Tanshinone IIA acts via p38 MAPK to induce apoptosis and the down-regulation of ERCC1 and lung-resistance protein in cisplatin-resistant ovarian cancer cells

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Abstract. Tanshinone IIA is known to induce apoptosis in several types of cancer cells. However, little is known about its activity in chemoresistant cells. The aim of this study was to investigate the anticancer properties of tanshinone IIA in cisplatin-resistant human ovarian cancer COC1/DDP cells *in vitro*. We used a variety of methods to measure cell viability, the resistance index (RI) of cisplatin, cellular apoptosis, p38 mitogen-activated protein kinase (MAPK) expression and phosphorylation, and the mRNA expression of several genes implicated in drug resistance including survivin, Caspase-3, excision repair cross-complementing gene 1 (ERCC1), multidrug resistance (MDR), lung resistance protein (LRP) and glutathione-S-transferase-π (GST-π). We found that tanshinone IIA time- and dose-dependently inhibited the proliferation of COC1/DDP cells and caused significant apoptosis. Western blotting revealed that tanshinone IIA also increased phospho-p38 MAPK in a time- and dose-dependent manner. After treatment by tanshinone IIA for 48 h, the RI of cisplatin and the mRNA expression of survivin, ERCC1 and LRP were all significantly decreased. Furthermore, blockade of p38 signal transduction decreased apoptotic cell rates and dramatically elevated the mRNA expression of the survivin, ERCC1 and LRP genes. We therefore conclude that tanshinone IIA induces apoptosis and reduces cisplatin resistance in COC1/DDP cells and thus causes significant growth inhibitory effects. This mechanism appears to involve p38-mediated downregulation of survivin, ERCC1 and LRP mRNA expression.

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Introduction

Ovarian cancer is the most commonly diagnosed and lethal gynecological malignancy. The high mortality rate is attributed in part to the lack of early detection systems, but is largely due to the development of chemoresistance (1,2). Although platinum-based compounds (e.g. cisplatin) in combination with taxanes are initially effective, the five-year survival rates are only about 50% (3). Although most ovarian tumors are initially sensitive to chemotherapy, the development of recurrent tumors that are resistant to cisplatin remains a major hurdle to successful therapy and is responsible for poor long-term overall survival (3,4). Cellular resistance to cisplatin is a complex phenomenon involving multiple adaptations such as increased defense mechanisms [e.g. multidrug resistance gene (MDR), lung resistance protein (LRP), glutathione-S-transferase-π (GST-π)], augmentation of DNA repair [e.g. excision repair cross-complementing gene 1 (ERCC1)], and inhibition of apoptosis (e.g. members of the caspase family) (5,6). Moreover, activation of cell pathways (e.g. the p38 mitogen-activated protein kinase (MAPK) pathway) is a common feature of cancer cells and could influence the cellular response after drug treatment (7,8).

Tanshinone IIA, a phenanthrenequinone derivative isolated from Danshen (*Salvia miltiorrhiza* bunge), has anti-oxidant properties, inhibiting the association of lipid peroxidation products with DNA by breaking the chain reactions of peroxidation by scavenging lipid free radicals (9-11). Recent studies have shown that tanshinone IIA has significant anti-proliferation effects by inducing apoptosis against multiple human cancer cell lines such as those derived from human breast cancer (12), hepatocellular carcinoma (13) and leukemia (14). Although the anti-cancer effects of tanshinone IIA have been studied in various cancer cells, it remains unknown whether it produces similar effects on drug-resistant cells such as cisplatin-resistant ovarian cancer cells. Therefore, the aim of this study was to elucidate the effects of tanshinone IIA on these cells and to identify its mechanism of action. Our results showed that tanshinone IIA induces apoptosis in cisplatin-resistant ovarian cancer cells and reduces resistance in these cells by downregulating survivin, ERCC1 and LRP. This downregulation was mediated by activation of p38

MAPK. Tanshinone IIA thus enhanced the apoptotic rate induced by cisplatin and attenuated cisplatin-induced upregulation of survivin and LRP expression in cisplatin-resistant ovarian cancer cells.

Materials and methods

Reagents. Tanshinone IIA was purchased from Xi'an Guanyu Bio-Tech Company. Cisplatin was obtained from Shandong Qilu Pharmaceutical Factory. Rabbit anti-phospho-p38 poly-clonal antibody and rabbit anti-p38 polyclonal antibody were from Santa Cruz Biotechnology (Santa Cruz, CA). The p38 inhibitor, SB203580, was supplied by Calbiochem (La Jolla, CA). RPMI-1640 medium was purchased from Hyclone Company (Logan, UT). MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] was obtained from Sigma (St. Louis, MO). All other chemicals and drugs were from Sigma Chemicals (St. Louis, MO).

