Original Article

Natural compound Tan-I enhances the efficacy of Paclitaxel chemotherapy in ovarian cancer

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Background: Paclitaxel is a widely used clinical first line chemotherapy drug for ovarian carcinoma. Tanshinone I (Tan-I) is one of the vital fat-soluble components, which derived from Chinese herbal medicine, Salvia miltiorrhiza Bunge. Herein, we evaluated whether Tan-I could enhance the efficacy of ovarian cancer to chemotherapy of Paclitaxel.

Methods: Ovarian cancer cells A2780 and ID-8 were exposed with Tan-I (4.8 μg/mL), Paclitaxel (0.1 μg/mL), or Tan-I combination with Paclitaxel for 24 hours. The cell proliferation was analyzed by CCK8 and EdU staining. Cell apoptosis was analyzed by the TUNEL assay and flow cytometry. The protein levels were determined by western blot. Cell migration was analyzed by Transwell and wound healing. Cell senescence was analyzed by senescence-associated b-galactosidase staining. Antitumor activity was analyzed by a subcutaneous tumor xenograft model of human ovarian cancer in nude mice. The protein expression and apoptosis level of tumor tissues were analyzed by immunohistochemistry and TUNEL staining.

Results: Tan-I treatment significantly elevated the Paclitaxel-cause reduction of A2780 and ID-8 cell proliferation and cell migration. Tan-I combination with Paclitaxel promotes apoptosis of cancer cells by promoting Bax expression and Bcl-2 expression. Besides, Tan-I treatment can notably increase Paclitaxel-inducing cell senescence by promoting DNA damage and senescence-associated proteins such as p21 and p16. Furthermore, the result of the transplanted tumor model indicated that Tan-I combination with Paclitaxel could inhibit tumor growth in vivo by inhibiting cell proliferation and inducing cell apoptosis.

Conclusions: Natural compound Tan-I enhances the efficacy of ovarian cancer to Paclitaxel chemotherapy. The results will help to supply the potential clinical use of ovarian carcinoma cells.

Keywords: Tanshinone I (Tan-I); Paclitaxel; ovarian carcinoma; apoptosis; cell migration; cell senescence; tumor growth

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Introduction

Ovarian cancer is the 10th most common type of cancer among women worldwide and the 5th most common cancer in China (1,2). Despite recent advances in clinical diagnosis, 75% of patients are still diagnosed at the late stage of the disease. The 5-year survival rate ranging from 15% to 25% is lower than that for many other leading cancers (1,3,4).

Chemotherapy is one of the methods of clinical therapy for ovarian cancer and most patients will attain a remission with initial treatment. Although the majority of ovarian cancer patients will respond to initial chemotherapy, most will ultimately develop disease recurrence (5). Paclitaxel

is a first-line preventive chemotherapy drug for ovarian carcinoma, which is widely used. Paclitaxel can kill tumor cells due to promoting the polymerization of tubulin by disrupting the healthy standard microtubule dynamics required for cell division and vital interphase processes and chromosome missegregation on multipolar spindles (6). The Paclitaxel alone or combination therapy achieves a CR ranging from 50% to 81% and median PFS range of 13.6 to 19.3 months in advanced ovarian cancer (7-9). Nevertheless, several clinical studies confirmed that the occurrence of Paclitaxel resistance and toxicities contributed to the failure of ovarian carcinoma treatment (10,11).

Tanshinone is the main pharmacologically active component of Salvia miltiorrhiza Bunge, Chinese herbal medicine with many pharmacological activities, such as cardiovascular safety, anti-inflammatory, anti-hepatic fibrosis and antitumor characteristics (12-15). The reported studies demonstrated that Tanshinone markedly inhibited the growth of leukemia, colorectal cancer, lung cancer, Glioma, and breast cancer *in vitro* and *vivo* by induction of apoptosis and anti-angiogenesis activity (16-21). Our first study showed that Tanshinone I (Tan-I) is a soluble component that can inhibit the growth of ovarian cancer *in vitro* and *vivo* by promoting apoptosis and inducing autophagic cell death (3). Besides, Tan-I exhibit less toxicity in healthy cells and tissues (22).

In this study, Tan-I and Paclitaxel have been administered simultaneously *in vivo* to the ovarian cancer cell lines *in vitro* and xenograft ovarian cancer model to evaluate the potential as a combination drug therapy in this research.

We present the following article in accordance with the ARRIVE reporting checklist (available at http://dx.doi.org/10.21037/atm-20-4072).

Methods

Mice

Experiments were performed under a project license (license number KS2020038) granted by the Animal Care Committee of Sichuan Agriculture University.

Female 4- to 6-week-old BALB/c nude mice were obtained from Nanjing model animal research centre, and mouse research procedures *in vivo* were performed according to the Animal Care Committee of Sichuan agriculture university. All mice were littermates and were maintained under specific pathogen-free (SPF) conditions in the Animal Center of Sichuan agriculture university (Sichuan, China). Experimental groups were n=10 in tumor xenograft experiments and n=6 in immunohistochemistry assay. Numbers of mice used in experimental groups in the survival studies are shown in the respective figures.

Cell culture

The human A2780 and mouse ID-8 cell lines were bought from American Type Culture Collection (ATCC, USA). A2780 and ID-8 cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (10% fetal bovine serum 10% FBS and 100 U/mL penicillin and streptomycin) in a cell incubator with 5% CO_2 at 37 ℃.

CCK8 assay

Following the manufacture's instructions, the cell viability was analyzed by the CCK8 kit (Beyotime, Shanghai, China). Cells were seeded in 96-well microplates at a density of 3×10^3/well in 100 μL of the medium. The cells were treated with Tan-I (4.8 μg/mL), Paclitaxel (0.1 μg/mL), or Tan-I combined with Paclitaxel for 24 hours. Then 10 μL of CCK-8 reagent was added to each well and then incubating for 2 hours. All experiments were performed three times. Using wells without cells as blanks, the absorbance was analyzed at 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

EDU staining assay

EdU staining was used to analyze cell proliferation by the following protocol. Briefly, A2780 and ID-8 cell lines were incubated with Tan-I (4.8 μg/mL), Paclitaxel (0.1 μg/mL), or Tan-I combined with Paclitaxel for 24 hours. The cells were incubated with EdU After treatment for 24 hours (20 mM) with 5% CO_2 at 37 ℃ for 2 hours and then cultured with Hoechest33342 to visualize the nuclei for 30 minutes. The cells were subsequently fixed with 4% paraformaldehyde for 20 minutes at room temperature. Proliferation was analyzed using the percentage of EdU positive cells in ten fields for each sample.

