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Original Article

Synergistic effect of Tripterygium glycosides and cisplatin on drug-resistant human epithelial ovarian cancer via ILK/GSK3β/Slug signal pathway

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Abstract: The side-effects of therapeutic drugs and the intrinsic or acquired cisplation resistance are considered impediments in the clinic treatment of human epithelial ovarian cancer, which contribute heavily to the startlingly high mortality. It is imperative to look for drugs to inhibit cancer and minimize the chemotherapy resistance safely and effectively from the Chinese herbal medicine. In the present study, we evaluated the anti-cancer effect of Tripterygium glycosides (GTW) and its sensitizing effect with cisplation (DDP) both in vitro and in vivo. The 5-ethynyl-2'-deoxyuridine (EdU) proliferation assay, transwell assay, and scratch wound healing assay demonstrated that GTW and DDP+GTW prominently inhibited the proliferation, migration, and invasion of SKOV3/DDP cells. In addition, treatment using GTW and DDP+GTW for 24 h significantly decreased the expression of ILK, p-AKT, p-GSK3β, N-Cadherin, and Slug, and markedly enhanced the expression of E-cadherin. Moreover, animal results confirmed that GTW and DDP+GTW significantly inhibited the tumor volume, increased the apoptosis of tumors cells and reduced the production of tumor markers CA125 and HE4 in mice serum. Similar to the results in vitro, GTW and DDP+GTW significantly inhibited the expression of proteins in epithelial-mesenchymal transition (EMT) and ILK/GSK3β/Slug signal pathway in tumors in vivo. In conclusion, our results indicated that GTW may be served as a potential therapeutic drug combination with DDP to treat drug resistant ovarian cancer via regulating ILK/GSK3β/Slug signal pathway.

Keywords: Tripterygium glycosides, drug-resistant human epithelial ovarian cancer, epithelial-mesenchymal transition, ILK/GSK3β/Slug signal pathway

Introduction

Ovarian cancer is a common gynecological malignancy with the highest mortality rate and epithelial ovarian cancer is the most common histological type of ovarian cancer [1, 2]. Although surgical excision, irradiation, and cisplatin-based chemotherapy are approved for the current clinical treatments, the five-year survival rate of epithelial ovarian cancer is only 25-30% due to the lack of effective early screening, chemoresistance, and particularly tumor invasion and metastasis [3, 4].

Therefore, it is significant to find more effective pharmacological interventions or a sensitizer for advanced or/and resistant epithelial ovarian cancer to cisplatin from traditional Chinese medicines for their reduced side effects. To improve life quality of patients with ovarian cancer, our group have long been committed to studying the anti-ovarian cancer effect and mechanism of triptolide (TPL) [5-8]. However, TPL is only allowed to be used as experimental supplies and far from being ready for use in actual patients for its short half-life in circulation, and poor aqueous solubility [9]. Nevertheless, the Gripterygium glycosides (GTW) containing the active ingredient of TPL has been widely used clinically in rheumatoid arthritis, primary glomerulonephritis, nephrotic syndrome, anaphylactic purpura nephritis, lupus erythematosus and other immune diseases for years in China [10, 11]. Moreover, recent studies indicated that GTW was able to potentially inhibit a variety of cancers, e.g., nasopharyn-

geal carcinoma and hematological malignancy, while few work has been done to treat epithelial ovarian cancer using GTW.

Epithelial-mesenchymal trasition (EMT) has been connected with the invasion and metastasis of epithelial ovarian cancer [12-15]. EMT is characterized by loss of cell polarity and adhesion (to each other and extra cellular matrix), down-regulation of epithelial molecule E-cadherin and up-regulation of mesenchymal markers N-cadherin, which is regarded as a key step during the tumor [16]. In addition, recent studies demonstrated that the transcriptional factors (Snail and Slug) play crucial role in promoting the EMT of ovarian cancer [17].

Integrin-linked kinase (ILK), a serine/threonine protein kinase, is involved in the regulation of cell survival, cycle, proliferation, adhesion and migration by adjusting the integrin signaling of various cells [18]. Researches indicated that ILK played its role in malignant ovarian cancer via phosphorylation in several key signaling pathways, and affected the expressions of protein kinase B (AKT) and glycogen synthase kinase 3β (GSK3β). In addition, studies have also shown that ILK could induce the EMT of epithelial ovarian cancer cells and then promote the invasion and metastasis of epithelial ovarian cancer [19, 20].

In the present study, we study the synergistic effect of GTW and DDP on suppressing the invasion and metastasis of epithelial ovarian cancer by inhibiting epithelial-mesenchymal transition and the ILK/GSK3β/Slug signal pathway both in vitro and in vivo.

Materis and methods

Reagents and antibodies

GTW (Chengdu Glip Biotechnology Co., Ltd, Chengdu, China) was dissolved in RPMI-1640 (Solarbio, Beijing, China) to obtain 12 mg/ml stock solution, filtrated through a 0.22 μm membrane, and kept at -20°C until diluted in medium to 12 mg/ml and 800 μg/ml for use. Ccisplatin (DDP, Jiangsu haosen pharmaceutical Co., Ltd, Jiangsu, China) was dissolved in RPMI-1640 to 0.4 mg/ml and 10 μg/ml. The antibodies for Cleaved-caspase-3 (Wuhan servicebio technology CO., LTD, Wuhan, China, no. GB11009-1), Bcl-2 (Wuhan servicebio technology CO., LTD, Wuhan, China, no. GB11008), Bax (Wuhan servicebio technology CO., LTD, Wuhan, China, no. GB11007), P53 (Wuhan servicebio technology CO., LTD, Wuhan, China, no. GB11029), Fas (Wuhan servicebio technology CO., LTD, Wuhan, China, no. GB11089), AKT (Cat. no. ab179463, Abcam), p-AKT (Cat. no. Ab8932, Abcam), ILK (Cat. no. Ab52480, Abcam), GSK3β (Cat. no. Ab32391, Abcam), p-GSK3β (Cat. no. Ab131097, Abcam), E-cadherin (Cat. no. Ab40772, Abcam), N-cadherin (Cat. no. Ab76011, Abcam), and Slug (Cat. no. Ab27568, Abcam) were used. The secondary antibodies used for western blot analyses were horseradish-peroxidase-conjugated anti-rabbit secondary antibody (Boster Biolocical Technology Co., Ltd, Wuhan, China). The secondary antibodies used for immunohistochemistry were biotin labeled goat-rabbit IgG (Wuhan servicebio technology CO., LTD, Wuhan, China).

