Targeted reversal of multidrug resistance in ovarian cancer cells using exosome-encapsulated tetramethylpyrazine

CHENGE ZHAO[1,2*], LULU QIU[1*], DI WU[1], MING ZHANG[1], WANYING XIA[1], HUIYI LV[1,3] and LICHUN CHENG[1]

[1]Department of Pharmacy, The Second Hospital of Dalian Medical University, Dalian, Liaoning 116027;
[2]Department of Pharmacy, The Fifth Affiliated Hospital of Jinan University, Heyuan, Guangdong 517000;
[3]Dalian Kexiang Technology Development Co. Ltd, Dalian, Liaoning 116044, P.R. China

Received July 20, 2023; Accepted November 24, 2023

DOI: 10.3892/mmr.2023.13148

Abstract. The objective of the present study was to develop exosomes (EXOs) encapsulating tetramethylpyrazine (TMP) for the reversal of drug resistance in ovarian cancer therapy. Human A2780 cells were incubated with TMP for 48 h. Purified TMP-primed EXOs (EXOs-TMP) were isolated through ultracentrifugation. The developed EXOs-TMP were characterized using techniques such as transmission electron microscopy, nanoparticle tracking analysis, Fluorescence microscopy and western blotting. Subsequently, MTT, western blotting and flow cytometry assays were performed to evaluate the biological effects in drug-resistant A2780T cells. The results demonstrated that the incorporation of TMP into EXOs exhibited an anti-ovarian cancer effect and markedly enhanced the antitumor efficacy of paclitaxel (PTX). Furthermore, it was identified that the ability of EXO-TMP to reverse cell resistance was associated with the downregulation of multidrug resistance protein 1, multidrug resistant-associated protein 1 and glutathione S-transferase Pi protein expression. Flow cytometry analysis revealed that EXO-TMP induced apoptosis in drug-resistant cells and enhanced the apoptotic effect when combined with PTX. EXOs are naturally sourced, exhibit excellent biocompatibility and enable precise drug delivery to target sites, thereby reducing toxic side effects. Overall, EXO-TMP exhibited direct targeting capabilities towards A2780T cells and effectively reduced their drug resistance. EXOs-TMP provide a novel and effective drug delivery pathway for reversing drug resistance in ovarian cancer.

Correspondence to: Professor Huiyi Lv or Dr Lichun Cheng, Department of Pharmacy, The Second Hospital of Dalian Medical University, 467 Zhongshan Road, Shahekou, Dalian, Liaoning 116027, P.R. China
E-mail: dmu_huiyilv@163.com
E-mail: chengdaojing-88@163.com

*Contributed equally

Abbreviations: EXO, exosome; EXO-TMP, exosome-tetramethylpyrazine; P-gp, P-glycoprotein; PTX, paclitaxel

Key words: exosome, tetramethylpyrazine, targeting, ovarian cancer, multidrug-resistance

Introduction

Ovarian cancer is a prevalent malignancy of the female reproductive system, carrying the highest mortality rate among gynecological tumors. The overall 5-year relative survival rate for ovarian cancer is <50% (1). The current standard of care for ovarian cancer involves a multimodal approach consisting of cytoreductive surgery and chemotherapy. The first-line chemotherapy regimen for advanced ovarian cancer remains the combination of paclitaxel (PTX) and cisplatin (2). Of patients who initially respond well to anticancer treatment experience, ~75% relapse within 2 years as they acquire resistance to existing chemotherapy drugs (3). Drug sensitivity in cells can be influenced by various factors and pathways, including efflux transporters, dysregulated apoptosis, autophagy, cancer stem cells, epigenetics and the unfolded protein response signaling network (4,5). Research has also revealed that the utilization of poly-ADP-ribose polymerase inhibitors markedly improves the prognosis of patients (6). Targeting a single region or pathway alone is insufficient to reverse drug resistance. Therefore, it is crucial to identify strategies that can effectively reduce drug resistance in tumor cells (7,8).

An increasing number of studies have demonstrated the inhibitory effects of Chinese medicine, including Chinese patent medicine and single Chinese herbs, on tumor metastasis. These studies have also investigated the molecular mechanisms underlying the anti-metastatic effects of Chinese medicine (9). Combining curcumin with platinum chemotherapy has been demonstrated to improve the survival rate of patients with non-small cell lung cancer (10). Ginsenoside has been found to attenuate breast tumor growth by inhibiting angiogenesis (11). Combination therapy has the potential to overcome drug resistance and reduce adverse reactions, ultimately enhancing treatment efficacy (12). Tetramethylpyrazine (TMP), one of the main bioactive components of ligustilide, exhibits inhibitory effects on tumor cell growth through various mechanisms (13-15). It has been demonstrated that TMP can reverse multidrug resistance in BEl-7402/ADM and Pumc-91/ADM cells towards Adriamycin (16,17). Danshensu is major bioactive ingredients from the Chinese herbs Salvia

miltiorrhiza Bge. Danshensu-Tetramethylpyrazine Conjugate DT-010 has shown efficacy in overcoming doxorubicin resistance in human breast cancer cells (18). In lung cancer cells, TMP induces S-phase arrest and inhibits pathological changes (19). However, limitations such as suboptimal targeting and alterations in drug elimination pathways, including liver and kidney metabolism, have been observed, affecting the effectiveness of TMP (20).

Exosomes (EXOs) are extracellular vesicles characterized by a diameter range of 40-160 nm (average, ~100 nm) and blast-like features (21). These vesicles have the ability to enter cells, release cargo and mediate various physiological and pathological processes. One of the notable advantages of EXOs is their 'natural' properties, which result in minimal, or even no, long-term accumulation in any organ or tissue, thereby minimizing potential toxic effects on the whole body (22). Compared with synthetic drug delivery systems such as liposomes, micelles, dendrimers and nanoparticles, EXOs, as membrane-derived vesicles with diverse origins, exhibit higher biocompatibility and targeting capabilities (23,24). Emerging studies have highlighted the promising potential of tumor cell-derived EXOs as drug carriers for cancer treatment. For instance, methotrexate-loaded extracellular vesicles derived from tumor cells have been used to alleviate biliary obstruction in patients with extrahepatic cholangiocarcinoma, while doxorubicin-loaded EXOs derived from liver cancer cells have demonstrated depletion of cancer stem cells in subcutaneous, orthotopic and metastatic tumor models (25-29). EXOs carrying LOC85009 regulate autophagy related 5-induced autophagy via the ubiquitin specific peptidase 5/upstream transcription factor 1 axis to suppress docetaxel resistance (30). In our previous study, a drug delivery system using folate-chitosan nanoparticles loaded with TMP was developed and its effective reversal of adriamycin resistance in human breast cancer cells was observed (31). Building upon these findings, the present study aimed to harness the abundance and practicality of tumor cell-derived EXOs to create a more efficient targeted delivery platform.

In the present study, a novel TMP formulation based on EXOs, termed EXOs-TMP, was successfully developed. The present findings demonstrated that EXOs-TMP effectively reversed the multidrug resistance of A2780T cells to PTX *in vitro*, as illustrated in Fig. 1. The incorporation of TMP into EXOs exhibited potent antitumor activity by suppressing the growth of tumor cells and enhancing the efficacy of PTX. Mechanistically, EXOs-TMP achieved this by downregulating the expression of drug resistance proteins and isoenzymes, while inducing cellular apoptosis.

Materials and methods

Cell culture. A2780 and A2780T human ovarian cancer cell lines and the A549 lung cancer cell line were obtained from Type Culture Collection of Shanghai Meixuan Biotechnology Co., Ltd (cat. nos. MXC020, MXC021 and MXC026). All cells were cultured in RPMI 1640 medium (Gibco, C11875500BT) at 37˚C in a 5% CO_2 humidified incubator. All media contained 10% FBS(AusGeneX, LV-FBSCN500S), 100 U/ml penicillin and 100 μg/ml streptomycin (Beyotime, C0222). A2780T cells were cultured in a drug-free medium for 7 days to avoid the interference of drug toxicity on the experimental results. Logarithmic growth phase cells were taken for the experiments.

EXO purification. EXOs were purified using the differential ultracentrifugation method. First, FBS used for cell incubation was centrifuged at 160,000 x g for 6 h at 4˚C to wipe out the existing EXOs. The precipitate was discarded. Subsequently, the supernatant was filtered and sterilized with a 0.22-μm syringe filter in the ultra-clean workbench, and frozen and stored at -20˚C for later use. A2780, A2780T or A549 cells were incubated in EXO-free RPMI 1640 for 48 h. The cell culture medium was collected and sequentially centrifuged at 300 x g for 10 min, 2,000 x g for 10 min and 100,00 x g for 30 min at 4˚C to remove cells and residual cell debris. EXOs were pelleted and washed with PBS, and recovered by centrifugation at 120,000 x g for 2 h at 4˚C.

