Bcl-2 Up-Regulation and P-p53 Down-Regulation Account for the Low Sensitivity of Murine L929 Fibrosarcoma Cells to Oridonin-Induced Apoptosis

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Drug resistance has been a major limitation to chemotherapy. There are many mechanisms that contribute to such resistance. In our study, we subcloned oridonin-sensitive and low sensitive L929 cells and both types of cells grew at almost the same growth rate. The acquired low sensitivity to oridonin-induced apoptosis was associated with Bcl-2 up-regulation and down-regulation of p53 phosphorylation. The p38 inhibitor SB203580 decreased Bcl-2 expression in the low sensitive L929 cells and made the cells more sensitive to oridonin. Moreover, a higher dose of oridonin promoted p53 phosphorylation, increased Bax expression and subsequently induced death of low sensitive L929 cells, however, it had no effect on Bcl-2 expression. The increased Bcl-2/Bax ratio in oridonin low sensitive L929 cells did not inhibit caspase-9 or -3 activation, but suppressed the cleavage of poly (ADP-ribose) polymerase (PARP), indicating the existence of caspase-9 or -3 independent PARP activation. These results indicated that in L929 cells, there was a relationship among the low sensitivity to oridonin, down-regulation of p53 phosphorylation and Bcl-2 up-regulation.

Key words oridonin; L929 cells; low sensitivity; Bcl-2; p53

The drug resistance of a tumor is a major problem in the treatment of cancer, and is the result of re-growth of a tumor that is no longer sensitive to the original drug. A number of mechanisms may contribute to cellular drug resistance, such as reduced intracellular drug concentrations, rapid inactivation of the drug by induction of certain enzyme systems and increased rate of DNA repair. 1) Dysregulation of apoptosis, a genetically controlled form of cell death, may also be important for drug resistance because the mechanism by which most chemotherapeutic agents induced cell death appeared to be apoptosis. 2) Identification of the gene related to apoptosis and gene products and investigation of the mechanisms of the apoptotic regulation have laid a foundation for the discovery of new drugs. It is well known that among the proteins involved in the regulation of cell death, the p53 and p53-regulated proteins, the caspase family, the mitochondrial Bcl-2 family and the mitogen-activated protein kinase (MAPK) family function as the key factors in inhibiting or promoting apoptosis. 3–6)

Herbal medicine, Donglingcao (Rabdosia rubescens), has been traditionally used in China. Diterpenoids are the major constituent of R. rubescens and oridonin (Fig. 1) is a diterpenoid isolated from R. rubescens, which has been reported to have various pharmacological and physiological effects of anti-inflammation, anti-bacteria and anti-tumor. 7,8) In our previous study, we had reported that oridonin had apoptosis-inducing activities in murine L929 fibrosarcoma cells through a caspase-independent but mitochondria- and MAPK-dependent pathway and that caspase inhibitor augmented the cell death. 9–10) In the process of investigating the cytotoxic effect of oridonin on L929 cells, we found that part of these cells had low sensitivity to oridonin, although the mechanism is still unclear. The focus of our current study was to investigate the mechanism that regulate apoptosis and induce low drug sensitivity, and for this purpose both oridonin-sensitive and low sensitive L929 cells were subcloned.

In this study, we for the first time demonstrated that the low sensitivity to oridonin in L929 cells was associated with suppression of drug-induced apoptosis, which was the result of up-regulation of Bcl-2 expression, down-regulation of p53 phosphorylation and inhibition of PARP fragmentation. The p38 inhibitor SB203580 decreased Bcl-2 expression and increased oridonin-induced cell death in the low sensitive L929 cells. Moreover, a high dose of oridonin promoted p53 phosphorylation and increased Bax expression in the same low sensitive cells.

MATERIALS AND METHODS

Chemical Material Oridonin was obtained from the Beijing Institute of Biological Products (Beijing China). The structure of oridonin was assigned by comparing the chemical and spectral data (1H-, 13C-NMR) with those reported in the literature. The purity of the oridonin was measured by HPLC and determined to be 99.2% and oridonin was dissolved in the medium.

