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Peroxiredoxin-1 as a molecular chaperone that regulates glutathione S-transferase P1 activity and drives mutidrug resistance in ovarian cancer cells

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ABSTRACT

Ovarian cancer is among the most prevalent gynecological malignancies around the globe. Nonetheless, chemoresistance continues to be one of the greatest obstacles in the treatment of ovarian cancer. Therefore, understanding the mechanisms of chemoresistance and identifying new treatment options for ovarian cancer patients is urgently required. In this study, we found that the mRNA and protein expression levels of PRDX1 were significantly increased in cisplatin resistant A2780/CDDP cells. Cell survival assays revealed that PRDX1 depletion substantially increased ovarian cancer cell sensitivity to cisplatin, docetaxel, and doxorubicin. Additionally, PRDX1 significantly increased GSTP1 activity, resulting in multidrug resistance. Biochemical experiments showed that PRDX1 interacted with GSTP1 through Cysteine 83, which regulated GSTP1 activity as well as chemotherapy resistance in ovarian cancer cells. Our findings indicate that the molecular chaperone activity of PRDX1 is a promising new therapeutic target for ovarian cancer.

1. Introduction

Ovarian cancer is the fifth leading cause of female deaths worldwide and is the gynecological tumor with the highest mortality rate [1]. Due to the insidious onset of ovarian cancer, about 75 % of patients have progressed to advanced stages by the time they are diagnosed, and only platinum-based chemotherapy can be used as the first-line treatment option. Multidrug resistance (MDR) is one of the main reasons for chemotherapy failure in ovarian cancer [2]. This resistance is related to the molecular activity and expression of drug transports [3], pH abnormalities in tumor cells, DNA damage repair capacity [4,5], apoptotic pathways [6], and the methylation of some genes [7,8]. MDR reduces the concentration of chemotherapeutic drugs in tumor cells, thereby reducing the sensitivity of these tumor cells to the drugs. A low level of ROS is one of the hallmarks of multidrug-resistant cells, but the mechanism still needs to be further investigated [9,10].

Peroxiredoxins (PRDXs) are a class of antioxidant proteins that play a crucial role in maintaining redox balance by catalyzing the conversion of hydrogen peroxide to water [11,12]. To date, a total of six distinct peroxiredoxins (PRDX1 to PRDX6) localized inside intracellular compartments have been documented [12]. One of the identified proteins is PRDX1, also known as peroxiredoxin 1, which is a 23-kDa redox protein found in macrophages that is generated by stress and exhibits many activities. PRDX1 expression is correlated with reactive ROS and the occurrence of malignant diseases [13]. In addition to its antioxidant enzyme, PRDX1 can function as a molecular chaperone with the ability to control the actions of multiple molecules. PRDX1 can interact with TRAF6 [14], p38α [15,16], TLR4 [17] and *C*-type lectin [18] to regulate inflammation, cancer invasion, and senescence. However, the role of the molecular chaperone function of PRDX1 in ovarian cancer chemotherapy tolerance has not yet been clearly studied.

In this study, we have observed that PRDX1 facilitates the enzymatic activity of GSTP1 through its interaction with GSTP1, independent of the antioxidant enzyme activity. This interaction subsequently leads to the promotion of ovarian cancer cell multidrug resistance. This work presents original findings suggesting that targeting the molecular chaperone activity of PRDX1 could be a potential therapeutic strategy in

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Abbreviations

CDDP	Cisplatin
Cys	Cysteine
GSH	glutathione
GST	glutathione-S-transferase
MDR	multidrug resistance
NADPH	nicotinamide adenine dinucleotide phosphate
PRDX1	peroxiredoxin 1
ROS	reactive oxygen species

the context of ovarian cancer chemotherapy.

2. Materials and methods

2.1. Cell culture

Human A2780 cells was purchased from the Cell Bank of the Chinese Academy of Sciences, Shanghai, China. The cisplatin resistant cell line A2780/CDDP was generated by exposing the parental cell line A2780 to cisplatin with the concentration of cisplatin gradually increasing from 0.5 μg/ml to 16 μg/ml. A2780 and A2780/CDDP cells were cultured in RPMI-1640 (Biological Industries) supplemented with 10 % FBS (Biological Industries), 100 U/ml penicillin, and 100 μg/ml streptomycin. All cells were cultured at 37 °C in a humid atmosphere (5 % CO_2 –95 % air).

2.2. Antibodies, and reagents

Antibodies for PRDX1 (No. 15816-1-AP), GAPDH (60004-1-Ig) and His-tag (No. 66005-1-Ig) were from Proteintech. Antibodies for GSTP1 (sc-66000) was from Santa Cruz. Peroxidase-labeled Anti-Mouse IgG (H + L) (No. 5220–0338) and peroxidase-labeled Anti-Rabbit IgG (H + L) (5220–0335) were from Seracare. Cisplatin (HY-17394), Docetaxel (HY–B0011) and Doxorubicin (HY15142A) were purchased from MedChemExpress.

2.3. Immunoprecipitation and immunoblot analysis

Cells were washed with ice-cold PBS and cell lysates were prepared using M-PER Mammalian Protein Extraction Reagent (Thermo Scientific) containing fresh protease inhibitor mixture (50 μg/ml aprotinin, 0.5 mM phenylmethanesulfonyl fluoride (PMSF), 1 mM sodium orthovanadate, 10 mM sodium fluoride and 10 mM β-glycerolphosphate). For immunoprecipitation, Cells were lysed with IP-buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1%NP-40, 1 mM EDTA, 10 mM *N*-Ethylmaleimide, and protease inhibitor mixture). The whole-cell lysates were incubated with desired antibodies, and the target protein was then pulled down with protein G agarose beads (Santa Cruze).