TMP-primed EXO collection and characterization. The toxic effect of TMP on A2780 cells was determined in a 48-h MTT assay (cytotoxicity test), and the cell viability of TMP was not significantly decreased at all concentrations, ranging between 0-200 μg/ml, and >85% of cells survived. Based on these results, A2780 cells were treated with TMP (50 μg/ml) for 48 h at 37˚C. Culture media were collected and the aforementioned method was used to concentrate EXO-TMP into a pellet. Morphological characteristics of EXO and EXO-TMP were observed after negative staining using a transmission electron microscope (JEM-1200EX; JEOL, Ltd.). A single-drop suspension (500 μg/ml) of the sample was applied onto a carbon-coated, 300 mesh copper grid and left to rest for 5 min or until it air-dried at ambient temperature. Subsequently, the sample was stained using a 1 M solution of phosphotungstic acetate for 5 min, after which any excess staining solution was carefully removed using filter paper. Then, the samples were placed in a transmission electron microscope for observation and photography. Nanoparticle tracking analysis (NanoSight NS300; Malvern Instruments, Inc.) was used to measure the concentration and size distribution of EXOs and EXOs-TMP. The amount of TMP loaded into EXO was measured by dissolving with methanol in ultrasound for 30 min to release TMP, and the content of TMP in EXOs was determined by high-performance liquid chromatography (HPLC). The chromatographic conditions were as follows: The chromatographic column was a C18 column (Dalian Yilite Analytical Instrument Co., Ltd.); mobile phase, methanol-water (60:40); flow, 0.8 $mg \cdot min^{-1}$; ultraviolet detection wavelength, 280 nm; room temperature; and sample volume, 10 μl. The analysis time was 9 min and retention time of TMP was 5.69 min. Exosomal proteins were analyzed using the BCA method and whole cells, the purified EXOs and EXOs-TMP were lysed in RIPA lysis buffer and then subjected to western blot analysis. The primary antibodies used included anti-CD63 (ab216130; Abcam), anti-tumor susceptibility 101 (TSG101; ab125011; Abcam) and anti-calnexin (ab22595; Abcam). All primary antibodies were diluted to 1:2,000.

Cancer EXOs homing to the mother cell line in vitro. A2780T or A549 cells were seeded into 24-well plates at a density of $1x10^5$ cells/ml. After 24 h of culture at 37˚C, the medium

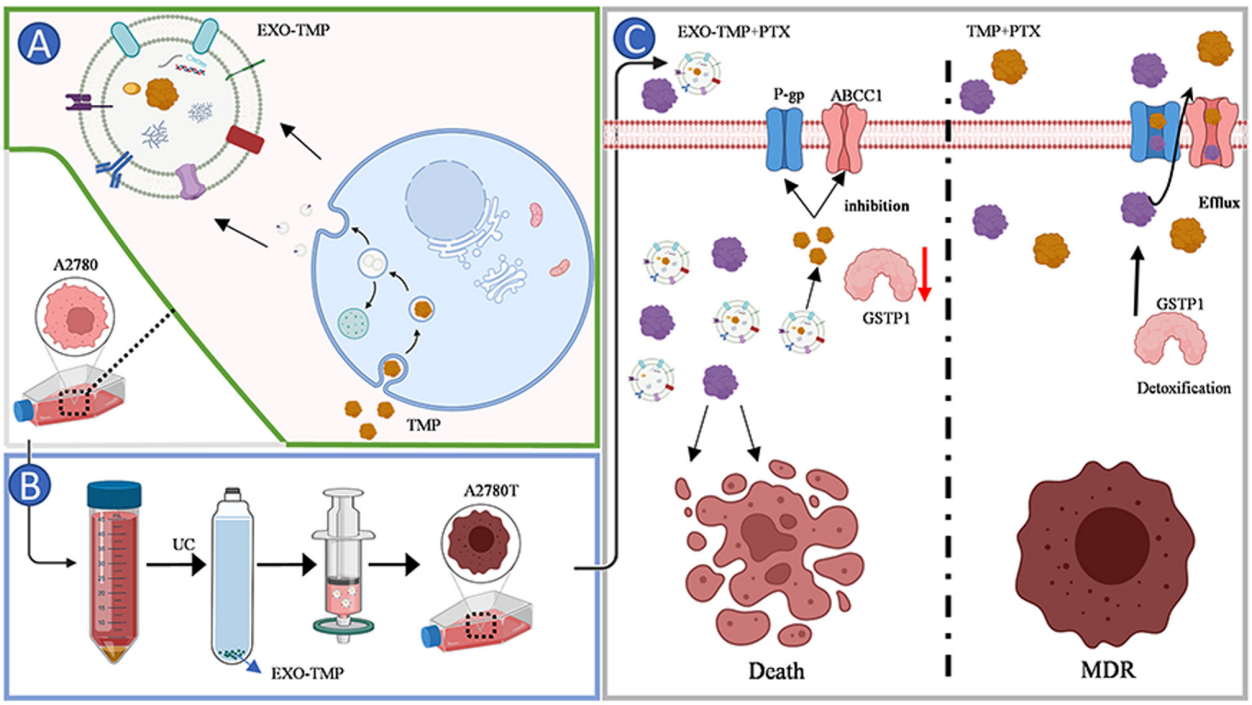

Figure 1. Schematic illustration of EXO-TMP as drug carriers for reversed tumor drug resistance. Schematic illustration of the preparation of EXO-TMP. (A) TMP was internalized by the cancer cells after incubation, then located in MVBs. After MVBs fused with the cell membrane, EXOs-TMP were exocytosed into the extracellular space. (B) Schematics showing EXO-TMP acquisition. (C) Schematics showing how EXO-TMP efficiently reversed tumor drug resistance. EXO-TMP bypassing the transporter P-gp and ABCC1 to allow more TMP to enter the cell, and then releasing TMP in the cell to close the transporter channel and reduce GSTP1 to reverse the drug resistance of the cell, allowing more PTX to remain in the cell and improving the effectiveness of PTX. ABCC1, multidrug resistant-associated protein 1; EXO, exosome; TMP, tetramethylpyrazine; MVB, multivesicular body; P-gp, P-glycoprotein; ABC, ATP-binding cassette; GSTP1, glutathione S-transferase π1; PTX, paclitaxel.

was replaced with 1640 medium containing 100 μg/ml Dil (C1991S-; Beyotime Institute of Biotechnology)-labeled A2780 EXOs or A549 EXOs. Cells were cultured for an additional 12 h, washed three times with PBS, fixed with 4% paraformaldehyde for 10 min at 37˚C and stained with Antifade Mounting Medium with DAPI (P0131; Beyotime Institute of Biotechnology). A fluorescence microscope (Leica DM6B THUNDER; Leica Microsystems GmbH) was used to capture images. LAS AF Lite (Leica Microsystems GmbH, version 3.3.0-10134) was used for fluorometric measurements.

EXOs-TMP reduce the expression of resistant proteins in PTX A2780T cells. The cytotoxic effect of EXOs-TMP on A2780T cells was evaluated using an MTT assay at 37˚C. The cells were inoculated at $1x10^5$ cells per well on a 96-well dish and adhered to the wall overnight. Subsequently, the cells were treated with different concentrations of TMP, PTX and EXOs-TMP in the culture medium for 48 h. Afterwards, 50 ml MTT was added and the resulting formazan crystals were dissolved in 150 μl DMSO. The absorbance was measured at 570 nm per well with a 96-well plate reader and cell viability was expressed as the percentage of untreated controls.

Western blot analysis. The cells were seeded in a 6-well plate with $2.5x10^5$ cells/well and allowed to adhere overnight. Subsequently, 8 μg/ml TMP, 0.6 μg/ml PTX, 8 μg/ml E-TMP (TMP 8 μg/ml), PTX + TMP, PTX + E-TMP and EXO + TMP + PTX was added to the culture medium to treat the cells for 48 h. The medium in the 6-well plate was discarded after 48 h. Pre-cooled PBS was added to rinse the cells twice. The prepared protein lysis solution (RIPA:PMSF, 100:1) was added to lyse the cells for 30 min (shaking the 6-well plate every 10 min). The cells were quickly scraped off with a cell scraper to collect the cells and centrifuged using a high-speed refrigerated centrifuge at 4˚C at 12,000 g/min for 15 min. The supernatant was aspirated and the BCA protein assay kit was used to determine the total protein concentration. Equal quantities of protein samples (20 μg/lane) were added to each lane and separated on 10% SDS-PAGE gels. Subsequently, the proteins were transferred onto 0.45 μm PVDF membranes and blocked using 5% non-fat milk for 60 min at room temperature. The PVDF membranes were then incubated overnight at 4˚C with primary antibodies. The lysate was subjected to western blotting and incubated with ATP-binding cassette sub-family C member 1 (ABCC1; diluted 1:1,000; WL01027; Wanleibio Co., Ltd.), multidrug resistance protein 1 (P-gp; diluted 1:10,000; ab170904; Abcam), glutathione S-transferase π (GSTP1; diluted 1:5,000; 66715-1-lg; Proteintech Group, Inc.) and GAPDH (diluted 1:5,000; AB8245; Abcam) antibodies. Following washing with TBS buffer containing 0.1% Tween-20 (Beyotime Institute of Biotechnology; cat. no. T1082), the PVDF membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (Thermo, XD345904, WE324878) at 37˚C for 60 min. The Odyssey CLX (LI-COR) was used to capture images of the protein bands. Image Studio software (LI-COR,4.0.21) was used for the optical density measurement of band intensity.

Apoptosis induction by EXO-TMP in ovarian cancer cells. A2780T cells were cultured in 6-well dishes ($4x10^5$ cells/well) overnight and then treated with 8 μg/ml TMP, 0.6 μg/ml PTX, PTX + TMP mixture, 8 μg/ml EXOs-TMP, PTX + E-TMP mixture or PTX + EXO + TMP mixture for 48 h. Next, the cells were collected by trypsinization, rinsed, resuspended in binding buffer and double stained using an Annexin V-FITC/PI kit (KeyGEN BioTECH, KGA108). Cell apoptosis was measured by flow cytometry using a BD FACSCalibur (BD Biosciences) flow cytometer according to the manufacturer's instructions. A total of $1x10^4$ gated events were recorded per sample. Data were analyzed using BD FACSDiva 8.0.1 software (BD Biosciences).

