PDCD1 strengthens the sensitivity of ovarian cancer to cisplatin chemotherapy by promoting apoptosis

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Summary

Purpose: *The primary purpose of this study was to make clear the role of PDCD1 in the occurrence and progression of ovarian cancer, and explain its mechanism.*

Methods: *RT-PCR, westem blot, MTT and immunohisto-chemistry were used to detect the expression levels of PDCD1 mRNA and protein in human ovarian cancer cell lines (SKOV3, 3AO, CAOV3 and OVCAR3), human normal ovary, serous cystadenoma, and serous cystadenocarcinoma tissue. SKOV3, SKOV3-MOCK, and SKOV3-PDCD1 cells were sub-cutaneously injected into the armpit of nude mice to observe the effect of PDCD1 expression on tumorigenic ability. Cis-platin, carboplatin, cyclophosphamide, etoposide and pacli-taxel were used in the experiments.*

Results: *PDCD1 was lowly expressed in SKOV3 and 3AO, moderately expressed in CAOV3, and highly expressed in OVCAR3. PDCD1 significantly inhibited the proliferation and clone formation ability of the ovarian carcinoma cell line SKOV3. In the SKOV3-PDCD1 group, the tumor forma-*

tion rate decreased significantly and the tumor formation time prolonged significantly. The CAOV3 and OVCAR3 cells with high expression of PDCD1 were more sensitive to cispla-tin. The SKOV3 and 3AO cells with low expression of PDCD1 were less sensitive to cisplatin. Compared with the SKOV3-MOCK control group, the apoptosis rate and the expression levels of the caspase-3/8 proteins activity increased signifi-cantly in the PDCD1 overexpression group. The expression levels of caspase-9 and Bax increased slightly. No significant changes were observed in the expression of Bcl-2.

Conclusion: *The expression of PDCD1 decreased signifi-cantly in human ovarian cancer. Overexpression of PDCD1 can inhibit the proliferation capacity of ovarian cancer. PDCD1 strengthens the sensitivity of ovarian cancer to cis-platin by promoting cisplatin-induced apoptosis.*

Key words*: chemotherapy sensitivity, cisplatin, ovarian cancer, PDCD1*

Introduction

Ovarian cancer is a common gynecological malignant tumor [1]. It has many types. Primary ovarian epithelial cancer accounts for 85.90% of malignant tumors of the ovary. Clinically, ovarian cancer is treated primarily by surgery and com-bination chemotherapy dominated by platinum derivatives [2]. However, tumor cells can become easily resistant to chemotherapeutic drugs and their efficacy is compromised. The 5-year sur-vival rate for women with ovarian cancer is still around 30% [3].

Programmed Cell Death-1 (PDCD1) is a new

cancer suppressor gene. It can inhibit the growth of tumor cells by inhibiting protein transcription and translation processes [4]. Overexpression of PDCD1 can inhibit malignant transformation of cells. Moreover, experiments [5] on transgenic mice showed that the PDCD1 can not only inhib-it the formation of the epidermic papilloma and decrease the incidence of skin cancer but also im-pede the malignant transformation from epider-mic papilloma to skin cancer. Research has shown that 84% of mice without the PDCD1 are able to spontaneously produce malignant B cell lympho-

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ma. Therefore, PDCD1 is identified as a cancer suppressor gene. In addition, results of the research on human tumors have indicated that the loss rate of PDCD1 is 83% in primary lung cancer and it is closely associated with the grade of malignancy [6]. Highly frequent loss and downregulation of PDCD1 exist not only in lung cancer but also in other malignant tumors such as colon cancer, glioma, pancreatic cancer, breast cancer etc [7,8].

Under the action of chemotherapeutic drugs, changes in the apoptotic ability of tumor cells are one of the major causes of chemotherapy resistance in ovarian cancer. Research has indicated that chemotherapy drug-induced apoptosis is not only associated with downregulation of cell survival genes but also with upregulation of apoptosis-promoting molecules or cancer suppressor genes [9]. PDCD1 may play different roles in different tumor cells. Research on breast cancer has found that apoptosis may be induced after the T-47D and MDA-MB-231 tumor cell lines are transfected with PDCD1, indicating that PDCD1 plays an important role in mediating apoptosis of breast cancer cell lines [10]. PDCD1 can also induce apoptosis of the liver cancer cell line Huh7. It has also been found that PDCD1 participates in TGF-β1 induced apoptosis of liver cancer cell lines. TGF-β1 significantly increases the expression of PDCD1 in the Huh7 cells. After Huh7 cells are transfected with the antisense PDCD1, they resist the TGF-β1 induced growth inhibition and the number of induced apoptotic cells also decreases significantly [11].

In conclusion, starting with cisplatin administration *in vivo* and *in vitro*, the research verifies the role of PDCD1 in strengthening cisplatin sensitivity in ovarian cancer and discusses its effect on other common chemotherapeutic drugs such as cyclophosphamide, paclitaxel etc [12], creating also a basis for discussing the mechanism of PDCD1 in strengthening the sensitivity to chemotherapy.

In the present study we attempted to investigate the mechanism of PDCD1 in strengthening chemotherapy sensitivity both theoretically and experimentally by studying whether PDCD1 plays a role in apoptosis and signal transduction pathways.

Methods

Tumor specimens, cells, and laboratory animals

Thirty-seven patients with serous cystadenocarcinoma, 23 patients with serous cystadenoma, and 30 normal control ovariectomized patients (without ovarian diseases) aged 35-74 years (mean 53) treated at the Department of Obstetrics & Gynecology of Binzhou People's Hospital (Shandong Province) between 2009-2014 were selected and studied. No patient had received radiotherapy, chemotherapy, or immunotherapy before operation. The human ovarian carcinoma cell line SKOV3 was purchased from the Shanghai Cellular Biological Research Institute of Chinese Academy of Sciences. 3AO was purchased from the Institute of Fundamental Research, Shandong Medical University. CAOV3 and OVCAR3 were purchased from the Wuhan Culture Collection Center. Female nude Balb/c mice aged 4-6 weeks and weighing 18-22 g were purchased from the Laboratory Animal Center, Inner Mongolia Medical University (Hohhot, Inner Mongolia, China).

Experiment on tumor formation in nude mice

The nude mice were subcutaneously injected with ovarian carcinoma cells SKOV3 (10^6 each mouse). Small tumor nodules appeared 1 week after. The mice were randomly divided into 6 groups: normal saline control group, empty vector group, PDCD1 group, cisplatin group, empty vector+cisplatin group, and PDCD1+cisplatin group. The mice weight was measured three times a week. All nude mice in the 6 groups were sacrificed after 6 weeks by cervical vertebral dislocation. The tumors were excised and weighed for later use.