Cell culture. COC1 (cisplatin-sensitive human ovarian epithelial carcinoma cell line) and COC1/DDP (cisplatin-resistant cell line) cells were obtained from China Center for Type Culture Collection) and cultured in RPMI-1640 containing 10% fetal bovine serum (FBS) and penicillin/streptomycin (100 U/ml) in a humidified 37°C incubator. For the inhibition experiments, cells were pretreated with the p38 MAPK inhibitor, SB203580, for 1 h prior to stimulation with tanshinone IIA at 8 mg/ml for 48 h.

Cell viability. Cell viability was assayed using the MTT assay. Briefly, $1x10^4$ COC1/DDP cells per well were dispensed into 96-well culture plates in 100 μl volumes. A range of concentrations of tanshinone IIA (1, 2, 4, 8 and 16 μg/ml) was added to designated wells. Vehicle buffer was added to an additional group of wells to act as a control sample. Each of the groups (treated and control) contained four adjacent wells. Plates were incubated for 1, 2, 3, 4, 5 and 6 d prior to addition of 20 μl of MTT working solution (5 mg/ml) and a further 4 h incubation. Plates were then centrifuged (10 min, 2000 x g) and culture medium supernatant was removed and replaced with 100 μl of DMSO to solubilize the cells. The absorbance (A value) of each well was measured using a microplate reader at 570 nm. Cell viability was calculated according to the formula:

$$\text{Viability rate} = \frac{100 \text{ x } A_t}{A_{con}}$$

where A_{con} is the A value of the control group and A_t is the A value of the treatment group. All experiments were repeated independently at least 3 times.

Resistance index (RI) of cisplatin. The RI of cisplatin in COC1/DDP cells was also assayed using the the MTT assay. COC1 and COC1/DDP cells were seeded in 96-well plates at $1x10^4$ cells per well. A range of concentrations of cisplatin (10, 20, 40, 80 and 100 nmol/ml) was then applied to designated wells, and buffer applied to control wells. Each condition was repeated in quadruplicate. After a 48 h

incubation period, cells were incubated for a further 4 h following addition of 20 μl MTT working solution (5 mg/ml). Cells were then solubilized and prepared for absorbance reading as described above. Cell inhibitory rate was calculated according to the formula:

$$\text{Inhibitory rate} = \frac{100 \text{ x } (A_{con} - A_t)}{A_{con}}$$

where A_{con} is the A value of the control group and A_t is the A value of the treatment group. The 50% inhibitory concentration (IC_{50}) was determined from dose-response data from at least three independent experiments. The IC_{50} values were calculated in each independent experiment and an average value obtained by calculating the mean of these IC_{50} values. The COC1/DDP cell RI of cisplatin was calculated according to the formula:

$$\text{RI} = \frac{IC50_{COC1/DDP}}{IC50_{COC1}}$$

where $IC50_{COC1/DDP}$ is the IC_{50} value in the cisplatin-resistant COC1/DDP cells and $IC50_{COC1}$ is the IC_{50} in the cisplatin-sensitive COC1 cells. This assay was then repeated in the presence of tanshinone IIA (8 μg/ml). All experiments were repeated at least three times independently.

Flow cytometry analysis for cell apoptosis. For FCM analysis, $1x10^6$ cells treated with cisplatin, SB203580 or different concentrations of tanshinone IIA were harvested, collected by centrifugation (10 min, 2000 x g) and washed three times with phosphate-buffered saline (PBS) at 4°C. Prior to analysis, cells were resuspended in binding buffer (10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl). Cells were then incubated with 5 μl Annexin V-FITC for 3 min and with 20 ng/ml propidium iodide in the dark for 15 min. This suspension was analyzed by flow cytometry (Becton-Dickinson, San Jose, CA). All data were collected and analyzed by Lysis II™ software (Becton-Dickinson). The experiments were repeated three times independently and the results were presented as the mean ± standard deviation.

Preparation of cytosolic proteins and Western blot analysis. Following treatment, cells were washed twice with ice-cold PBS and pelleted by centrifugation (3 min, 2000 x g) before homogenization in ice-cold fractionation buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 150 mM NaCl, 1% TritonX-100, 1 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, 10 μg/ml aprotinin, 1 mM sodium orthovanadate [Na_3VO_4], 10 mM sodium pyrophosphate [$Na_4P_2O_7$] and 50 mM sodium fluoride [NaF]). This cell lysate was incubated on ice for 15 min and centrifuged at 20,000 x g for 30 min at 4°C. The cytosolic fraction was collected and subjected to SDS-PAGE with a 10% running gel. Protein concentrations were determined using a BCA Protein assay kit (Pierce, Rockford, IL). The proteins were transferred to a polyvinylidene fluoride membrane which was then blocked by incubation with 5%

Table I. Forward and reverse primers used for quantitative RT-PCR.