Proteins were quantified using the BCA protein assay kit (Beyotime). Total protein concentrations were normalized in all samples. Then proteins were heated at 95 °C for 10 min in the loading buffer (Beyotime). For non-reducing gel, samples were heated in DTT and 2-Mercaptoethanol free loading buffer. SDS-PAGE was used to separate protein samples. Then proteins were transferred to a nitrocellulose (NC) membrane (PALL), and blocking with 5 % bovine serum albumin, the membranes were incubated overnight at 4 °C with primary antibodies. The membranes were washed three times with TBST and incubated with peroxidase-labeled secondary antibody for 1 h at room temperature. After the second round of wash, the Pico PLUS Chemiluminescent Substrate reagent (Thermo Scientific) was used to visualize protein bands.

2.4. CCK-8 assay

Cells were seeded on a 96-well plate at a density of 5000 cells per well. The cells were treated with drugs for 24 h and then treatment with 10 μl of Cell Counting Kit-8 solution (HY–K0301, MedChemExpress). After 2 h of incubation at 37 °C, the absorbance was measured at 450 nm using a microplate reader. For drug treatment, CDDP was used at final concentrations of 1.25 μg/ml, 2.5 μg/ml, 5 μg/ml and 10 μg/ml. Docetaxel was used at final concentrations of 50 μM and 100 μM [19]. Doxorubicin was used at final concentrations of 5 μg/ml and 10 μg/ml [20].

2.5. Plasmid, siRNAs and transfection

The human gene *PRDX1* was cloned into the pcDNA3.1 expression vector. The cysteine mutanted PRDX1 plasmids were constructed using Takara MutanBEST kit (No. R401) according to the manufacturer's instructions. The specifically targeting small interfering RNAs (siRNAs) siPRDX1, siGSTP1 and a negative control (siNC) were purchased from GenePharma (Shanghai, China) and were transfected into cells using jetPrime Transfection Reagent (Polyplus).

2.6. GSH concentration determination

GSH and GSSG were quantified with GSH Assay Kit (S0053, Beyotime). Briefly, A2780 cells were collected and resuspended in a protein removal agent to collect supernatant. Total GSH and GSSG were measured after the addition of 5,5′-dithio-bis-(2-nitrobenzoic acid) (DNTB) at an absorbance of 405 nm. GSSG was selectively measured after assaying samples in which GSH was abolished by treatment with GSH removal reagent. The difference between the two values gave the GSH level in the cells.

2.7. NADPH concentration determination

NADP+/NADPH Assay with WTS-8 Kit (S0179, Beyotime) per the manufacturer's instructions. Briefly, A2780 cells were collected and resuspended in ice cold NADP+/NADPH Extraction Buffer. Glucose-6-phosphate (G6P) is oxidized into 6-phosphogluconate (6-PG) by the glucose-6-phosphate dehydrogenase (G6PDH), during which NADP+ is reduced to NADPH. In the presence of 1-MPMS (1-methoxy-5-methylphenazinium Methyl Sulfate), the generated NADPH reduces WST-8 to an orange water soluble formazan with a maximum absorption at 450 nm. The formazan formed in the reaction mixture is proportional to the total NADP+/NADPH in the sample. After incubating the sample at 60 °C for 30 min, NADP+ in the sample is degraded while NADPH remains. Therefore, the formazan generated in the reaction mixture represents the content of NADPH. The ratio of NADP+/NADPH can be calculated based on the amount of total NADP and NADPH obtained above.

2.8. GSTs activity assay

GSTs activity were quantified with Glutathione S-transferase (GSH-ST) assay kit (Colorimetric method) (A004-1-1, Nanjing Jiancheng Bioengineering Institute) per the manufacturer's instructions. Briefly, A2780 cells were collected and resuspended in GSTs assay Buffer. GSTs can catalyze the binding of GSH to 1-chloro-2,4-dinitrobenzene (CDNB). Within a certain reaction time, its activity is linearly related to the change of substrate concentration before and after the reaction. Therefore, measured the GSH concentration to reflect the GST activity, and the more the GSH concentration decreased, the more the GST activity increased. The more the concentration of GSH (substrate) decreases, the more the GST activity increases.

2.9. ROS assay

Intracellular ROS levels were assessed using a 2′,7′-dichlorofluorescein diacetate (DCFH-DA) fluorescent probe (S0033S, Beyotime). Cells were incubated with 10 μM DCFH-DA at 37 °C for 30 min. Then cells were washed, collected, resuspended in PBS, and analyzed immediately using Backman CytoFLEX Flow Cytometer. Data were analyzed using CytExpert software (Backman).

2.10. Quantitative PCR (qPCR) assays

For quantitative PCR (qPCR) assays, cDNA was synthesized using ReverTra Ace qPCR RT Kit (TOYOBO). qPCR was performed using SYBR Green and ABI Q5 System. Changes in the mRNA levels of desired genes were normalized to the level of β-actin (ACTB). The primers used to amplify target genes are listed below:

ACTB: Forward CATGTACGTTGCTATCCAGGC Reverse, CTCCTTAATGTCACGCACGAT.

PRDX1: Forward CCACGGAGATCATTGCTTTCA, Reverse AGGTGTATTGACCCATGCTAGAT.

PRDX2: Forward GAAGCTGTCGGACTACAAAGG,
Reverse TCGGTGGGGCACACAAAAG.

PRDX3: Forward ACAGCCGTTGTCAATGGAGAG,
Reverse ACGTCGTGAAATTCGTTAGCTT.