Flow cytometry assay

A2780 and ID-8 cells (1×10^5 cells/mL) were cultured in 10% FBS high glucose DMEM complete medium for 24 hours in 6-well plates. Then the cells were co-cultured with Tan-I (4.8 μg/mL), Paclitaxel (0.1 μg/mL), or Tan-I combined

with Paclitaxel. Next, the treated cells were digested by 0.25% Trypsin-no EDTA after treatment for 24 hours and washed with cold PBS buffer. At 4 ℃ for 30 minutes in the dark, the cells were resuspended in 400 μL binding buffer and stained with 5 μL Annexin V-FITC combined with 5 μL propidium iodide (Beyotime, Shanghai, China). The stained cells were washed by binding buffer three times and then resuspended in 500 μL binding buffer. The percentages of apoptosis were recorded by flow cytometry (BD, FACSCalibur, USA). The experiment was repeated in triplicate.

TUNEL assay

The TUNEL assay in cells and tissues was performed according to the manufacturer's instructions (Vazyme Biotech Co., Ltd, China). For cells, A2780 and ID-8 cells were treated with Tan-I (4.8 μg/mL), Paclitaxel (0.1 μg/mL), or Tan-I combined with Paclitaxel for 24 hours. The cells were first incubated in a bright red labeling mix for 45 minutes at 37 ℃, followed by treatment with Click-iT reaction cocktail. The nucleus was stained with DAPI. For tissues, the sections of tumor tissue were deparaffinized in xylene and rehydrated in PBS buffer. The sections were incubated with Biotin-dUTP Labeling Mix for 1 hour at 37 ℃ in the dark and then were covered in Streptavidin-HRP for 30 minutes. The slides were visualized by the DAB substrate and looked at using a microscope (OLYMPUS, Japan). TUNEL-positive cells and the apoptotic index was calculated as a ratio of (apoptotic cell number)/(total cell number) in each field.

Transwell assay

Holding 10% FBS for 24 hours on 6-well plates A2780 and ID-8 cells (1×10^5 cells/mL) were grown in DMEM. Then the cells were treated with Tan-I (4.8 μg/mL), Paclitaxel (0.1 μg/mL), or Tan-I combined with Paclitaxel for 24 hours. These administrated cells were digested and suspended with a serum-free medium at a density of 1×10^6 cells/mL for cell migration. Cells were seeded into the upper chamber of a 24-well plate (Corning, Corning, NY, USA) with a volume of 100 μL, before which 500 μL of medium with 10% FBS was added into the lower chamber. The cells were washed with PBS three times after incubation for 24 hours, fixed 100% ice-cold methanol for 10 minutes, and stained with 0.4% crystal violet (Sigma-Aldrich) for 5 minutes. The non-migrated cells were cleared through using a wet cotton swab. For the assessment of cell migration, ten fields were randomly chosen and calculated the number of migrations under a Nikon microscope. Each experiment was repeated at least three times in triplicate.

Wound healing

A2780 and ID-8 cells (2×10^6 cells/mL) in a 6-well plate were treated with Tan-I (4.8 μg/mL), Paclitaxel (0.1 μg/mL) or Tan-I combined with Paclitaxel. After the treatment for 24 hours, a straight scratch was made in individual wells with a 200 mL pipette tip. This point was considered 0 hours. The width of the wound was photographed at 0 and 48 hours. Wound healing was measured by calculating the reduction of the width of the wound after incubation and comparing it to 0 hours, which were set at 100%.

Senescence assay

Holding 10% FBS for 24 hours at a density of 5×10^4 cells/well in 6-well plates, A2780, and ID-8 cells were grown in DMEM. Then the cells were treated with Tan-I (4.8 μg/mL), Paclitaxel (0.1 μg/mL), or Tan-I combined with Paclitaxel. The treated cells were fixed with 4% formaldehyde in PBS for 15 minutes at room temperature and incubated with SA b-gal staining solution at 37 ℃ overnight after the treatment for 24 hours. The SA b-gal positive cells were evaluated by counting 100 cells per dish. Data were expressed as the percentage of cells on each dish that was SA b-gal positive.

Tumor xenograft assay

Mouse research was performed according to the Animal Care Committee of Sichuan agriculture university. Female 4–6 weeks old BALB/c nude mice were obtained from the Nanjing model animal research center. The A2780 cells were injected bilaterally and subcutaneously into the flanks of the nude mice (100 μL, 2×10^7 cells). The mice were randomly divided into control groups (DMSO), Tan-I group (30 mg/kg), and Tan-I (30 mg/kg) combined with Paclitaxel (10 mg/kg) group. Three groups were administered by i.p. an injection every three days. Tumor weight was measured after treatment for 30 days.

Western blotting assay

The RIPA buffer (Beyotime, Shanghai, China) extracted the total protein used for western blot, and BCA Protein Assay Kit (Beyotime, Shanghai, China) quantified them. The protein bands were separated by sodium dodecyl sulfate

polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a Nitrocellulose (NC) membrane (Millipore). After blocking with 5% bovine serum albumin (BSA) for 1 hour, the NC membrane was incubated with primary antibodies for 2 hours at room temperature. After washing by PBST for three times. Next, the NC membrane was incubated with horseradish peroxidase conjugated secondary antibodies for 1 hour at room temperature. Finally, enhanced chemiluminescence was visualized the immunoblot by Bio-Imaging System. β-actin was used as an internal control, and protein expression was determined by ImageJ software.

