

Original Article

STAT3-mediated Twist1 upregulation contributes to epithelial-mesenchymal transition in cisplatin resistant ovarian cancer

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Abstract: Epithelial-mesenchymal transition (EMT) is a key process contributing to the invasion and chemoresistance in many tumors, and the critical roles of signal transducer and activator of transcription 3 (STAT3) and Twist basic helix loop helix transcription factor 1 (Twist1) in the progress of epithelial ovarian cancer have been demonstrated. Herein, the relationship between STAT3, Twist1 and EMT pathways and their effect on chemoresistance in ovarian cancer was studied. A cisplatin-sensitive human epithelial ovarian cancer cell line (A2780) and the resistant variant (A2780/DDP) were applied and quantitative real-time PCR (qRT-PCR) and Western blot were performed to detect the mRNA and protein expression levels in A2780/DDP and A2780. Loss-of-function assays were performed to analyze the effects of STAT3 and Twist1 knockdown on the sensitivity of ovarian cancer cells to DDP and the underlying molecular mechanisms in vitro. We found that the activity of STAT3 could contribute to the expression of Twist1 in mRNA and protein levels and further regulate the occurrence of EMT. Furthermore, knocking down the expression of STAT3 could significantly reverse Cisplatin resistance in A2780/DDP cells. However, knocking down Twist1 expression barely affects the expression of STAT3 and simply regulates the process of EMT and sensitivity to Cisplatin. In conclusion, the results suggest that STAT3-mediated Twist1 upregulation may contribute to Cisplatin resistance in epithelial ovarian cancer via EMT, which provides support for a potential novel gene therapy targeting the STAT3-Twist1 signaling network for the treatment of cisplatin resistant ovarian cancer.

Keywords: Epithelial ovarian cancer, STAT3, Twist1, EMT, cisplatin resistance

Introduction

Epithelial ovarian cancer is the most common lethal gynecologic malignance, accounting for approximately 5% of mortality from all female cancers [1]. The current standard treatments are appropriate surgical staging and cytoreduction followed by a platinum agent-based chemotherapy, which has led to some improvement in overall survival (OS). Nevertheless, the contribution of treating women with advanced, persistent, or recurrent epithelial ovarian cancer has remained greatly unsatisfactory during past few decades [2], the high mortality rate of the patients is due to treatment failure under such circumstances of progressive or recurrent disease that is resistance to chemotherapies based on DDP [3]. Therefore, understanding the molecular mechanism involved in DDP che-

moresistance will make a great difference for the treatment of chemoresistant ovarian tumor.

Epithelial-mesenchymal transition (EMT), a shift toward the mesenchymal state with the adhesion molecules expressed by the cells being modified, allows the cells to adopt invasive and invasive behaviors [4]. EMT plays an important role in normal embryonic development. Actually, increasing studies have presented that occurrence of EMT validly promotes to metastasis and progression in various cancer types including epithelial ovarian cancer [5-7]. Therefore, the essential parallels between cell plasticity in embryo-fetal development and cancer progression led to the assumption that EMT is a critical driver of epithelial-derived carcinoma malignancies. Twist1 is referred to as a key regulator of EMT, which contributes cancer

The role of STAT3-Twist1 axis in A2780/DDP cells

cells to transform phenotype from epithelial to Mesenchymal. Recently, few studies have demonstrated that STAT3-mediated Twist1 upregulation could contribute cancer cells to metastasis and progression [8-10], and some researches have suggested that STAT3 may exert a critical influence on establishing cell polarity during directed cancer cells progression, processes significant for EMT programs [11].

Although STAT3 and Twist1 in the cancer micro-environment could both induce occurrence of EMT, the relationship between the two factors and further synergistic effect on mediating acquisition of chemotherapy resistance through initiating EMT are unclear, especially in ovarian cancer. In this study, we explored the underlying mechanisms involve transcriptional regulation of Twist1 by STAT3 in cisplatin resistant ovarian cancer cell line, and further investigated whether STAT3-mediated Twist1 upregulation contributes to Cisplatin resistance in epithelial ovarian cancer via EMT. The results derived from the study could provide a critical insights into the biology of the aberrant expression of STAT3 and Twist1, and present a theoretical basis for STAT3-Twist1 signaling as an important regulative target for reversing drug resistance of ovarian neoplasm.