PRDX4: Forward AGAGGAGTGCCACTTCTACG,
Reverse GGAAATCTTCGCTTTGCTTAGGT.

PRDX5: Forward GCTGCAAAGCCAGTTCTGTG.
Reverse CCACTGAGGGAATGGCATCTC.

PRDX6: Forward GACTCATGGGGCATTCTCTTC.
Reverse CAAGCTCCCGATTCCTATCATC.

2.11. Bioinformation analysis

The data for the TCGA cohort of Ovarian cancer were downloaded via https://xenabrowser.net/. Correlation analysis were performed by using Corrplot tools in Hiplot Pro (https://hiplot.com.cn/), a comprehensive web service for biomedical data analysis and visualization. Spearman's Rank-Order Correlation coefficient was applied to determine correlation coefficients R. Survival analysis were downloaded by www.kmplot.com, and the hazard ratio with 95 % confidence intervals and logrank P value are calculated [21].

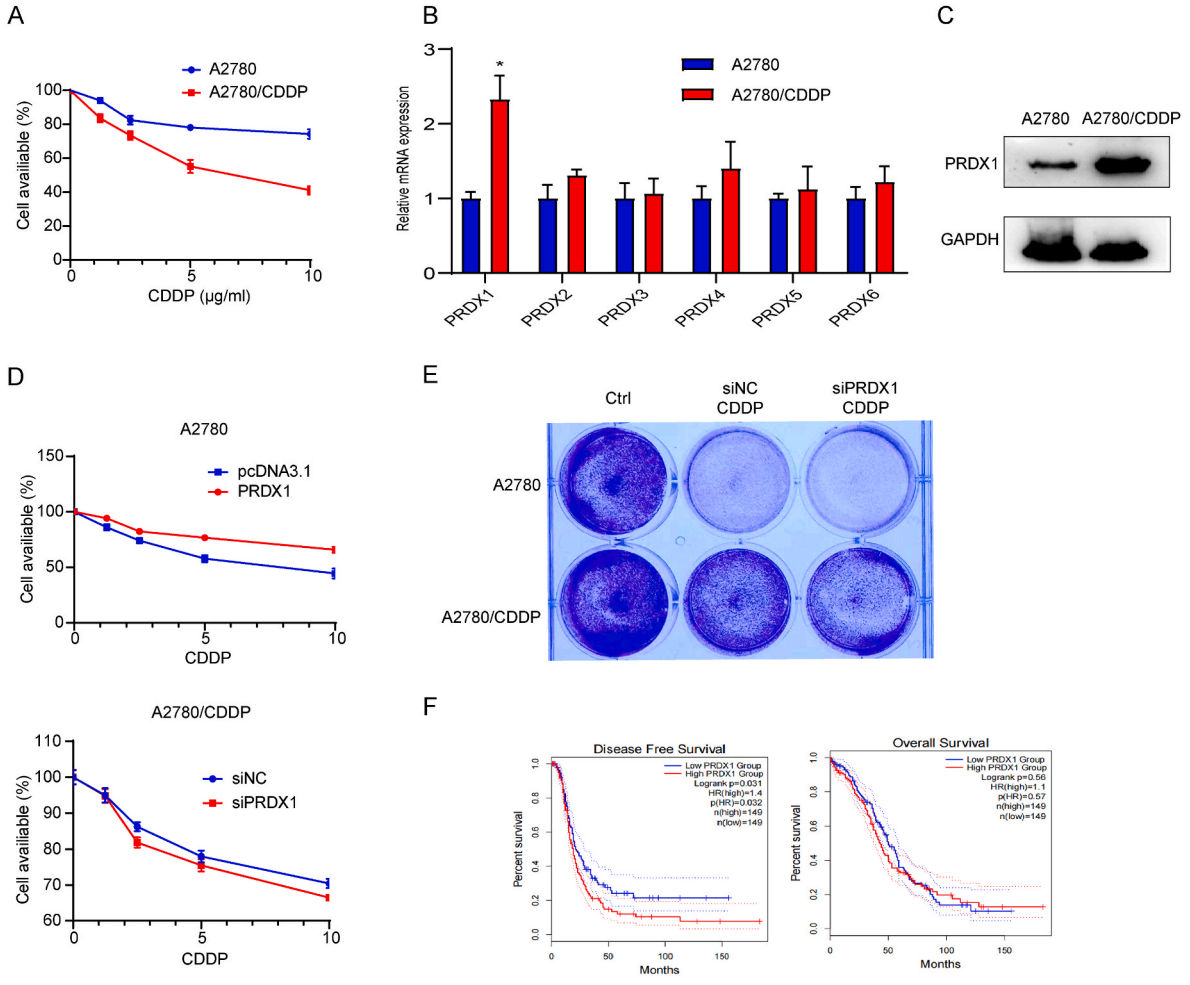

Fig. 1. High expression of PRDX1 promotes cisplatin resistance in A2780 cells
A CCK-8 detection of cisplatin sensitivity in A2780 cisplatin-resistant cells and sensitive cells; **B** PRDX family mRNA expression in A2780 cisplatin-resistant cells and sensitive cells; **C** Western Blotting detection of PRDX1 expression in A2780 cisplatin-resistant cells and sensitive cells; **D** CCK-8 detection of PRDX1 expression on cisplatin sensitivity of A2780 cisplatin-resistant cells and sensitive cells; **E** Crystal violet staining of A2780 cisplatin-resistant cells and sensitive cells downregulaton PRDX1 cultured with or without cisplatin for 48 h; **F** Kaplan-Meier analysis showed an association between PRDX1 and disease free survival and overall survival in ovarian cancer patients. Data are the mean ± SEM; *p < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.12. Statistical analysis

The data are presented as means ± SEM and were analyzed with GraphPad Prism software 8.0 (GraphPad). Student's *t*-test was used to comparisons between two groups and one-way ANOVA with Dunnett's multiple comparisons test for comparisons among different groups. Kaplan-Meier analyses were exploited for survival analysis. All of the experiments were repeated at least three times, and $p < 0.05$ denoted statistical significance (indicated as *$p < 0.05$, **$p < 0.01$, and ns $p > 0.05$ in the Figures).