Statistical analysis. The data are expressed as mean ± standard error of the mean. Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software; Dotmatics). To compare differences between two groups, a two-way independent-sample t-test was employed. For comparisons involving three or more groups, we conducted one-way ANOVA) followed by Tukey's post-hoc test to assess significance. $P<0.05$ was considered to indicate a statistically significant difference.

Results

EXOs of tumor cells specifically home to their parental cells. To test whether tumor cell EXOs return to their parent cells *in vitro*, EXOs produced by A2780 and drug-resistant A2780T cells were selected as experimental subjects. A2780T cells were cocultured with Dil-labeled A2780 EXOs or Dil-labeled A2780T EXOs for 12 h and fluorescence microscopy was used to quantify EXO uptake in A2780T cells. No significant difference was observed in the uptake of the two EXOs in A2780T cells (Fig. 2A and B). Subsequently, Dil-labeled A2780 EXOs or Dil-labeled A549 EXOs were selected for co-culture with A2780T cells for 12 h, and fluorescence microscopy was used to quantify EXO uptake in A2780T cells. A2780T cells took ≥4-fold more A2780-derived EXOs compared with A459-derived EXOs (Fig. 2C and D). To ensure that this finding was not an A2780T-specific phenomenon, it was examined whether EXOs derived from A549 cells would also exhibit parental cell tropism. A549 cells were co-cultured with fluorescently labeled A549 EXOs and A2780 EXOs for 12 h. A549 cells were ~3 times more efficient in taking up A549 EXOs than A2780 EXOs (Fig. 2E and F). Thus, the present data suggested that EXOs from tumor cells preferentially returned to parental cells.

EXOs-TMP isolation and characterization. After confirming the homing ability of tumor cell-derived EXOs, A2780 cell-derived EXOs were selected as the drug delivery vehicle of TMP in order to inhibit ovarian cancer drug resistance. The EXOs were encapsulated with TMP endocytotically.

The typical structures of EXOs and EXOs-TMP were observed by transmission electron microscopy. EXOs and EXOs-TMP had normal morphological features, being cup-round in shape and measuring ~100 nm in diameter (Fig. 3A). Nanoparticle tracking analysis showed that the average size of EXOs and EXOs-TMP was 97.0±2.8 and 112.6±3.8 nm, respectively (Fig. 3B). These features indicated that the properties of the EXOs were not affected by the loaded drugs. The loading rate of the preparation was measured using HPLC and a loading efficiency of 20% was obtained (Fig. 3F). When the preparation was stored at -80˚C for 6 and 12 months, stability was demonstrated by nanoparticle tracking analysis showing that the average size remained <160 nm (Fig. 3C). EXOs-TMP were isolated from the collected cell culture supernatants using differential ultracentrifugation. EXO marker proteins were detected by western blotting and the results demonstrated that TSG101 and CD63 were positively expressed in cells and EXOs, while calnexin was expressed in cells but not detected in EXOs (Fig. 3D), confirming that EXOs-TMP were indeed isolated from cells. TMP and A2780 cells were co-cultured in 96-well plates for 48 h, and the toxicity of TMP in A2780 cells was detected by an MTT assay. The experimental results revealed that when the drug concentration gradient of TMP was 0-200 μg/ml, the survival rate of A2780 cells was >90%, which demonstrated that TMP had very low or no toxicity (Fig. 3E).

EXOs-TMP reduce cell resistance to PTX. After the EXOs-TMP drug delivery system was successfully constructed, in order to test whether TMP would affect the absorption of EXOs, EXOs and EXOs-TMP were incubated with A2780T cells for 12 h. Fluorescence microscopy demonstrated that there was no significant difference in the fluorescence intensity of EXOs and EXOs-TMP absorption by A2780T cells. It was demonstrated that loading TMP into EXOs did not affect cellular uptake (Fig. 4A). The IC_{50} of PTX in A2780T cells was 11 μg/ml, and that in A2780 cells was 0.65 μg/ml, and the drug resistance fold was ~17 times (Fig. 4B). The expression of P-gb was positive in A2780T cells but not in A2780 cells (Fig. 4C). The drug resistance stability of A2780T cells was confirmed. The cells were incubated with EXOs, free TMP and EXOs-TMP at drug concentrations of 3 and 8 μg/ml for 48 h and the cytotoxicity of EXOs-TMP was examined using an MTT assay. TMP had no effect on cell viability at the concentrations of 3 and 8 μg/ml. EXOs-TMP reduced the viability of A2780T cells by ~75% when the concentration of TMP was 8 μg/ml. Neither EXOs nor TMP alone was cytotoxic, while TMP was cytotoxic when loaded into EXOs, suggesting that the EXOs confer a novel therapeutic effect of TMP rather than just reducing cell resistance (Fig. 4D). The cytotoxicity of EXOs-TMP when combined with PTX in ovarian cancer resistant cells was next verified. Cells were incubated with PTX and TMP, EXO-TMP treatment for 48 h, and viability was examined using an MTT assay. The effect of TMP on drug resistance was very weak when a low concentration of TMP was combined with PTX. EXOs-TMP (E-TMP8) containing 8 μg/ml TMP in combination with PTX markedly inhibited cell proliferation compared with free PTX and PTX + TMP mixtures (Fig. 4E). It was hypothesized that the reversal effect of free TMP was not obvious due to the high drug resistance of A2780T cells. Next, the mechanism of its cytotoxicity will be investigated.

EXOs-TMP reduce the expression of resistant proteins in PTX A2780T cells. There is substantial evidence that the expression of ATP-binding cassette (ABC) transporters, especially P-gp and ABCC1, can confer resistance to cytotoxic and targeted chemotherapy (32). A2780T cells were incubated with PTX,

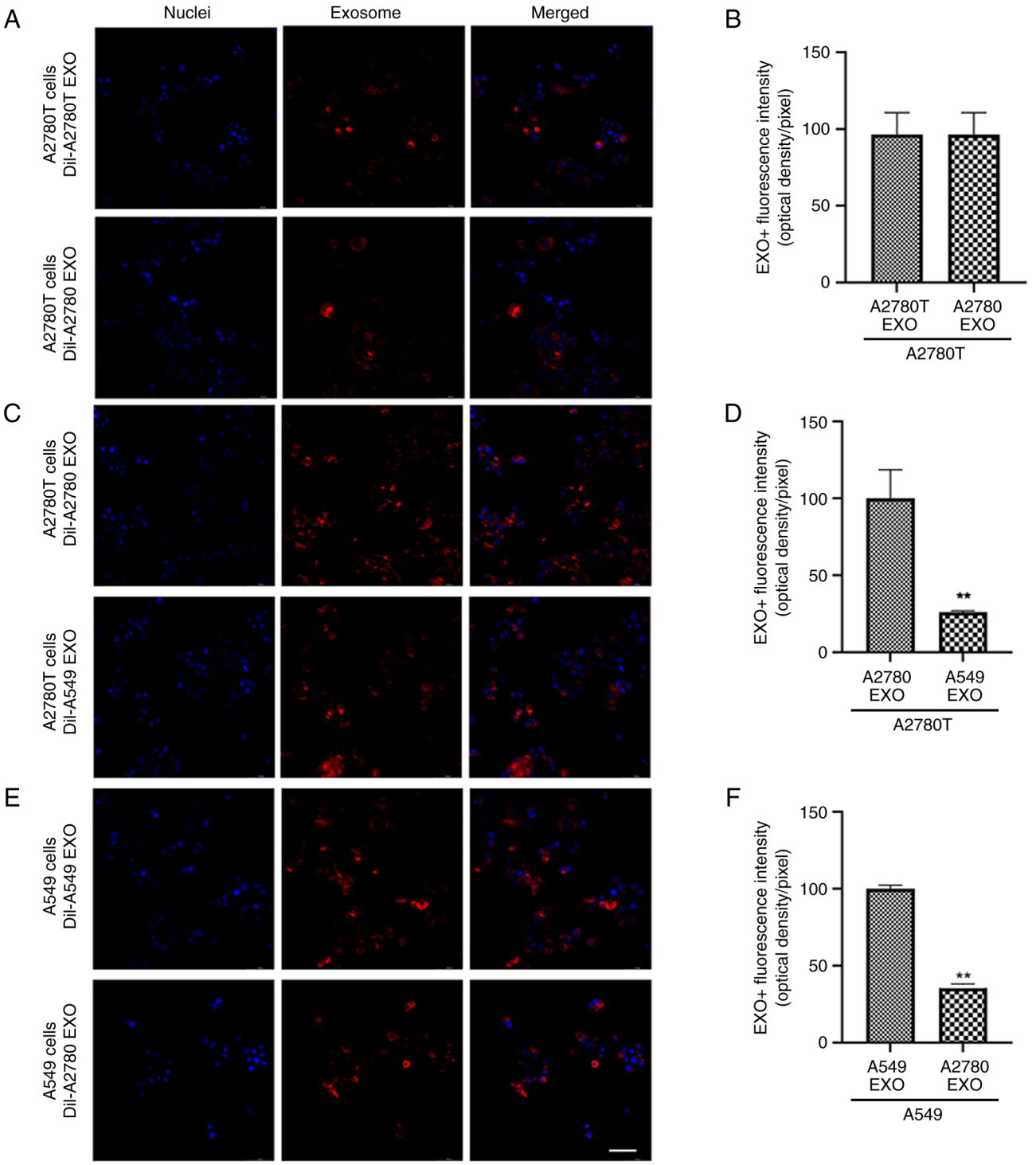

Figure 2. Cancer cell-derived EXOs preferentially fuse with their parent cells. (A) Representative micrographs showing uptake of Dil-labeled A2780 exosomes and A549 exosomes by A2780T cells. Endocytosed exosomes (red) can be seen around nuclei. Scale bar, 50 μm. (B) Quantification of A2780T EXO exosome uptake (n=3). (C) Representative micrographs showing uptake of Dil-labeled A2780 exosomes and A549 exosomes by A549 cells. Endocytosed exosomes (red) can be seen around nuclei. Scale bar, 50 μm. (D) Quantification of A549 EXO exosome uptake (n=3). (E) Representative micrographs showing uptake of Dil-labeled A2780T exosomes and A2780 exosomes by A2780T cells. Endocytosed exosomes (red) can be seen around nuclei. Scale bar, 50 μm. (F) Quantification of A2780T EXO exosome uptake (n=3). All data are are expressed as mean ± standard error of the mean, **P<0.01. EXO, exosome.