Cell cultures

Cisplatin-resistant human epithelial ovarian cancer cell line SKOV3/DDP was purchased from China Center for Type Cultre Collection (Wuhan, China). Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) (Biological Industries, Israel) and kept in a 5% humidified CO_2 atmosphere at 37°C. To maintain the acquired resistance to cisplatin, 0.375 μg/ml cisplatin was added into the culture media.

Cell proliferation assays

The cell proliferation ability was measured by the 5-ethynyl-2'-deoxyuridine (EdU) proliferation assay. Cells were resuspended and seeded in 96-well plates (1×10^4 cells/well) and continually cultured at 37°C for 24 h. Then, different drugs were added at the appropriate concentrations and incubated for another 24 h at 37°C. After being washed 3 times with PBS, cells were incubated for 4 h in serum-free RPMI-1640 supplemented with 50 μM EdU (Guangzhou RiboBio Biotechnology, Co., Ltd., Guangzhou, China). Next, cells were fixed with 4% polyformaldehyde in PBS at room temperature for 30 min. Finally, cells were subsequently incubated with Apollo staining solution and Hoechst 33342 for another 30 min. The percentage of EdU-positive cells relative to the total cell numbers was intended to represent the proliferation index.

Cell migration and invasion assays

Transwell assays were used to quantitate the effect of GTW on the migration and invasion of SKOV3/DDP cells. First, 5 × 10^4 SKOV3/DDP cells/well were added into the upper chamber of each well of 24-well plates containing 8.0 μm pore size membranes, and the bottom wells were added with 500 μl RPMI-1640 medium supplemented with 10% FBS. Then, different disposed RPMI-1640 medium were appended to the chambers, respectively. The crystal violet was used on stained cells that had reached the underside of the membrane after 24 h. Finally, 5 random fields were photographed and calculated. Transwell assays to assess the invasion were similarly performed except that 90 μl diluted matrigel was added into each well 3 h before cells were seeded in the upper chamber.

A scratch wound healing assay was used to examine the effect of GTW on the migration of SKOV3/DDP cells. Briefly, the SKOV3/DDP cells (1 × 10^6/well) were seeded in 6-well plates cultured with RPMI-1640 medium supplemented with 10% FBS, then a 200 μl pipette tip was used to make a straight scratch on the confluent monolayer of the cells attached to the bottom of the plates. The suspended cells were washed 3 times with PBS. FBS-free RPMI-1640 with 10 μg/ml DDP, 800 μg/ml GTW, 10 μg/ml DDP+800 μg/ml GTW were added to each well. The wound area was photographed under an inverted microscope at 0 h and 24 h.

Western blotting

Protein extraction and immunoblot analysis were performed on cells or tumor tissue lysed in RIPA buffer (Beijing Applygen Technologies Inc, Beijing, China) with protease and phosphatase inhibitors. The lysates were centrifuged and the supernatants were collected as the total lysate protein. The concentration of protein was determined by BCA protein assay kit (Solarbio, Beijing, China) according to the manufacturer's instructions. Equal amounts of total proteins were separated by SDS-PAGE and transferred onto 0.22 μm polyvinylidene difluoride (PVDF) membranes. After blocked in TBST (Tris-buffered solution, pH 7.6, 0.05% Tween-20) with 5% non-fat dry milk for 2 h, the membranes were further incubated with specific antibodies overnight at 4°C. After repeated washing, membranes were incubated with horseradish-peroxidase-conjugated anti-rabbit secondary antibody at room temperature for 1 h. Bands were visualized using an enhanced chemiluminescence system (US Everbright Inc.), and the expression levels of the proteins were compared to the control based on the relative intensities of the bands.

Mouse model of ovarian cancer

To establish primary tumor xenografts of ovarian cancer cells in mice, SKOV3/DDP cells were injected into the flanks of 24 4- to 6-week-old BABL/c nude mice in a volume of 100 μl of PBS, at 5 × 10^6 cells/site (n=6 for each group). When tumors reached approximately 100 mm^3, normal saline (0.5 ml normal saline for gavage every day, NC group), DDP (4 mg/kg/days for intraperitoneal injection, on the 1st and 8th days, DDP group), GTW (120 mg/kg/days for gavage every day, GTW group), DDP+GTW (4 mg/kg/days of DDP on the 1st and 8th days for intraperitoneal injection, 120 mg/kg/days of GTW for gavage every day, DDP+GTW group) were used to treat mice. During the total experimental periods, body weights and the diameter of each tumor were measured every two days. Finally, all mice were sacrificed. The tumor xenografts were removed, weighed, and photographed, and approximately half of the tumor xenografts were fixed in 4% paraformaldehyde buffer for further TUNEL staining and immunohistochemical analysis, while the other half were flash-frozen in liquid nitrogen and kept at -80°C for western blotting analysis.