Immunohistochemistry assay

After mice were sacrificed, the tumor tissues were isolated. The tissues were at once fixed in 4% paraformaldehyde for 24 hours and embedded in paraffin. The embedded sections were sliced into 5 μm sections. Caspase-3 and Ki67 staining were performed as previously described (22).

Statistical analysis

The data from these experiments are presented as mean ± SD. The statistical analyses were assessed using the SPSS software version19.0. The statistical significance for the comparisons among these groups of either two or three was analyzed using the Student *t*-test or one-way ANOVA, respectively. A P value of <0.05 showed a statistically significant result.

Results

Tan-I combined with Paclitaxel inhibit proliferation of ovarian cancer cell lines

CCK-8 assays and EDU staining were used to detect the viability of ovarian cancer cells (A2780 and ID-8) after treatment with alone Tan-I, Paclitaxel, or Tan-I combined with Paclitaxel for 24 hours to explore the activity of Tan-I Combination with Paclitaxel in the proliferation of ovarian cancer cells. As showed in *Figure 1A*, Tan-I and Paclitaxel significantly inhibited the growth of ovarian cancer cells than that of the control group. Compared with Tan-I and Paclitaxel, Tan-I combined with Paclitaxel further induced about 50% growth inhibition in A2780 and ID-8 ovarian cancer cells. Western blot assay indicated that the treatment of Tan-I combined with Paclitaxel markedly reduced the Ki67 protein expression in A2780 cells and ID-8 cells than that of alone Tan-I or Paclitaxel (*Figure 1B*). Furthermore, the EdU positive cells markedly inhibited by alone Tan-I or Paclitaxel treatment than that of the control group (P<0.05) (*Figure 1C,D*). Compared with alone Tan-I or Paclitaxel treatment, the EdU positive cells significantly reduced by treatment of Tan-I combined with Paclitaxel. These results suggested that Tan-I combined with Paclitaxel could significantly suppress cell proliferation in A2780 cells and ID-8 cells.

Tan-I combined with Paclitaxel promote apoptosis of cancer cell

Flow cytometry and TUNEL staining were performed in A2780 and ID-8 cells to analyze the apoptosis level of Tan-I combined with Paclitaxel. After being treated with alone Tan-I or Paclitaxel for 24 hours, the apoptotic level significantly increased in A2780 and ID-8 cells compared with the control group. Tan-I combined with Paclitaxel markedly induce apoptosis of A2780 and ID-8 cells than that of alone Tan-I or Paclitaxel treatment (P<0.05) (*Figure 2A,B*). TUNEL staining showed that the number of apoptotic cells significantly increased with treatment of Tan-I or Paclitaxel than that of the control group (P<0.05), and then Tan-I combined with Paclitaxel further promote the apoptosis of A2780 and ID-8 cells compared to the treatment of Tan-I or Paclitaxel alone (*Figure 2C,D*). Besides, Tan-I combined with Paclitaxel significantly promoted Bax expression and reduced Bcl-2 expression in A2780 and ID-8 cells (*Figure 2E,F,G*). These data suggested that combined with Paclitaxel promoted apoptosis of cancer cells by inducing apoptosis-associated protein expression and cleavage.

Tan-I combined with Paclitaxel inhibits migration of cancer cells

We performed wound-healing assay and Transwell assay to investigate whether Tan-I combined with Paclitaxel had an impact on the migration of cancer cells. The distance of cell migration significantly shorted in the Tan-I combined with Paclitaxel group than that of either alone group after 24 hours of treatment with Tan-I or Paclitaxel alone or Tan-I combined with Paclitaxel (P<0.05) (*Figure 3A,B*). The wound-healing assay showed that the number of cells passing through the membrane significantly decreased in the Tan-I combined with the Paclitaxel group compared to that in either alone group (P<0.05) (*Figure 3C,D*). These data illustrated that Tan-I could inhibit the migration of

