relationship between miR-132 and DDP resistance in ovarian cancer. Bmi-1 gene, a proto-oncogene discovered in 1991, is a widely expressed nucleoprotein, directly involved in regulating cell cycle, proliferation, and aging[15,16]. As an epigenetic modification protein, it participates in the maintenance of tumor stem cells and plays a key role in tumor formation, invasion, and metastasis[17]. Research has shown that Bmi-1 exhibits a high expression level in some malignant diseases including gastric cancer, lymphoma, colon cancer, ovarian cancer, and breast cancer.

Zhang et al[18] detected the expression of the Bmi-1 protein in 47 cases of epithelial ovarian cancer by the immunohistochemical streptavidin-peroxidase method, which showed that the positive expression rate was 80.85%, and the strong positive expression rate was 46.81%. The Bmi-1 protein expression level has a correlation with the clinical stage, suggesting that the Bmi-1 expression may indicate invasion and metastasis of ovarian cancer[18]. Liu et al[10] showed that miR-132 can target and inhibit the expression of Bmi-1, thus promoting the sensitivity of cervical cancer cells to radiotherapy. Based on the above findings, we sought to explore the role of miR-132 in DDP resistance in ovarian cancer and its related mechanisms. The difference in the miR-132 expression between DDP-resistant SKOV3/DDP cell line and SKOV3 cell line was detected through qRT-PCR. It was found that the miR-132 expression level in SKOV3/DDP cell line was significantly lower than that in the maternal SKOV3 cell line, suggesting that the down-regulation of miR-132 may be involved in the drug resistance of ovarian cancer cells. To verify this hypothesis, SKOV3/DDP cell line was transfected with miR-132 mimic, which revealed that its sensitivity to DDP was enhanced, its apoptosis rate was increased, and its invasion and metastasis abilities were inhibited. The results of qRT-PCR and Western blotting demonstrated that the overexpression of miR-132 in SKOV3/DDP cells can suppress the expression of Bmi-1. The following luciferase reporter gene assay verified the binding site between miR-132 and Bmi-1, and the results confirmed that miR-132 could indeed negatively regulate the expression of Bmi-1 through the predicted site in SKOV3/DDP cells.

Conclusions

We indicated that the low expression of miR-132 can promote the resistance of ovarian cancer to DDP, and its mechanism may be to inhibit cell apoptosis through the targeted regulation of the Bmi-1 expression. We provide a theoretical basis for the treatment strategy of reversing DDP resistance and inhibiting cell invasion and metastasis in ovarian cancer by targeted regulation on miR-132.

Conflict of interest

The authors declare no conflicts of interest.

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