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The iron chelator desferrioxamine synergizes with chemotherapy for cancer treatment

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ABSTRACT

Background: Cisplatin (CDDP) resistance remains a major obstacle for treatment of ovarian cancer. Iron contributes to the growth and reproduction of malignant cells, thus iron chalators can inhibit the growth of tumor cells by depleting the intracellular iron pool. The iron chelator, desferrioxamine (DFO), has performed anticancer in previous study. The aim of our study is to determine the correlation between iron-deprivation and tumor chemosensitivity in ovarian cancer.

Methods: To investigate the prognostic value of ferritin light (FTL), ferroportin (FPN), hepcidin (HAMP) and divalent metal-ion transporter-1 (DMT1) in ovarian cancer, the Kaplan–Meier analysis and the Gene Expression Profiling Interactive Analysis (GEPIA) were used. The ovarian cancer cell lines (SKOV-3 and OVCAR-3) were exposed to a gradient concentration of DFO (10, 20, 50, 100, 200 μM) and CDDP (1, 5, 10, 50,100 μM) for 24 h. The protein expression of FTL was tested. The expression of cancer stem cell (CSC) markers, including Sox2, Nanog and C-myc, were downregulated with treatment of DFO. Also, the mamosphere formation and the plation of $CD44^{+/high}/CD133^{+/high}$ and Aldehyde dehydrogenase $(ALDH)^{+/high}$ SKOV-3 cells were reduced after treatment for 7d. Furthermore, we detected the expression of p53, BCL-2, BAX, and caspase-8.

Results: The survival analysis revealed that high expression of FTL, DMT1, HAMP, showed poor overall survival (OS) in ovarian cancer patients. Our combined data found that DFO could effectively inhibit CSCs, improve the resistance to chemotherapy, and significantly enhanced the efficacy of CDDP therapy in vitro in promoting apoptosis. Besides, targeting molecular targets, including BAX, BCL-2, p53 and caspase-8 could serve as the clinical biomarkers to evaluate the effects of ovarian cancer. It is reasonable to believe that DFO adjuvant therapy in combination with CDDP chemotherapy can promote the improvement of treatment response in ovarian cancer patients.

Conclusion: Our research suggests the experimental evidence for DFO and CDDP as a new effective combination therapy to enhance the efficacy of chemical therapy in ovarian cancer.

1. Introduction

Ovarian cancer is one of the most lethal gynecological malignancy [1]. The overall 5-year survival rate for patients with advanced ovarian cancer is only about 40%, and has remained essentially static for the past 20 years [2,3]. Current treatment standard is cytoreductive surgery and platinum-based combination chemotherapy. However, approximately 90% of ovarian cancer deaths are caused by chemotherapy resistance [4]. Urgent measures should be taken to reduce the resistance of chemotherapy. Therefore, exploring the mechanism of chemotherapy resistance in ovarian cancer and finding effective treatment may help to improve the treatment effect.

Cisplatin (CDDP), a platinum-containing metal compound, is widely used in the chemotherapy of a variety of tumors, including ovary, testis, head and neck tumors. In addition, CDDP is the first platinum-derived compound approved by Food and Drug Administration (FDA) for clinical cancer treatment. CDDP is one of the most effective and generally used chemotherapeutic drugs for ovarian cancer [5]. However,

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resistance to CDDP is responsible for the failure of ovarian cancer treatment. In order to enhance the therapeutic effect, the combination of CDDP and other drugs is expected to be a new treatment strategy for ovarian cancer [6].

Iron can promote the growth and reproduction of malignant cells. Cancer cells undergoing rapid self-metabolism have a great need for intracellular iron concentrations, during which high levels of ferritin (including light and heavy ferritin) are expressed for long-term storage of iron [7,8]. In addition, it has been found that light ferritin is involved in the acquired drug resistance of cancer cells [9]. In recent years, studies have shown that intracellular iron levels, including possible iron pools and iron storage protein ferritin, serve as key factors in the development of chemotherapy resistance and tumor prognosis in cancer patients [10]. Deferoxamine (DFO) is the iron chelator widely used in clinic to reduce iron overload [11]. In addition, DFO inhibits the growth of tumor cells by depleting the intracellular iron pool necessary for its enzyme activation, to produce anticancer activity [12,13].

In this study, we present the strong evidence of the correlation between iron-deprivation and tumor chemosensitivity in ovarian cancer cell line SKOV-3 and OVCAR-3. Furthermore, we detected the efficacy of DFO and the possible improved mechanism of CDDP chemotherapy therapy in vitro. Our combined data uncovered the enhanced efficacy of combination of iron-chelating and chemotherapy, which may also create a new path for enhancing cancer therapeutics with DFO.

2. Materials and methods

2.1. Cell culture and reagents

Human ovarian cancer cell line SKOV-3 and OVCAR-3 were obtained from the American Type Culture Collection (Manassas, VA, USA), and cells were cultured in phenol red-free RPMI-1640 medium (Gibco BRL Life Technologies Inc, USA), coculture with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Invitrogen) in a humidified supplied with 5% CO_2 at 37 °C.

2.2. Iron binding studies of cancer cells

Iron binding studies of cancer cells at 0 and 24 h, after the cancer cells treated with DFO/DFX (Sigma-Aldrich) for 24 h. Cancer cells were tested after the cells washed with PBS and cocultured with Calcein-AM (ATT Bioquest, Inc.) for 20 min at 1.0 μM. Cancer cells were washed with PBS, following with the iron binding efficacy detected through flow cytometry.