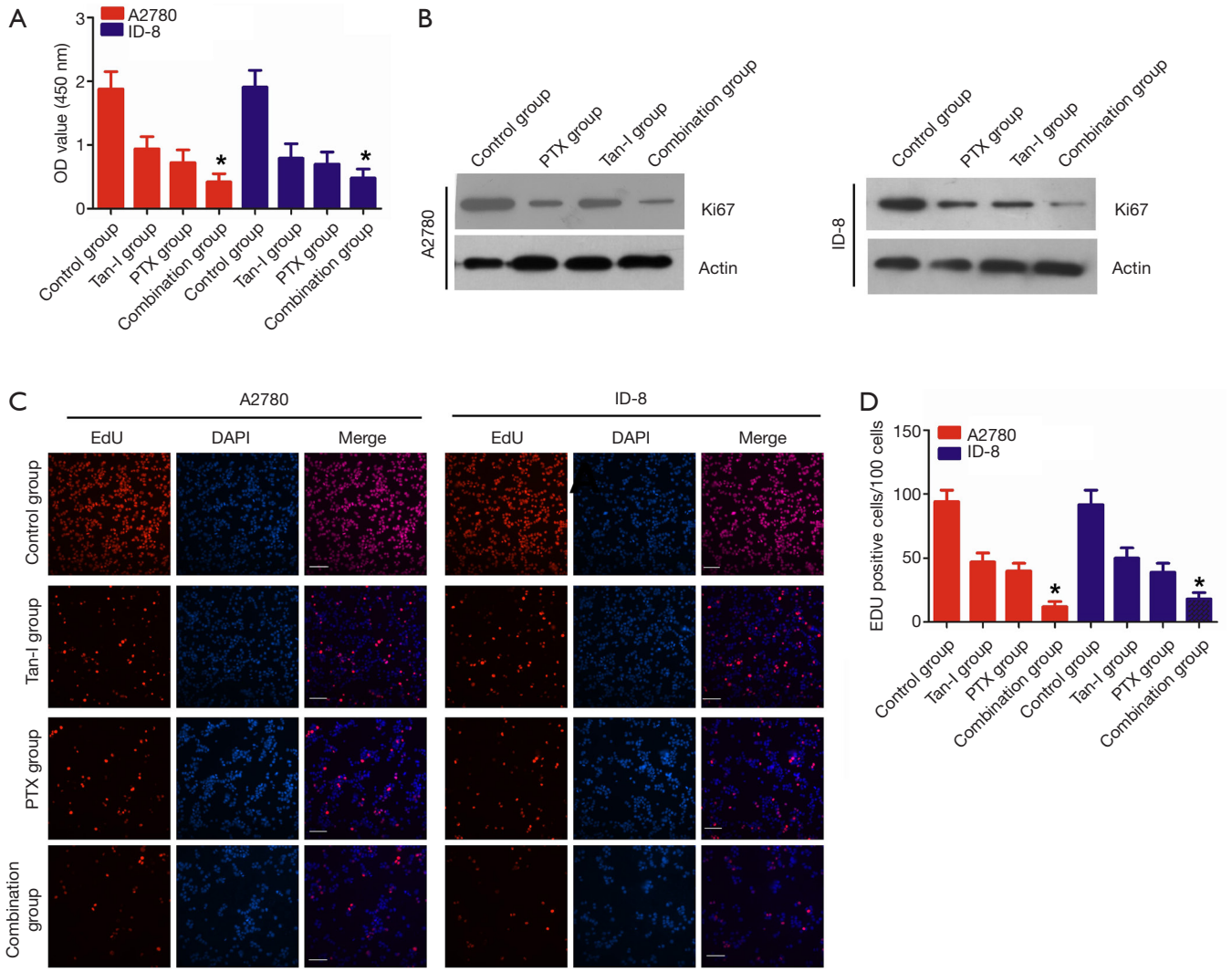

Figure 1 Tan-I combined with Paclitaxel inhibit proliferation of ovarian cancer cell lines. (A) Cells proliferation assay of A2780 and ID-8 cells by CCK-8 assay in control group, Tan-I group, PTX group, and combination group. A2780 and ID-8 cells were treated with Tan-I (4.8 μg/mL), Paclitaxel (0.1 μg/mL), or Tan-I combined with Paclitaxel for 24 hours. Data are presented as the mean ± SD of three independent experiments. *, P<0.05 versus which Tan-I group or Paclitaxel group. (B) Ki67 protein expression of A2780 and ID-8 cells by western blot in the control group, Tan-I group, PTX group, and combination group. (C) Cells proliferation assay of A2780 and ID-8 cells by EdU staining in control group, Tan-I group, PTX group, and combination group (magnification 100×). (D) Percentage of EdU positive cells of A2780 and ID-8 cells. Data are shown as mean ± SD of three independent experiments. *, P<0.05 versus which Tan-I group or Paclitaxel group.

ovarian cancer cells when combined with Paclitaxel.

Tan-I combined with Paclitaxel accelerate senescence of cancer cells

Cell senescence was evaluated by SA-β-Gal staining to evaluate the effect of Tan-I combined with Paclitaxel on the senescence of cancer cells. The results showed that Tan-I combined with Paclitaxel treatments caused evident cellular senescence than that of Tan-I or Paclitaxel alone (P<0.05) (*Figure 4A,B*). To further insights into the molecular mechanism of Tan-I combined with Paclitaxel during the senescence development, we evaluated DNA damage response (DDR) signals and senescence-associated proteins