3. Result

3.1. Upregulation of PRDX1 promotes cisplatin resistance in A2780 cells

To investigate the molecular mechanism of cisplatin resistance in ovarian cancer, we constructed ovarian cancer cisplatin-resistant cells: A2780/CDDP. compared with the sensitive strain A2780, A2780/CDDP showed significant resistance to cisplatin (Fig. 1A), suggesting that the construction of resistant cells was successful. Subsequently, we performed Q-PCR screening for PRDX family expression. The results showed that the mRNA of PRDX1 was significantly up-regulated in cisplatin-resistant cells A2780/CDDP, while the expression of other members of the family did not change significantly (Fig. 1B). The same result was obtained by WB results, which showed that the protein expression of PRDX1 was significantly upregulated in A2780/CDDP cells (Fig. 1C). Therefore, we hypothesized that PRDX1 upregulation is involved in cisplatin resistance in ovarian cancer. To test our hypothesis, we constructed PRDX1 overexpression vectors to overexpress PRDX1 in A2780 cells or used small interfering RNA to down-regulate PRDX1 expression in A2780/CDDP, and subsequently detected the sensitivity of cells to cisplatin. As shown in Fig. 1D, overexpression of PRDX1 promoted cellular resistance to cisplatin, whereas A2780/CDDP cells with down-regulated PRDX1 had increased sensitivity to cisplatin. Cell staining experiments illustrated the same results (Fig. 1E). The above data suggest that high PRDX1 expression may be one of the mechanisms