2.3. Sphere formation of ovarian cancer

The tumor spheres of ovarian cancer were cultured in the ultra-low attachment plates with 5000 cells/mL, supplemented with the DMEM/F-12 medium containing epidermal growth factor (EGF) (20 ng/mL), insulin (4 μg/mL), basic fibroblast growth factor (FGF) (20 ng/mL) and B27 (0.4%). Tumor spheres of ovarian cancer were counted after culture for 7 d.

2.4. The expression of $CD44^+/CD133^+$

Ovarian cancer cells treated with or without DFO were tested with 10% FBS for 30 min, following with anti-CD133 and anti-CD44 antibodies (BioLegend) for 20 min. Cells were then washed with PBS for three times, flowing with the test with cytometry.

2.5. ALDH level analysis

Ovarian cancer cells were treated with methanol for 5 min, following with 0.1% PBS-Tween for 10 min. Cancer cells were then incubation with first-antibody (ALDH) for 60 min and following with FITC-conjugated anti-rabbit secondary antibody for 30 min. The expression of ALDH were then texted with flow cytometry by the NovoCyte 1040 flow cytometer.

2.6. Western blot analysis

The western blot analysis was performed to detect the expression levels of various proteins in cells. Cells were harvested, after washing with PBS, and lysed with RIPA lysis buffer for 40 min on ice, following with centrifuged at 12,000*g*for 15 min at 4 °C. The total protein was tested by the BCA protein assay kit (Solarbio Science & Technology Co., Ltd., Beijing, China). The equal amounts (50 μg) of each sample was subjected to SDS-PAGE electrophoresis, following with the transfer to polyvinylidene fluoride (PVDF) membranes (Millipore). The membranes of protein were blocked with 10% of non-fat milk for 1 h in room temperature. Following with the primary antibodies (Table S1) and the secondary antibodies. β-actin was used as a loading control for normalization.

2.7. CCK-8 cell viability assay

Cell Counting Kit-8 (CCK-8) w tested by the manufacturer's protocol (Solarbio Science & Technolo Co., Ltd., Beijing, China). 20,000 cells were inoculated into 96-well plates, and cancer cells were subjected to different concentration of DFO/DFX and CDDP at different concentration for 24 h. The cell viability of cancer cells was determined following the provided reagents.

2.8. Cell growth assays

Cell growth was determined by the CCK8 assay following the manufacture's protocol (Solarbio Science & Technology Co., Ltd., Beijing, China). Overall, 5000 cancer cells were seeded onto 96-well plates with medium of 10% serum overnight. Cell viability was tested after CDDP treatment with or without DFO pretreatment at 50 μM for 24 h. Thereafter, cancer cells were washed with phosphate-buffer saline (PBS) three times and then cultured with CCK8 for 3 h. The fluorescence intensity was tested with the microplate reader.

2.9. Assays of cancer cell death

SKOV-3 cells (1×10^5) were stained with anti-annexin V antibody, labeled in combination with propidium iodide (PI), following with the manufacturer's protocol (KeyGen Biotech, Nanjing, People's Republic of China). Cells were then analyzed in the fluorescence-activated cell sorting (BD, Franklin Lakes, USA). The death percentage of cells were corresponded to annexin V^+–PI^+, and the apoptotic death percentage of cells was shown with annexin V^+–PI^-.

2.10. Bioinformatics analysis

To determine the expression pattern of the iron metabolism genes (including TFRC, DMT1, FPN, FTL and HAMP) in ovarian cancer, the search of the Oncomine database (http://www.oncomine.com) was carefully conducted. Briefly, all of the 5 genes were shown in the database with the Box chart (mRNA level of iron metabolism). Also, the prognostic value of the 5 iron metabolism genes (including TFRC, DMT1, FPN, FTL and HAMP), the expression of mRNA in ovarian cancer was also analyzed by the Kaplan–Meier Plotter (http://kmplot.com/analysis/). Currently, the database of Kaplan–Meier Plotter includes 54,675 genes, also the effect of survival is assessed by 10,461 cancer samples [14]. The database was used as previously described. The genes were imported into the database of ovarian cancer to obtain Kaplan–Meier survival plots, with the database used as previously described. All of the Multivariate Cox regression model was shown with Hazard Rate (HR), and 95% confidence interval (CI). The log-rank p-

value were calculated through the database of ovarian cancer and displayed.

2.11. Immunofluorescence staining

Ovarian cancer cells were seeded in the 6-well plates, and subjected to different concentration after CDDP treatment with or without DFO pretreatment at 50 μM for 24 h. Then, cancer cells were incubated with the primary antibody against P53, followed with the fluorescence-conjugated secondary antibodies in succession. Dye 4′-6-Diamidino-2-phenylindole (DAPI) was used to show the nucleus. The photographs were taken at random fields, and results of the photographs were taken at random fields.

2.12. Real-time polymerase chain reaction (PCR) analysis

Ovarian cancer cells were treated with DFO at different concentrations for 24 h. Real-time PCR were performed to exam the expression of RNA. Total RNA was isolated with TRIzol reagent followed with the PrimeScript RT reagent kit for the first-strand complementary deoxyribonucleic acid (cDNA). The real-time PCR was conducted for the expression of the CSCs marker. β-actin was used as the invariant control. Results were from experiments in triplicate.