RT-PCR

10^6 ovarian carcinoma cell lines (SKOV3, 3AO, CAOV3, and OVCAR3) or 100mg *in situ* tumor tissue of ovarian cancer were subjected to conventional extraction of RNA. Fluorescent RT-PCR reaction solution 20 μL, DNA polymerase 1 μL, reverse transcriptase 0.35 μL, and template RNA 5 μL were well mixed and centrifuged for 10 s at 6000 r/min. Fluorescent RT-PCR amplification process: reversely transcribed for 3 min at 50°C; pre-denatured for 3 min at 95°C; denatured at 95°C for 15 s, annealed for 30 s at 50°C; extended for 30 min at 72°C, 5 cycles in total; denatured for 10 s at 95°C; annealed for 40 s at 55°C; 40 cycles in total; the information on primers were as follows: PDCD1: upstream 5'TCA GCG ACA GTG GGA GT 3', downstream 5'AGC ACG GTA GCC TTA TC 3' (purchased from Sangon Biotech Co., Ltd., Shanghai, China).

Western blot

1 mg of ovarian carcinoma cells and *in situ* tumor protein of ovarian cancer were subjected to SDS-PAGE and 2 pieces of gel were removed carefully after SDS-PAGE. A spongy cushion, filter paper, gel, membrane, filter paper, and a spongy cushion were spread in sequence for preparation of gel transfer interlayer. The bubbles were expelled with a glass rod. The membrane was placed in an electrophoretic transfer tank (100 V 350mA) and transferred for 60-90 min. The membrane

was moved to a plate containing 25 mL blocking buffer and gently shaken on a decoloration shaker for 2 hrs. Then, the primary anti-PDCD1 antibody (Abcam, Cambridge, UK) was added. The membrane was incubated overnight at 4°C and washed 3 times x 5 min with tris-buffered saline tween (TBST) on a shaker at room temperature. The appropriate secondary antibody (Zhongshan Jinqiao, Beijing, China) was then added. The membrane was incubated for 1 hr on the shaker at 37°C. The membrane was washed 3 times x 5 min with TBST and was developed for observation.

Immunohistochemistry

The paraffin sections of the ovarian cancer tissue were dewaxed, washed 3 times x 3 min with phosphate buffered saline (PBS), for 2 min with antigen (98°C) or digested with protease, washed 3 times x 3 min with PBS, allowed to stand still for 20 min at room temperature after endogenous peroxidase was inhibited by 0.3% H_2O_2, washed 3 times x 3 min with PBS, incubated for 30 min after dropwise addition of normal goat serum (1:20), not washed, incubated overnight at 4°C after dropwise addition of the specific primary antibody anti-PDCD1 (Abcam, Cambridge, UK), rewarmed on the next day, washed 3 times x 3 min with PBS, washed 3 times x 3 min with PBS after dropwise addition of the biotinylated secondary antibody (Zhongshan Jinqiao, Beijing, China, 1:200, 37°C, 30 min), washed 3 times x 3 min with PBS after dropwise addition of streptavidin-HRP (1:400) (Boster Biotechnology Co., Ltd., Wuhan, China, 37°C, 30 min), developed with diaminobenzidine (DAB), restained with hematoxylin, dehydrated, mounted with gum, and observed under microscope [13].

Cell transfection

The cells were inoculated into a 24-well microplate and cultured in a humidifying incubator containing 5% CO_2 at 37°C until the cells reached 80-90% confluence. In each well, 1 µg transfected PDCD1 plasmid (Sangon Biotech Co., Ltd., Shanghai, China) was diluted with culture modified Eagle medium (MEM) until the final volume reached 50 µl: 2-50 µl lipidosome was diluted with the same culture medium and was mixed thoroughly via pipetting up and down and allowed to stand still for 25 min at room temperature. The old culture medium was removed from the culture plate inoculated on the previous day. 500 µL MEM culture medium was added. The above mixture (100 µl/well) was added to the cell wells. The culture plate was shaken back and forth several times and the mixture was uniformly distributed onto the cell surface. Cells were cultured for 6 hrs in a humidifying incubator containing 5% CO_2 at 37°C. The solution was changed to appropriate culture medium containing 10% fetal bovine serum and cells were then cultured in 5% CO_2 at 37°C for 48 hrs.

MTT method

The ovarian carcinoma cells in the logarithmic growth phase were digested with 0.25% pancreatin, counted, and inoculated into a 96-well microplate enabling the cell density in each well to reach 1×10^4. Different chemotherapy drugs (cisplatin, carboplatin, etoposide, cyclophosphamide and paclitaxel (Qilu Pharmaceutical Co., Ltd., Shandong, China) were used. Four parallel wells were set in each group and blank control without cells was set for each group. The cells were cultured for 24 hrs at 37°C until cell adherence. The supernatant was pipetted and 100 µL culture medium containing MTT reagent was added to each well (Sigma Aldrich, St. Louis, MO, USA). The cells were cultured for 1-8 hrs and the optical density (OD) value was detected at 1, 2, 4, and 8 hrs at different wavelengths. During detection, 100 µL 10% sodium dodecyl sulfate (SDS) were added to each well and shaken to dissolve the formazan crystals [14]).

Small interfering RNA (siRNA) transfection

The cells were digested and diluted to 1×10^5/ mL with MEM for preparation of cell suspension. Opti-MEM was used to dilute siPORTTM NeoFXTM Transfection Agent (Amersham Pharmacia Biotech, Uppsala, Sweden) and PDCD1siRNA (Amersham Pharmacia Biotech, Uppsala, Sweden) was added at 25 µl/well. Cells were incubated for 10 min at room temperature. The diluted siRNA and siPORTTM NeoFXTM Transfection Agent were mixed. Cells were incubated for 10 min and added to the culture plate with 50 µL per well. Cell suspension (450 µL) was added to each well. The cell suspension was gently applied onto the surface of the transfection compound, it was well mixed by gently shaking the plate and cells were placed in an incubator containing 5% CO_2 at 37°C. The cells were collected 8-72 hrs after transfection and the interference result was detected with RT-PCR and Western blot [15].