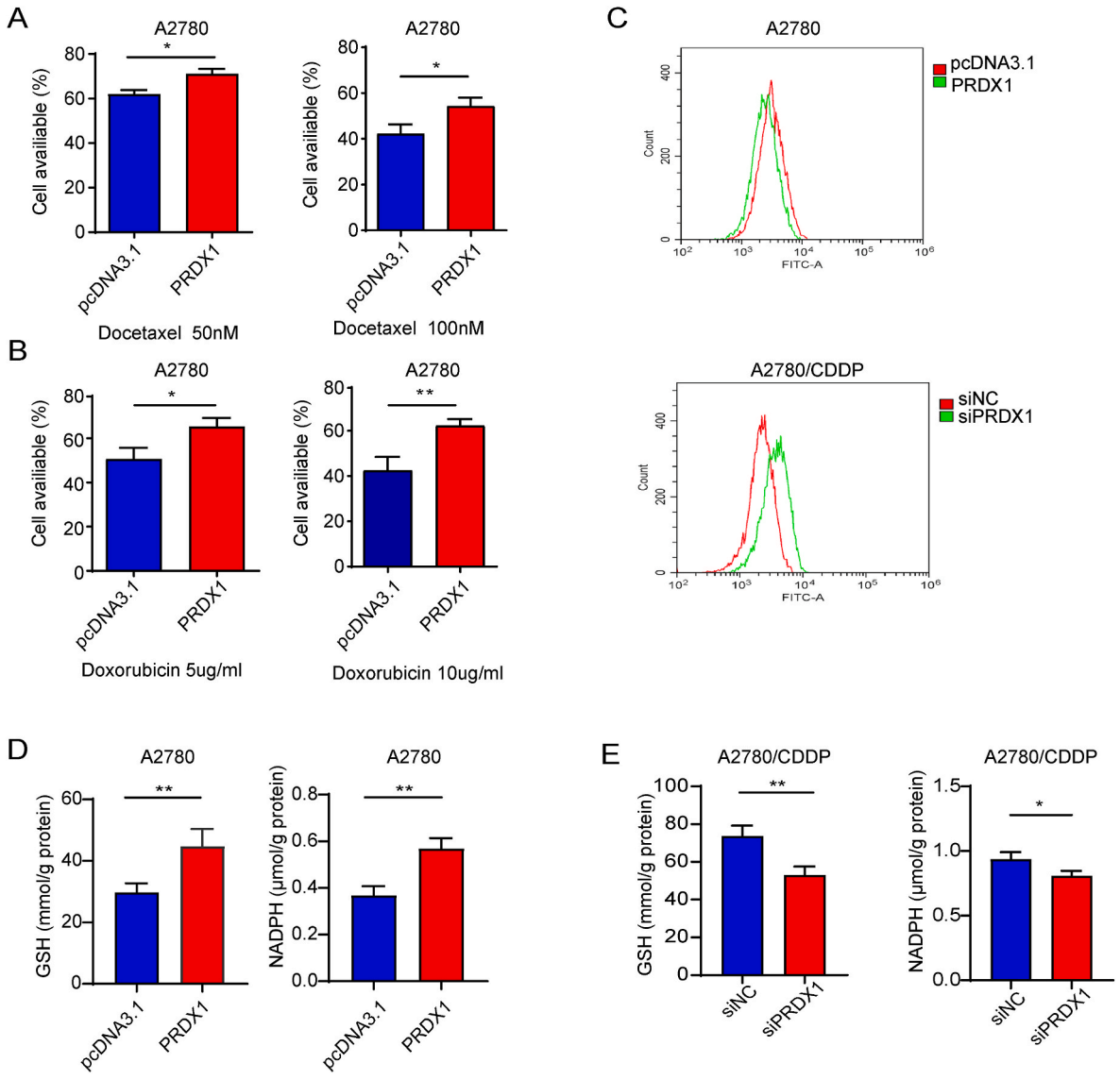

Fig. 2. PRDX1 upregulation mediates multidrug resistance in A2780 cells
A CCK-8 assay is used to detect the sensitivity of A2780 cells overexpressing PRDX1 to docetaxel; **B** CCK-8 assay is used to detect the sensitivity of A2780 cells overexpressing PRDX1 to doxorubicin; **C** Flow cytometry to detect the effect of PRDX1 expression on cellular accumulation of doxorubicin; **D** Overexpression of PRDX1 in A2780 cells detects glutathione and NADPH concentration; **E** Down-regulation of PRDX1 expression in A2780/CDDP cells detecting glutathione and NADPH. Data are the mean ± SEM; *$p < 0.05$, and **$p < 0.01$.

of cisplatin resistance in ovarian cancer. Database analysis revealed that Disease Free Survival (DSF) was significantly reduced in patients with high expression of PRDX1, while overall survival was not affected, suggesting that PRDX1 affected the therapeutic efficacy (Fig. 1F).

3.2. PRDX1 upregulation mediates multidrug resistance in A2780 cells

Multidrug resistance is a common challenge in ovarian cancer therapeutic. While the data suggest that PRDX1 is involved in cisplatin resistance in ovarian cancer, whether PRDX1 is involved in

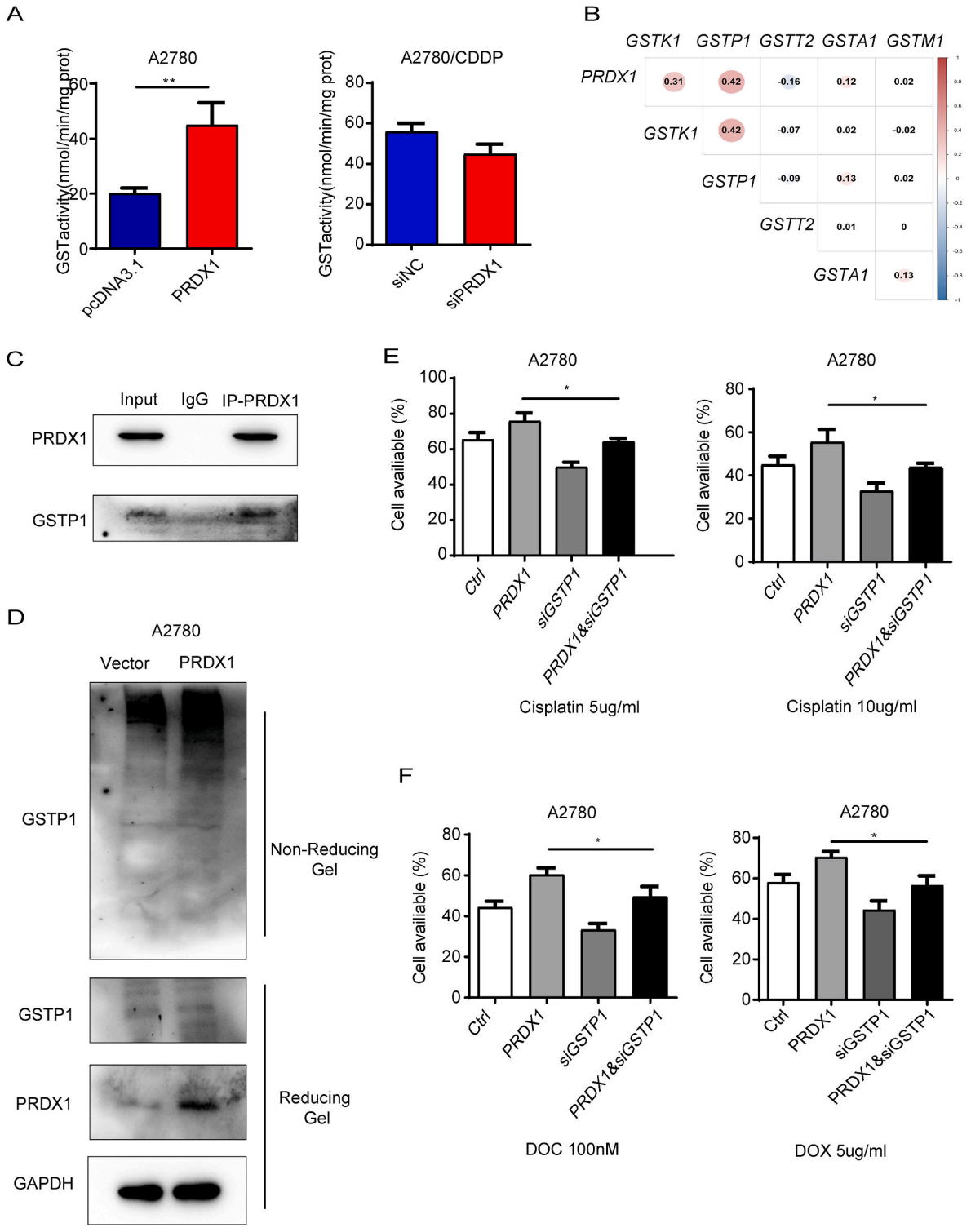

Fig. 3. PRDX1 mediates multidrug resistance in A2780 cells through GSTP1

A Overexpression or downregulation of PRDX1 expression to detect cellular GST enzyme activity; **B** Correlation between PRDX1 and GST family expression analyzed using the TCGA OV database; **C** PRDX1-GSTP1 interaction detected by immunoprecipitation; **D** Non-reducing gel detection of oxidized aggregation of GSTP1 in A2780 cells overexpressing PRDX1; **E** Down-regulation of GSTP1 rescues cisplatin resistance mediated by overexpression of PRDX1 in A2780 cells; **F** Down-regulation of GSTP1 rescues overexpression of PRDX1-mediated multidrug resistance in A2780 cells. Data are the mean ± SEM; *p < 0.05, and **p < 0.01.