2.13. Statistical analysis

The Independent *t*-test and One-Way ANOVA test were used to analyze the experimental data. Data were presented in mean ± standard deviation (SD). Here, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Survival analysis of iron metabolism genes of ovarian cancer

To investigate the prognostic value of FTL, FPN, HAMP and DMT1 in ovarian cancer, the Kaplan–Meier plotter database (http://kmplot.com/analysis/) was used. The survival analysis revealed that high expression of FTL, DMT1, HAMP, showed poor OS in ovarian cancer patients (Figs. 1A and S1). Also, FPN mRNA high expression was correlated to better OS. To consistent with the Kaplan–Meier analysis of FTL, the GEPIA analysis was carried out. GEPIA analysis is a newly established gene expression analysis web server [15], which includes both TCGA and GTEx databases, with about 9736 cancers and 8587 normal samples, showing the various differential analyses. We investigated the relative expression of FTL expression patterns with GEPIA web tool. The results showed higher expression of FTL in patients with ovarian cancer.

3.2. DFO inhibits the intracellular iron concentration in dose-dependent manner

The ovarian cancer cells were exposed to a gradient concentration of DFO (0, 10, 20, 50, 100, 200 μM), DFX (0, 10, 20, 50, 100 μM) and CDDP (0, 1, 5, 10, 50,100 μM) for 24 h, as shown in Figs. 2A–D and S1. The SKOV-3 and OVCAR-3 cells were treated with DFO and DFX at 50 μM, at which concentration, DFO and DFX showed no significant cytotoxicity to ovarian cancer cells. The protein expression of FTL was tested. As shown in Fig. 3A and B, DFO significantly reduced the protein level of FTL at different concentration of DFO with dose-dependent manner both in SKOV-3 and OVCAR-3 cells. As shown in Fig. 3A and B, DFO (50 μM) treatment significantly reduced the protein level of FTL. The intracellular iron concentration treated with DFO was determined by the Calcein-AM probe with the fluorescent intensity. Ovarian cancer cells were treated with DFO for 24 h, the fluorescent intensity significantly increased compared to the control groups (Fig. 3C). All these data indicated that DFO can significantly reduce the intracellular iron concentration in SKOV-3 and OVCAR-3 cells.

3.3. DFO depletes the CSCs of ovarian cancer

Next, we investigated the potential impact of the iron chelator on the reduction of CSCs, which is considered to be closely related to chemoresistance [16,17]. DFO reduced the intracellular iron concentration, with the reduction of FTL and labile iron pool (LIP), showed in Fig. 3A–C, with sublethal concentration at 50 μM. A few markers were thought to be closely related with chemoresistance, including Sox2, Nanog and C-myc (Fig. 3C). The expression of these genes was tested with quantitative real-time PCR (q-PCR) and the CSC-associated genes reduced remarkably compared to the control group. To this end, our data supported the rationale for iron chelator to deplete the CSCs of ovarian cancer by reducing the intracellular iron.

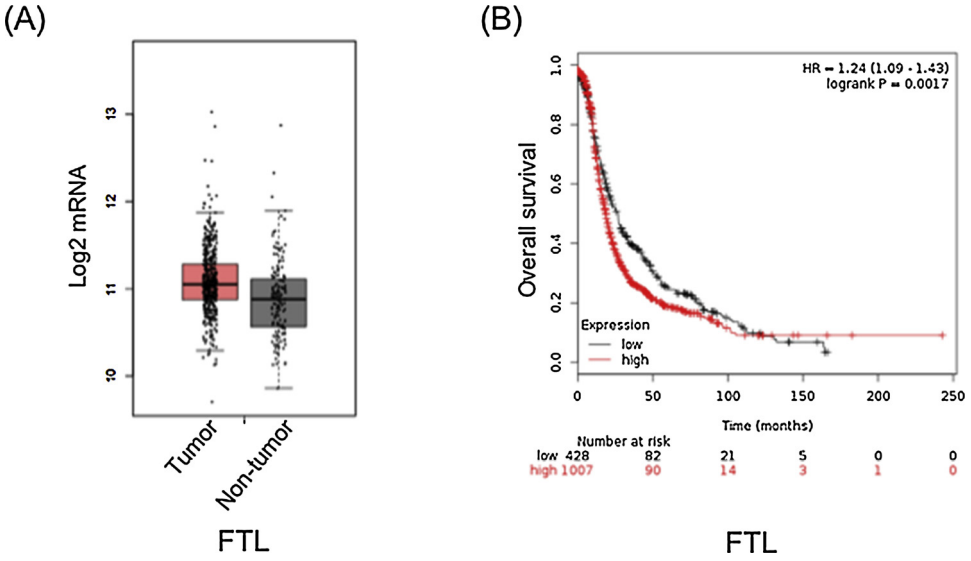

Fig. 1. Iron contributes to progression in ovarian patients. (A) Box and whiskers plots of Gepia database on ferritin light (FTL) mRNA levels in ovarian cancer and normal tissues. (B) The prognostic value of FTL expression in ovarian cancer patients (N = 216). Pound (#) denotes $P < 0.001$, compared to untreated cells.