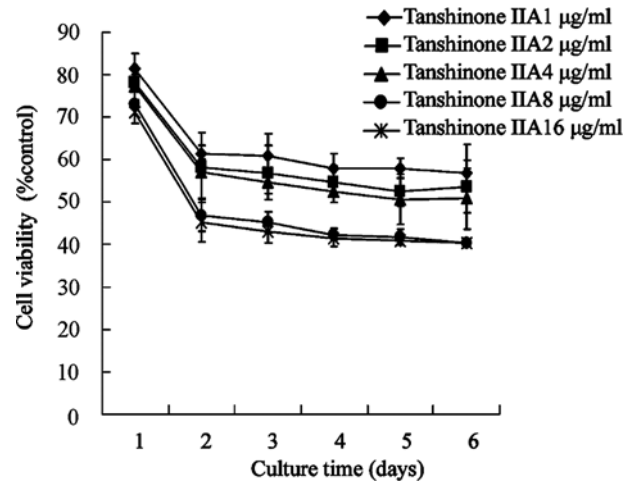| Gene	Sequence
Human MDR	Forward: 5'-GGAGCGGTTCTACGA-3'
	Reverse: 5'-ACGATGCCCAGGTGT-3'
Human ERCC1	Forward: 5'-ACCGTGAAGTCAGTCAACAAA-3'
	Reverse: 5'-TCGTGCAGGACATCAAACA-3'
Human GST-π	Forward: 5'-CTGGAAGGAGGAGGTGGTG-3'
	Reverse: 5'-GACGCAGGATGGTATTGGAC-3'
Human LRP	Forward: 5'-GGCTCCTTCCGCTACGT-3'
	Reverse: 5'-GCCGAGACCGCTCAATAC-3'
Human Caspase-3	Forward: 5'-GTGGCATTGAGACAGAC-3'
	Reverse: 5'-GGCACAAAGCGACTG-3'
Human survivin	Forward: 5'-CTGCGGAGAAAGTGCG-3'
	Reverse: 5'-GGGAATAAACCCTGGAAG-3'
Human ß-actin	Forward: 5'-AAGGCTGTGGGCAAGG-3'
	Reverse: 5'-TGGAGGAGTGGGTGTCG-3'

Figure 1. Tanshinone IIA inhibits the growth of cisplatin-resistant ovarian cancer cells. COC1/DDP cells were treated with different concentrations of Tanshinone IIA, and viability was determined using the MTT assay as described in Materials and methods. Values are expressed as mean ± SD (n=3).

bovine serum albumin in Tris-buffered saline containing 0.05% Tween-20 (TTBS) at room temperature for 1 h. Different primary antibodies (in TTBS) were applied at 4˚C for 12 h followed by horseradish peroxidase-conjugated secondary antibody at room temperature for 1 h. Membrane was washed extensively with TTBS between blocking, primary and secondary antibody stages.

RNA extraction and quantitative real-time PCR analysis. Total RNA was isolated using Trizol reagent (Sigma Chemical) according to the manufacturer's instructions. Total RNA (2 µg) was reverse-transcribed using a Promega (Madison, WI) reverse transcription system. The forward and reverse PCR primers used are summarized in Table I.

Reaction mixture (1 µl) was subjected to PCR. The amount of PCR product formed in each cycle was evaluated on the basis of SYBR Green I fluorescence. All amplification reactions were performed using the Mx3000 Multiplex Quantitative PCR system (Stratagene, La Jolla, CA) with the following cycle conditions: one cycle at 95˚C for 5 min, followed by 40 cycles at 95˚C for 30 sec, 58˚C for 30 sec and 72˚C for 30 sec. Results were analyzed with Stratagene Mx3000 software and the mRNA level for each gene was normalized to ß-actin levels. PCR reactions were performed in duplicate and each experiment was repeated independently three times.

Statistical analysis. Quantitative data are presented as the mean ± SD determined from the indicated number of experiments. Statistical analysis was based on Student's t-test for comparison of two groups or one-way ANOVA followed by a least squares difference (LSD) test for comparison of multiple groups.

Results

Cell growth rate of COC1/DDP was inhibited by tanshinone IIA. Tanshinone IIA (1, 2, 4 µg/ml) had little inhibitory effect on COC1/DDP cells. However, higher concentrations of this agent (8 and 16 µg/ml) significantly inhibited their proliferation. The inhibitory rate of tanshinone IIA at concentrations between 8-16 µg/ml is much higher than that of lower concentrations (p<0.05). Viability was relatively high on the first day but decreased significantly from day 2 onwards (p<0.05) (Fig. 1). The IC$_{50}$ values after 1, 2, 3, 4, 5 and 6 days of treatment were 98.14, 7.23, 5.45, 3.82, 3.23 and 3.09 µg/ml, respectively.

RI of cisplatin in COC1/DDP cells is reduced after treatment with tanshinone IIA. The inhibition of cell growth by cisplatin is appreciably reduced in the cisplatin-resistant COC1/DDP cells compared with the cisplatin-sensitive COC1 cells. However, in the presence of tanshinone IIA, the sensitivity to cisplatin-mediated growth inhibition appears to be restored to a level similar to that in COC1 cells (Fig. 2A). Furthermore, the resistance index (RI) of cisplatin the COC1/DDP cells is significantly reduced by the presence of tanshinone IIA (Fig. 2B), confirming that tanshinone dramatically reduces the resistance of these cells to cisplatin cytotoxicity.