chemotherapy multidrug resistance is a question we explored further. We overexpressed PRDX1 in A2780 cells and treated the cells with docetaxel and doxorubicin, which are commonly used drugs for ovarian cancer chemotherapy, and the results showed that high expression of PRDX1 mediated cellular resistance to docetaxel and doxorubicin (Fig. 2A and B). It is suggested that PRDX1-mediated resistance in ovarian cancer is characterized by multidrug resistance. Multidrug-resistant cells usually have low levels of ROS to maintain their stem cell properties, and we next tested whether PRDX1 regulates ROS production in ovarian cancer cells. The experimental results showed that overexpression of PRDX1 in A2780 cells decreased ROS in the cells, while down-regulation of PRDX1 expression in drug-resistant cells A2780/CDDP increased ROS in the cells (Fig. 2. C). To further validate that PRDX1 regulates ROS levels in ovarian cancer drug-resistant cells, we examined the effect of PRDX1 on the levels of GSH and NADPH, the reducing substances in cells. The results showed that up-regulation of PRDX1 expression resulted in a significant increase in the content of GSH and the reducing power of NADPH in A2780 cells (Fig. 2. D). While knockdown of PRDX1 expression in drug-resistant cells resulted in a significant decrease of GSH and NADPH in the cells (Fig. 2. E). The above data suggest that PRDX1 regulation of cellular ROS levels mediates multidrug resistance in ovarian cancer.

3.3. PRDX1 mediates multidrug resistance in A2780 cells through GSTP1

Our previous data suggest that overexpression of PRDX1 in ovarian cancer cells promotes ROS-associated multidrug resistance. The glutathione-S-transferase (GST) is an important mechanism for the development of multidrug resistance [22]. We further investigated whether PRDX1 mediates multidrug resistance in ovarian cancer cells through GSTs. First, we examined the correlation between PRDX1 expression and cellular GST enzyme activity. The results showed that overexpression of PRDX1 significantly increased GST activity in A2780 cells (Fig. 3. A). Similarly, knockdown of PRDX1 expression in A2780/CDDP cells significantly decreased GST activity in the cells (Fig. 3. A). It is suggested that PRDX1 may mediate multidrug resistance in ovarian cancer by promoting cellular GST activity. By analyzing the correlation between PRDX1 and GSTs through the TCGA database, we found that PRDX1 had the highest correlation with GSTP1 (Fig. 3. B). In addition to peroxidase activity, PRDX1 also has a molecular chaperone function, which can promote the activity of substrate proteins through interaction with proteins. Then we first tested whether PRDX1 interacts with GSTP1. The immunoprecipitation results showed that PRDX1 interacted with GSTP1 (Fig. 3. C). Since PRDX1 is able to regulate protein activity by forming disulfide bonds, we speculated that PRDX1 might form disulfide bonds with GSTP1 to regulate GST activity. We used non-reducing gel experiments to test this hypothesis. As shown in Fig. 3D, overexpression of PRDX1 enhanced the oxidative aggregation of GSTP1. We further examined the effect of GSTP1 on PRDX1 mediated chemoresistance. Overexpression of PRDX1 in A2780 cells resulted in cellular resistance to cisplatin, whereas A2780 cells with knockdown of GSTP1 showed enhanced sensitivity to cisplatin (Fig. 3. E). We found that A2780 cells overexpressing PRDX1 while knocking down GSTP1 were significantly more sensitive to cisplatin than cells overexpressing PRDX1 (Fig. 3. E). The above data suggest that down-regulation of GSTP1 can rescue cisplatin resistance mediated by high PRDX1 expression. Moreover, we found that down-regulation of GSTP1 also inhibited docetaxel and doxorubicin resistance mediated by overexpression of PRDX1 (Fig. 3. F). The previous data suggest that PRDX1 promotes chemoresistance in ovarian cancer cells by enhancing the enzymatic activity of GSTP1.

3.4. PRDX1 promotes GSTP1 enzymatic activity and chemoresistance in A2780 cells through molecular chaperone functions

The oxidative aggregation of GSTP1 was greatly increased by PRDX1. This process needs cysteines to form disulfide bonds. We further explored the molecular mechanism by which PRDX1 promotes the oxidative aggregation of GSTP1. PRDX1 contains four cysteines: Cys52, Cys71, Cys83, and Cys173. Cys52 and Cys173 are the enzyme activation centers of PRDX1, whereas the functions of Cys71 and Cys83 have not yet been clarified. By mutating the cysteine of PRDX1 and using immunoprecipitation to detect the interaction of PRDX1 with GSTP1, we found that Cys83-mutated PRDX1 could not interact with GSTP1 (Fig. 4. A). Additionally, by non-reducing gel, we found that Cys83-mutated PRDX1 was not increasing the oxidative aggregation of GSTP1 (Fig. 4. B), suggesting that PRDX1 oxidizes GSTP1 via Cys 81 cysteine of GSTP1. Further, we examined the effect of Cys83-mutated PRDX1 on GSTP1 enzyme activity. The results showed that overexpressing PRDX1 in A2780 cells increased the activity of GSTP1, but there was no difference between the control group and PRDX1 C83S (Fig. 4. C). Similarly, PRDX1 C83S did not affect the concentration of GSH in the A2780 cells (Fig. 4. D). By cell viability assay, we found that overexpression of PRDX1 promoted resistance to cisplatin in A2780 cells, whereas there was no difference in sensitivity to cisplatin between overexpression of PRDX1 C83S and control cells (Fig. 4. E). Since PRDX1 caused multidrug resistance, we tested the sensitivity of A2780 cells to doxorubicin and docetaxel and similarly found that PRDX1 C83S did not affect the sensitivity of cells to these two drugs (Fig. 4 E and F). The above data suggest that PRDX1 interacts with GSTP1 through Cys83, thereby promoting the enzymatic activity of GSTP1 with multidrug resistance in A2780 cells.