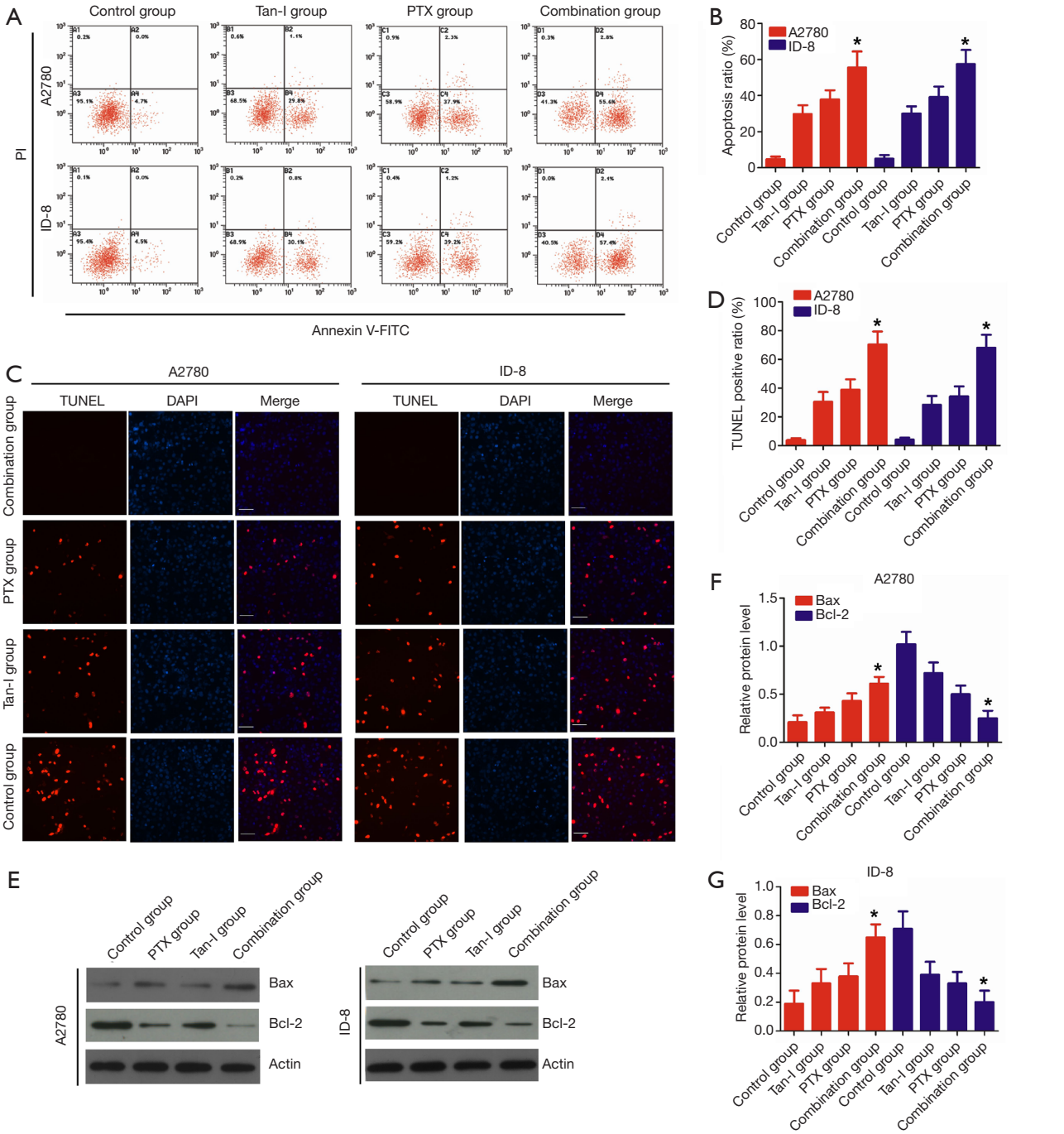

Figure 2 Tan-I combined with Paclitaxel promote apoptosis of cancer cell. (A) Apoptosis assay of A2780 and ID-8 cells by flow cytometer in the control group, Tan-I group, PTX group, and combination group. A2780 and ID-8 cells were treated with Tan-I (4.8 μg/mL), Paclitaxel (0.1 μg/mL), or Tan-I combined with Paclitaxel for 24 hours. (B) The apoptosis-positive cells of A2780 and ID-8 cells in A. Data are shown as mean ± SD. *, P<0.05 versus which Tan-I group or Paclitaxel group. (C) Apoptosis assay of A2780 and ID-8 cells by TUNEL staining in control group, Tan-I group, PTX group, and combination group (magnification 100×). (D) The apoptosis-positive cells of A2780 and ID-8 cells in (C). Data are shown as mean ± SD. *, P<0.05 versus which Tan-I group or Paclitaxel group. (E) Bcl-2 and Bax expression level in A2780 and ID-8 cells by western blot in the control group, Tan-I group, PTX group, and combination group. (F) and (G) Relative fold change in the expression of Bcl-2 and Bax in comparison with Actin, and the results were analyzed by densitometric using image J software. Data are presented as the mean ± SD of three independent experiments. *, P<0.05 versus which Tan-I group or Paclitaxel group.