free TMP (8 μg/ml) and EXOs-TMP for 48 h, and the expression of drug resistance proteins was detected by western blotting. In the present study, a low dose of TMP could not reduce the expression of P-gp and ABCC1, while EXOs loaded with TMP could reduce the expression of P-gp and ABCC1 to 68.2 and 72.9%, respectively. When TMP and PTX were combined, the protein expression levels of P-gp and ABCC1 were decreased, and the protein expression levels of the EXO-TMP + PTX group were decreased from 72.9 and 81.8% to 51.5 and 55.2% in the free TMP + PTX group. The efficacy of EXOs-TMP in reversing the multidrug resistance of A2780T cells was demonstrated. GSTP1 is involved in the

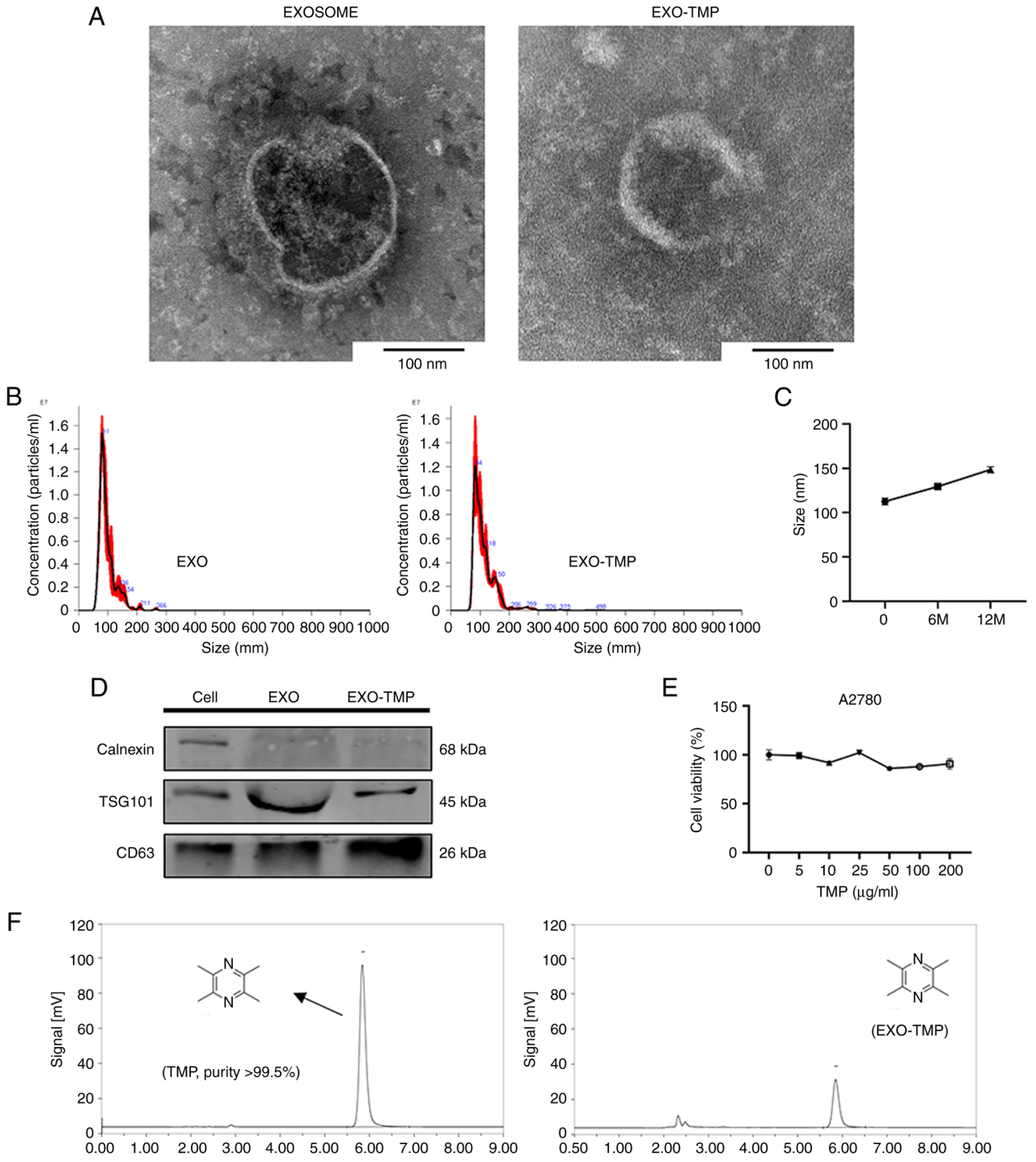

Figure 3. Characterization of EXO-TMP. (A) Transmission electron microscopy showing morphology of EXOs and EXO-TMP. (B) Size distributions of EXOs and EXO-TMP obtained by NanoSight particle tracking analysis (n=3). (C) Particle size of EXO-TMP stored at -80˚C for 0, 6 and 12 months. (D) Western blotting showed positive expression of EXO labeled proteins CD63 and TSG101 in the EXO and EXO-TMP groups, but negative protein expression of calnexin. This indicated that EXO-TMP was successfully prepared. (E) Effects of TMP (0-200 μg/ml) on the viability of A2780 cells detected using an MTT assay. (F) High-performance liquid chromatography chromatograms of TMP and EXO-TMP. EXO, exosome; TMP, tetramethylpyrazine; CD, cluster of differentiation; TSG101, tumor susceptibility 101.

anti-apoptosis and metabolism of numerous chemotherapeutic drugs. Platinum drugs have been found to be metabolized by GSTP1, resulting in the expression of GSTP1 in ovarian tumors (33). Therefore, GSTP1 can be used as a target gene and candidate response biomarker for platinum-based chemotherapy. In addition, it has been found that GSTP1 serves a role in the metabolism of PTX in ovarian cancer cells. However, to the to the best of the authors' knowledge, the effect of TMP on the expression of GSTP1 has not yet been reported. In the present study, EXOs-TMP were found to reduce the expression of GSTP1 and the combination of EXOs-TMP with PTX was more effective than free TMP. Together, these results suggested that EXOs-TMP reversed PTX resistance by reducing GSTP1 expression (Fig. 5A and B).