Enzyme-linked immunosorbent assays (ELISA)

The levels of CA125 and HE4 in the serum of mice were measured using corresponding ELISA kits for CA125 (Uscn Kit Inc, Wuhan, China) and HE4 (Cusabio Biotech Co., Ltd, Wuhan, China) according to the manufacturer's protocols. The optical density was determined at 450 nm using an absorbance microplate reader (Bio Tek, USA), and the levels of CA125 and HE4 were calculated from the OD450 values according to the ELISA curve within diluent standards.

Immunohistochemical staining

Paraffin-embedded tumor sections were sliced and deparaffinized in xylene before hydrated in

gradient alcohol. Then, slides were treated with 1 mM EDTA buffer in a microwave for 3 min for antigen retrieval. After that, slides were equably blocked in 3% bovine serum albumin for 30 min at room temperature, and were incubated with primary antibody in a humidified chamber at 4°C overnight. After incubation with biotin labeled goat-rabbit IgG, slides were visualized using diaminobenzidine (DAB), and redyed with hematoxylin. Images were taken by an optical microscope and the immunohistochemistry accumulates optical density (IOD) of each image was calculated by Image-pro plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA).

Terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) assays

For confirming the cells of tumor xenografts of apoptosis, the TUNEL assays were performed with an in situ cell death detection kit, Fluorescein (Roche, Mannheim, Germany), according to the manufacturer's instructions. Five random fields were photographed and analyzed. The nucleated cells were counted which were stained as blue, and the nuclei of apoptotic cells were counted which were stained as green. Finally, the apoptotic index (AI) was calculated as the percentage of TUNEL-positive cells: AI= number of apoptotic cells/total number of nucleated cells.

Statistical analysis

Values were expressed as mean ± SD (standard deviation) of three independent determinations. All data were calculated using GraphPad Prism software version 7.0 (GraphPad Software Inc., La Jolla, CA, USA). Two-tailed independent t-test was applied for comparison of means between two groups, and one-way ANOVA was applied for comparison of means among groups. A $P<0.05$ was considered as a significant statistical difference.

Results

GTW inhibits proliferation of SKOV3/DDP cells

EdU proliferation assay was used to detect the cell viability after SKOV3/DDP cells were incubated with DDP, GTW, DDP+GTW for 24 h. As shown in **Figure 1A**, 10 μg/ml DDP (27.37±0.02%), 800 μg/ml GTW (18.91±0.02%), and 10 μg/ml DDP+800 μg/ml GTW (7.04±0.01%) significantly inhibited the proliferation of SKOV3/DDP cells compared with control group (39.08±0.01%) ($P<0.05$, **Figure 1A**). In addition, significant decrease of cell viability was noticed in DDP+GTW group compared with the GTW or DDP monotherapy group.

GTW inhibits migration as well as invasion of SKO3/DDP cells

As we know, migration and invasion play an important role for the death of tumor sufferers [21]. As shown in **Figure 1B**, the migration vs. invasion of SKOV3/DDP cells at 24 h in DDP, GTW, and DDP+GTW groups was 35.46±0.65% vs. 58.02±1.26%, 29.79±0.61% vs. 30.27±0.71%, and 16.19±0.69% vs. 12.07±1.32, which was lower than that in control group ($P<0.001$, **Figure 1B**). Moreover, the inhibitory effect of DDP+GTW was better than that in GTW and DDP groups.

Then, wound healing assay was also used to confirm the inhibition of the cell migration ability of GTW. The results turned out that DDP (74.0%), GTW (77.7%), and DDP+GTW (94.3%) markedly suppressed the wound healing of SKOV3/DDP cells compared with NC group (55.3%) ($P<0.05$, **Figure 1C**). Moreover, migration of SKOV3/DDP cells was more significantly suppressed in DDP+GTW group compared with that in DDP or GTW group.

GTW alters the expression of EMT-related genes in SKOV3/DDP cells

To further investigate the inhibition mechanism of GTW in migration and invasion of SKOV3/DDP cells, EMT-associated protein expressions were evaluated by western blot analysis. In SKOV3/DDP cells, DDP, GTW, and DDP+GTW notably up-regulated the expression of epithelial-like cell marker of E-cadherin ($P<0.001$, **Figure 2**), while down-regulated the expression of mesecnchymal-like cell marker of N-caderin and transcription factor Slug ($P<0.01$, **Figure 2**).

Furthermore, to test whether GTW can inhibit the epithelial ovarian cancer via ILK/GSK3β/Slug signaling pathway, protein expressions of ILK, AKT, p-AKT, GSK3β, and p-GSK3β were also evaluated. As shown in **Figure 2**, the expressions of ILK, p-AKT, and p-GSK3β were significantly down-regulated after DDP, GTW,