Material and methods

Cell lines and culture

A cisplatin-sensitive human epithelial ovarian cancer cell line (A2780) and the resistant variant (A2780/DDP) were purchased from China Center for Type Culture Collection. Cells were maintained in RPMI-1640 (Jenom, Hangzhou, China) complete medium with 100 U/ml penicillin/streptomycin (Beyotime Institute of Biotechnology, Haimen, China) and 10% fetal bovine serum (FBS; Gibco-BRL, Invitrogen Life Technologies). Under 37°C in a humidified atmosphere containing 5% CO₂.

RNA interference

Small interfering (siRNA) sequences directed against STAT3 and Twist1 were designed and synthesized by the Shanghai Genechem Company (Shanghai, China). The sequences of STAT3 and Twist1 were: 5'-GCAAAGAATCACATGCCACTT-3'; 5'-TCCGCAGTCTTACGAGGAGCT-3',

respectively. And the empty vector as the control. For transfection, the cells were resuspended at a density of 5×10^4 cells/ml and seeded in six plates. When the cells were 60-80% confluent, they were transfected using Lipofectamine TM 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. The stably transfected cells were named A2780/DPP/si-STAT3, A2780/DPP/si-Twist1 and A2780/DPP/si-NC, respectively. Forty-eight hours later, cells were harvested for total RNA extraction.

Cell growth and viability assay

Cell viability and IC50 values (drug concentration causing 50% inhibition of cell growth) were measured by the CCK-8 assay (Beyotime Institute of Biotechnology, Haimen, China). Briefly, Cells were suspended with RPMI-1640 medium at a density of 5×10^4 cells/ml and seeded into 96-well plates, after being incubated for 24 h at 37°C. On the one hand, the CCK-8 assay was performed before transfection and then at 24 h, 48 h, 72 h and 96 h following transfection. On the other hand, the cells in 96-well plates were transfected and incubated for 24 h at 37°C, following removed the spent culture medium and 100 µL of prepared medium containing various concentrations of DDP (0, 5, 10, 20, 40, 80, 160 µM) was added. Each sample was loaded in triplicate. Following incubation at 37°C for 24 h. For the assay, 10 µL CCK-8 solution was added to each well and the cells were incubated for 2 h. The absorbance of the wells was then measured at 450 nm using a microplate spectrophotometer (Victor 3 1420 Multilable Counter; PerkinElmer, Inc., Waltham, MA, USA). The viability of the treated group was expressed as a percentage of the untreated control group, which was designated as 100%. Cellular growth curves were plotted as follows: Cellular growth (%) = OD450 of experimental well/OD450 of control well.

Plate clone formation assay

Cells were resuspended at a density of 400 cells/well and seeded in six plates. Each group loaded in triplicate. Following being incubated at 37°C for 2 weeks, the cells were washed three times with PBS and stained with Crystal Violet Staining Solution (Wuhan Goodbio Technology Co., Ltd., Wuhan, China). The num-

The role of STAT3-Twist1 axis in A2780/DDP cells

ber of colonies containing more than 50 cells were counted using a microscope and the plate clone formation efficiency was calculated as follows: plate clone formation efficiency = (number of colonies/number of cells inoculated) × 100%.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis

Total RNA was extracted through TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) from cultured cells. cDNA synthesis was performed by a RevertAid First Strand cDNA Synthesis kit (Fermentas; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. qPCR was performed using SYBR Green labeled probes (Takara Bio, Inc., Tokyo, Japan) and the gene expression levels were detected by an ABI 7500 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The expression levels of the target mRNAs were calculated and normalized to the mRNA level of GAPDH. The following primers (Sangon Biotech Co., Ltd., Shanghai, China) were used: Twist1, primers 5'-GTC CGC AGTCTT ACG AGG AG-3' and reverse 5'-CCA GCT TGA GGG TCT GAA TC-3'; STAT3, primers 5'-GAAGAATCCAA-CAACGGCAG-3' and reverse, 5'-TCACAATCAGG-GAAGCATCAC-3'; GAPDH, primers 5'-GCA CCG TCA AGG CTG AGA AC-3' and reverse, 5'-TGG TGA AGA CGC CAG TGGA-3'.