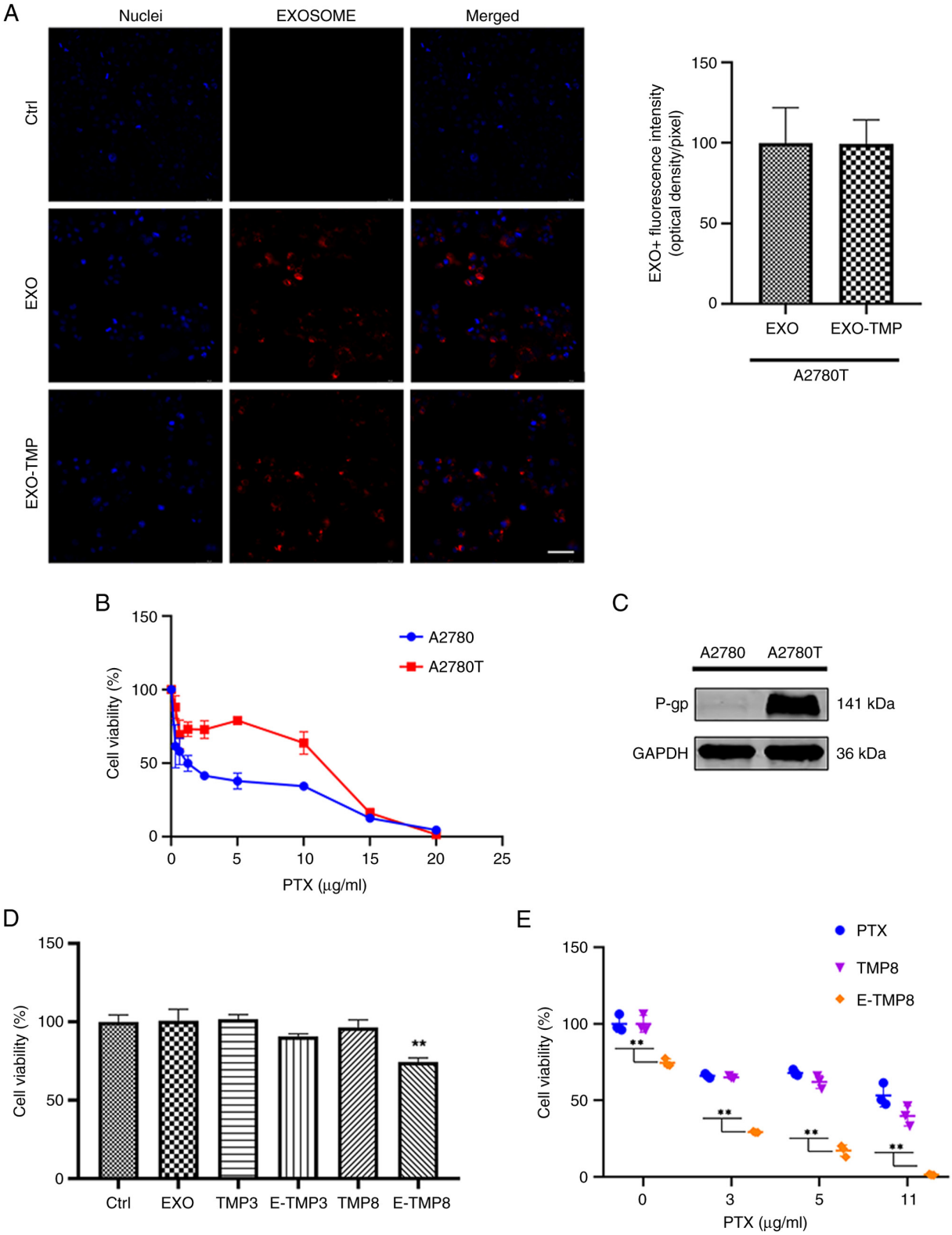

Figure 4. EXOs-TMP reduce resistance to PTX in drug-resistant cells. (A) Representative micrographs showing absorption of Dil-labeled EXO and EXO-TMP by A2780T cells. The endocytic EXOs are seen around the nucleus (red). Scale bar, 50 μm. (B) PTX (0-20 μg/ml) was added to A2780 and A2780T cells, which were 17 times more resistant. (C) P-gp expression of A2780T cells. (D) A2780T cells were co-incubated with free TMP (3 or 8 μg/ml), EXO and EXO-TMP (TMP 3 or 8 μg/ml) for 48 h, and cell viability was determined using the MTT method (n=3). (E) A2780T cells were co-incubated with free PTX, TMP (8 μg/ml) + PTX and EXO-TMP (TMP 8 μg/ml) + PTX for 48 h, and cell viability was determined using the MTT method (n=3). All data are expressed as mean ± standard error of the mean. **P<0.01. EXO, exosome; TMP, tetramethylpyrazine; PTX, paclitaxel; P-gp, multidrug resistance protein 1.

Apoptosis induction by EXOs-TMP in ovarian cancer cells. Induction of apoptosis can increase the sensitivity of drug-resistant cells to PTX (34). TMP combined with PTX treatment markedly promoted the apoptosis induced by PTX in A2780T ovarian cancer cells. To demonstrate the apoptotic effect of EXOs-TMP on drug-resistant cells, apoptosis was assessed by

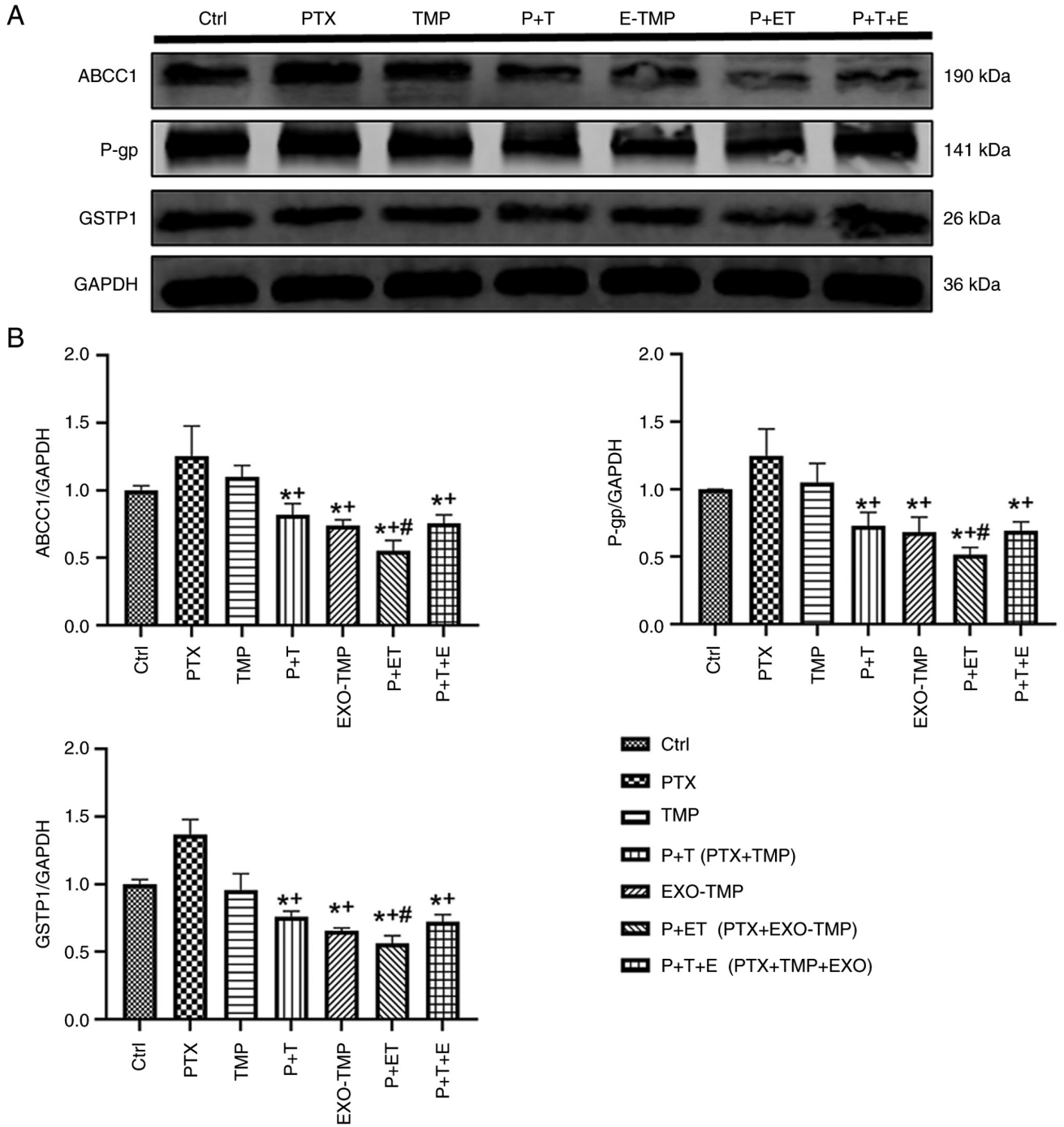

Figure 5. EXOs-TMP reduce the expression of drug resistance proteins in PTX-resistant A2780T cells. (A and B) A2780T cells were co-incubated with free TMP (3 or 8 μg/ml), EXO and EXO-TMP (TMP concentration is 3 or 8 μg/ml) for 48 h, respectively. The protein levels of ABCC1, P-gp and GSTP1 were determined by western blot analysis, and the optical density of the proteins was calculated. All data are expressed as mean ± standard error of the mean. *P<0.05 vs. untreated controls; $^{+}$P<0.05 vs. TMP; $^{\#}$P<0.05 vs. EXO-TMP. EXO, exosome; TMP, tetramethylpyrazine; PTX, paclitaxel; ABC, ATP-binding cassette; P-gp, P-glycoprotein; GSTP1, glutathione S-transferase π1.

flow cytometry in A2780T cells treated with 0.6 μg/ml PTX in coculture with TMP or EXOs-TMP for 48 h. The treated cells were double stained with Annexin V-FITC/PI. The results demonstrated that the apoptosis rates induced by each treatment (calculated by adding the values in the upper and lower right quadrants) were consistent with the cytotoxicity results. There was no apoptotic effect of TMP on A2780T cells, but the apoptosis rate of A2780T cells treated with PTX was increased by 1.9 times. EXOs-TMP increased the apoptosis rate of A2780T cells by 1.6 times. These results indicated that the EXO loading of TMP bestowed a novel antitumor effect to TMP in inducing apoptosis of drug-resistant ovarian cancer cells. Furthermore, EXOs-TMP + PTX exhibited a much higher apoptosis rate than both free PTX and PTX + TMP mixtures. The apoptosis rate induced by P + ET was ~3 times higher than that of the control group, and ~2 times higher than that induced by P + T, indicating the superiority of the EXOs-TMP drug delivery system. The results