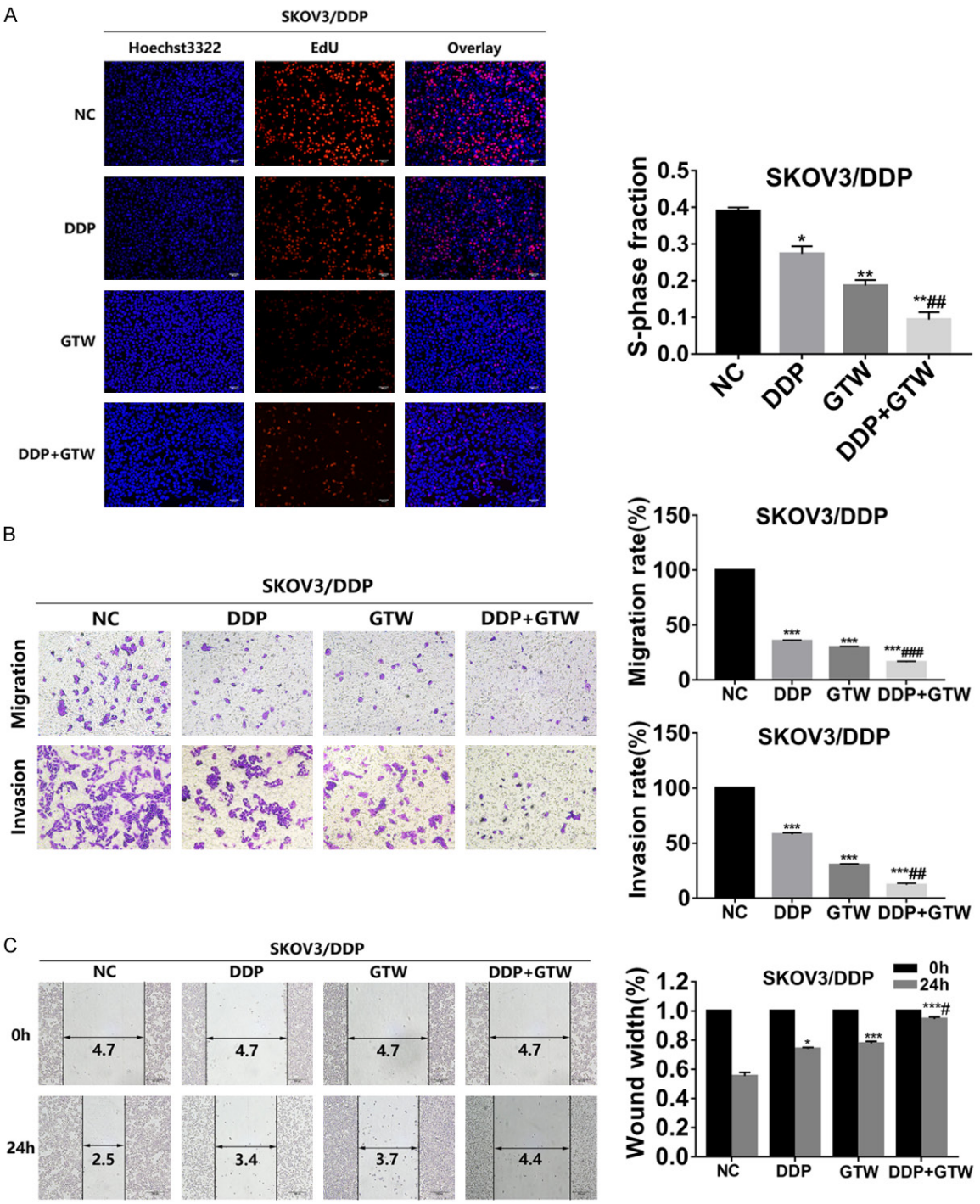


Figure 1. Effect of GTW on the proliferation, migration, and invasion of SKOV3/DDP cells. A. EdU proliferation assay was used to assess the proliferation of SKOV3/DDP cells treated with DDP, GTW, and DDP+GTW (× 100 magnification). B. A transwell assay was used to estimate the migration and invasion of SKOV3/DDP cells which were treated as described in materials and methods (× 200 magnification). C. A scratch wound healing assay was applied to evaluate the migration of SKOV3/DDP cells. The bar graphs represent the mean ± SD of three independent experiments (× 100 magnification). *P<0.05, **P<0.01, ***P<0.001 compared with control group; #P<0.05, ##P<0.01, ###P<0.001 compared with DDP group; &P<0.05, &&P<0.01, &&&P<0.001 compared with GTW group.

DDP+GTW were used (P<0.05). Interestingly, the inhibition of EMT-related genes expression in DDP+GTW group was better than that in DDP group or GTW group.