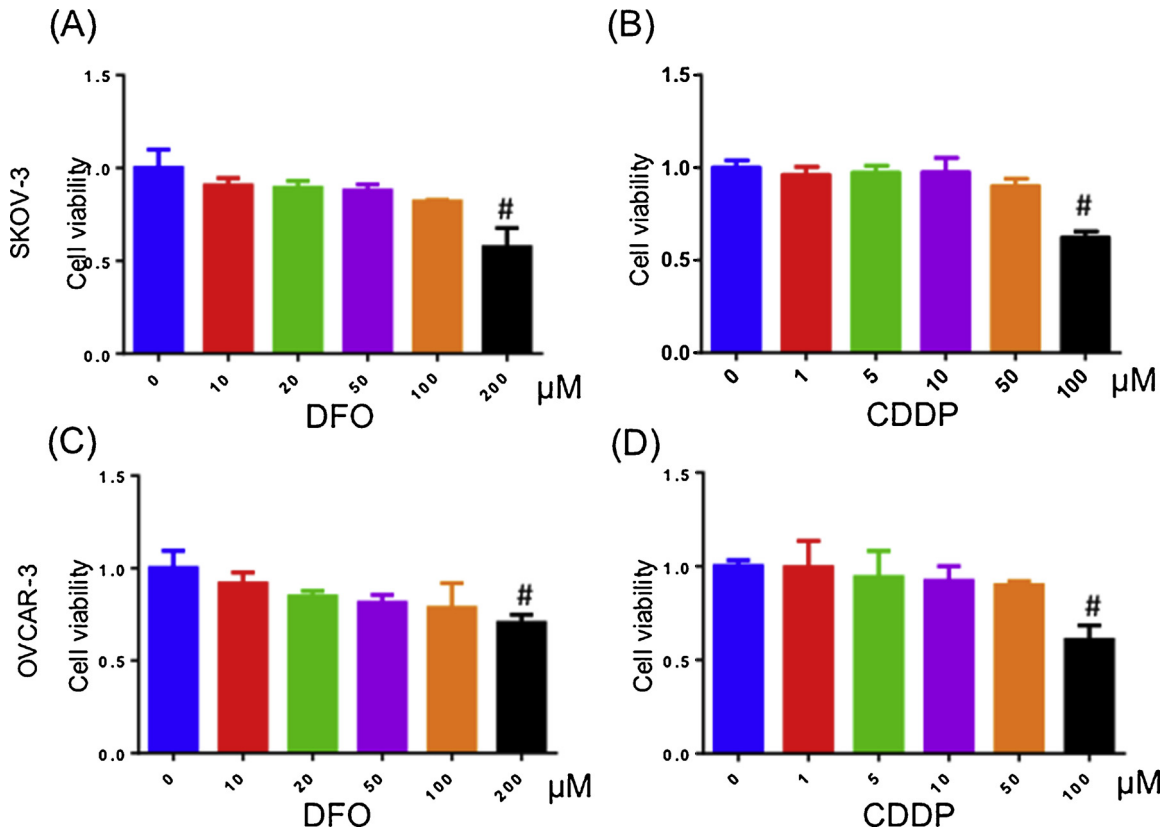

Fig. 2. Desferrioxamine (DFO) and cisplatin (CDDP) inhibit the proliferation of ovarian cancer cells. (A, B) Cell viability of ovarian cancer cell line (SKOV-3) after DFO (0,10,20,50,100,200 μM) and CDDP (0,1,5,10,50,100 μM) treatment for 24 h (n = 5). (C, D) For comparison, ovarian cancer cells (OVCAR-3) were also individually treated with DFO and CDDP at the same concentrations(n = 5). The experiment was repeated five times, and data were reported as the means ± SD. Pound (#) denotes P < 0.001, compared to untreated cells.