Western blot analysis

Total protein was extracted from cells using RIPA buffer (Beyotime Institute of Biotechnology) and the quantity was examined by the Bicinchoninic Acid Protein Assay kit (Beyotime Institute of Biotechnology). The protein samples were separated by SDS-PAGE gels and transferred onto polyvinylidene fluoride (PVDF) membranes (EMD Millipore, Billerica, MA, USA). The blots were blocked at room temperature for 1 h with 5% non-fat milk solution, following being incubated overnight at 4°C with diluted primary antibody and the blots were incubated at room temperature for 1 h with different horseradish peroxidase-conjugated secondary antibodies (1:10,000 dilution; LICOR Biosciences, Lincoln, NE, USA). Finally, the protein intensity were visualized and quantified by an Odyssey imaging system (LICOR Biosciences, Lincoln, NE, USA). Experiments were performed in triplicate. The following primary antibodies

were used: mouse polyclonal STAT3 antibody (1:1000; cat. no. 9139), mouse polyclonal E-cadherin antibody (1:1000; cat. no. 14472), mouse polyclonal N-cadherin antibody (1:1000; cat. no. 13116), rabbit monoclonal Vimentin antibody (1:1000; cat. no. 5741), all obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA); rabbit monoclonal Twist1 antibody (1:1000; cat. no. ab50581) were obtained from abcam, Inc. (Cambridge, USA). β -actin antibody (1:1000 dilution; cat. no. 4970; Cell Signaling Technology, Inc.) served as an endogenous reference. Images of the blots were captured using a GelDoc XR imaging system (BioRad Laboratories, Inc., Hercules, CA, USA) and they were analyzed using Quantity One 4.62 software (BioRad Laboratories, Inc.).

Annexin V/PI assay

A2780 cells were seeded into 6 six plates at a density of 5×10^5 cells/well and cultured overnight at 37°C. When the cells were 60-80% confluent which were transfected and incubated for 24 h at 37°C. Each sample was loaded in triplicate. Annexin V/PI assay was performed according to the manufacturer's instructions (Beyotime Institute of Biotechnology). Briefly, the samples were washed with phosphate-buffered saline (PBS, Jenom, Hangzhou, China) and resuspended at a density of 1×10^6 cells/ml. Following being incubated with 10 μ L Annexin V-FITC for 15 min at room temperature in the dark. Next, 5 μ L PI was added to incubate for 5 min, subsequently, 400 μ L 1 × binding buffer was added into the sample and analyzed by the FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). The percentage of early apoptotic, late apoptotic and necrotic cells was analyzed using BD FACS DIVA software (BD Biosciences).

Statistical analysis

The differences observed between the control and treated groups for cell proliferation, cell cycle, apoptosis and the level of the protein were analyzed using either One-way ANOVA or unpaired Student t-test (two-tailed) using the statistical Package for Social Science (SPSS Release 22.0; SPSS Inc, Chicago, IL, USA). The results were expressed as mean \pm standard deviation from triplicate experiments and a value of $p < 0.05$ was considered statistically significant.

The role of STAT3-Twist1 axis in A2780/DDP cells

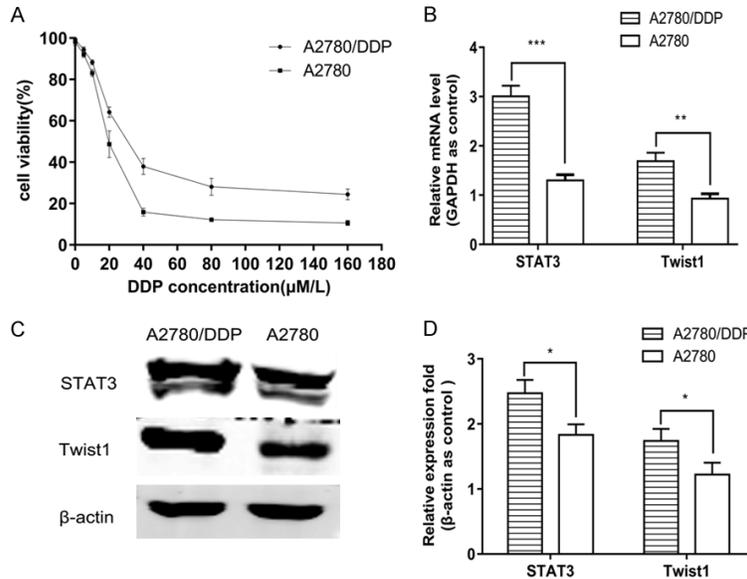


Figure 1. The expression of STAT3 and Twist1 were upregulation in A2780/DDP cells. (A) The cytotoxic effect of cisplatin on A2780 and A2780/DDP cells following treatment with a wide concentration range of cisplatin (5, 10, 20, 40, 80, 160 μM/L) were assessed by CCK-8 assay. (B) The mRNA levels of STAT3 and Twist1 in A2780 and A2780/DDP cells were examined by qPCR, GAPDH was co-amplified as the internal control. (C) Protein expression of STAT3 and Twist1 in A2780 and A2780/DDP cells were detected using western blot analysis in total cell extracts. β-actin was reported to confirm equal protein loading. (D) The quantitative analysis for (C). Data are expressed as the mean ± SD of three independent experiments (bars represent S.D.). (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Results