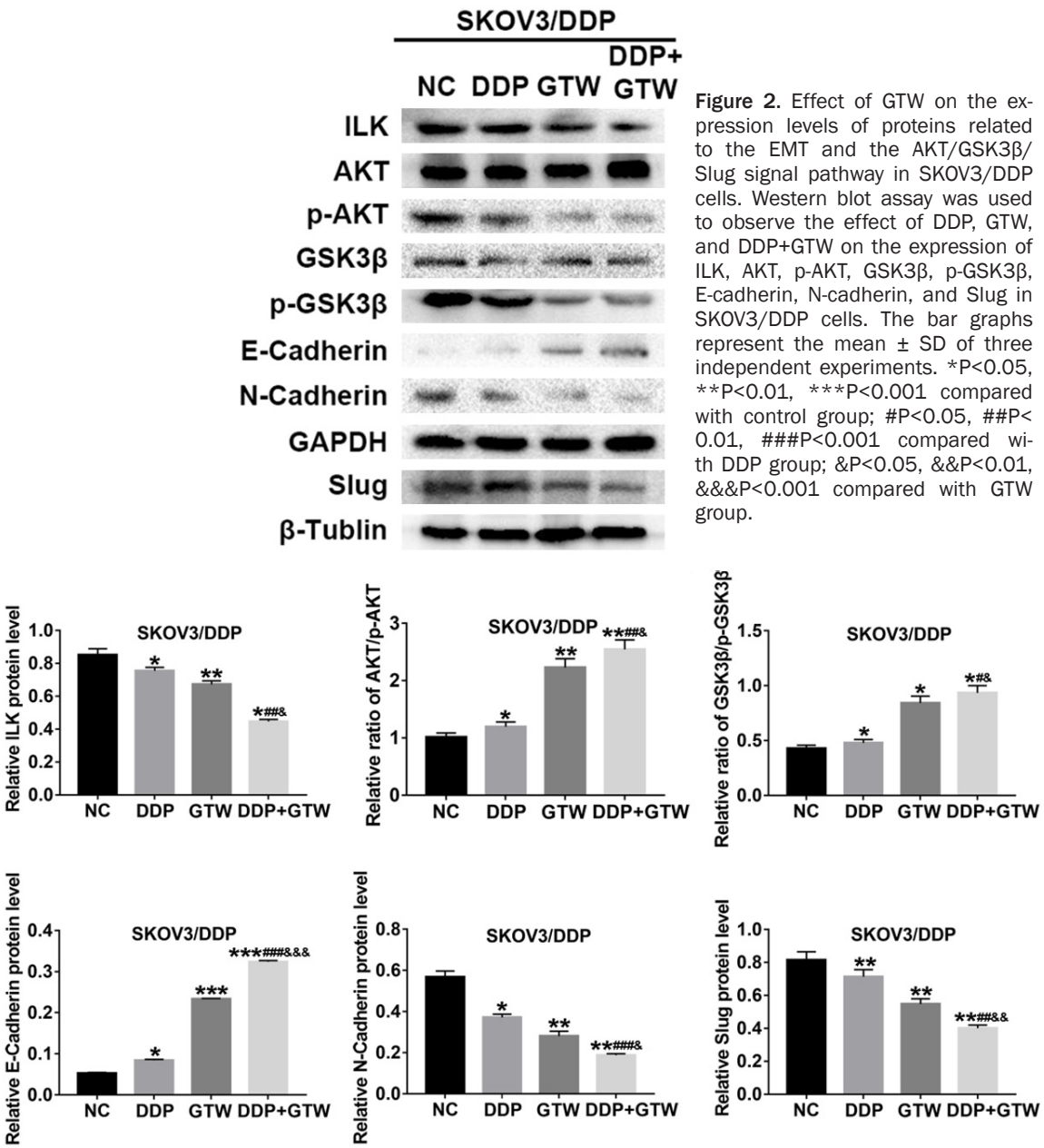


Figure 2. Effect of GTW on the expression levels of proteins related to the EMT and the AKT/GSK3β/Slug signal pathway in SKOV3/DDP cells. Western blot assay was used to observe the effect of DDP, GTW, and DDP+GTW on the expression of ILK, AKT, p-AKT, GSK3β, p-GSK3β, E-cadherin, N-cadherin, and Slug in SKOV3/DDP cells. The bar graphs represent the mean ± SD of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 compared with control group; #P<0.05, ##P<0.01, ###P<0.001 compared with DDP group; &P<0.05, &&P<0.01, &&&P<0.001 compared with GTW group.

GTW inhibits tumor growth in nude mouse models of xenograft tumor

We established nude mouse models bearing inoculated SKOV3/DDP tumors, and investigated the anti-tumor effect of GTW in vivo. The mouse treated with 4 mg/kg/d DDP (690.96±83.83 mm^3), 120 mg/kg/d GTW (773.85±137.60 mm^3), and 4 mg/kg/d DDP+120 mg/kg/d GTW (585.04±220.90 mm^3) displayed attenuated tumor growth compared with mouse treated with normal saline (1449.81±441.12 mm^3) (P<0.001, **Figure 3A**), while no statistically significant changes were observed between DDP+GTW group and DDP group. However, the overall weight of the tumors in the DDP (1.59±0.52 g), GTW group (1.55±0.39 g), and DDP+GTW group (1.19±0.39 g) was obviously lower than that in NC group (2.92±0.99 g) (P<0.05, **Figure 3B**, **3C**).

GTW controls the expression of CA125 and HE4 in serum

Research indicated that CA125 and HE4 had application value for diagnosis and clinical staging of ovarian malignant tumors [22]. Therefore, the effects of DDP, GTW, and

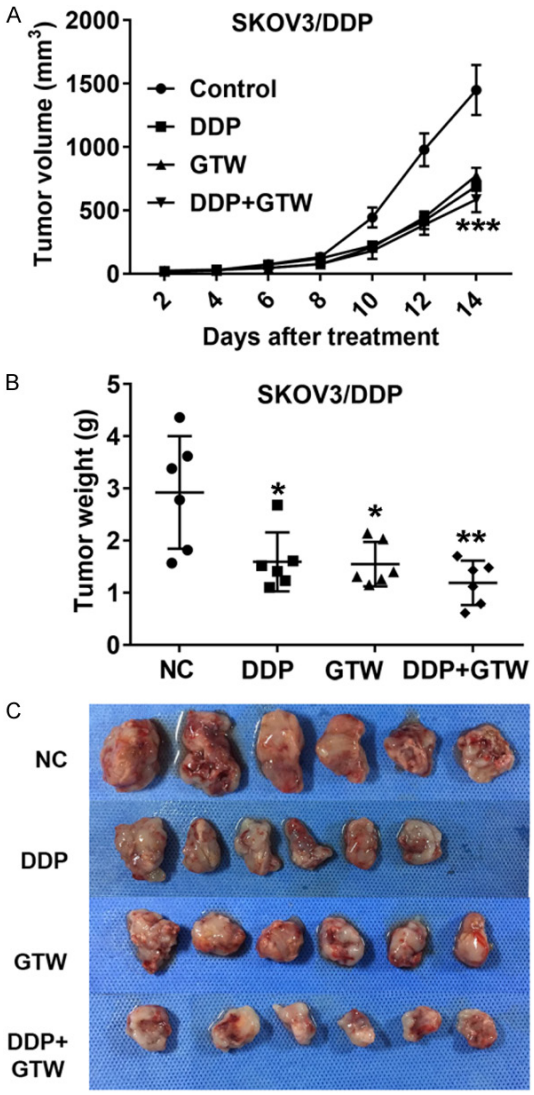


Figure 3. Effect of GTW on the tumor growth in xenografts SKOV3/DDP models in vivo. A. Tumor volumes were measured every two days after treating with DDP, GTW, and DDP+GTW. B. Tumor weights were measured after the tumor xenografts SKOV3/DDP were removed. C. The photos of the tumor tissues taken on day 16 after treating with DDP, GTW, and DDP+GTW. *P<0.05, **P<0.01, ***P<0.001 compared with control group.