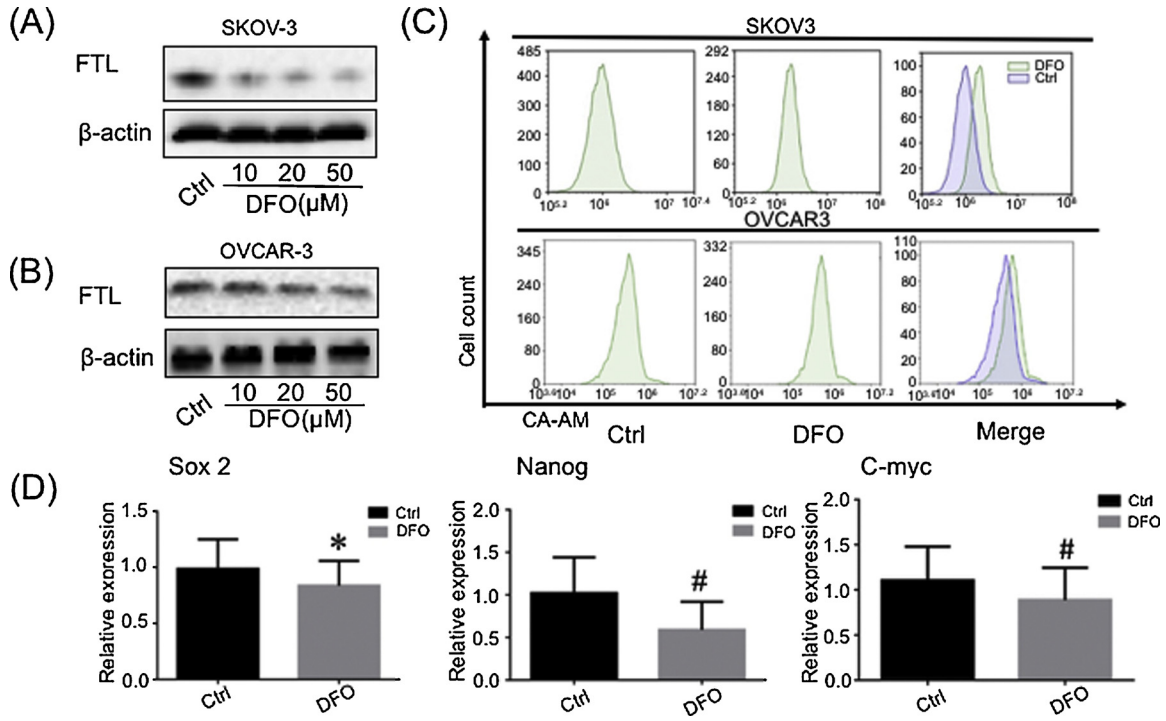

Fig. 3. Desferrioxamine (DFO) showed the ability of depleting cancer stem cells (CSCs). (A, B) Protein level of ferritin light (FTL) in ovarian cancer cell lines (SKOV-3 and OVCAR-3) were tested after treated with DFO at 10, 20, and 50 μM for 24 h. (C) Calcein-AM fluorescent intensity was tested for the intracellular concentration. The SKOV-3 and OVCAR-3 cells were treated with 50 μM of DFO for 24 h. Quantified data were shown in the right panel (n = 3). (D) The mRNA level of Sox2, Nanog and C-myc in SKOV-3 cells were treated with DFO at 50 μM for 24 h through PCR. The experiment was repeated three times, and data are presented as the means ± SD. Asterisk (*) symbols P < 0.05, pound (#) denotes P < 0.001, relative to control.

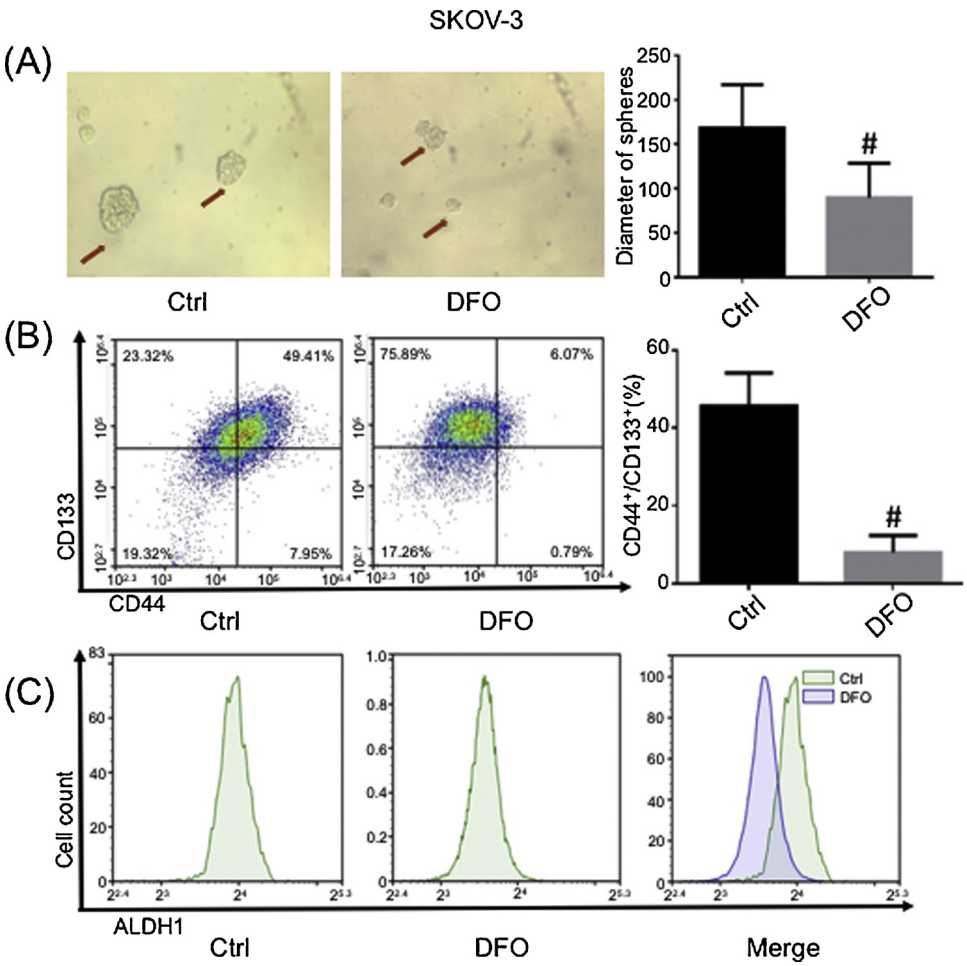

Fig. 4. Desferrioxamine (DFO) showed great capability of suppressing cancer stem cells (CSCs) in ovarian cancer cell line (SKOV-3). (A)The ability of sphere formation assay following with treatment of DFO for 7d. Red arrows show the sphere of CSCs. Quantified data of three independent experiments were shown in the right panel. (B) The percentage of $CD44^{+}/CD133^{+}$ subpopulation of SKOV-3 cells with treatment of DFO. Quantified data were presented in the right panel (n = 3). (C) Expression of Aldehyde dehydrogenase (ALDH) in SKOV-3 cells with/without treatment of DFO. Pound (#) denotes $P < 0.001$, compared to untreated cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3.4. DFO inhibits the subpopulation of $CD44^{+/high}/CD24^{-/low}$ and $ALDH^{+/high}$

The mamosphere formation of ovarian cancer was tested after the treatment for 7d. The average sphere size of SKOV-3 cells treated with DFO and DFX were shown in Fig. 4A and S2, showing a great decrease of the sphere formation. The subpopulation of $CD44^{+/high}/CD133^{+/high}$ and $ALDH^{+/high}$ can show the ability of CSCs [18,19]. The fluorescent of $CD44^{+/high}/CD133^{+/high}$ and $ALDH^{+/high}$ was tested following the treatment of DFO. Meanwhile, we observed a greater reduction of DFO in SKOV-3 derived mamospheres, confirming the greater capability of DFO. The mean fluorescent intensity (MFI) of ALDH in SKOV-3 and OVCAR-3 cells was measured by flow cytology, showing the inhibition of the iron chelator (Figs. 4B, C and S4).