The expression of STAT3 and Twist1 were up-regulation in cisplatin-resistant ovarian cancer cells

First, we examined the cytotoxic effect of cisplatin on A2780 and A2780/DDP cells by an CCK-8 assay. IC₅₀ values were used to represent the levels of cytotoxicity. The IC₅₀ values in A2780 and A2780/DDP cells were $23.11 \pm 0.97 \mu\text{M}$ and $39.43 \pm 1.19 \mu\text{M}$, respectively, which suggested that the A2780/DDP cells were more resistant to cisplatin-induced cytotoxicity compared with A2780 cells (**Figure 1A**). Next, to investigate the association of STAT3 and Twist1 expression with cisplatin resistant ovarian cancer, western blot and qPCR assay were performed to determine the expression levels of STAT3 and Twist1 in cisplatin-sensitive ovarian cancer cell line (A2780) and the resistant variant (A2780/DDP). qPCR results suggested that the relative mRNA expression levels of STAT3 and Twist1 in A2780/DDP cells

were significantly higher than those A2780 cells (**Figure 1B**). According to the assay by western blot, the protein expression level of STAT3 in A2780 and A2780/DDP cells was detectable and relative absorbance values were 1.83 ± 0.16 and 2.47 ± 0.21 , respectively. The protein expression level of Twist1 in A2780 was lower 1.42 folds than those in A2780/DDP (**Figure 1C and 1D**).

Knocking down STAT3 expression could decrease the expression of Twist1 in cisplatin resistant ovarian cancer cells

To further investigate the relationship between STAT3 and Twist1 in cisplatin resistant ovarian cancer, the siRNA was designed to intervene the expression of the both genes. qPCR assays verified the stable knockdown of endogenous STAT3 and Twist1 in A2780/DDP/si-STAT3 and

A2780/DDP/si-Twist1, respectively, which were compared with A2780/DDP/si-NC and A2780/DDP cell line (**Figure 2A**). Interesting, it was observed that the protein expression level of STAT3 and Twist1 was decreased by 0.80 and 1.00 folds in A2780/DDP/si-STAT3 cells compared with A2780/DDP cells, respectively. Nevertheless, the Twist1 expression was detached to significantly lower and the protein expression level of STAT3 was no prominently alter compared to A2780/DDP/si-NC and A2780/DDP cells in A2780/DDP/si-Twist1 (**Figure 2B and 2C**). The similar results were also observed in qPCR assays (**Figure 2A**).

Knocking down STAT3 expression could reverse the resistance to cisplatin in A2780/DDP cells

The results above have suggested that the protein expression level of Twist1 was regulated by STAT3, to further explore whether STAT3 mediates Twist1 expression contribute to chemotherapy resistance in ovarian cancer, a wide