DDP+GTW on CA125 and HE4 in serum were further evaluated. As shown in **Figure 4A** and **4B**, the tumor markers CA125 and HE4 were reduced in DDP, GTW, and DDP+GTW groups compared with NC group.

GTW promotes cell apoptosis in nude mouse model of xenograft tumor

The effects of GTW on the apoptosis of xenograft tumor and the expression of apoptosis-

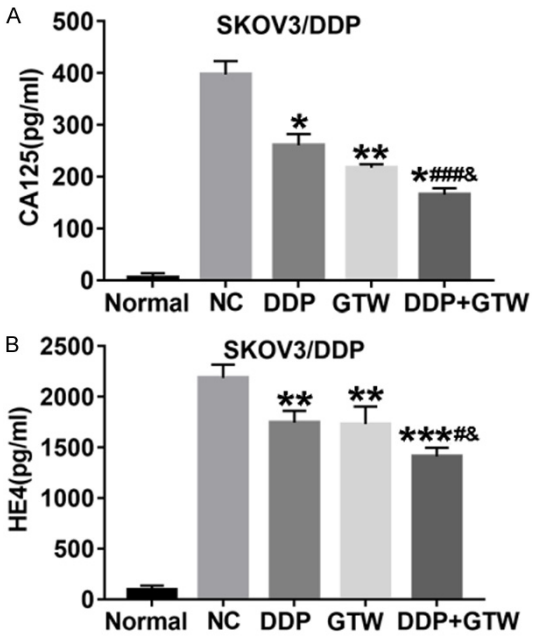


Figure 4. Effect of GTW on the serum content of tumor markers. A. ELISA was used to detect the expression of CA125 in the serum. B. ELISA was used to detect the expression of HE4 in the serum. *P<0.05, **P<0.01, ***P<0.001 compared with control group; #P<0.05, ###P<0.001 compared with DDP group; &P<0.05 compared with GTW group.

associated proteins were tested using immunohistochemical staining and TUNEL staining. The expression of inhibitor of apoptosis protein Bcl-2 was reduced when treated with DDP, GTW, and DDP+GTW compared with the NC group, while the level of pro-apoptotic proteins cleaved-caspase-3 protein, Fas protein, P53 protein, and Bax protein was increased in the DDP, GTW, and DDP+GTW groups (DDP+GTW> GTW>DDP; **Figure 5A**). A raised apoptosis rates in DDP (2.42±0.24%), GTW (2.99±0.06%), and DDP+GTW groups (8.40±0.17%) were also observed using TUNEL staining (P<0.001, **Figure 5B**). Similar to the previous results, DDP+GTW group possessed the best treatment effect than other groups.

GTW alters the expression of EMT-related genes in nude mouse model of xenograft tumor

To better understand whether the inhibition of GTW on epithelial ovarian cancer was connected with ILK/GSK3β/Slug signal pathway, we investigated the effect of GTW on EMT in nude mouse model of xenograft tumor. Our results indicated that the expression of E-cadherin was