Cell apoptosis detection via Hoechest33258 method

After the cells were inoculated into the plate, cisplatin (Qilu Pharmaceutical Co., Ltd., Shandong, China) was added to induce cell apoptosis. The cells were washed twice with cold PBS and 1 ml of 4% formaldehyde solution was added. The cells were fixed for 10 min at 4°C and were washed twice with PBS. 100 µL Hoechst33258 (KeyGEN BioTECH Co., Ltd., Nanjing, China) working solution was dropwise added to each well. The cells were stained for 10 min at room temperature, cleaned with water, and air-dried. Fluorescence was excited with ultraviolet light at 340 nm wavelength and observation was performed under fluorescence microscope [16].

Detecting late apoptosis with the Tunnel method

The treated cells were collected, counted, cen-

trifuged, and washed twice with PBS. The cells were fixed for 60 min with 2% paraformaldehyde at room temperature, centrifuged at 1000 r/min for 5 min, and washed once with PBS after the fixing solution was discarded. Holes were drilled for 2 min at 0°C. The cells were washed twice with PBS and were resuspended after the 50 μL Tunnel reaction solution (Roche Innovatis, Bielefeld, Germany) was added to each tube. The cells were stained for 60 min at 37°C in the dark, and washed twice with PBS. PBS was added to resuspend the cells. Then, the cells were transferred to a flow tube and the final volume reached 250-500 mL PBS. Flow cytometer (BD Pharmingen, San Diego, CA, USA) was used for detection of cell apoptosis [17].

Statistics

The experimental result was analyzed with SPSS19.0. T-test was used to compare differences in cell proliferation, tumor volume, and weight between the experimental and the control group. For numerical variables the t-test was used and for categorical vari-

ables the Fisher exact probability test for statistical analysis was used. Differences were considered significant at p<0.05.

Results

Expression of PDCD1 in human ovarian carcinoma cell lines and primary ovarian cancer tissue

The expression of PDCD1 in the ovarian carcinoma cell lines was detected with RT-PCR and Western blot to identify the role of PDCD1 in ovarian cancer. The results indicated that PDCD1 mRNA was lowly expressed in SKOV3 and 3AO cells, moderately expressed in the CAOV3 cells, and highly expressed in the OVCAR3 cells (Figure 1A). The expression of the PDCD1 protein in the 4 types of ovarian carcinoma cells coincided with the above results (Figure 1B). The expression levels of the PDCD1 protein in 20 cases of normal ovary, 26 cases of serous cystadenoma,
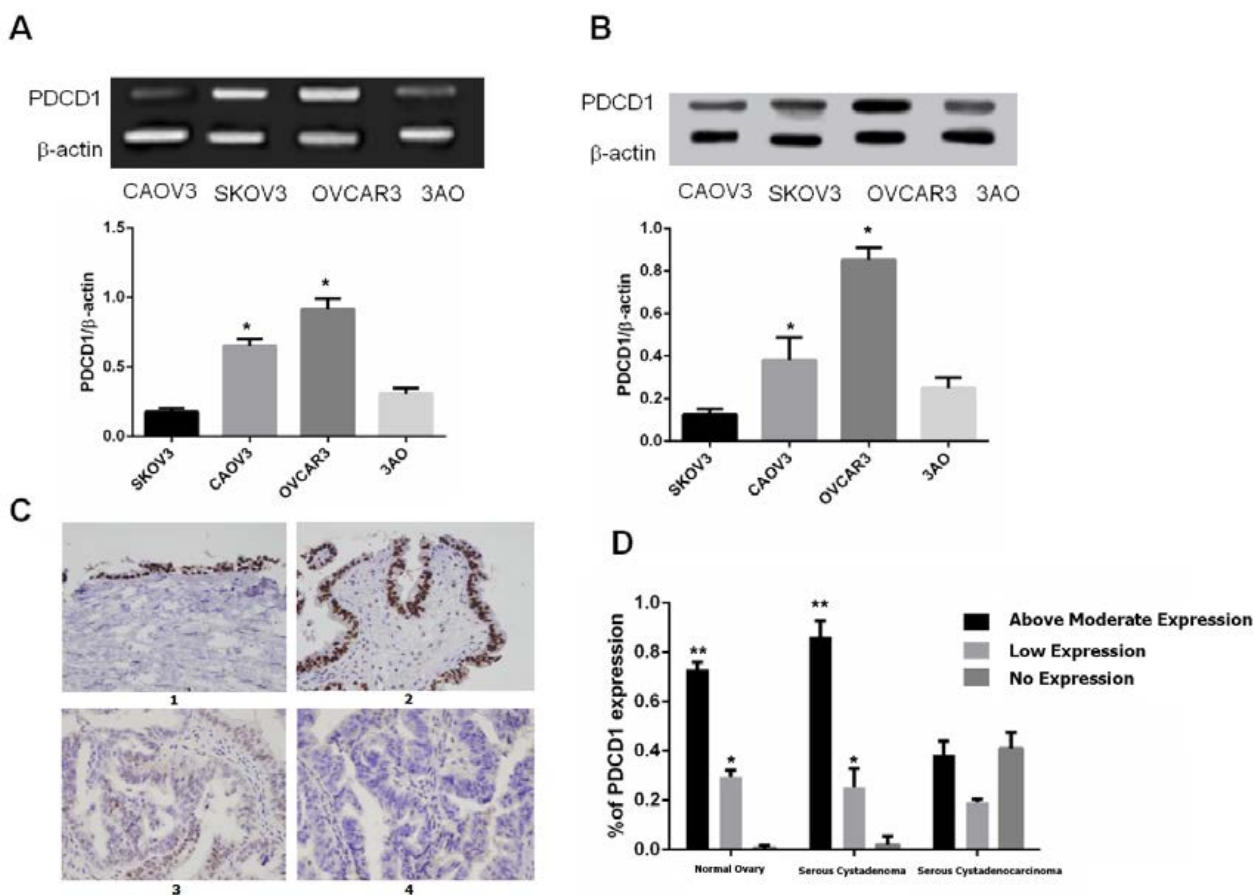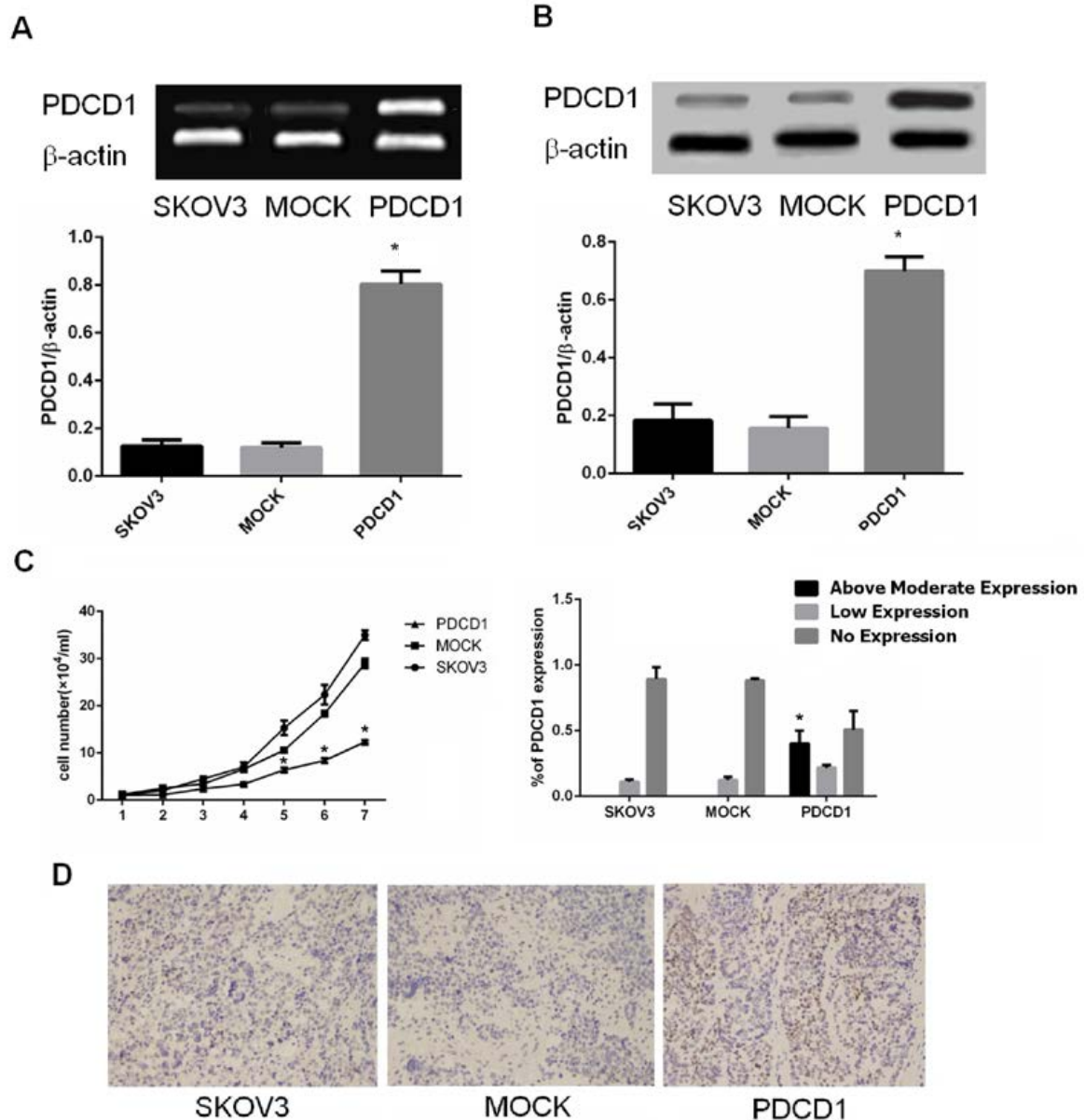