COC1/DDP cell apoptosis caused by tanshinone IIA. As shown in Fig. 3, the presence of tanshinone IIA for 24-72 h caused an increase in the rate of apoptosis in COC1/DDP cell cultures. At 24 h, there was a small but significant (p<0.05) increase in apoptosis induced by 8 µg/ml tanshinone IIA (Fig. 3). This effect was considerably enhanced at 48 and 72 h (p<0.01). A higher concentration (16 µg/ml) of tanshinone IIA also significantly (p<0.01) increased apoptosis rate at 48 h (Fig. 3) but a lower concentration (4 µg/ml) had no significant effect.

In COC1/DDP cells treated with cisplatin (20 µM) in combination with tanshinone IIA (8 µg/ml), the rate of
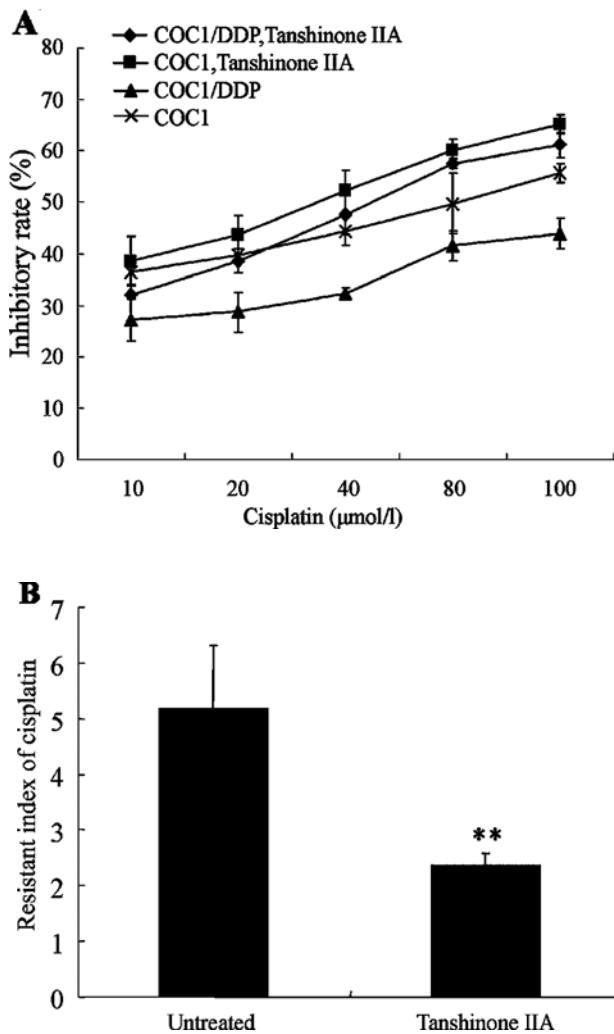
A

B

Figure 2. Modulation of cisplatin-mediated growth inhibition and resistance index by tanshinone IIA. COC1 and COC1/DDP cells were exposed to increasing concentrations of cisplatin for 48 h in the presence or absence of tanshinone IIA (8 μg/ml). Cell growth was assessed using an MTT assay. (A) The rate of growth inhibition in each cell line in the presence and absence of tanshinone IIA. (B) Resistance index (RI) of cisplatin in COC1/DDP cells in the presence and absence of tanshinone IIA. Values are expressed as mean ± SD (n=3). Data in (B) were analyzed using Student's t-test. **p<0.01, statistically significant difference.
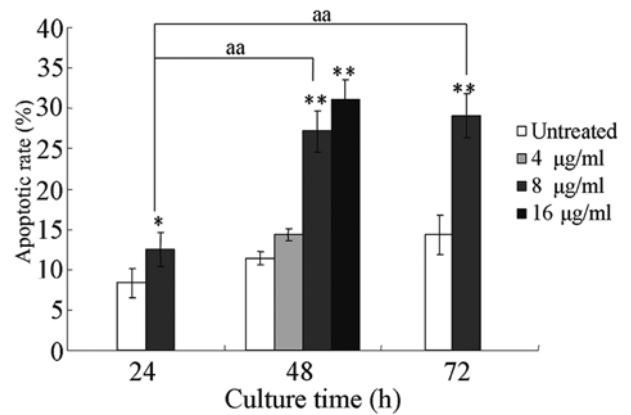