The role of STAT3-Twist1 axis in A2780/DDP cells

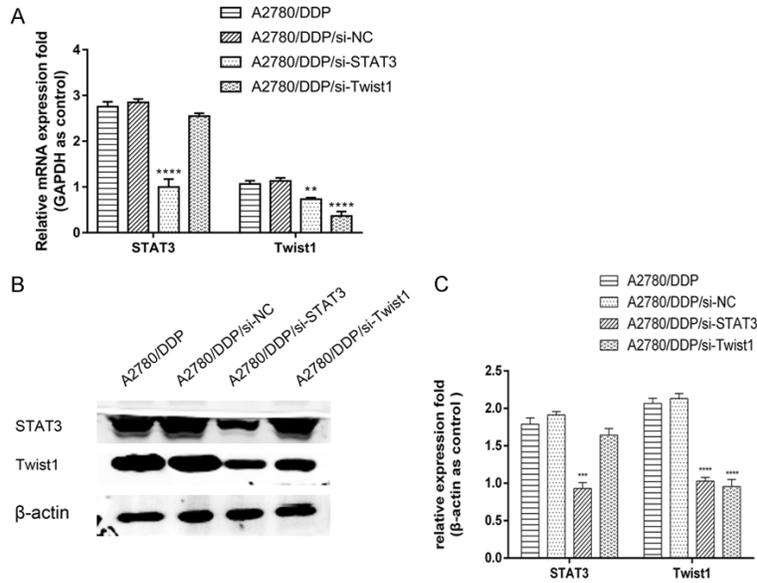


Figure 2. STAT3 mediates Twist1 expression in A2780/DDP cells. (A) After STAT3-siRNA and Twist1-siRNA were transfected into A2780/DDP cells, respectively, qPCR was detected the mRNA levels and confirmed the effectivity of transfection, mRNA levels of STAT3 and Twist1 in A2780/DDP/si-STAT3 and A2780/DDP/si-Twist1 cells was compared with A2780/DDP cells. (B) Protein expression of STAT3 and Twist1 in A2780/DDP, A2780/DDP/si-NC, A2780/DDP/si-STAT3 and A2780/DDP/si-Twist1 cells were detected by Western blot analysis, Protein levels of STAT3 and Twist1 in A2780/DDP/si-STAT3 and A2780/DDP/si-Twist1 cells was compared with A2780/DDP cells. (C) The quantitative analysis for (C). Data are expressed as the mean \pm SD of three independent experiments (bars represent S.D.) (** $P < 0.001$, **** $P < 0.0001$).

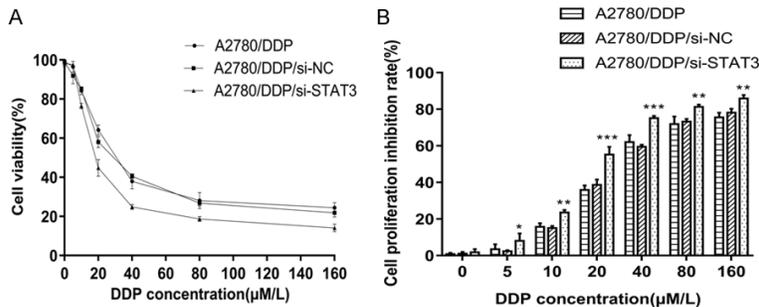


Figure 3. The resistance to Cisplatin could be reversed by Knocking down STAT3 expression in A2780/DDP cells. A. A2780/DDP, A2780/DDP/si-NC, A2780/DDP/si-STAT3 cells were treated with cisplatin (0, 5, 10, 20, 40, 80, 160 mM/L) for 48 h, After the treatment, cell viability was determined by CCK-8 assay. B. A2780/DDP, A2780/DDP/si-NC, A2780/DDP/si-STAT3 cells proliferation inhibition following treatment with cisplatin (0, 5, 10, 20, 40, 80, 160 mM/L) assessed by CCK-8 assay, A2780/DDP/si-STAT3 cells proliferation inhibition was compared with A2780/DDP cells. Data are expressed as the mean \pm SD of three independent experiments (bars represent S.D.). (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

concentration range of Cisplatin (0, 5, 10, 20, 40, 80, 160 μ M) was designed, CCK-8 assay demonstrated the IC₅₀ of A2780/DDP/si-STAT3 was decreased by 0.33 and 0.31 folds

compared to A2780/DDP/si-NC and A2780/DDP cells, which suggested that knocking down the expression of STAT3 could significantly reverse the resistance to Cisplatin and Twist1 may play an essential role in the process of acquisition of drug resistance in ovarian cancer cells (Figure 3A). What's more, according to the cellular growth curves as follows by CCK-8 assay, it was observed that the cell proliferation was remarkably suppressed after knocking down the STAT3 in A2780/DDP cells compared to blank control group and negative control group ($p < 0.05$, Figure 3B). Which further indicted the significance of STAT3 and Twist1 for affecting the sensitivity of cisplatin in ovarian cancer.

Knocking down STAT3 expression could slow down cell growth and promote cisplatin-induced cell apoptosis in ovarian cancer

We have defined the role of aberrant expression of STAT3 and Twist1, to make clear how the two genes affect the cells growth and the possible mechanism responsible for the STAT3-mediated upregulation of Twist1 to the proliferation of Cisplatin-resistant ovarian cancer cells. Plate clone formation assay suggested that the capability of proliferation was enormously restrained in A2780/DDP/si-STAT3 cells compared with A2780/DDP/si-NC and A2780/DDP cells (Figure 4A and 4B), combination with the results above, we have great confidence to define that the

viability of cisplatin-sensitive ovarian cancer cells could be directly promoted by the aberrant expression of STAT3 and Twist1. Furthermore, Annexin V-FITC and PI double stain-