Figure 1. Expression of PDCD1 in human ovarian carcinoma cell lines and primary ovarian cancer tissue. **A:** Expression of PDCD4 mRNA in different ovarian carcinoma cell lines. The expression level was highest in OVCAR3, moderate in CAOV3, and lowest in SKOV3 lines (*compared with SCOV3, p<0.05); **B:** Expression of PDCD1 mRNA in different ovarian carcinoma cell lines (*compared with SCOV3, p<0.05). The expression level was highest in OVCAR3, moderate in CAOV3, and lowest in SKOV3 lines; **C:** Expression of the PDCD4 protein in normal ovary and serous tumor (×400) (1): PDCD1 was highly expressed in the epithelial cells of the normal ovary; (2) PDCD1 was highly expressed in the epithelial cells of the serous cystadenoma; (3) and (4): PDCD1 was lowly expressed in the serous cystadenocarcinoma cells; **D:** Analytical results of overall expression levels of PDCD1 in all normal ovaries and serous tumors (**compared with no expression, p<0.01).

and 43 cases of serous cystadenocarcinoma tissue was detected using the immunohistochemical method. The loss rate of PDCD1 in the serous cystadenocarcinoma tissue was 39.5% and the low expression rate was 18.6%. Normal ovarian (80%) and serous cystadenoma tissue (70%) exhibited moderate or high expression of PDCD1. No loss of PDCD1 was observed (Figure 1C-D).

Expression of exogenous PDCD1 significantly inhibited the growth of ovarian carcinoma cells

The cell line SKOV3 derived from serous cystadenocarcinoma with low expression of PDCD1 was transiently transfected with the empty vector and the PDCD1 recombinant expression vector *in vitro*. Stably expressed SKOV3-PDCD1 and SKOV3-MOCK cell lines were established after screening

Figure 2. Expression of exogenous PDCD1 significantly inhibited the growth of ovarian carcinoma cells. **A-B:** Expression levels of PDCD1mRNA and protein in SKOV3 cells after the empty vector or PDCD1 expressed vector was stably transfected. *: The comparison between SKOV3 and SKOV3-PDCD1 indicated p<0.05; the comparison between #SKOV3-MOCK and SKOV3-PDCD1 cells indicated p<0.05; **C:** Overexpression of PDCD1 on growth of the SKOV3 cells: SKOV3-PDCD1 cells grew significantly slowly compared with SKOV3 and SKOV3-MOCK cells (*compared with MOCK group, p<0.05); **D:** Expression levels of PDCD1 in the tumors in the SKOV3 group, SKOV3-MOCK group, and SKOV3-PDCD1 group: The expression of PDCD1 in the tumor in the SKOV3-PDCD1 group was significantly higher than that in the other two groups; overexpression of PDCD1 in the SKOV3 cells could decrease their tumorigenic ability in the nude mice and slow down the tumor growth (*compared with MOCK group, p<0.05).

with G418 at a final concentration of 300 mg/ml at 24 hrs.