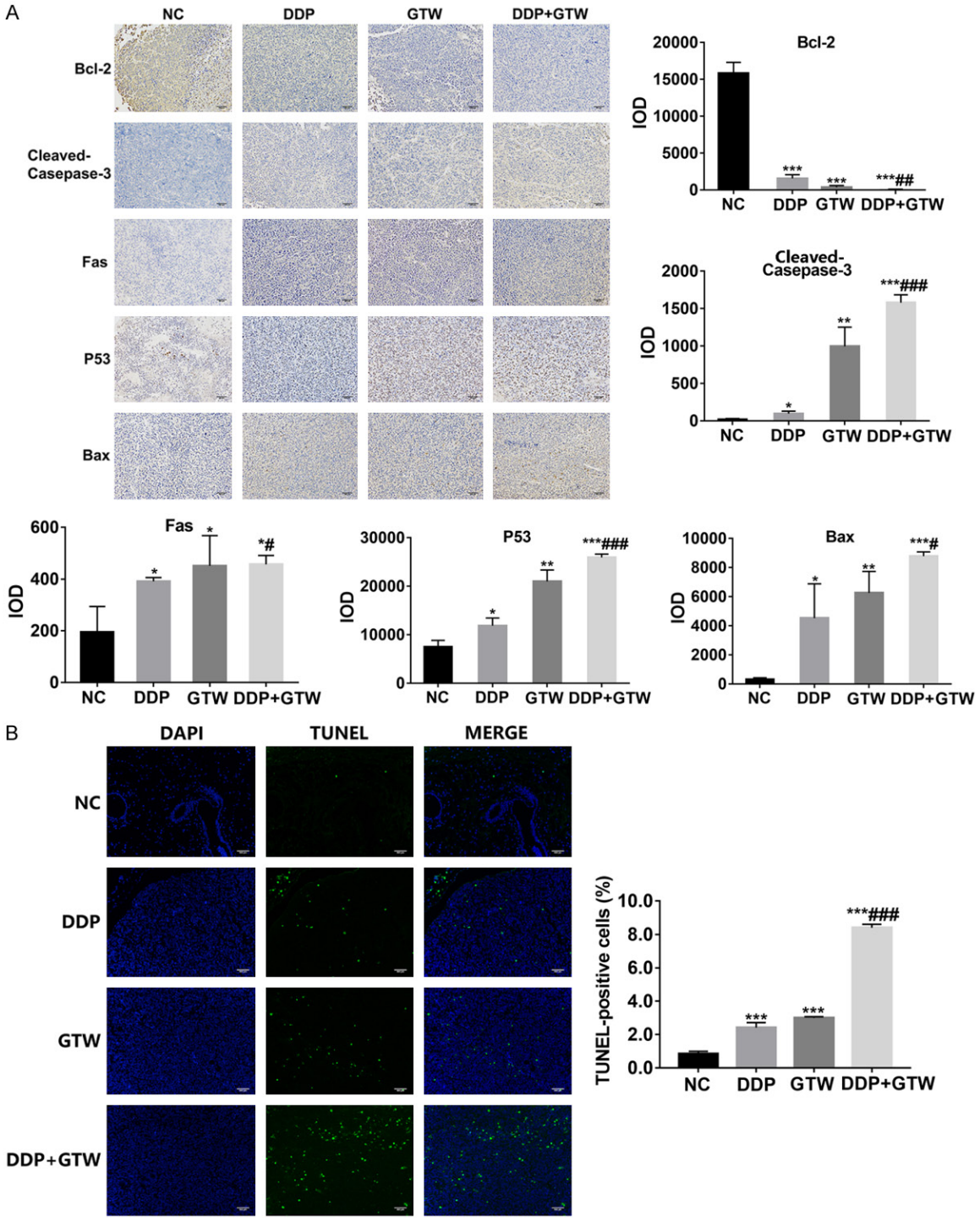


Figure 5. Effect of GTW on the cell apoptosis in the tumor tissues. A. Immunochemistry staining was applied to test the expression of Bcl-2, cleaved-casepase-3, Fas, P53, and Bax in each group (× 40 magnification). B. TUNEL assay was used to show the apoptosis cells in the tumor tissues which were treated with DDP, GTW, and DDP+GTW, respectively. The bar graphs represent the mean ± SD of three independent experiments (× 40 magnification). *P<0.05, **P<0.01, ***P<0.001 compared with control group; ###P<0.001 compared with DDP group; &P<0.05, &&P<0.01, &&&P<0.001 compared with GTW group.

up-regulated, and the expression of ILK, p-AKT, p-GSK3β, N-cadherin, and Slug was down-regulated in vivo, which was consistent with the in vitro results (**Figure 6**).

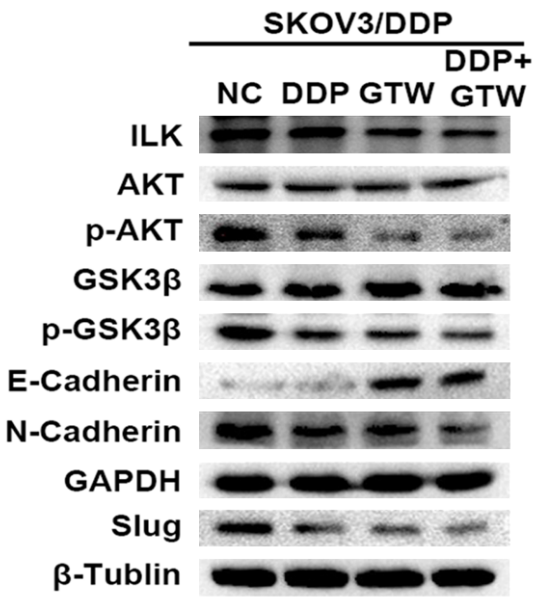
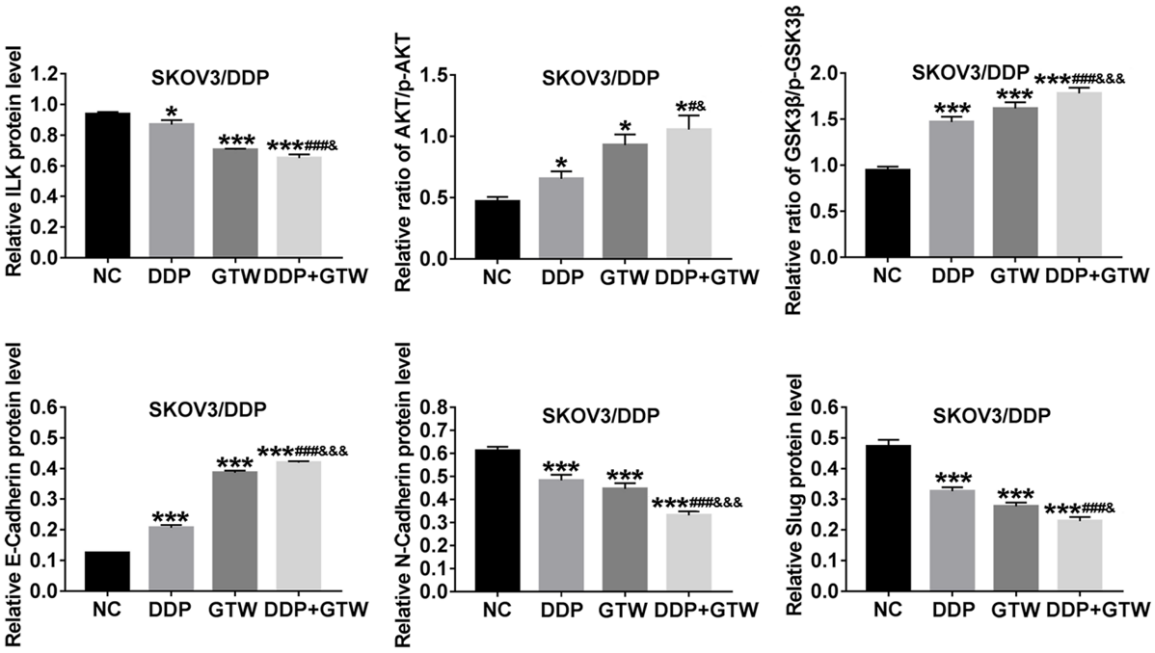