3.5. DFO synergizes with CDDP for chemotherapy in vitro

For the intracellular iron concentration is associated with the expression of CSC markers, including Sox2, Nanog and C-myc, which are closely related with the cell proliferation and chemoresistance [20,21]. Given that high concentration of iron contributes to the development of ovarian cancer, we suppose that the iron chelator also has the inhibition of the proliferation and/or the chemoresistance. To verify this hypothesis, we treated ovarian cancer cells with the iron chelator, DFO, for 24 h and examined its cytotoxicity in vitro. The cell counting was tested with CCK-8 assay, the result showed that compared with the blank control, DFO synergizes with CDDP effectively suppressed the proliferation of ovarian cancer cells at the concentration in Fig. 5C and D. All these results showed the iron chelator could improve the therapeutic effects of CDDP. For the following experiments, we tested that DFO was used for improving therapeutic responses to CDDP in ovarian cancer. After 24 h of treatment with 10 μM of CDDP and/or 50 μM of DFO, we observed that the proportion of apoptotic cells with of CDDP (10 μM) and DFO (50 μM) increased significantly, with great increase than the treatment of CDDP and DFO alone, respectively (Fig. 5A and B). In agreement with our hypothesis, we can confirm that DFO and CDDP has the ability to induce apoptosis of ovarian cancer cells by reducing the expression of the CSC markers, which was considered to have closely related to chemoresistance.

3.6. DFO improves the therapeutic effects of CDDP with regulation of p53

To explore the mechanism of results, we explored the expression of p53 under the treatment of CDDP with/without DFO. As shown in Fig. 6A, expression of p53 protein in SKOV-3 cells decreased. Furthermore, we detected the expression of BCL-2, BAX, and caspase-8. As shown in Fig. 6B and C, Protein expression levels of BCL2 were significantly down-regulated in SKOV-3 and OVCAR-3 cells treated with CDDP and DFO. On the contrary, BAX was shown to be highly expressed. Moreover, the protein level of caspase-8 was high in our research, which indicated that the activity of p53, BAX and caspase-8 was deeply activated when cells were pretreated with DFO. These results also reflected the interaction between iron metabolism and chemoresistance in ovarian cancer cells. Our data also anticipated that iron chelator is more effective against chemoresistance in ovarian cancer cells.