RT-PCR and Western blot were used for identification of PDCD1 mRNA and protein levels. The results indicated that the expression of PDCD1 mRNA and protein was significantly upregulated in the SKOV3-PDCD1 cells (Figure 2A-B). SKOV3-PDCD1 cells grew significantly slowly compared with SKOV3 and SKOV3-MOCK cells (Figure 2C, p<0.001). SKOV3, SKOV3-MOCK, and SKOV3-PDCD1 cells were subcutaneously injected into the armpit of each nude mouse. Immunohistochemical detection of the expression of PDCD1 in the tumor indicated that the expression of PDCD1 in the tumor in the SKOV3-PDCD1 group was significantly higher than that in the other two groups (Figure 2D). These results indicated that the overexpression of PDCD1 in the SKOV3 cells could decrease its tumorigenic ability in the nude mice and slow down the tumor growth.

Relationship between overexpression of PDCD1 and the chemotherapy sensitivity in ovarian cancer

Cisplatin was used to treat the above four types of cells. The MTT result indicated that the CAOV3 and OVCAR3 cells with high expression of PDCD1 were more sensitive to cisplatin whereas the SKOV3 and 3AO cells with low expression of PDCD1 were less sensitive (Figure 3A). To further verify the effect of PDCD1 on the chemotherapy sensitivity to ovarian cancer, PDCD1 was found overexpressed in the SKOV3 and CAOV3 cell lines. The cell survival rate was detected with the MTT assay at 24 hrs after cisplatin of different concentrations was added. The results indicated that the cell survival rate of SKOV3-PDCD1 was significantly lower than that of SKOV3-MOCK under the action of the same concentration (Figure 3B). To further verify whether the expression of PDCD1 can potentiate the sensitivity of the ovarian carcinoma cells to cisplatin we used the other 4 types of chemotherapy drugs (carboplatin, etoposide, cyclophosphamide and paclitaxel) to conduct the above experiment. The results showed that PDCD1 could also potentiate the chemotherapy sensitivity of another platinum drug, carboplatin (Figure 3C), but PDCD1 had no significant effect on the sensitivity of cyclophosphamide (Figure 3D), etoposide (Figure 3E), and paclitaxel (Figure 3F), suggesting that PDCD1 improved the sensitivity to chemotherapy possibly due to its specificity of platinum compounds.
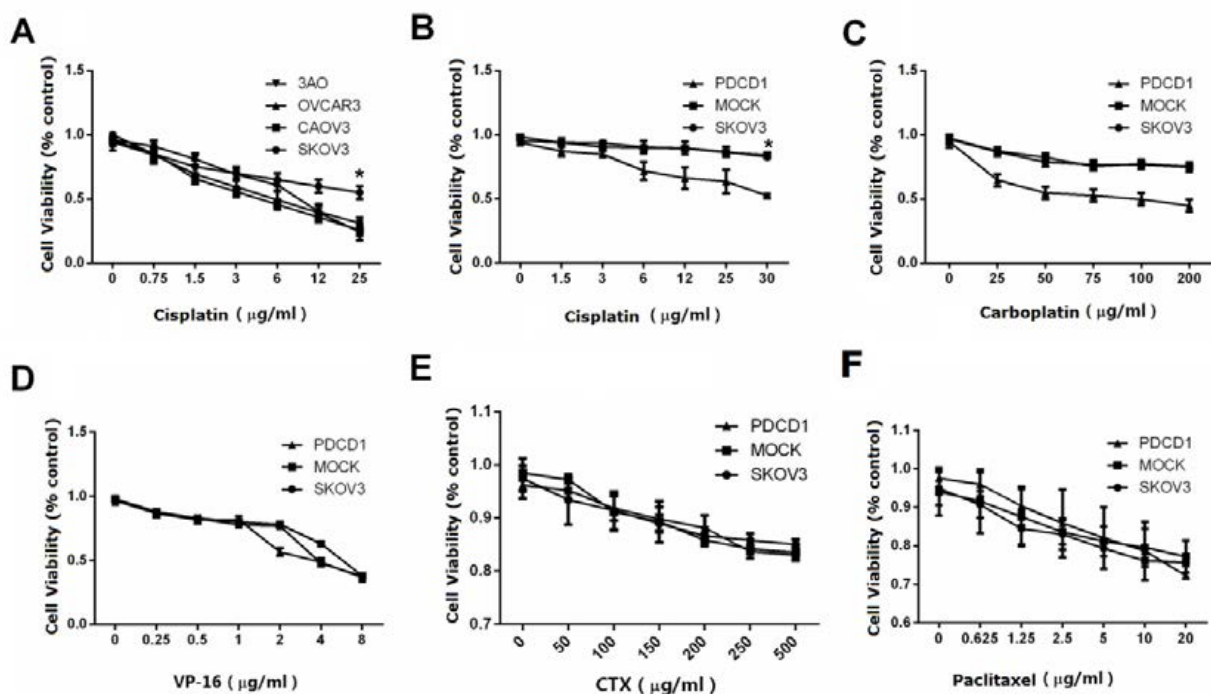
Figure 3. Relationship between the expression level of PDCD1 and chemotherapy sensitivity to ovarian cancer. **A:** Chemotherapy sensitivity of 4 types of ovarian carcinoma cell lines to cisplatin was detected with the MTT method (*compared with CAOV3, p<0.05); **B:** Cell survival rates of SKOV3, SKOV3-MOCK, and SKOV3-PDCD1 at 24hrs after treatment with cisplatin of different concentrations: the cell survival rate of SKOV3-PDCD1 was significantly lower than that of SKOV3-MOCK under the action of cisplatin of the same concentration; (*compared with PDCD1, p<0.05); **C-F:** Survival rates of SKOV3, SKOV3-MOCK, and SKOV3-PDCD1 cells at 24hrs after treatment with carboplatin, cyclophosphamide, VP-16, and cyclophosphamide: PDCD1 had no significant effect on the chemotherapy sensitivity of these drugs.