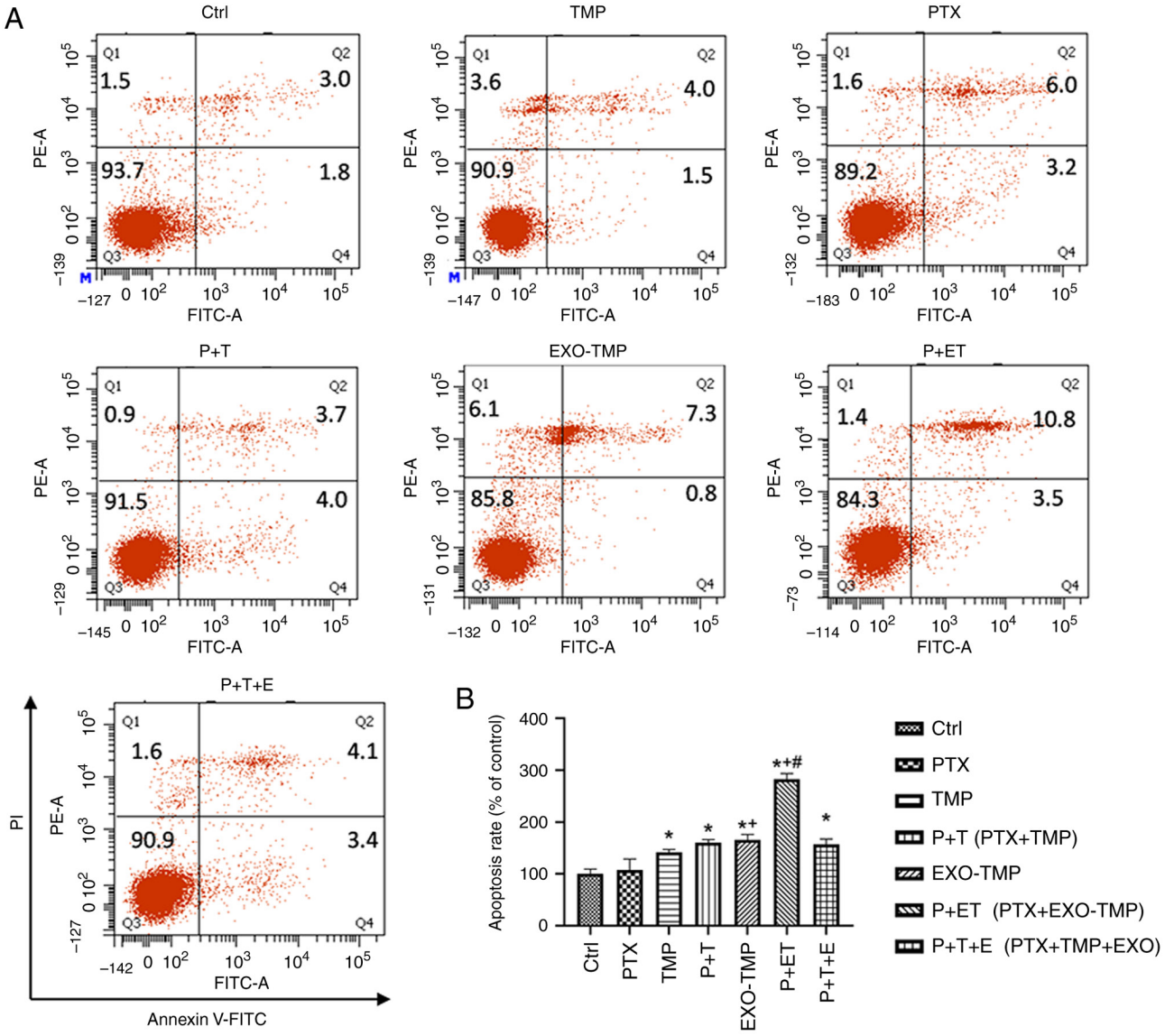

Figure 6. EXOs-TMP induce apoptosis *in vitro*. (A and B) A2780T cells were incubated with 8 μg/ml free TMP, free PTX, PTX + TMP mixture, PTX + EXO-TMP and PTX + TMP + EXO for 48 h, and then analyzed. Apoptotic cells detected by flow cytometry were stained by Annexin V-FITC/PI double staining. All data are expressed as mean ± standard error of the mean. *P<0.05 vs. untreated controls; +P<0.05 vs. TMP; #P<0.05 vs. EXO-TMP. EXO, exosome; TMP, tetramethylpyrazine; PTX, paclitaxel.

demonstrated the enhanced apoptotic effect of EXOs-TMP, and in particular, EXOs conferred an apoptotic effect of TMP on ovarian cancer cells (Fig. 6A and B).

In conclusion, the present findings suggested that ovarian cancer EXOs had the ability to return to their parent cells. Compared with free TMP, the loading of TMP into EXOs not only showed novel characteristics of toxicity to ovarian cancer cells, but also markedly promoted the antitumor effect of PTX. After investigating the mechanism of action of EXOs-TMP, it was revealed that EXOs-TMP could markedly reduce the expression of drug resistance proteins, including P-gp, ABCC1 and GSTP1, to enhance the sensitivity of drug-resistant cells to PTX. Furthermore, EXO-TMP itself exhibited cytotoxicity because EXOs-TMP caused apoptosis and also enhanced the pro-apoptotic effect of PTX. Based on the present results, EXOs-TMP enhanced the antitumor activity of PTX, and thus, should be further developed as a potential anticancer candidate.

Discussion

Ovarian cancer is a disease with high incidence and mortality rate, posing a significant threat to human health (35). Chemotherapy and radiotherapy still represent the common and established methods for treating patients with advanced cancer (36). As a complementary or alternative therapy, the combination of Chinese herbal medicine with other drugs shows promise (37). To address the side effects, drug resistance and unsatisfactory treatment outcomes in clinical cancer treatment, numerous studies on combination therapy have been conducted, with strategies aimed at improving efficacy or reducing toxicity gaining increased attention (38). Combined treatment is a feasible strategy for the development of Chinese herbal medicine. For example, *Ginkgo biloba* extract inhibits the proliferation, invasion and migration of gastric cancer cells (39). Dihydroartemisinin, a sesquiterpene lactone extracted from *Artemisia annua*, can induce apoptosis and

inhibit proliferation, invasion and migration of ovarian cancer cells by inhibiting the hedgehog signaling pathway (40). Baicalin, a flavonoid extracted from *Scutellaria baicalensis*, inhibits epithelial-mesenchymal transition and angiogenesis through the PI3K/Akt/mTOR signaling pathway (41).

TMP is an alkaloid monomer extracted from *Ligusticum chuanxiong*. A series of studies have demonstrated that TMP had a variety of antitumor effects, including the inhibition of tumor cell proliferation, invasion and drug resistance. For example, TMP inhibits angiogenesis and tumor growth in lung cancer in a dose- and time-dependent manner by blocking the bone morphogenetic protein/Smad/inhibitor of DNA binding 1 signaling pathway (42). Tmp-betulinic acid derivative inhibits the growth and metastasis of bladder cancer cells (T24) by interfering with glutathione metabolism and activating glycerophosphatidylcholine metabolism to block angiogenesis (43). TMP can inhibit the proliferation and migration of ovarian cancer cells by regulating miR-211 (44). In addition, we have confirmed that TMP can reduce the expression of multidrug-resistance-1 and GST-π at the mRNA level (45). However, due to the different pharmacokinetics of the drugs and their nonspecific biodistribution and membrane transport properties, combination therapy is far from ideal (46). Therefore, the preparation of a novel drug delivery regimen by loading TMP into EXOs was considered.

A variety of synthetic drug delivery systems have been developed over the past decades (47). However, the application of such systems is limited due to inefficiency, cytotoxicity and/or immunogenicity (48). EXOs are small vesicles produced by fusion and exocytosis between multivesicles (MVBs) and the plasma membrane, with a diameter range of 40-160 nm (49). The negative charge on the surface of EXOs ensures stability in the circulation system and the ability to deliver biomolecules to recipient cells makes them suitable drug delivery carriers (50). For example, PTX encapsulated in EXOs derived from human bone marrow mesenchymal stem cells exhibited marked cytotoxic and tumor growth inhibition effects against triple-negative breast cancer cells in *in vitro* and *in vivo* experiments (51). Gemcitabine-loaded EXOs were evaluated in mice with pancreatic tumors and were associated with inhibition of tumor growth, minimal damage to normal tissues and prolonged mouse survival (52). A study found that EXOs produced by HeLa and HT1080 cells had the property of homing to maternal tumors, and drug-loaded cancer EXOs could be used for targeted cancer therapy (53). In the present study, the selection of cell lines was based on validating the homing effect of exosomes derived from ovarian cancer cells on parental drug-resistant ovarian cancer cells. The choice of A549 cells was merely based on the consideration that they are not of the same lineage as ovarian cancer cells. Therefore, EXOs from A2780 ovarian cancer cells and A549 lung cancer cells were selected to observe whether they also have homing properties. When the mother cells were A2780T cells, the uptake rate of A2780 cell EXOs was three times higher than that of A549 EXOs and *vice versa*, confirming the ability of cancer-derived EXOs to home to their maternal tumors. Nevertheless, EXOs as drug carriers offer numerous advantages, but they also come with certain disadvantages and limitations. For example, they have limited drug-loading capacity, pose challenges in production and purification and are relatively fragile, making them susceptible to damage during storage and transportation. The research objective of this project is to develop an EXO-TMP drug delivery system and investigate its *in vitro* targeted drug delivery capabilities. The absence of validation through *in vivo* experiments is a limitation of this study. In future research, it is planned to enhance the purity of exosomes and conduct comprehensive *in vivo* experiments.

In the present study, cytotoxicity of TMP in A2780 and A2780T ovarian cancer cell lines was investigated. Serial concentrations up to 200 μg/ml of TMP did not show any inhibitory effect, even at the concentration of 200 μg/ml. This is consistent with a previous study (54). However, treatment of EXOs-TMP with TMP reduced the cell survival rate to <80% at the concentration of 8 μg/ml. To the to the best of the authors' knowledge, this is the first report of TMP inhibiting ovarian cancer cells. It is possible that EXOs confer a novel anticancer effect of TMP. It was found that 11 μg/ml PTX reduced the cell viability to ~50%, and the survival rate of A2780T cells was almost zero when combined with EXO-TMP. Its role and mechanism are worthy of further study.