4. Discussion

Recent studies have shown that the regulation of iron metabolism in

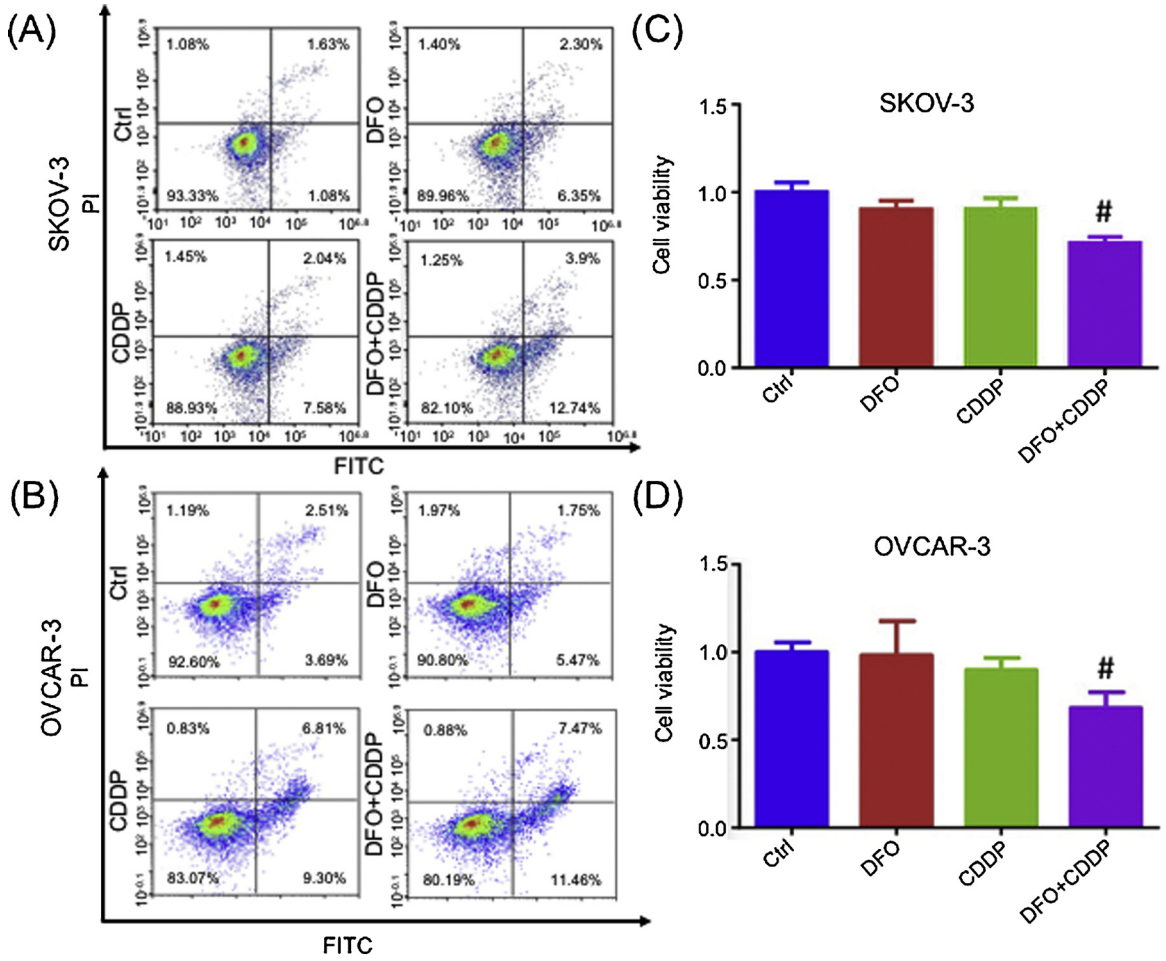

Fig. 5. Synergistic antitumor activity of desferrioxamine (DFO) and cisplatin (CDDP) on ovarian cancer cells in vitro. (A, B) Flow cytometry assay of apoptosis in ovarian cancer cell lines (SKOV-3 and OVCAR-3) after treatment of DFO, CDDP and combination of both. (C, D) Cell viability of SKOV-3 and OVCAR-3 cells after treatment of DFO, CDDP or combination of both. Pound (#) denotes $P < 0.001$, compared to untreated cells.

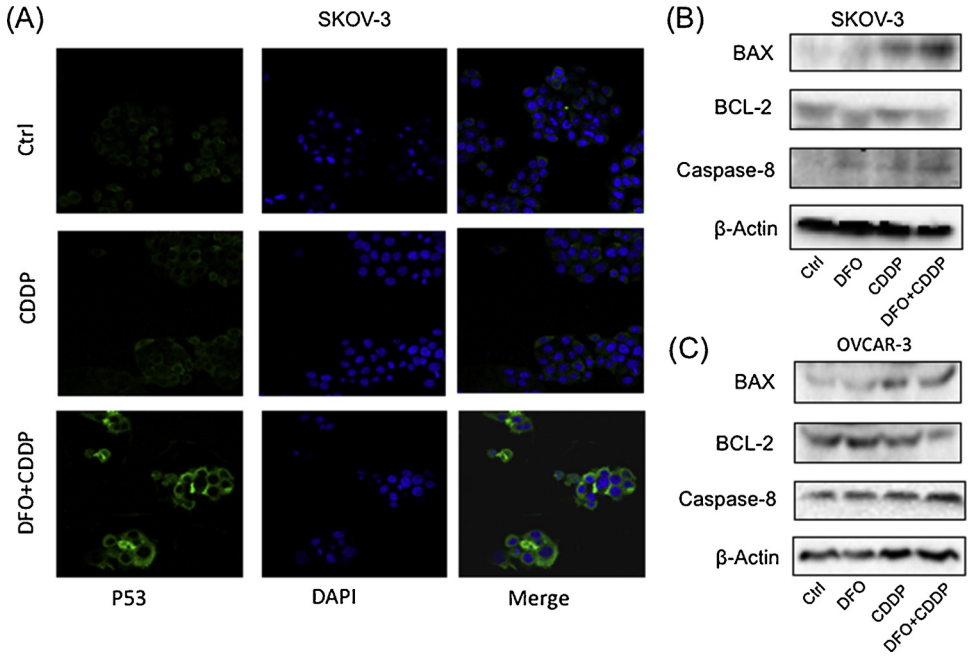

Fig. 6. Desferrioxamine (DFO) synergizes with cisplatin (CDDP) through p53/BAX/BCL-2 signal pathway. (A) Cells were treated with DFO, CDDP or combination of both for 24 h. Expression of P53 was tested with immunofluorescence staining. (B) Protein levels of BAX, BCL-2 and Caspase-8 in ovarian cancer cell lines (SKOV-3 and OVCAR-3) after treatment of CDDP with or without DFO pretreatment for 24 h through Western blot analysis.

ovarian cancer plays an important role in promoting cell proliferation, and many functional gene changes affect the process of iron metabolism [22]. Increased expression of TFR1, DMT1 and HAMP (Fig. S1), decreased FPN levels, which also resulted in high intracellular iron concentrations, as well as high levels of FTL [23–25]. Iron concentration was higher in tumor compared to normal tissue in numerous studies and it promoted the development of advanced tumor. Furthermore, the concentration of FTL is up-regulated in cancer tissues, which is associated with chemical resistance [10]. Before commencing this experiment, we chose two different analytical methods Kaplan–Meier analysis and GEPIA analysis and we found that patients with high expression of FTL, DMT1, HAMP and other genes had lower OS, and patients with high expression of FPN mRNA had higher OS in analyzing the survival of ovarian cancer. We conclude that FTL expression levels are high in patients with ovarian cancer. This is consistent with all previous studies.

DFO, the iron chelator, is a commonly used iron chelator in clinical treatment of iron overload disease. Iron reduction is a novel strategy for cancer treatment. The iron chelator, DFO, has an anti-proliferative effect on DNA synthesis and energy metabolism [26]. However, few studies have focused on the synergistic anti-tumor effects of iron depletion and CDDP in ovarian cancer cells. The clinical application of CDDP is limited because it has side effects of dose-dependent toxicity. Iron chelator can be a safe chemotherapeutic agent by increasing sensitivity to chemical agents and reducing toxicity of CDDP. The therapeutic effect of DFO have been proven in breast cancer [27]. Iron deprivation caused by DFO can improve the prognosis of breast cancer patients, especially in the TNBC subtype. In addition, many studies have shown that it has antiproliferative activity against a variety of tumor cell lines. For example, DFO inhibits the growth of esophageal carcinoma cells in vitro and in vivo studies by blocking their proliferation during the S phase of the cell cycle [28]. In our study, the iron concentration of ovarian cancer cells decreased with different concentrations of DFO. Therefore, we suspect that DFO may play a role in the treatment of ovarian cancer.