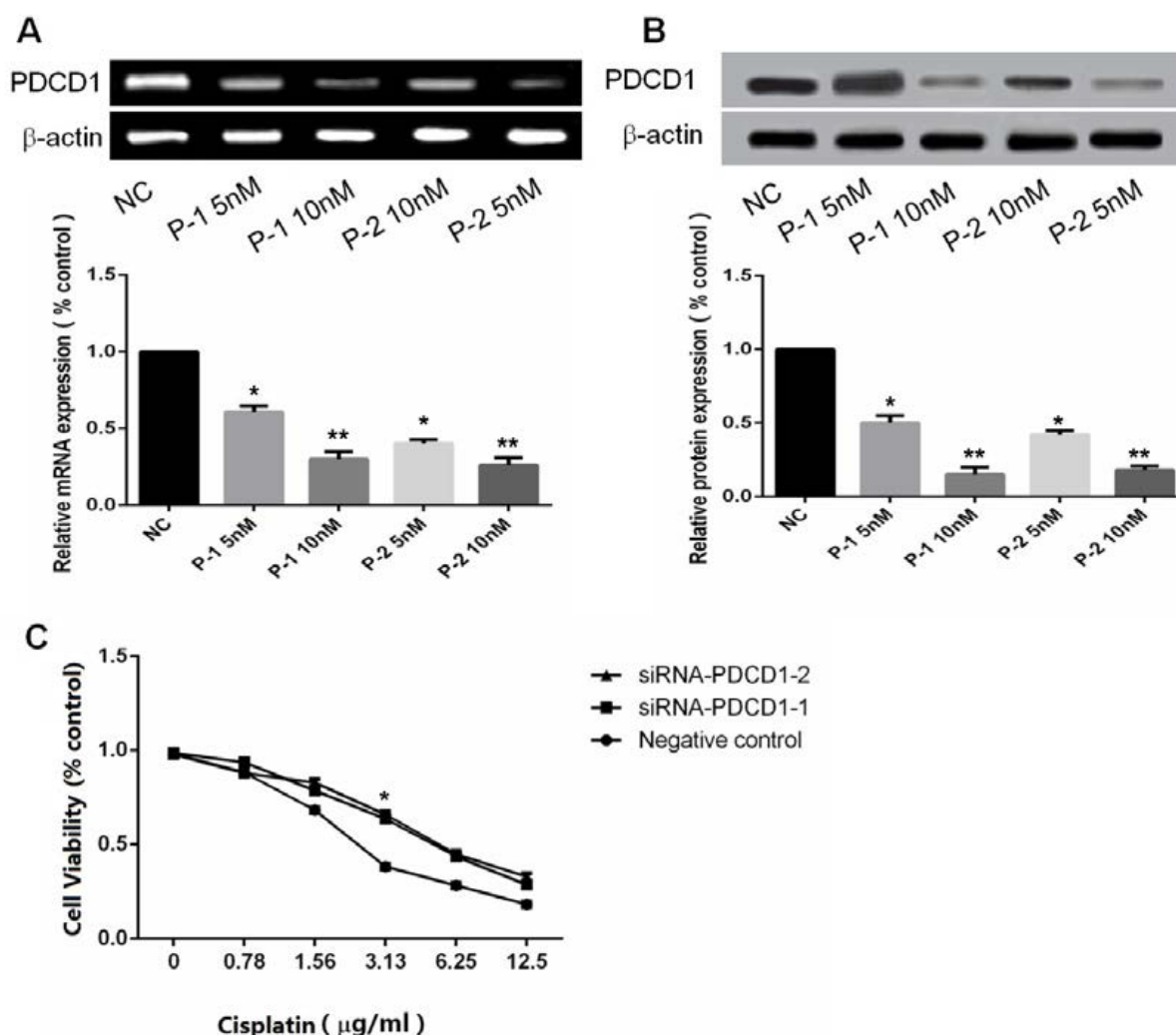*Silent PDCD1 decreased the sensitivity of ovarian cancer to cisplatin*

To further determine the effect of PDCD1 on chemotherapy sensitivity of ovarian cancer, two PDCD1 specific siRNA fragments were designed and synthesized. siRNA was transfected into the ovarian cancer cell line OVCAR3 with high expression of PDCD1. The cells were collected at 48 hrs and the interference result was detected with RT-PCR and Western blot.

The results indicated that both siRNA fragments could significantly silence the PDCD1 gene. The expression rates of siRNA-1 and siRNA-2 in inhibiting PDCD1 mRNA were 77% and 76% and the expression rates of siRNA-1 and siRNA-2 in inhibiting PDCD1 protein were 80% and 88% (Figure 4A-B). The two siRNA fragments were

transfected into the OVCAR3 cells and different concentrations of cisplatin were added at 48 hrs. Then, the cell survival rate was detected with the MTT method. The results showed that both siRNA fragments could significantly increase the survival rate of the ovarian carcinoma cells and decrease the sensitivity to cisplatin when compared with the control group (Figure 4C).

Combination of PDCD1 and cisplatin significantly inhibited the growth of ovarian cancer

The tumor growth in the PDCD1 and cisplatin combined treatment group was inhibited significantly when compared with that in the PDCD1+empty vector group. The difference was statistically significant (Figure 5A). All nude mice in the 6 groups were sacrificed and the tumors were

Figure 4. Silent PDCD1 decreased sensitivity of ovarian cancer cells to cisplatin. **A-B:** Results of PDCD1 siR-NA in silencing the expression of PDCD1 mRNA and protein in the OVCAR3 cells: Both siRNA fragments could significantly silence the PDCD1 gene (*compared with NC group, $p<0.05$; **compared with NC group, $p<0.01$); **C:** Changes in chemotherapy sensitivity of ovarian carcinoma cells to cisplatin following silent expression of PDCD1: Both siRNA fragments could significantly increase the survival rate of ovarian carcinoma cells and decrease the chemotherapy sensitivity to cisplatin when compared with the control group (*compared with NC group, $p<0.05$);. NC: Non-specific nucleotide fragment, P-1: PDCD1 specific siRNA fragment 1, P-2: PDCD1 specific siRNA fragment 2.

excised and weighed. The results showed that the weight of tumor in the PDCD1+cisplatin group (0.0300±0.0044g) was significantly lower than that in the cisplatin group (0.0860±0.0204g), the PDCD1 group (0.0533±0.0042g), or the empty vector +cisplatin group (0.07667±0.0126 g) (Figure 5B). The immunohistochemical result indicated that the expression of PDCD1 in the tumor tissue in the treatment group with the PDCD1 vector was significantly higher compared with the other control groups (Figure 5C).