Figure 3. Rate of cell apoptosis caused by tanshinone IIA. COC1/DDP cells were treated with different concentrations of tanshinone IIA and cell apoptosis was analyzed by flow cytometry at various time points. Values are expressed as the mean ± SD (n=3). Statistical significance was assessed by one-way ANOVA followed by least squares difference (LSD) analysis. *p<0.05 vs. untreated cells; **p<0.01 vs. untreated cells; aap<0.01 vs. cells treated by tanshinone IIA (8 μg/ml, 24 h).
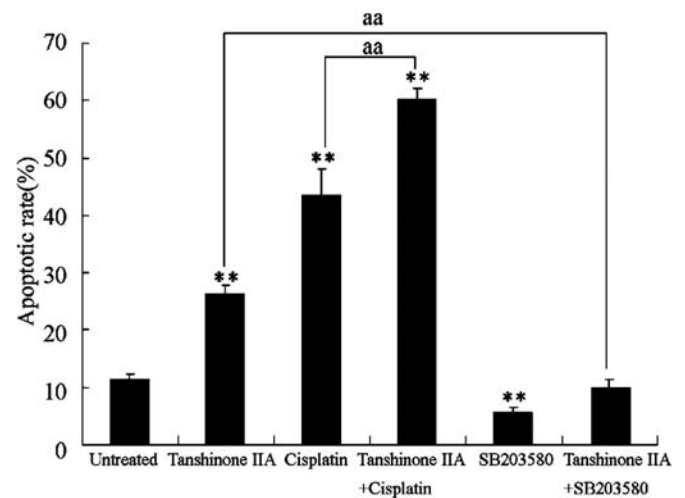

Figure 4. Modulation of apoptosis by tanshinone IIA and cisplatin, and the role of p38. COC1/DDP cells were treated with cisplatin (20 μM), tanshinone IIA (8 μg/ml), or a combination of these two compounds. In addition, some cells were treated with vehicle or tanshinone IIA in the presence of the p38 inhibitor, SB203580 (10 μM). Cellular apoptosis was then assessed by flow cytometry. Values are expressed as the mean ± SD (n=3). Statistical significance was assessed by one-way ANOVA followed by least squares difference (LSD) analysis. **p<0.01 vs. untreated cells; aap<0.01 between column pairs as indicated.

apoptosis was significantly (p<0.01) higher than that in the presence of cisplatin alone (Fig. 4). A 1 h pre-treatment with the p38 MAPK inhibitor, SB203580 (10 μM), resulted in a total blockade of the tanshinone IIA-mediated increase in apoptotic rate. This suggests that tanshinone IIA employs a p38-dependent signaling mechanism to modulate cellular apoptosis.

Tanshinone IIA enhances p38 MAPK activation of COC1/DDP cells. We investigated the effect of tanshinone IIA on p38 MAPK activity in COC1/DDP cells using a Western blot technique. Phosphorylation of p38 MAPK in COC1/DDP cells was significantly increased by tanshinone IIA in a time-dependent (Fig. 5) and dose-dependent (Fig. 6) manner.

Treatment of COC1/DDP cells with a combination of tanshinone IIA (8 μg/ml) and cisplatin (20 μM) caused a

significantly (p<0.01) greater increase in phospho-p38 than either agent alone (Fig. 7).

Survivin, ERCC1 and LRP mRNA expression in COC1/DDP cells is downregulated by Tanshinone IIA. To investigate the mechanism of Tanshinone IIA's effect on COC1/DDP cells, we used real-time PCR analysis to examine the mRNA expression of survivin, Caspase-3, ERCC1, LRP, MDR and GST-π, after treatment with tanshinone IIA (8 (g/ml) for 48 h. The results showed significant downregulation in the
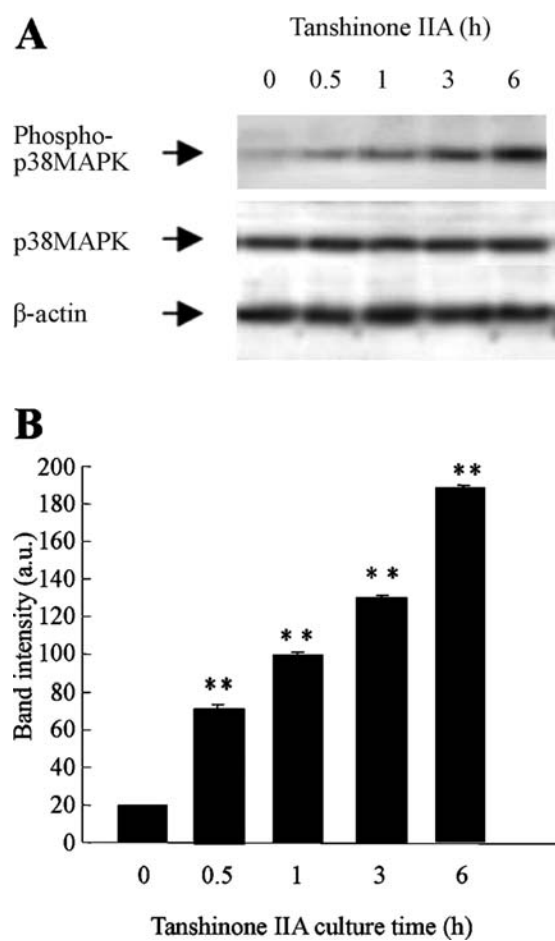
Figure 5. Western blot analysis of p38 MAPK and phospho-p38 MAPK cultured with tanshinone IIA for various time periods. (A) a representative image of a Western blot from this experiment. (B) densitometric data quantifying band density in our blots. Values are expressed as the mean ± SD (n=3). Data were analyzed statistically using one-way ANOVA followed by least squares difference (LSD) analysis. **p<0.01 vs. control.
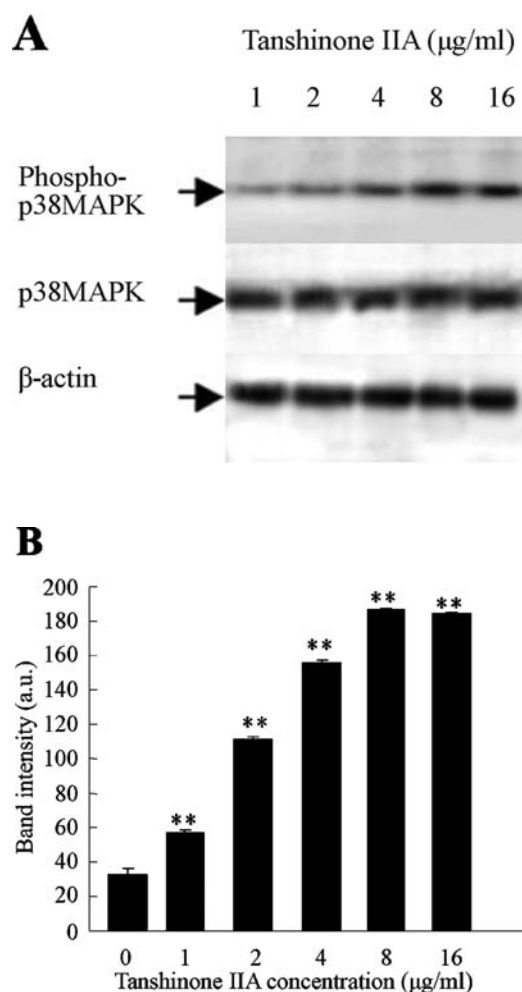