At present, the treatment of ovarian cancer is mainly combined with tumor cytoreductive surgery and platinum-based chemotherapy. A considerable number of malignant tumors have been transformed from incurable to partially treatable, but the reality is that once ovarian cancer recurs and metastasizes, the mortality rate is extremely high. Nowadays, the recurrence and metastasis of malignant tumors gradually rise to the main contradiction, and one of important causes of recurrence and metastasis is drug resistance. The development of modern medicine and the progress of molecular biology have made us realize that cancer stem cell cells are the most fundamental cause of tumor resistance [29,30]. As we all know, tumor stem cells can be defined as a kind of cell population with self-renewal ability and can produce heterogeneous tumor cells in tumor tissues with self-renewal, multi-directional differentiation ability and the characteristics of DNA repair capability. Therefore, CSC is associated with development of drug resistance. In order to overcome this problem, we try to eliminate CSCs of ovarian cancer cells with iron chelator. The CSC related genes were detected by q-PCR, including CD44, CD133, ALDH, SOX-2, C-myc, Nanog, etc. Although DFO showed weak cytotoxicity in ovarian cancer cells, DFO effectively induced the inhibition of cancer stem cells. Previous study found that iron depletion by the iron chelators deferasirox and DFO suppressed the proliferation of miPS-LLCcm cells and the expression of stemness markers [31]. And in Raggi's research [32], they also verified iron chelator DFO decreased 3D spheres forming efficiency and inhibited the growth of CSCs. These results are consistent with ours. Iron deprivation can be a potential mechanism for the synergism with CDDP in our research. At the molecular level, DFO treatment resulted in a significant decrease in the expression of a panel of key cancer stem cell markers including CD44, CD133, ALDH, Nanog, Sox2, and C-myc. Here we demonstrated that DFO could deplete the CSCs of ovarian cancer. Down-regulation of CSCs marker expression under DFO treatment restored the chemosensitivity of ovarian cancer cells, which was associated with chemoresistance, ultimately inhibiting cell proliferation and increasing CDDP sensitivity [33,34].

Inducing tumor cell apoptosis is an important mechanism for the cytotoxicity of many therapeutic drugs. A recent study found that irreversible cell stress in C26 colorectal adenocarcinoma can activate both caspase-dependent apoptosis and p21waf1 mediated growth arrest pathways, likely to be driven by the upregulated nuclear p53 protein [35]. The p53 gene and p53-mediated up-regulation of the gene also contributed to the decline of SKOV-3 cells to a certain extent [36,37]. The Bcl-2 gene (apoptosis suppressor gene), BAX gene (apoptosis-promoting gene), and caspase zymogen activation were involved in cell death and drug-resistance [38–40]. Cell apoptosis was detected by examining protein levels of p53, BAX, BCL-2 and Caspase-8 in SKOV-3 cells after treatment of CDDP with or without DFO. All these results are consistent with current research. The expression of BAX is regulated by p53, leading to the apoptosis of cancer cells. The transfer of p53 from the cytoplasm to the mitochondrial and destruction of the Bcl-2 / Bax ratio ultimately leads to releasing cytochrome C, which activates the release of pro-apoptotic factors. On the other hand, Bcl-2, which is located on the outer membrane of mitochondria, inhibits apoptosis by reducing pro-apoptotic proteins, thereby inhibiting the induction of apoptosis cascade. Therefore, the level of Bcl-2 and Bax protein determines the resistance of cells to apoptosis [41]. In the present study, the expression of pro-apoptotic proteins (Bax and caspase 8) was up-regulated for the synergism of DFO and CDDP [42]. Notably, DFO exacerbated p53-induced up-regulation of pro-apoptotic proteins and down-regulation of anti-apoptotic proteins compared to DFO and CDDP alone.

In order to explore the role of DFO in ovarian cancer chemotherapy, further research is needed to conduct a comprehensive scrutiny from the level of gene, molecular, protein and clinical trials. In this study, we demonstrated that possible mechanisms for the use of CDDP to produce ovarian cancer resistance. This suggested the feasibility of iron chelator promoting chemotherapy sensitivity in ovarian cancer cell lines. We may conclude that DFO improve the treatment effects of CDDP with regulation of p53. Although its efficacy remains to be confirmed, this finding has brought a new Strategy to improve the survival rate of patients with refractory advanced ovarian cancer, which is bound to create a new path for the treatment of ovarian cancer and bring a new hope for them.

5. Conclusions

To summarize, our study deciphered the existence of dysregulation of iron metabolism of ovarian cancer, which can be exploited as a new therapeutics target to cancer treatment. Also, it can serve as a therapeutic target to design the effective treatment for ovarian cancer. Our combined data found that DFO could effectively inhibit CSCs, enhance the ability of CDDP in promoting tumor cell apoptosis, and finally improve the ability of cisplatin to overcome resistance of the used cancer cells to chemotherapy. Besides, targeting molecular targets, including BAX, BCL-2, P53 and caspase-8 could serve as clinical biomarkers to evaluate the effects of ovarian cancer [43,44]. It is plausible to consider that DFO adjuvant therapy with CDDP chemotherapy could promote the improvement of treatment response in ovarian cancer patients. Taken together, our study revealed a strong theoretical basis for the application of DFO, an FDA-approved clinical drug, to enhance CDDP chemotherapy and treat ovarian cancer. However, further studies would be required to confirm our conclusions in clinical trials involving CDDP and DFO.

Declaration of Competing Interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jtemb.2019.07.008.

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