4. Discussion

Our study demonstrated that PRDX1 was highly expressed in cisplatin-resistant ovarian cancer cells A2780/CDDP and promoted ROS clearance with multidrug resistance. Mechanistically, we found that PRDX1 promotes the enzymatic activity of GSTP1 by interacting with GSTP1 in an enzyme activity-independent manner, which in turn promotes multidrug resistance. This study demonstrates for the first time that the molecular chaperone function of PRDX1 is a potential target for effective ovarian cancer chemotherapy. This study presents novel findings indicating that the molecular chaperone activity of PRDX1 may serve as a therapeutic target in ovarian cancer chemotherpy.

Cisplatin is the first line of chemotherapy for ovarian cancer, and cisplatin resistance is an important cause of treatment failure in ovarian cancer. At present, the underlying mechanism of chemotherapy resistance in ovarian cancer has not been fully elucidated. Redox reprogramming is an important cause of tumor chemoresistance [23]. Low level of ROS is a hallmark of tumor drug resistance [9]. The PRDX family is an important ROS scavenger in cells. We first examined the expression of the PRDX family in cisplatin-resistant cells of ovarian cancer. The results revealed that PRDX1 was significantly overexpressed in drug-resistant cells. Through cellular experiments, we found that PRDX1 expression was indeed associated with drug resistance in ovarian cancer. Upregulation of PRDX1 in A2780 cells mediated cellular resistance to cisplatin, while overexpression of PRDX1 increased the sensitivity of resistant cells A2780/CDDP to cisplatin. By analyzing the survival of ovarian cancer patients, we found that progression-free survival of patients with high PRDX1 expression had a significant reduction. These data suggest that upregulation of PRDX1 expression is associated with cisplatin resistance in ovarian cancer. Since multidrug resistance is an important cause of chemotherapy tolerance in tumors, we further examined whether PRDX1 mediated tolerance to other chemotherapeutic agents. We found that A2780 cells overexpressing PRDX1 were also resistant to docetaxel and doxorubicin, which is consistent with the multidrug resistance observed in the clinic. Since PRDX1 is an important molecule for maintaining cellular redox homeostasis, we further examined the effect of PRDX1 on cellular redox. First, we found that PRDX1 expression showed negative correlation with ROS in A2780 and A2780/CDDP. And we found that PRDX1 expression was regulating the