The role of STAT3-Twist1 axis in A2780/DDP cells

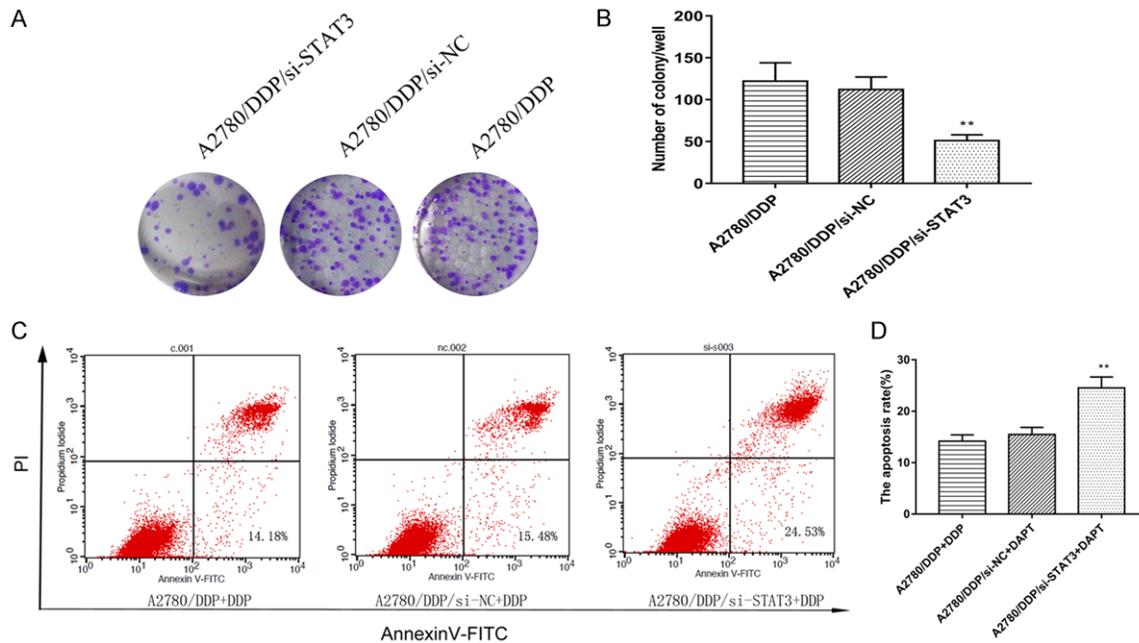


Figure 4. STAT3 regulates cisplatin-induced apoptosis and cell growth in ovarian cancer. A and B. The capability of proliferation of A2780/DDP, A2780/DPP/si-NC, A2780/DPP/si-STAT3 cells were evaluated by Plate clone formation assay, the colony number of A2780/DPP/si-STAT3 cells was statistically significant compared with A2780/DDP cells. C and D. A2780/DDP, A2780/DPP/si-NC, A2780/DPP/si-STAT3 cells were treated for 48 h with 20 mM/mL cisplatin. The cells were collected sequentially stained with Annexin-V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) followed by flow cytometric analysis, the apoptosis rate of A2780/DPP/si-STAT3 + DDP group was statistically significant compared with A2780/DDP + DDP group. Data are expressed as the mean \pm SD of three independent experiments (bars represent S.D.). (** $P < 0.01$).

ing of A2780/DDP cells knocked down STAT3 observed significant increase in the number of cisplatin-induced apoptotic cells compared with the control group (Figure 4C and 4D). The apoptosis rates were $24.53 \pm 0.93\%$, $15.48 \pm 1.47\%$ and $14.18 \pm 2.19\%$ for A2780/DPP/si-STAT3 cells, A2780/DPP/si-NC and A2780/DDP, respectively. The enhance number cells in apoptosis was statistically significant ($p < 0.05$).

STAT3-mediated Twist1 upregulation contributes to epithelial-mesenchymal transition in cisplatin resistant ovarian cancer

Previous studies have well revealed that Twist1 as a key regulator of EMT and plays an important role in the process of cancer development [12, 13]. The protein expression levels of special genes relative to EMT in ovarian cancer were examined by Western blot assay. It was observed that the expression of E-cadherin was markedly decreased while N-cadherin was increased in A2780/DDP cells, and the protein expression of Vimentin was increased by 0.30

folds compared to A2780 cells (Figure 5A and 5B). Which was suggested that the occurrence of EMT may involve in acquisition of cisplatin resistance in ovarian cancer. Next, we further confirmed the expression levels of E-cadherin, N-cadherin and Vimentin when A2780/DDP was transiently transfected with NC-siRNA and STAT3-siRNA, respectively. Compared with the A2780/DDP and A2780/DDP/NC-siRNA cells, the expression of N-cadherin and Vimentin was markedly downregulated in A2780/DPP/si-STAT3 cells protein level. By contrast, E-cadherin protein expression were increased by 0.17 folds and 0.19 folds in A2780/DDP cells transfected with STAT3-siRNA, when compared with blank control group and negative control group cells (Figure 5C and 5D).