Figure 6. Western blot analysis of p38 MAPK and phospho-p38 MAPK cultured with various concentrations of tanshinone IIA. (A) a representative image of a Western blot from this experiment. (B) densitometric data quantifying band density in our blots. Values are expressed as the mean ± SD (n=3). Data were analyzed statistically using one-way ANOVA followed by least squares difference (LSD) analysis to compare each column to the control column. **p<0.01 vs. control.

mRNA expression of survivin (p<0.05), ERCC1 (p<0.01), LRP (p<0.01) and GST-π (p<0.01) in response to tanshinone IIA compared with control cells (Fig. 8), while Caspase-3, MDR and was not.

p38-MAPK inhibitor, SB203580, attenuated tanshinone IIA-induced downregulation of survivin, ERCC1 and LRP expression in COC1/DDP cells. We incubated cells with tanshinone IIA (8 μg/ml) in the presence of SB203580 (10 μM) to investigate whether p38 MAPK was involved in the tanshinone IIA-induced downregulation of survivin, ERCC1 and LRP expression. As shown in Fig. 8, SB203580 not only significantly blocked tanshinone IIA-induced downregulation of survivin, ERCC1 and LRP expression (p<0.01) but indeed causes a huge upregulation of the expression of these mRNAs (Fig. 8).

Tanshinone IIA attenuated cisplatin-induced upregulation of survivin and LRP expression in COC1/DDP cells. As shown in Fig. 8, tanshinone IIA significantly attenuated the cisplatin-induced upregulation of survivin (p<0.05) and LRP (p<0.01) expression.

Discussion

The present study demonstrated that tanshinone IIA can induce apoptosis and downregulation of drug resistance genes in cisplatin-resistant ovarian cancer cells via p38 MAPK.

Resistance to drug therapy represents a major limitation to the efficacy of platinum-based therapies in different malignancies. In ovarian cancers, >70% of patients initially respond to therapy with platinating agents. However, these patients often quickly develop resistance: the five-year survival rate for ovarian carcinoma is <25% (15). Resistance can develop as a result of decreased influx or increased efflux of drug (MDR, LRP), glutathione or metallothionein conjugation (GST-π), drug detoxification, DNA repair, or skipping lesions during DNA replication (ERCC1), and inhibition of apoptosis (5,6). Moreover, activation of survival pathways is a common feature of cancer cells and could influence the cellular response after drug treatment (p38 MAPK) (7,8).