Overexpression of PDCD1 significantly strengthened apoptosis induced by cisplatin

The cells were stained with Hoechst at the same concentration (12.5g/ml or 25g/ml) after been subjected to cisplatin treatment. The SKOV3 cells with overexpression of PDCD1 exhibited 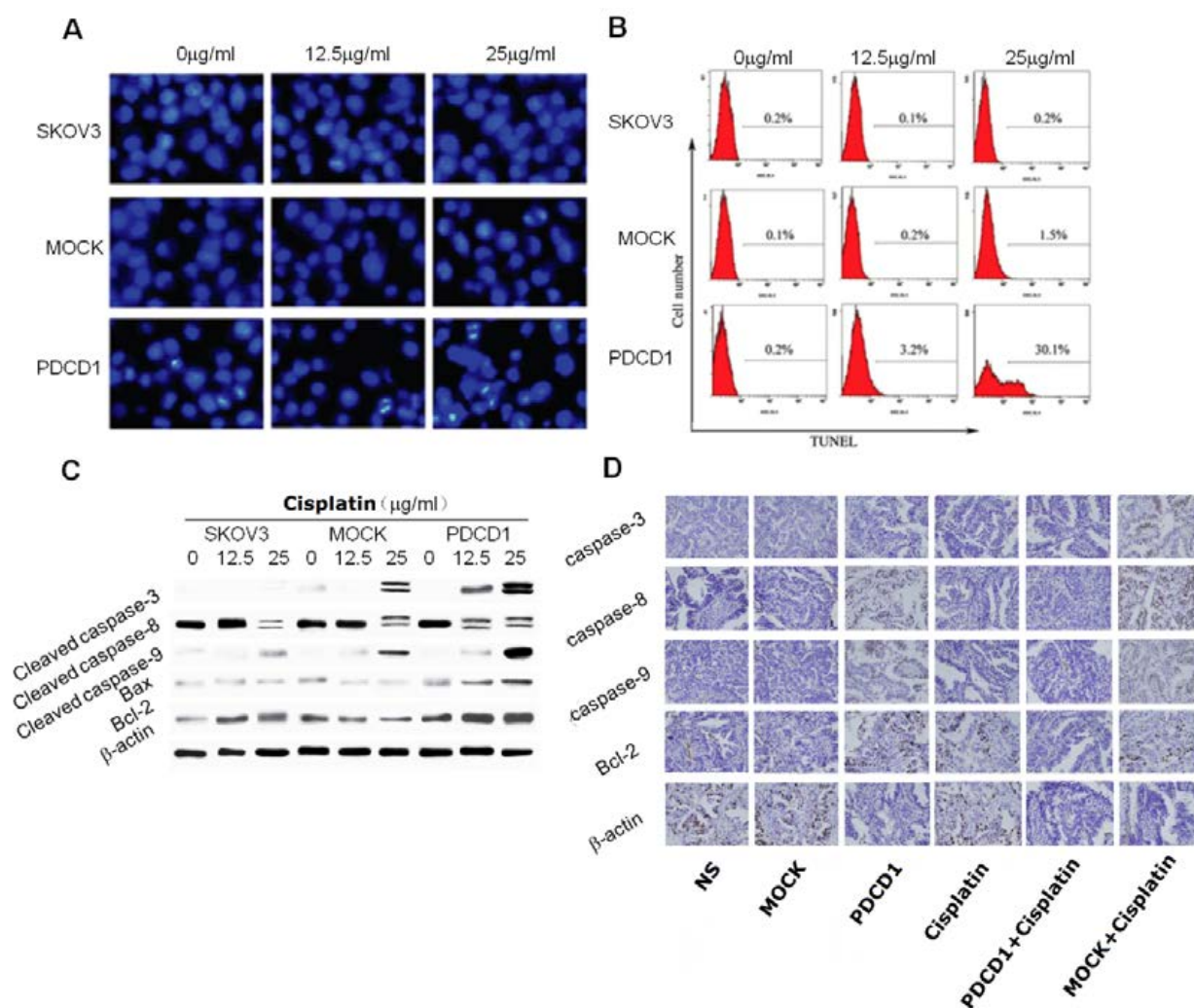significant apoptosis, karyopyknosis, and high ag-glomeration of chromatin, whereas the apoptotic cells in the empty vector control group were fewer (Figure 6A). When the concentration of cisplatin was 12.5μg/ml, the apoptosis rate in the PDCD1 transfection group reached 3.2%, whereas no significant late-stage apoptosis occurred in the empty vector control group. Similarly, when the concentration of cisplatin was 25μg/ml, the apoptosis rate in the PDCD1 stable transfection group was 30.1%, much higher than that in the empty vector control group (1.5%) (Figure 6B).

The Western blot method was used to detect the changes in the apoptosis-related molecules caspase-3,8,9, Bcl-2, and Bax. The expression levels of the caspase-3 and caspase-8 activity proteins treated with cisplatin in the PDCD1 over-expression group increased significantly when compared with those in the empty vector control group, whereas the expression levels of caspase-9
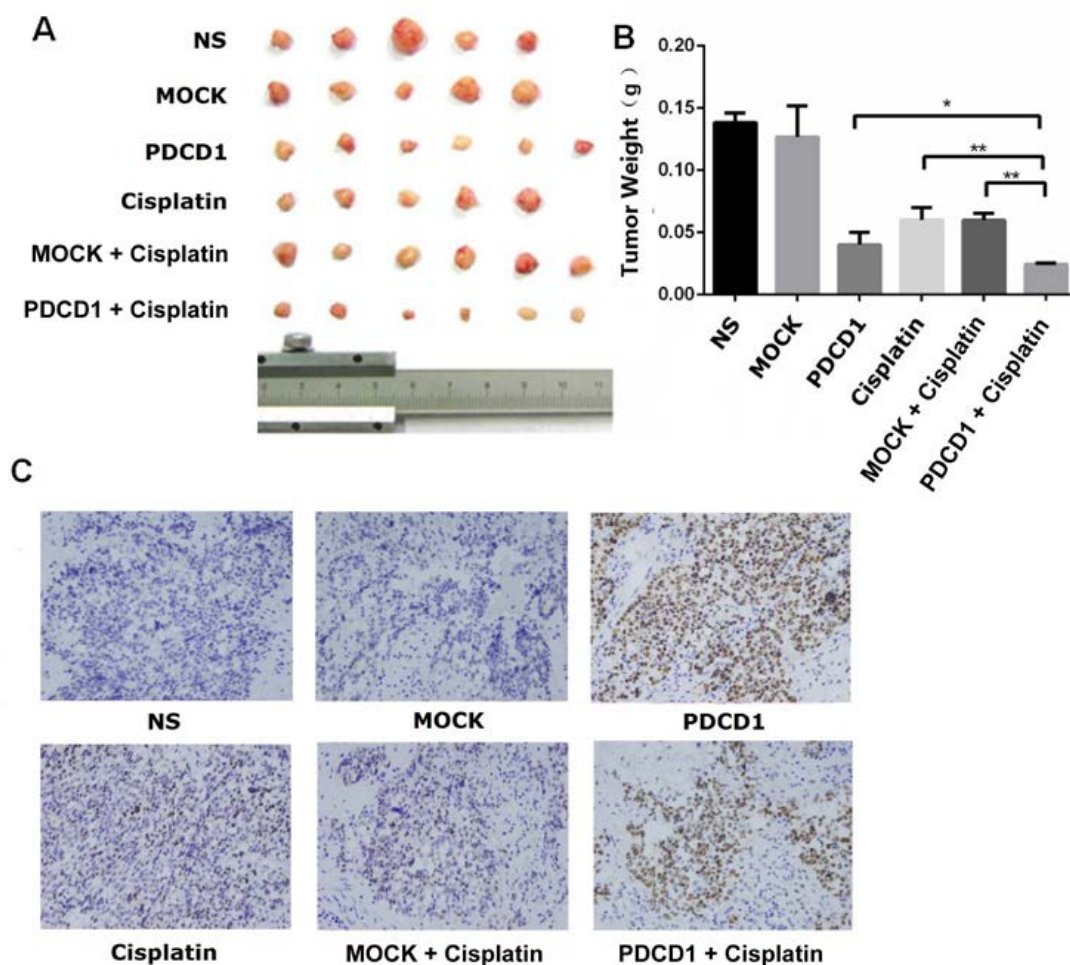
Figure 5. Combination of PDCD1 and cisplatin significantly inhibited growth of ovarian cancer cells. **A:** General appearance of tumors in different treatment groups in tumor-bearing nude mice; **B:** Weight of tumors in different treatment groups in tumor-bearing nude mice: The tumor growth in the PDCD1 and cisplatin combined treatment group was inhibited significantly when compared with that in the PDCD1+empty vector group and the difference was statistically significant (*p<0.05, **p<0.01); **C:** Expression of PDCD1 in the tumors in different treatment groups (immunohistochemistry, ×400): The expression of PDCD1 in the tumor tissue in the treatment group with the PDCD1 vector was significantly higher than that in other control groups.