Discussion

The most major obstacle for successful chemotherapy of recurrent tumor is the development of acquired chemoresistance, although research has illuminated that the cancer cells exert drug resistance effect though enhance

The role of STAT3-Twist1 axis in A2780/DDP cells

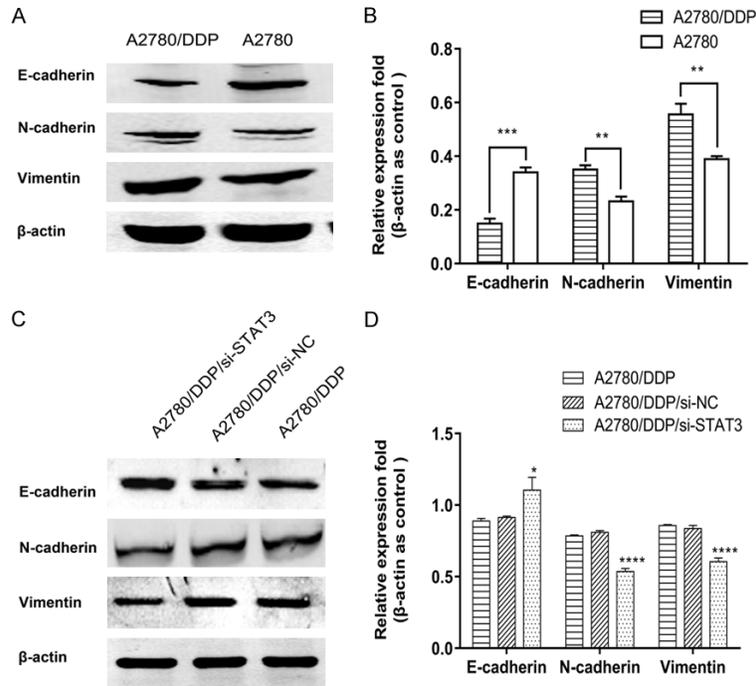


Figure 5. STAT3 contributes to epithelial-mesenchymal transition in A2780/DDP. (A) Protein expression of E-cadherin, N-cadherin and Vimentin in A2780 and A2780/DDP cells were detected using western blot analysis in total cell extracts. (B) The quantitative analysis for (A). (C) The protein expression levels of A2780/DDP, A2780/DDP/si-NC, A2780/DDP/si-STAT3 cells were examined using western blot analysis. β -actin was reported to confirm equal protein loading, the EMT marker protein levels in A2780/DDP/si-STAT3 cells was statistically significant compared with A2780/DDP cells. (D) The quantitative analysis for (C). Data are expressed as the mean \pm SD of three independent experiments (bars represent S.D.). (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

survival pathways against apoptosis signals and inactivating apoptotic factors [14]. The detailed molecular mechanisms remain poorly understood and need putting in greater efforts for exploring. There is no doubt that cisplatin centered systemic chemotherapy remains the essential method of ovarian tumor therapy, therefore, finding out the novel molecular target for reversing the DDP chemoresistance will bring bright for suffering for progression and recurrence ovarian neoplasm. Undertaking to explore the role of aberrant expression of STAT3 in EMT in the current study, we discovered that A2780/DDP cells with high STAT3 expression/activity undergo Twist1-induced EMT, and this phenotypic transition is associated with acquisition of chemotherapy resistance in human epithelial ovarian cancer.

The impacts of EMT on the embryonic development have been well studied, and increasing

evidence are indicating its essential role in early-stage dissemination and invasion of cancer cells. More recent, by establishing an EMT lineage-tracing system to surveil this process and inhibiting EMT via overexpressing the microRNA miR-200 in spontaneous breast-to-lung metastasis mice models, it was observed that EMT has no effect on lung metastasis development, but contributes to chemoresistance [15]. At the same time, the similar results were put forward by utilizing mouse models of pancreatic ductal adenocarcinoma (PDAC) with deficiency of Snail or Twist [16], which strongly support the perception that EMT may promote a cellular ability to evade the effects of cisplatin centered systemic chemotherapy and highlight the significance of combining EMT suppression with chemotherapy. In the current study, by validating via hallmarks of EMT, it was observed that the protein expression levels of E-cadherin decreased markedly while N-cadherin and Vimentin increased in A2780/DDP cells, compared to cisplatin-sensitive ovarian cancer cells. which was strongly suggested that the occurrence of EMT is associated with the resistance of cisplatin and in line with the argument that EMT pathway is of enormous therapeutic interest in the treatment of malignancy [17].