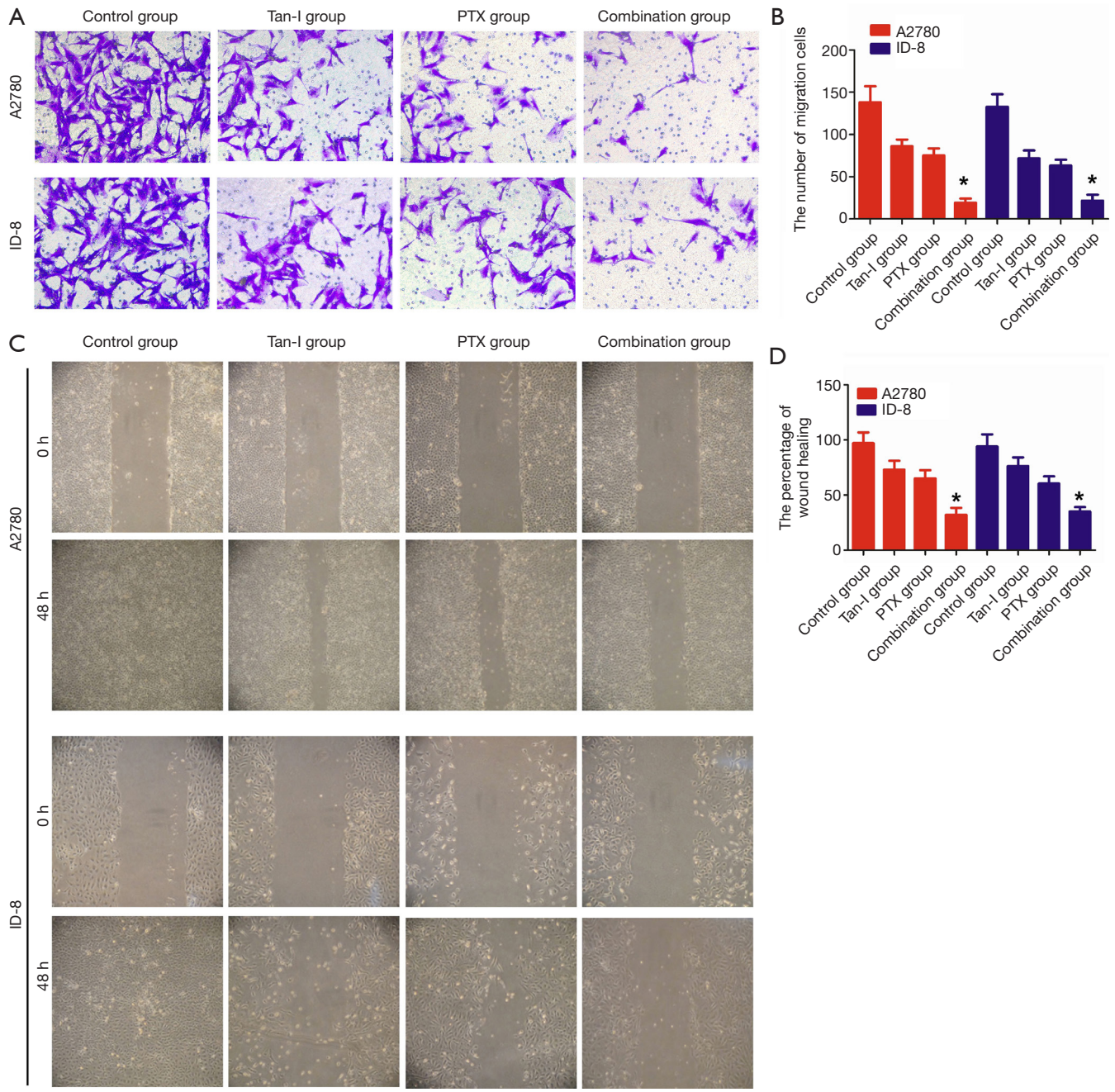

Figure 3 Tan-I combined with Paclitaxel inhibit cell migration of cancer cells. (A) Cell migration assay of A2780 and ID-8 cells by Transwell in control group, Tan-I group, PTX group, and combination group. A2780 and ID-8 cells were treated with Tan-I (4.8 μg/mL), Paclitaxel (0.1 μg/mL), or Tan-I combined with Paclitaxel for 24 hours. Cells were stained with 0.4% crystal violet. Magnification 100×. (B) Quantitative analysis in (A). Data are presented as the mean ± SD of three independent experiments. *, P<0.05 versus which Tan-I group or Paclitaxel group. (C) Migration assay of A2780 and ID-8 cells by wound healing in the control group, Tan-I group, PTX group, and combination group (magnification 100×). (D) The percentage of cell migration in (C). Data are presented as the mean ± SD of three independent experiments. *, P<0.05 versus which Tan-I group or Paclitaxel group.

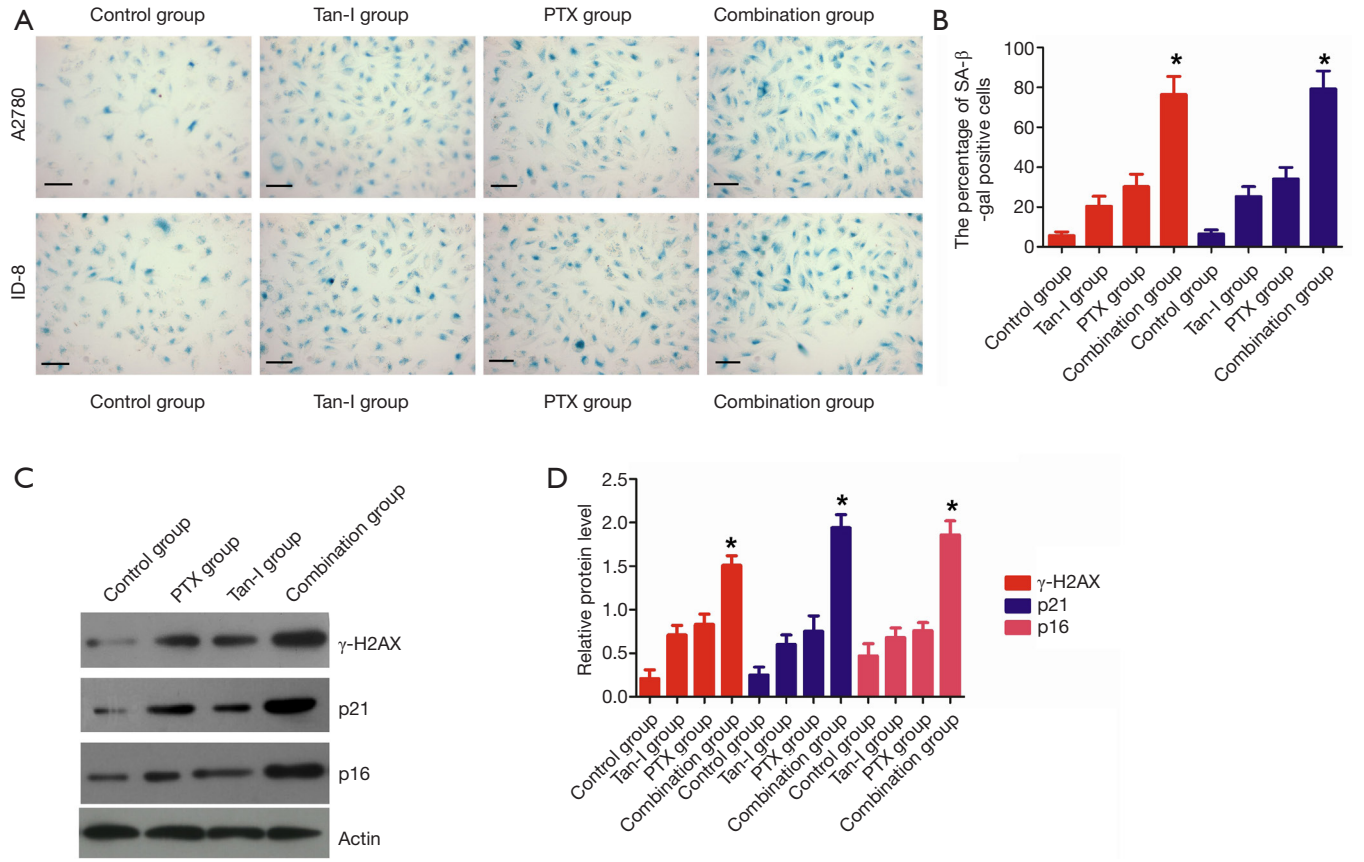

Figure 4 Tan-I combined with Paclitaxel accelerate cell senescence of cancer cells. (A) SA-b-gal activity assay of A2780 and ID-8 cells in the control group, Tan-I group, PTX group, and combination group. A2780 and ID-8 cells were treated with Tan-I (4.8 μg/mL), Paclitaxel (0.1 μg/mL), or Tan-I combined with Paclitaxel for 24 hours. Cells were stained with SA b-gal. Magnification 100×. (B) Quantification of SA-β-gal positive cells in (A). Data are presented as the mean ± SD of three independent experiments. *, P<0.05 versus which Tan-I group or Paclitaxel group. (C) The protein level of DNA damage response (DDR) signals and senescence-associated proteins by western blot in the control group, Tan-I group, PTX group, and combination group. (D) Relative expression levels in (C) were analyzed by densitometric using image J software. Data are presented as the mean ± SD of three independent experiments. *, P<0.05 versus which Tan-I group or Paclitaxel group.

by western blot. We found that Tan-I combined with Paclitaxel treatment almost simultaneously with activation of the DDR machinery (phosphorylated histone H2AX (γ-H2AX)) and senescence-associated proteins (p21 and p16) (P<0.05) (*Figure 4C,D*).