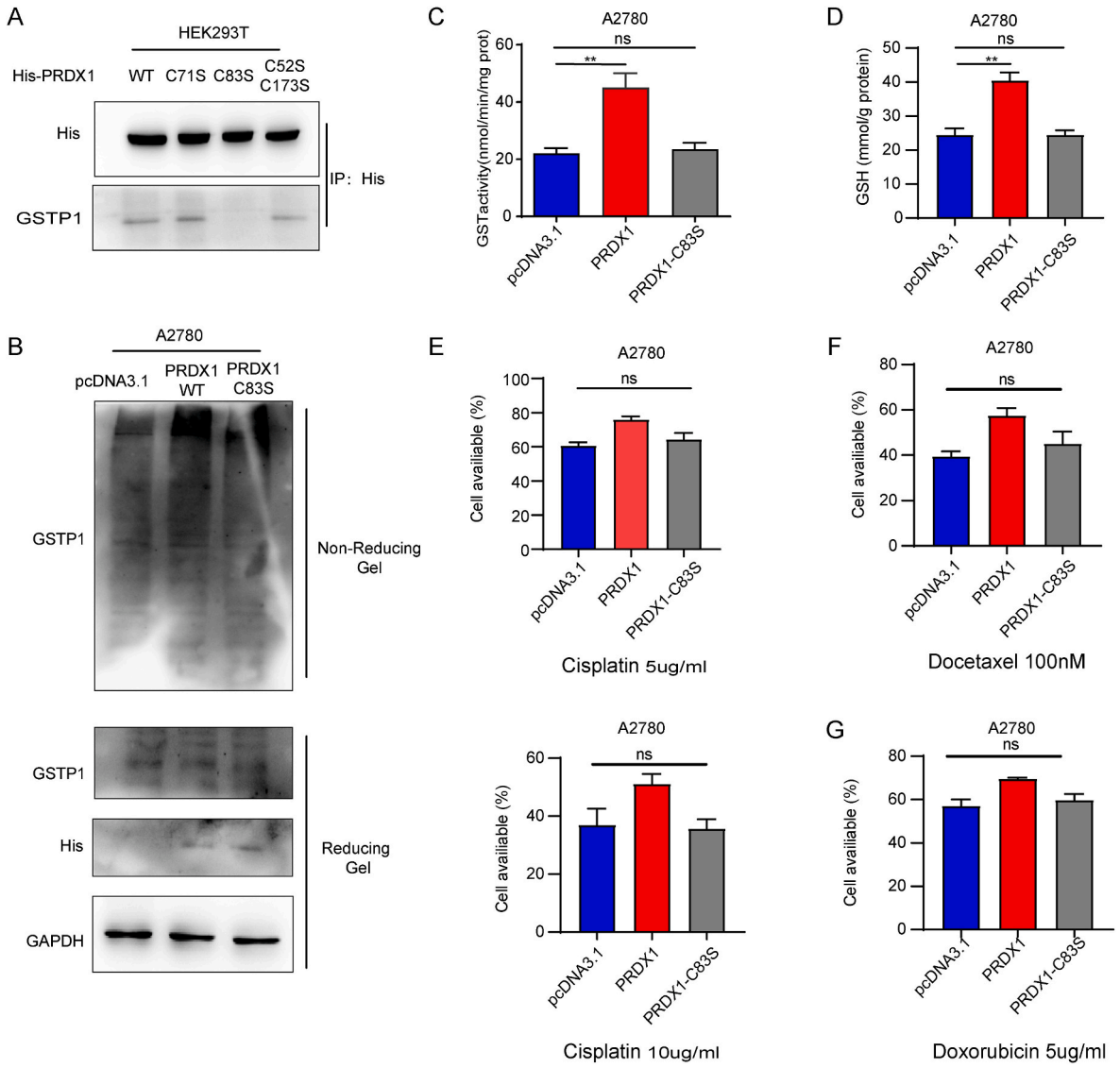

Fig. 4. PRDX1 promotes GSTP1 enzymatic activity and chemoresistance in A2780 cells through molecular chaperone functions
A Immunoprecipitation to detect the interaction of wild-type or cysteine mutant PRDX1 with GSTP1; **B** Non-reducing gel detects oxidative polymerization of GSTP1 in A2780 cells overexpressing wild-type or cysteine-mutated PRDX1; **C** Overexpression of wild-type or C83S mutant PRDX expression assays GST enzyme activity in A2780 cells; **D** Overexpression of wild-type or C83S mutant PRDX1 expression assays A2780 cell GSH concentration; **E** Overexpression of wild-type or C83S mutant PRDX1 expression detects cisplatin sensitivity in A2780 cells; **F** Overexpression of wild-type or C83S mutant PRDX1 expression detects docetaxel sensitivity in A2780 cells; **G** Overexpression of wild-type or C83S mutant PRDX1 expression detects doxorubicin sensitivity in A2780 cells. Data are the mean ± SEM; *p < 0.05, **p < 0.01, and ns p > 0.05.

concentration of GSH and NADPH, the major reducing substances in cells. Therefore, PRDX1 promotes multidrug resistance in ovarian cancer cells by regulating redox.

Multidrug resistance is the resistance of tumor cells to multiple chemotherapeutic drugs with different structures and targets. The most important mechanism is the transport of the drug outside the cell by ABC transporters. Because of the large structural differences between different drugs, cells will modify the drugs by glutathionylation via glutathione-S-transferase to make them negatively charged and transported to the outside of the cell by Multidrug Resistance Proteins (MRPs) [24]. And GSTs also have a regulatory effect on cellular redox homeostasis. Our previous data showed that overexpression of PRDX1 in ovarian cancer cells promoted ROS-associated multidrug resistance. We therefore speculated whether PRDX1 mediates multidrug resistance in ovarian cancer cells through GST. We found that the enzyme activities of GSTs were significantly increased in drug-resistant cells. With database analysis, we found that PRDX1 was correlated with GSTP1. Further, we found that PRDX1 interacted with GSTP1 and that this interaction promoted oxidative aggregation of GSTP1, which may be related to the mechanism by which PRDX1 promotes GSTP1 enzyme activity. Subsequently, we determined that down-regulation of GSTP1 rescued PRDX1-mediated resistance to A2780 cisplatin, doxorubicin, and doxorubicin, confirming that PRDX1 mediates multidrug resistance in ovarian cancer cells by increasing the enzyme activity of GSTP1. PRDX1 possesses not only peroxidase activity but also molecular chaperone activity [25,26]. Through biochemical experiments, we determined that the regulation of GSTP1 by PRDX1 does not depend on the peroxidase activity of PRDX1, but rather promotes the enzymatic activity of GSTP1 through the exercise of molecular chaperone function by Cys83 of PRDX1. We found that Cys83-mutated PRDX1 lost its function of regulating GSH concentration and promoting tolerance to chemotherapeutic agents in A2780 cells. Thus, our results suggest that PRDX1 promotes multidrug resistance in ovarian cancer cells by regulating the enzymatic activity of GSTP1 through molecular chaperone function.

In conclusion, this is the first study to show that increasing PRDX1 in ovarian cancer cells mediates GSTP1-dependent multidrug resistance. This was achieved through the PRDX1 molecular chaperone function. In our report, targeting the molecular chaperone activity of PRDX1 rather than peroxidase activity could be a new target for ovarian cancer chemotherapy.

CRediT authorship contribution statement

Chengling Fan: Writing – review & editing, Writing – original draft, Investigation. **Shubin Yuan:** Investigation, Formal analysis. **Yuemei Zhang:** Investigation, Formal analysis. **Yinmei Nie:** Investigation. **Li Xiang:** Formal analysis, Data curation. **Tianchao Luo:** Investigation. **Qi Xi:** Data curation. **Yaqin Zhang:** Investigation. **Zixiang Gu:** Investigation. **Peng Wang:** Writing – original draft, Investigation, Formal analysis, Data curation. **Hongxia Zhou:** W.

Declaration of competing interest

The authors disclose no conflicts of interest.

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