Tanshinone IIA, a derivative of phenanthrenequinone isolated from Danshen, is now widely used in the treatment
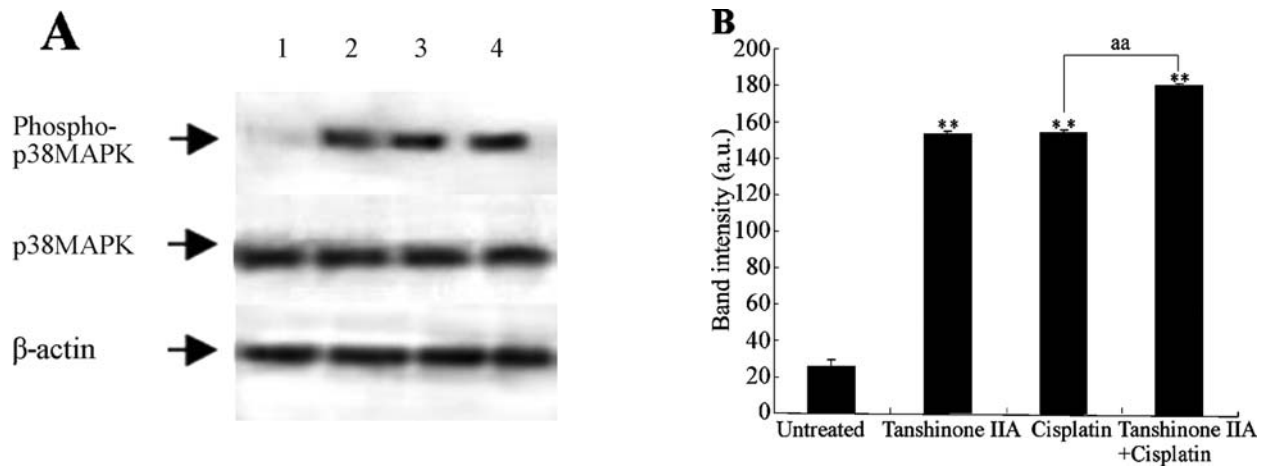
Figure 7. (A) Lane 1, untreated; lane 2, tanshinone IIA (8 µg/ml); lane 3, cisplatin (20 µM); lane 4, Tanshinone IIA (8 µg/ml) + cisplatin (20 µM). Western blot analysis of p38 MAPK and phospho-p38 MAPK in COC1/DDP cells following treatment with tanshinone IIA (8 µg/ml) and/or cisplatin (20 µM. Values are expressed as the mean ± SD (n=3). Data were analyzed using one-way ANOVA followed by least squares difference (LSD) analysis to compare all pairs of columns. **p<0.01 vs. untreated cells; aap<0.01 between indicated pairs.

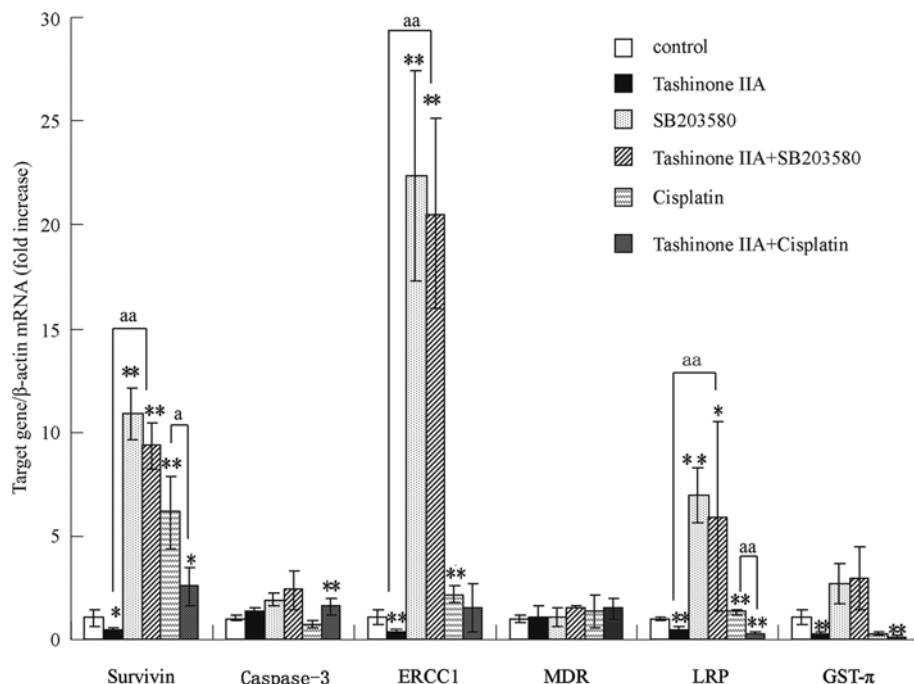
Figure 8. Expression of survivin, Caspase-3, ERCC1, MDR, LRP and GST-π mRNA expression in COC1/DDP cells following treatment with tanshinone IIA, cisplatin and/or SB203580. We used quantitative RT-PCR to measure the mRNA levels of various drug resistance genes in COC1/DDP cells treated with tanshinone IIA (8 µg/ml), cisplatin (20 µM) and/or SB203580 (10 µM). Values are expressed as the mean ± SD (n=3). Statistical analysis was by one-way ANOVA followed by least squares difference (LSD) analysis between all columns. *p<0.05 vs. control; **p<0.01 vs. control; ap<0.05 between indicated pairs; aap<0.01 between indicated pairs.

of inflammatory and cardiac diseases. Previous studies have shown that tanshinone IIA has a large variety of pharmacological activities such as inhibition of clotting (16), inhibition of NO synthase (17) and dose-dependent inhibition of the basic fibroblast growth factor (bFGF)-induced human smooth muscle cell (SMC) proliferation (18). Recent data have demonstrated that tanshinone IIA has anti-cancer activity on a large variety of cancer cells including solid tumors (12,13) as well as certain types of leukemia cells (14,19). Tanshinone IIA has also been reported to induce apoptosis in human liver

cancer cells via a p38-mediated mechanism of upregulated Fas/Caspase-3 expression (20). However, the molecular mechanism underlying the chemopreventive effect of tanshinone IIA has not been fully elucidated.