Figure 6. Effect of GTW on the expression levels of proteins related to the EMT and the AKT/GSK3β/Slug signal pathway in transplanted SKOV3/DDP neoplasia. Western blot assay was utilized to observe the effect of DDP, GTW, and DDP+GTW on the expression of ILK, AKT, p-AKT, GSK3β, p-GSK3β, E-cadherin, N-cadherin, and Slug in xenografts. The bar graphs represent the mean ± SD of three independent experiments. *P<0.05, ***P<0.001 compared with control group; #P<0.05, ###P<0.001 compared with DDP group; &P<0.05, &&&P<0.001 compared with GTW group.



Discussion

Epithelial ovarian cancer is characterized by insidious onset, the lack of typical symptom, distant organs metastasis, invasive fast-growing, high recurrence rate and post-treatment resistance [23]. In particular, the emergence of drug resistance after chemotherapy had become one of the main obstacles in the treatment of epithelial ovarian cancer [24, 25]. In the present study, we evaluated the effect of GTW on the proliferation, migration, invasion, and apoptosis of drug-resistant human epithelial ovarian cancer both in vivo and in vitro. According to the pre-experiment results, the concentrations of 800 μg/ml GTW, 10 μg/ml DDP and 10 μg/ml DDP+800 μg/ml GTW were used to evaluate the effect of these agents on proliferation, migration and invasion of drug-resistant epithelial ovarian cancer cells after 24 h of treatment using EdU proliferation assay, transwell assay, and scratch wound healing assay, and our results indicated that 10 μg/ml DDP+800 μg/ml GTW significantly inhibited the cellular proliferation, migration and invasion in vitro. Moreover, our in vivo results also con-

firmed that 120 mg/ml/kg GTW and 4 mg/ml/kg DDP+120 mg/ml/kg GTW significantly inhibited the growth of xenograft, reduced the CA125 and HE4 in serum of mice, induced apoptosis of cancer cells and reduced the expression of ILK, p-AKT, p-GSK3β, N-Cadherin, and Slug in ILK/GSK3β/Slug signal pathway.

As we know, the weakened adhesion and enhanced movement between tumor cells are the basis of local invasion and distant metastasis of tumors which are characteristic of malignant tumors [26]. It is a complex process of tumor invasion and metastasis involving multiple genes and multiple steps, and the invasion and metastasis depend on the interaction of internal environmental factors, among which EMT is one of the main factors [27]. EMT refers to the transition of epithelial cell into mesenchymal cells with stronger ability to migrate, invade and resist apoptosis under certain physiological and pathological conditions [28]. And the evolution process of EMT is characterized by lost or down-regulated epithelial markers E-cadherin, α-catenin, and β-catenin as well as up-regulated mesenchymal markers N-cadherin, vimentin, and α-smooth muscle actin [29]. Therefore, it is crucial for the treatment of ovarian cancer to block the development of EMT [30, 31]. The sound promotion effect on E-cadherin expression and inhibition effect on N-cadherin of GTW and DDP+GTW both in vivo and in vitro indicated that GTW and DDP+GTW could effectively inhibit the migration and invasion of epithelial ovarian cancer cells by reducing the EMT of cancer.

ILK is a multifunctional intracellular signal protein, which is an important molecular skeleton at the junction of cell-extracellular matrix [32]. It can regulate cell growth, proliferation, differentiation, adhesion, migration, invasion and apoptosis by phosphorylating the downstream substrate protein kinase B (PKB/AKKT) and glycogen synthase kinase 3 beta (GSK3β) [33]. Phosphorylated PKB/AKT promotes the cell development and progression, cell proliferation, and apoptosis resistance of tumors through devitalizing proapoptotic molecules and precrossing transcription factors. In addition, the decreased activity of phosphorylated GSK3β reduced degradation of β-catenin, induced the expression of target genes and promoted the occurrence of EMT [34]. Interestingly, it is believed that ILK had carcinogenic gene properties in a variety of malignant tumors including gastric cancer, prostate cancer, colon cancer, breast cancer, ovarian cancer and melanoma [35]. In addition, recent studies have shown that ILK is highly expressed in ovarian malignant tumor tissues, and the higher the tumor grade, the stronger the ILK expression is [18, 36]. Thus, increased ILK expression promotes the development of ovarian cancer [37].

Moreover, we also studied the effect of GTW and DDP+GTW on transcription factor Slug. As a kind of zinc finger protein, Slug can compete with Smad-interacting protein for E-box in the promoter binding region, inhibiting the expression of E-cadherin, promoting the expression of vimentin, and inducing the occurrence of EMT, and this protein also can promote the occurrence of EMT by forming a transcription complex called β-catenin-T-cell factor-4 [38]. Studies indicated that the expression of Slug could regulate AKT and GSK3β [39]. As GTW and DDP+GTW markedly down-regulated the expression of ILK, p-AKT, p-GSK3β and Slug both in vivo and in vitro, it indicated that the anti-cancer effect of GTW and DDP+GTW might be ILK/AKT/GSK3β dependent.

In summary, this research shows that GTW can suppress the occurrence of EMT by modulating the ILK/GSK3β/Slug signaling pathway and thereby inhibit the proliferation, migration and invasion of drug-resistant epithelial ovarian cancer cells both in vivo and in vitro. Therefore, GTW may be potentially used for the treatment of drug-resistant epithelial ovarian cancer as a natural Chinese medicine.

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Disclosure of conflict of interest

None.

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