Resistance to chemotherapy remains one of the most important obstacles to successful treatment of cancer. One of the most intensively studied mechanisms of multiresistance is the upregulation of expression of proteins from the ABC transporter superfamily (55). P-gp and ABCC1, in particular, efflux widely used chemotherapy drugs such as PTX, vincristine and doxorubicin (32). P-gp and ABCC1 in tumor cells cause multidrug resistance by pumping drugs out of the cell and altering drug metabolism. These efflux transporters diminish drug efficacy by reducing intracellular drug concentrations. Previous work has demonstrated that TMP could reverse the resistance of cancer cells to chemotherapeutic drugs by reducing drug resistance proteins (16). The present study revealed that the protein expression of P-gp and ABCC1 decreased to 72.9 and 81.8% when PTX was combined with TMP. A low dose of TMP did not reduce the expression of drug resistance proteins. However, EXOs-TMP reduced the resistance protein to ~70%. It was hypothesized that EXOs are composed of cell membranes, which can fuse with the plasma membrane or endocytic membrane and deliver TMP, bypassing P-gp-mediated efflux. Thus, the TMP worked. Furthermore, when EXOs-TMP were combined with PTX, the expression of drug resistance proteins was reduced to 50-60%. The effect was more significant than that of free TMP + PTX. GSTP1 serves an important regulatory role in detoxification, anti-oxidative damage and the occurrence of various diseases (56). GSTP1 is involved in the anti-apoptosis and metabolism of numerous chemotherapeutic drugs. In ovarian cancer, highly expressed GSTP1 serves a major role in the metabolism of cisplatin and carboplatin (57). To the to the best of the authors' knowledge, the present study was the first to demonstrate that EXO-TMP also exerted an inhibitory effect on GSTP1. The trend was consistent with the resistance proteins. Therefore, loading TMP into EXOs can exert an improved effect on drug-resistant cells.

As one of the most crucial mechanisms for triggering cell death, the effective elimination of cancer cells through apoptosis has been a primary objective in clinical cancer therapy. Dioscin inhibits the growth of human osteosarcoma

by inducing apoptosis in *in vitro* and *in vivo* settings (58). TMP can induce apoptosis in a variety of tumor cells (59,60). However, no apoptotic effects in A2780T ovarian cancer cells were observed in the present study. While TMP has been found to enhance the apoptosis-inducing effect of PTX in A2780 cells, the present experiments demonstrated that free TMP did not enhance the apoptotic effect of PTX on drug-resistant cells (54). It was hypothesized that TMP was lost to induce apoptosis through ABC transporter and its isoenzyme GSTP1, which was consistent with the failure of free TMP to reduce the expression of drug resistance proteins in resistant cells. Nonetheless, EXOs-TMP itself could promote the apoptosis of A2780T cells. TMP alone has not been shown to induce apoptosis in ovarian cancer cells. The combination of EXO-TMP and PTX increased the apoptosis rate by ~threefold, highlighting the potential of EXO-TMP as a reversal agent for overcoming drug resistance in tumor cells. Due to the stability in bodily fluids, good biocompatibility, and strong targeting capability of EXOs, the present study confirmed their potential as carriers for traditional Chinese medicine formulations. This provided a novel drug delivery pathway for overcoming multidrug resistance in tumors.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Dalian Traditional Chinese Medicine Scientific Research Project (grant no. 21Z12008), the Dalian Key Field Innovation Team Project (grant no. 2021RT14) and In-Hospital Cultivation Project of the Second Hospital of Dalian Medical University (grant no. dy2yynpy202210).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CZ, LQ, DW, HL, LC contributed to the study conception and design. CZ, LQ, LC wrote the manuscript and collected and analyzed data. CZ and LC critically revised the final manuscript. MZ, WX interpreted data, CG was responsible for photographing and organizing data images. CZ, LQ, DW, MZ, WX, CG, HL and LC confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Miller KD, Nogueira L, Mariotto AB, Rowland JH, Yabroff KR, Alfano CM, Jemal A, Kramer JL and Siegel RL: Cancer treatment and survivorship statistics, 2019. CA Cancer J Clin 69: 363-385, 2019.
2. Bodurka-Bevers D, Sun CC and Gershenson DM: Pharmacoeconomic considerations in treating ovarian cancer. Pharmacoeconomics 17: 133-150, 2000.
3. Yang ZJ, Zhao BB and Li L: The significance of the change pattern of serum CA125 level for judging prognosis and diagnosing recurrences of epithelial ovarian cancer. J Ovarian Res 9: 57, 2016.
4. Xiao R, You L, Zhang L, Guo X, Guo E, Zhao F, Yang B, Li X, Fu Y, Lu F, *et al*: Inhibiting the IRE1α axis of the unfolded protein response enhances the antitumor effect of AZD1775 in TP53 mutant ovarian cancer. Adv Sci (Weinh) 9: e2105469, 2022.
5. Miller EM, Samec TM and Alexander-Bryant AA: Nanoparticle delivery systems to combat drug resistance in ovarian cancer. Nanomedicine 31: 102309, 2021.
6. Chiappa M, Guffanti F, Bertoni F, Colombo I and Damia G: Overcoming PARPi resistance: Preclinical and clinical evidence in ovarian cancer. Drug Resist Updat 55: 100744, 2021.
7. Norouzi-Barough L, Sarookhani MR, Sharifi M, Moghbelinejad S, Jangjoo S and Salehi R: Molecular mechanisms of drug resistance in ovarian cancer. J Cell Physiol 233: 4546-4562, 2018.
8. Eisenhauer EA: Real-world evidence in the treatment of ovarian cancer. Ann Oncol 28 (Suppl 8): viii61-viii65, 2017.
9. Huang MY, Zhang LL, Ding J and Lu JJ: Anticancer drug discovery from Chinese medicinal herbs. Chin Med 13: 35, 2018.
10. Wang X, Liu Z, Sui X, Wu Q, Wang J and Xu C: Elemene injection as adjunctive treatment to platinum-based chemotherapy in patients with stage III/IV non-small cell lung cancer: A meta-analysis following the PRISMA guidelines. Phytomedicine 59: 152787, 2019.
11. Zhang E, Shi H, Yang L, Wu X and Wang Z: Ginsenoside Rd regulates the Akt/mTOR/p70S6K signaling cascade and suppresses angiogenesis and breast tumor growth. Oncol Rep 38: 359-367, 2017.
12. Malyutina A, Majumder MM, Wang W, Pessia A, Heckman CA and Tang J: Drug combination sensitivity scoring facilitates the discovery of synergistic and efficacious drug combinations in cancer. PLoS Comput Biol 15: e1006752, 2019.
13. Guo SK, Chen KJ, Qian ZH, Weng WL and Qian MY: Tetramethylpyrazine in the treatment of cardiovascular and cerebrovascular diseases. Planta Med 47: 89, 1983.
14. Chen Z, Zhang C, Gao F, Fu Q, Fu C, He Y and Zhang J: A systematic review on the rhizome of *Ligusticum chuanxiong* Hort. (Chuanxiong). Food Chem Toxicol 119: 309-325, 2018.
15. Zhao Y, Liu Y and Chen K: Mechanisms and clinical application of tetramethylpyrazine (an interesting natural compound isolated from *Ligusticum Wallichii*): Current status and perspective. Oxid Med Cell Longev 2016: 2124638, 2016.
16. Wang S, Lei T and Zhang M: The reversal effect and its mechanisms of tetramethylpyrazine on multidrug resistance in human bladder cancer. PLoS One 11: e0157759, 2016.
17. Wang XB, Wang SS, Zhang QF, Liu M, Li HL, Liu Y, Wang JN, Zheng F, Guo LY and Xiang JZ: Inhibition of tetramethylpyrazine on P-gp, MRP2, MRP3 and MRP5 in multidrug resistant human hepatocellular carcinoma cells. Oncol Rep 23: 211-215, 2010.
18. Zhou X, Wang A, Wang L, Yin J, Wang L, Di L, Hoi MP, Shan L, Wu X and Wang Y. A danshensu-tetramethylpyrazine conjugate DT-010 overcomes multidrug resistance in human breast cancer. Front Pharmacol 10: 722, 2019.
19. Huang HH, Liu FB, Ruan Z, Zheng J, Su YJ and Wang J: Tetramethylpyrazine (TMPZ) triggers S-phase arrest and mitochondria-dependent apoptosis in lung cancer cells. Neoplasma 65: 367-375, 2018.
20. Alotaibi BS, Buabeid M, Ibrahim NA, Kharaba ZJ, Ijaz M, Noreen S and Murtaza G: Potential of nanocarrier-based drug delivery systems for brain targeting: A current review of literature. Int J Nanomedicine 16: 7517-7533, 2021.
21. Kalluri R and LeBleu VS: The biology, function, and biomedical applications of exosomes. Science 367: eaau6977, 2020.