Tan-I combination with Paclitaxel inhibits tumor growth in vivo

To analysis, the antitumor effect of Tan-I combined with Paclitaxel in vivo, A2780 tumor-bearing xenograft was constructed and treated. The results showed that tumor weight in Tan-I combined with Paclitaxel group markedly decreased than that of Tan-I group and Paclitaxel group (P<0.05) (*Figure 5A,B*). Caspase-3 positive cells in Tan-I combined with Paclitaxel group significantly enhanced than that of Tan-I group and Paclitaxel group (P<0.05) (*Figure 5C*). Nevertheless, the Ki67 positive ratio in Tan-I combined with the Paclitaxel group significantly inhibited compared to the Tan-I group and Paclitaxel group (P<0.05) (*Figure 5D*). TUNEL positive cells in Tan-I combined with Paclitaxel group significantly enhanced than that of Tan-I group and Paclitaxel group (P<0.05) (*Figure 5E*). The dates showed that Tan-I combination with Paclitaxel inhibits tumor growth *in vivo*.

Discussion

At a five-year survival rate, ovarian carcinoma has become

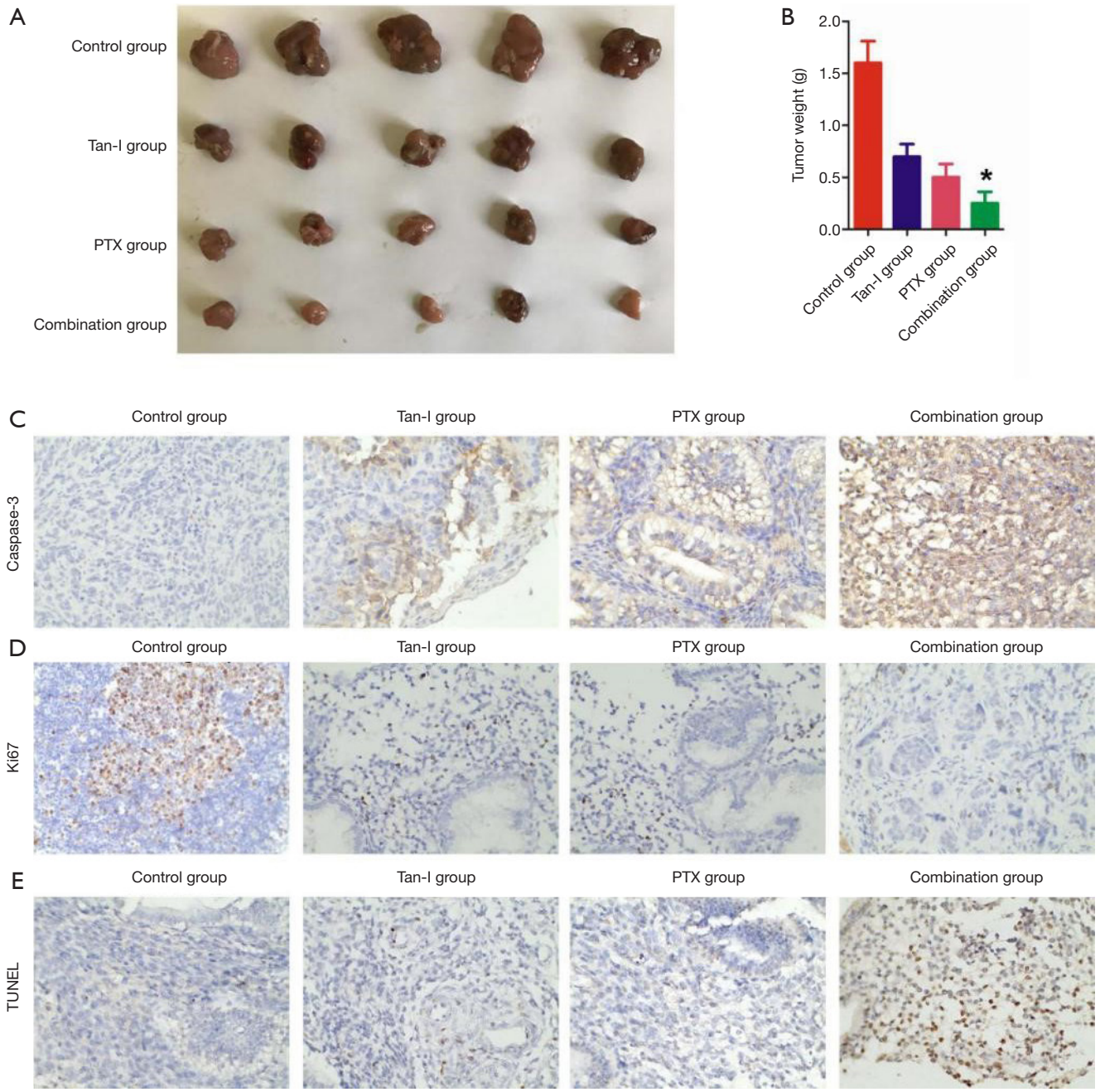

Figure 5 Tan-I combination with Paclitaxel inhibits tumor growth in vivo. (A) Tumors in the control group, Tan-I group, PTX group, and combination group were isolated and pictured after four weeks' treatment. (B) Tumors' weight was calculated in the control group, Tan-I group, PTX group, and combination group. Data are shown as mean ± SD. *, P<0.05 versus which Tan-I group or PTX group. (C) Caspase-3 protein expression in tumor tissues of the control group, Tan-I group, PTX group, and combination group was analyzed by immunohistochemistry (magnification, 200×). (D) Ki67 protein expression in tumor tissues of the control group, Tan-I group, PTX group, and combination group was analyzed by immunohistochemistry (magnification, 200×). (E) Apoptosis assay in tumor tissues of the control group, Tan-I group, PTX group, and combination group was analyzed by TUNEL staining (magnification, 200×).

the most aggressive genital system tumor in women for the resistance of chemotherapy (23,24). It is an essential value to chemotherapy that can enhance efficacy and alleviate side effects. We, in our study, analyzed the efficacy of Tan-I combination with Paclitaxel on ovarian carcinoma A2780 and ID-8 cells. Inducing cell apoptosis and promoting cell senescence, the results showed that Tan-I enhanced the efficacy of ovarian cancer to Paclitaxel chemotherapy by inhibiting cell proliferation and cell migration.