Figure 6. Overexpression of PDCD1 significantly strengthened apoptosis induced by cisplatin. **A:** Effect of over-expression of PDCD1 on morphological changes in cisplatin-induced apoptosis (Hoechst,×400): the SKOV3 cells with overexpression of PDCD1 exhibited significant apoptosis, karyopyknosis, and high agglomeration of chromatin whereas the apoptotic cells in the empty vector control group were fewer; **B:** Effect of overexpression of PDCD1 on the changes in cisplatin-induced later stage apoptosis: when the concentration of cisplatin was 12.5 µg/mL or 25 µg/mL, the apoptosis rate in the PDCD1transfection group was significantly higher than that in the empty vector control group; **C:** Effect of overexpression of PDCD1 on the changes in cisplatin-induced apoptosis-related protein: the expression levels of the caspase-3 and caspase-8 active proteins treated with cisplatin in the PDCD1 overexpression group increased significantly when compared with those in the empty vector control group. The expression levels of caspase-9 and Bax increased slightly. No significant changes were observed in the expression of Bcl-2; **D:** Changes in the expression of apoptosis-related proteins in the tumor tissue in different treatment groups.

and Bax increased slightly. No significant changes were observed in the expression of Bcl-2 (Figure 6C). In addition, similar results were obtained with the immunohistochemical detection of the expression of the above protein molecules in the paraffin sections (Figure 6D).

Discussion

PDCD1 is a cancer suppressor gene recently identified which can inhibit tumor growth and metastasis by inhibiting translation and transcription of tumor cells [2,3]. Our research found that the expression of PDCD1 is decreased significantly or lost in ovarian cancer and that PDCD1 can significantly inhibit the proliferation and clone formation ability of the ovarian carcinoma cell line SKOV3 *in vitro*, while inhibiting the tumorigenic ability of the SKOV3 cells in the nude mice *in vivo*. All of these experimental results indicate that PDCD1 plays a very important role in the incidence and progression of ovarian cancer. The threatening activity of a tumor comes from its rapid proliferation and it has been reported that PDCD1 is able to inhibit the proliferation of ovarian cancer cells [5,18,19]. The S6K1 and 6TRCP-mediated PDCD1 degradation can promote protein translation and cell proliferation [20,21]. These results indicate that PDCD1 possibly plays its role by inhibiting the proliferation of

ovarian cancer cells. Our results also showed that the overexpression of PDCD1 in the SKOV3 cell line can effectively inhibit the proliferation of the ovarian carcinoma cells, suggesting that PDCD1 can inhibit the activity of ovarian cancer cells *in vitro*. However, the mechanism of action of PDCD1 as a cancer suppressor gene still remains unclear [6,22].

The combination chemotherapy dominated by platinum derivatives is always the common treatment plan for ovarian cancer but the resistance of ovarian cancer cells limits its efficacy [23]. The present study has found that the cancer suppressor gene PDCD1 significantly strengthens the sensitivity of ovarian cancer to platinum derivatives in the *in vitro* culture system or the *in vivo* experiment, but had no significant effect on the sensitivity of other drugs such as cyclophosphamide, etoposide, and paclitaxel. At present, the mechanism of PDCD1 in strengthening the sensitivity of chemotherapy still remains unclear. It has been reported that overexpression of PDCD1 can strengthen the geldanamycin-induced G2-M phase blocking and apoptosis [24]. It has also been reported that PDCD1 strengthens the sensitivity of paclitaxel possibly by downregulating the expression of YB-1 [25]. Our research has found that overexpression of PDCD1 can effectively strengthen apoptosis induced by cisplatin and demonstrated that PDCD1 does play a role in inducing apoptosis. *In vitro* overexpression of PDCD1 can induce

apoptosis of the breast cancer cell line T-47D [26]. We demonstrated that overexpression of PDCD1 can increase the cisplatin-induced apoptosis by the Hoechst staining and the Tunnel experiment but the exact pathway needs to be further elucidated.

The present study indicated that the overexpression of PDCD1 can significantly increase the expression of the caspase-3 and caspase-8 active proteins. The expression levels of caspase-9 and Bax increased slightly, while no significant changes were observed in the expression of Bcl-2. These results indicate that the pathway by which PDCD1 promotes cisplatin-induced apoptosis is mainly the death receptor pathway. Inhibiting caspase-8 can block the role of PDCD1 in strengthening the sensitivity to cisplatin, which further verifies our results. However, the mechanism of PDCD1 in activating the death receptor pathway warrants further studies.

In conclusion, this study showed that PDCD1 can significantly strengthen the sensitivity of ovarian cancer to platinum derivatives by promoting cisplatin-induced apoptosis. These results provide strong evidence for application of PDCD1 as a chemotherapy sensitizer in the clinical treatment ovarian cancer.

Conflict of interests

The authors declare no confict of interests.

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