22. Antimisiaris SG, Mourtas S and Marazioti A: Exosomes and exosome-inspired vesicles for targeted drug delivery. Pharmaceutics 10: 218, 2018.
23. Kim MS, Haney MJ, Zhao Y, Mahajan V, Deygen I, Klyachko NL, Inskoe E, Piroyan A, Sokolsky M, Okolie O, *et al*: Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells. Nanomedicine 12: 655-664, 2016.
24. Elsharkasy OM, Nordin JZ, Hagey DW, de Jong OG, Schiffelers RM, Andaloussi SE and Vader P: Extracellular vesicles as drug delivery systems: Why and how? Adv Drug Deliv Rev 159: 332-343, 2020.
25. Chinnappan M, Srivastava A, Amreddy N, Razaq M, Pareek V, Ahmed R, Mehta M, Peterson JE, Munshi A and Ramesh R: Exosomes as drug delivery vehicle and contributor of resistance to anticancer drugs. Cancer Lett 486: 18-28, 2020.
26. Ma J, Zhang Y, Tang K, Zhang H, Yin X, Li Y, Xu P, Sun Y, Ma R, Ji T, *et al*: Reversing drug resistance of soft tumor-repopulating cells by tumor cell-derived chemotherapeutic microparticles. Cell Res 26: 713-727, 2016.
27. Gao Y, Zhang H, Zhou N, Xu P, Wang J, Gao Y, Jin X, Liang X, Lv J, Zhang Y, *et al*: Methotrexate-loaded tumour-cell-derived microvesicles can relieve biliary obstruction in patients with extrahepatic cholangiocarcinoma. Nat Biomed Eng 4: 743-753, 2020.
28. Rao Q, Zuo B, Lu Z, Gao X, You A, Wu C, Du Z and Yin H: Tumor-derived exosomes elicit tumor suppression in murine hepatocellular carcinoma models and humans in vitro. Hepatology 64: 456-472, 2016.
29. Yong T, Zhang X, Bie N, Zhang H, Zhang X, Li F, Hakeem A, Hu J, Gan L, Santos HA and Yang X: Tumor exosome-based nanoparticles are efficient drug carriers for chemotherapy. Nat Commun 10: 3838, 2019.
30. Yu Z, Tang H, Chen S, Xie Y, Shi L, Xia S, Jiang M, Li J and Chen D: Exosomal LOC85009 inhibits docetaxel resistance in lung adenocarcinoma through regulating ATG5-induced autophagy. Drug Resist Updat 67: 100915, 2023.
31. Shieh MJ, Hsu CY, Huang LY, Chen HY, Huang FH and Lai PS: Reversal of doxorubicin-resistance by multifunctional nanoparticles in MCF-7/ADR cells. J Control Release 152: 418-425, 2011.
32. Bruckmueller H and Cascorbi I: ABCB1, ABCG2, ABCC1, ABCC2, and ABCC3 drug transporter polymorphisms and their impact on drug bioavailability: What is our current understanding? Expert Opin Drug Metab Toxicol 17: 369-396, 2021.
33. Cui J, Li G, Yin J, Li L, Tan Y, Wei H, Liu B, Deng L, Tang J, Chen Y and Yi L: GSTP1 and cancer: Expression, methylation, polymorphisms and signaling (review). Int J Oncol 56: 867-878, 2020.
34. Jiang L and Hou R: Tetrandrine reverses paclitaxel resistance in human ovarian cancer via inducing apoptosis, cell cycle arrest through β-catenin pathway. Onco Targets Ther 13: 3631-3639, 2020.
35. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
36. Jayson GC, Kohn EC, Kitchener HC and Ledermann JA: Ovarian cancer. Lancet 384: 1376-1388, 2014.
37. Yang Z, Zhang Q, Yu L, Zhu J, Cao Y and Gao X: The signaling pathways and targets of traditional Chinese medicine and natural medicine in triple-negative breast cancer. J Ethnopharmacol 264: 113249, 2021.
38. Meyer CT, Wooten DJ, Paudel BB, Bauer J, Hardeman KN, Westover D, Lovly CM, Harris LA, Tyson DR and Quaranta V: Quantifying drug combination synergy along potency and efficacy axes. Cell Syst 8: 97-108.e116, 2019.
39. Fu Z, Lin L, Liu S, Qin M, He S, Zhu L and Huang J: *Ginkgo biloba* extract inhibits metastasis and ERK/nuclear factor kappa B (NF-κB) signaling pathway in gastric cancer. Med Sci Monit 25: 6836-6845, 2019.
40. Liu Y, Gao S, Zhu J, Zheng Y, Zhang H and Sun H: Dihydroartemisinin induces apoptosis and inhibits proliferation, migration, and invasion in epithelial ovarian cancer via inhibition of the hedgehog signaling pathway. Cancer Med 7: 5704-5715, 2018.
41. Li CY, Wang Q, Wang X, Li G, Shen S and Wei X: Scutellarin inhibits the invasive potential of malignant melanoma cells through the suppression epithelial-mesenchymal transition and angiogenesis via the PI3K/Akt/mTOR signaling pathway. Eur J Pharmacol 858: 172463, 2019.
42. Yi M, Jiao D, Qin S, Chu Q, Wu K and Li A: Synergistic effect of immune checkpoint blockade and anti-angiogenesis in cancer treatment. Mol Cancer 18: 60, 2019.
43. Cui H, Guo W, Zhang B, Li G, Li T, Yuan Y, Zhang N, Yang Y, Feng W, Chu F, *et al*: BA-12 inhibits angiogenesis via glutathione metabolism activation. Int J Mol Sci 20: 4062, 2019.
44. Zhang H, Ding S and Xia L: Ligustrazine inhibits the proliferation and migration of ovarian cancer cells via regulating miR-211. Biosci Rep 41: BSR20200199, 2021.
45. Ma H, Deng C, Zong X, He Y, Cheng L, Fan Q, Shao M, Lin Y, Zhao C, Li G and Zhang C: Reversal of doxorubicin-resistance by delivering tetramethylprazine via folate-chitosan nanoparticles in MCF-7/ADM cells. Int J Clin Exp Med 9: 5439-5448, 2016.
46. Li FS and Weng JK: Demystifying traditional herbal medicine with modern approach. Nat Plants 3: 17109, 2017.
47. Shi J, Kantoff PW, Wooster R and Farokhzad OC: Cancer nanomedicine: Progress, challenges and opportunities. Nat Rev Cancer 17: 20-37, 2017.
48. He H, Liu L, Morin EE, Liu M and Schwendeman A: Survey of clinical translation of cancer nanomedicines-lessons learned from successes and failures. Acc Chem Res 52: 2445-2461, 2019.
49. Namee NM and O'Driscoll L: Extracellular vesicles and anti-cancer drug resistance. Biochim Biophys Acta Rev Cancer 1870: 123-136, 2018.
50. Batrakova EV and Kim MS: Using exosomes, naturally-equipped nanocarriers, for drug delivery. J Control Release 219: 396-405, 2015.
51. Kalimuthu S, Gangadaran P, Rajendran RL, Zhu L, Oh JM, Lee HW, Gopal A, Baek SH, Jeong SY, Lee SW, *et al*: A new approach for loading anticancer drugs into mesenchymal stem cell-derived exosome mimetics for cancer therapy. Front Pharmacol 9: 1116, 2018.
52. Li YJ, Wu JY, Wang JM, Hu XB, Cai JX and Xiang DX: Gemcitabine loaded autologous exosomes for effective and safe chemotherapy of pancreatic cancer. Acta Biomater 101: 519-530, 2020.
53. Qiao L, Hu S, Huang K, Su T, Li Z, Vandergriff A, Cores J, Dinh PU, Allen T, Shen D, *et al*: Tumor cell-derived exosomes home to their cells of origin and can be used as Trojan horses to deliver cancer drugs. Theranostics 10: 3474-3487, 2020.
54. Zou L, Liu X, Li J, Li W, Zhang L, Li J and Zhang J: Tetramethylpyrazine enhances the antitumor effect of paclitaxel by inhibiting angiogenesis and inducing apoptosis. Front Pharmacol 10: 707, 2019.
55. Wang JQ, Yang Y, Cai CY, Teng QX, Cui Q, Lin J, Assaraf YG and Chen ZS: Multidrug resistance proteins (MRPs): Structure, function and the overcoming of cancer multidrug resistance. Drug Resist Updat 54: 100743, 2021.
56. Lei X, Du L, Yu W, Wang Y, Ma N and Qu B: GSTP1 as a novel target in radiation induced lung injury. J Transl Med 19: 297, 2021.
57. Sawers L, Ferguson MJ, Ihrig BR, Young HC, Chakravarty P, Wolf CR and Smith G: Glutathione S-transferase P1 (GSTP1) directly influences platinum drug chemosensitivity in ovarian tumour cell lines. Br J Cancer 111: 1150-1158, 2014.
58. Ding Q, Zhang W, Cheng C, Mo F, Chen L, Peng G, Cai X, Wang J, Yang S and Liu X. Dioscin inhibits the growth of human osteosarcoma by inducing G2/M-phase arrest, apoptosis, and GSDME-dependent cell death in vitro and in vivo. J Cell Physiol 235: 2911-2924, 2020.
59. Mohammad RM, Muqbil I, Lowe L, Yedjou C, Hsu HY, Lin LT, Siegelin MD, Fimognari C, Kumar NB, Dou QP, *et al*: Broad targeting of resistance to apoptosis in cancer. Semin Cancer Biol 35 (Suppl): S78-S103, 2015.
60. Wu X, Wang Z, Wu G, Xu X, Zhang J, Li Y, Zhang H and Guo S: Tetramethylpyrazine induces apoptosis and inhibits proliferation of hypertrophic scar-derived fibroblasts via inhibiting the phosphorylation of AKT. Front Pharmacol 11: 602, 2020.