Cell apoptosis is an essential manifestation of cell death, which is implicated with the development of tumors (25). The studies had shown that Paclitaxel causes cell death by stabilization of microtubule dynamics resulting in activation of the spindle assembly checkpoint and apoptosis (26-28). The first study had shown that Tan-1 could inhibit the growth of ovarian cancer by promoting cell apoptosis (22). We postulated that the combination therapy of Tan-1 and Paclitaxel further promoted the apoptosis of tumor cells. The results manifested that Tan-1 can significantly promote cell apoptosis and inhibit cell proliferation further compared with Tan-1 or Paclitaxel alone combined with Paclitaxel. This result is consistent with our hypothesis.

Tumor cell migration is a crucial process for cancer cell dissemination and metastasis that is controlled by extracellular matrix (ECM) remodeling, and dynamic reorganization of cell adhesions with neighboring cells and with the underlying connective tissue (29,30). Paclitaxel inhibited numbers of tumor cell migration by suppressing microtubule dynamics (31,32). Our study first showed that Tan-1 could inhibit the migration of ovarian cancer cells. Then, the combination therapy of Tan-1 and Paclitaxel could block cell migration. Migration-associated proteins such as FAK, ROCK1, p-AKT, and uPA were more inhibited to determine the probable mechanisms of combining Tan-1 and Paclitaxel and treatment and Tan-1 or Paclitaxel alone.

Cellular senescence is elicited in response to endogenous or exogenous stress signals, which is an irreversible cell-cycle arrest (33,34). The senescence of cancer cells is widely recognized as a potent tumor-suppressive mechanism. The reports showed that mice with tumors capable of TIS had a much better prognosis following chemotherapy (35). Another study further showed that sunitinib treatment of OS-RC-2 RCC xenografts inhibited tumor formation by promoting SA-β-Gal activity (36). The study reported that senescence was induced in various cancer cell lines following treatment with doxorubicin, irinotecan, methotrexate, 5-fluorouracil, oxaliplatin, or Paclitaxel (37). Our study showed that Paclitaxel could significantly induce senescence of ovarian carcinoma. Furthermore, Tan-1, combined with Paclitaxel, promoted senescence of ovarian carcinoma cells compared with Tan-1 or Paclitaxel alone.

Also, the toxicity of antitumor therapy not only brings great pain to patients; however, it reduces compliance with patients. Anemia, nausea, vomiting, fatigue, diarrhea, cardiotoxicity, and leukopenia for myeloid inhibition are the main side effect of chemotherapy. Paclitaxel showed a significant side effect in healthy ovarian cells, rather than Tan-I. The treatment of Tan-I combination with Paclitaxel did not enhance cell cytotoxicity of Paclitaxel in healthy ovarian cells (*Figure S1*). In the tumor model, Paclitaxel treatment caused the reduction of body weight in bearing tumor mice; however, the treatment of Tan-I combination with Paclitaxel significantly alleviate cell cytotoxicity of Paclitaxel *in vivo* (*Figure S2*).

Base on the above mechanism, we found that Tan-I combination with Paclitaxel inhibits tumor growth in vivo. In summary, Tan-I enhances the efficacy of ovarian cancer to Paclitaxel chemotherapy by inhibiting cell proliferation, migration, and senescence. Therefore, Tan-I is a potential antitumor compound in ovarian cancer for low toxicity, and it may supply the potential clinical use to ovarian carcinoma.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at http://dx.doi.org/10.21037/atm-20-4072

Data Sharing Statement: Available at http://dx.doi.org/10.21037/atm-20-4072

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-20-4072). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all

aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Experiments were performed under a project license (license number KS2020038) granted by the Animal Care Committee of Sichuan Agriculture University. Mouse research procedures *in vivo* were performed according to the guideline of the Animal Care Committee of Sichuan Agriculture University.

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Supplementary

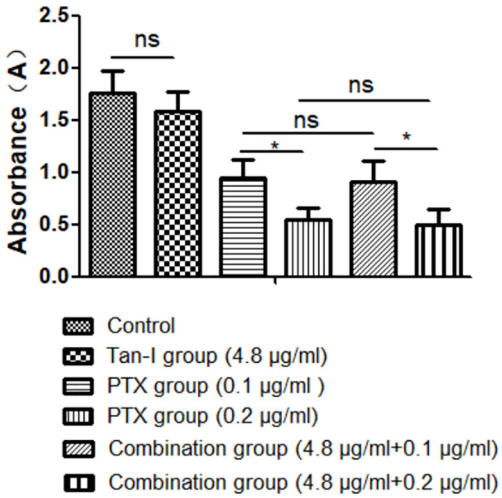

Figure S1 The cytotoxicity of HOSEpic cell in different treatment, Data are presented as the mean ± SD of three independent experiments. *, P<0.05.

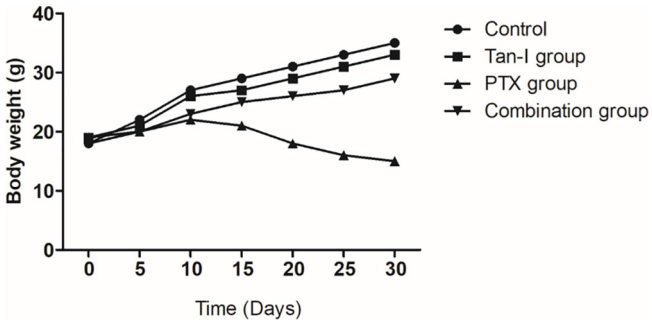

Figure S2 The body weight of bearing tumor mice in the control group, Tan-I group, PTX group, and combination group. Data are presented as the mean ± SD of three independent experiments.