The emergence of EMT is orchestrated by integrated networks of signaling pathways and EMT-related transcription factors which mediate the expression of genes associate with cell differentiation, adhesion and motility. The bHLH factors Twist1 have been identified as the EMT inducers [18]. Twist1 protein could directly combine with the E-box sequence of the pivotal epithelial hallmark E-cadherin and result in suppressing the trans-activation of E-cadherin promoter, which consequently promotes EMT [19]. The aberrant Twist1 expres-

sion is associated with the resistance of cisplatin and in line with the argument that EMT pathway is of enormous therapeutic interest in the treatment of malignancy [17].

sion is frequently detected in various types of neoplasm, including epithelial ovarian cancer, and always along with the emergence of EMT [12, 20]. In our study, we have found that the A2780/DDP cells characterized by EMT more likely upregulate the protein expression levels of Twist1 and the characteristics of EMT of A2780/DDP cells were remarkably retreated after silencing Twist1 gene expression. At the same time, the resistance to cisplatin was also noteworthy reversed in A2780/DDP/Twist1-siRNA, which was in line with the standpoint of Zheng in the PDAC with deficiency of Snail or Twist [16]. Therefore, we could conclude that Twist1 gene plays an important role as a trigger of EMT and confers resistance to chemotherapy.

We would further interrogate what's the initial factor to mediate the aberrant Twist1 expression and the Cisplatin tolerance in ovarian cancer. Increasing evidence has demonstrated that the overexpression of Twist1 followed by the upregulation of STAT3 in some malignancies [8, 9]. What's more, Lo et al. have put forward that constitutively active STAT3 could significantly activate the Twist1 promoter, nevertheless the JAK/STAT3 inhibitor and dominant-negative STAT3 inhibited Twist1 promoter [10]. Similar viewpoint has been put forward in gastric cancer [21], which strongly indicates that STAT3 mediates aberrant expression of Twist1 promotes resistance to chemotherapy in ovarian cancer. Different from other STATs, STAT3 can turn into the nucleus after being phosphorylated. Nuclear collection of activated STAT3 followed by increased nuclear import and decreased nuclear export is extraordinary dependent on tyrosine residue (Tyr-705) phosphorylation [22]. Constitutive activation of STAT3 has been observed to drive unarranged gene transcription whose products subsequently contribute to cancer development and progression [23]. In this study, inflated expression levels of STAT3 was observed in A2780/DDP, and the ability of proliferation was higher, while the cisplatin induced apoptosis rate was lower than A2780. Through abrogation of the STAT3 signaling cascade could result in an apparent downregulation of Twist1 and the mesenchymal marker N-cadherin and Vimentin, as well as the cisplatin induced apoptosis rate also increased. Whereas by silencing the Twist1 signaling cascade just results in altering the expression of E-cadherin, N-cadherin and

Vimentin, the STAT3 expression was not apparently down or over expression, What's more, the IC50 of cisplatin was simultaneously obviously decreased, which was not only suggested that STAT3 is the essential checkpoint of Twist1, but also indicated that STAT3 could mediate Twist1 upregulation and contribute to cisplatin resistance, the molecular mechanism of acquisition of drug resistance may through the EMT network.

On the basis of the results described above, we illuminated the underlying mechanisms of STAT3-induced occurrence of EMT and the effects of STAT3 on Twist1 expression and ovarian cancer resistance to cisplatin. We demonstrate that down-regulated expression of STAT3 facilitated cisplatin-apoptosis and STAT3-mediated Twist1 upregulation contributes to Cisplatin resistance in epithelial ovarian cancer via epithelial-mesenchymal transition. Worthwhile, further elucidation of functional characterization is essential. The results of the current study provide support for this potential novel gene therapy with STAT3-Twist1 signaling network for the treatment of cisplatin resistant ovarian cancer.

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Disclosure of conflict of interest

None.

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The role of STAT3-Twist1 axis in A2780/DDP cells

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