In this study, we found that tanshinone IIA inhibited the growth of cisplatin-resistant human ovarian cancer COC1/DDP cells and caused significant apoptosis in a time- and dose-dependent manner. Marked changes in cell apoptosis were clearly observed after the cells had been exposed to tanshinone IIA (8 µg/ml) for 48 h. Tanshinone IIA signi-

ficantly reduced the resistance index (RI) for cisplatin of COC1/DDP cells, and the apoptotic rate was considerably higher (>50%) than that without tanshinone IIA. Western blotting showed that increases in the phosphorylation of p38 MAPK in COC1/DDP cells was also stimulated by tanshinone IIA in a time- and dose-dependent manner. The p38 MAPK pathway is implicated in cancer cell apoptosis and is induced by several chemotherapeutic drugs including cisplatin (21). The mechanism of p38 MAPK downstream signaling to apoptosis has long been studied, including the involvement of the p38/p53/p21 pathway (22-25), the Bcl-2 family (26,27) and the Fas/FasL pathway (28). Studies on the mechanism of p38 MAPK actions resulting in cisplatin-resistance have focused mainly on cell apoptosis, but have neglected to assess the effects of this signaling system on the expression of genes related with resistance. In our study, we examined the mRNA expression of the survivin, Caspase-3, ERCC1, MDR, GST-π and LRP. We found that after treatment with tanshinone IIA (8 μg/ml for 48 h), mRNA expressions of survivin, ERCC1, GST-π and LRP were downregulated, while Caspase-3 and MDR were not. When SB203580 (10 μM) was included, it significantly blocked these effects of tanshinone IIA. We therefore concluded that the cell apoptosis induced by tanshinone IIA was caused by the downregulation of surviving, and the decreased RI of COC1/DDP cells for cisplatin was induced by downregulation of the resistance genes ERCC1 and LRP via p38 MAPK.

Survivin, a member of the apoptosis inhibitor family, is expressed in most human malignancies and is implicated in mitosis regulation and preservation of cell viability (29). Previous data have revealed that survivin is one of the genes most consistently over-expressed in tumor cells and plays important roles in both cell proliferation and cell death (30). Downregulation of survivin expression may lead to programmed cell death (31), indicating that it may be an appealing new target for novel therapy in cancer (32). Our results show that tanshinone IIA may trigger apoptosis in COC1/DDP cells by downregulating survivin levels via activation of p38 MAPK. Activated p38 MAPK might therefore inhibit the expression of survivin.

ERCC1 is a rate-limiting DNA repair protein in the nucleotide excision repair pathway that recognizes and removes cisplatin-induced DNA adducts (33-36). ERCC1 is also important for repairing interstrand cross-links in the DNA and in recombination processes (37-39). In ovarian cancer, ERCC1 expression was shown to be increased in the tumors of patients resistant to platinum treatment (40,41). Our results revealed that tanshinone IIA treatment of cisplatin-resistant human ovarian cancer COC1/DDP cells caused downregulation of the expression of ERCC1 mRNA in a p38 MAPK-dependent manner. We conclude that tanshinone IIA-induced activation of p38 MAPK might inhibit the expression of ERCC1 protein in cisplatin-resistant human ovarian cancer cells.

LRP has been identified as the major human vault protein (42). Structural studies of vaults have indicated that these may play a role in cytoplasmic redistribution and the nucleocytoplasmic transport of various substrates. LRP has been reported to be a marker for drug resistance *in vitro*, both for MDR-related drugs (doxorubicin, vincristine) and for other

agents (cisplatin, carboplatin and melphalan) (43). In this study, our results showed that tanshinone IIA treatment of COC1/DDP cells downregulated the expression of LRP via p38 MAPK. We conclude that tanshinone IIA-induced activation of p38 MAPK might inhibit the expression of LRP protein in cisplatin-resistant human ovarian cancer cells.

In summary, our results demonstrate that tanshinone IIA has significant anti-proliferative effects on cisplatin-resistant human ovarian cancer COC1/DDP cells by inducing apoptosis and downregulating cisplatin-resistance genes. Tanshinone IIA-induced apoptosis in COC1/DDP cells is mainly related to the downregulation of the anti-apoptotic protein, survivin, and decreased cisplatin-resistance was caused by the downregulation of the resistance genes, ERCC1 and LRP, via p38 MAPK. These results indicate that tanshinone IIA may serve as a potential therapeutic agent for the treatment of cisplatin-resistant human ovarian cancer.

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