Biological Material 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), ribonuclease (RNase), proteinase K, propidium iodide (PI) and Hoechst 33258 were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Caspase-9 inhibitor (z-LEHD-fmk) and caspase-3 inhibitor (z-DEVD-fmk) were purchased from Enzyme Systems.

![Fig. 1. Chemical Structure of Oridonin](image)
(Sacramento, CA, U.S.A.). ERK inhibitor (PD98059), p38 inhibitor (SB203580) and JNK inhibitor (SP600125) were obtained from Calbiochem (CA, U.S.A.). Polyclonal antibodies against p38, phospho-p38, PARP, β-actin, and horseradish peroxidase-conjugated secondary antibodies (goat-anti-rabbit and goat-anti-mouse) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Antibodies against p53, Bcl-2 and Bax were obtained from Oncogene Research Products (Boston, MA, U.S.A.). Caspase-9 colorimetric activity assay kit was purchased from Chemicon International (CA, U.S.A.) and caspase-3 apoptosis kit was from Santa Cruz Biotechnology.

**Cell Culture**

The oridonin sensitive and oridonin resistant subclonal cells were derived from the murine fibrosarcoma L929 cells purchased from the American Type Culture Collection (#CRL-2148, ATCC, Manassas, VA, U.S.A.). The cells were cultured in RPMI-1640 medium (GIBCO, NY, U.S.A.) supplemented with 10% FBS (Shengma Yuanheng, Beijing, China), 100 mg/l streptomycin, 100 IU/ml penicillin, and 0.03% L-glutamine and maintained at 37 °C with 5% CO₂ in a humidified atmosphere.

**Subclone of L929 Cells**

L929 cells in RPMI-1640 supplemented with 10% fetal bovine serum were seeded into 96-well plates at the density of 3×10² cells/well to form single-cell clone in one well. Thirty-six single-cell clones were obtained using a microscope and cultured in RPMI-1640 medium containing 10% FBS for 5 min, washed by PBS, and then incubated in RPMI-1640 medium containing 10% FBS for 15000 g centrifugation for 15 min. The supernatant was incubated with 20 mg/ml RNase A (2 µl) and 20 mg/ml proteinase K (2 µl) at 37 °C for 1 h, then kept at 0.5 M NaCl (20 µl) and isopropanol (120 µl) at −20 °C overnight, and centrifuged at 15000 g for 15 min. DNA was dissolved in TE buffer [10 mM Tris (pH 7.4), 10 mM EDTA (pH 8.0)], subjected to 2% agarose gel electrophoresis at 50 V for 40 min and stained with ethidium bromide.

**Caspase Activity Assay**

L929 cells were cultured at 1×10⁶ cells and treated with or without oridonin for different time periods. The enzymatic reaction for caspase-3 activity was carried out with caspase-3 substrate (DEVD-AFC) according to the manufacturer’s instructions. The activity of caspase-9 was measured by a caspase-9 colorimetric activity assay kit.

**Westernblot Analysis**

L929 cells were treated with oridonin for different time periods. Both adherent and floating cells were collected, and then Westernblot analysis was carried out as previously described with some modification. Briefly, the cell pellets were resuspended in lysis buffer consisting of Heps 50 mmol/l pH 7.4, Triton-X 100 1%, sodium orthovanadate 2 mmol/l, sodium fluoride 100 mmol/l, edetic acid 1 mmol/l, PMSF 1 mmol/l, aprotinin (Sigma, MO, U.S.A.) 10 mg/l and leupeptin (Sigma) 10 mg/l and lyzed at 4 °C for 60 min. After 13000 g centrifugation for 15 min, the protein content of the supernatant was determined by a protein assay reagent (Bio-Rad U.S.A.). The protein lysates were separated by electrophoresis in 12% SDS polyacrylamide gel and blotted onto a nitrocellulose membrane. Proteins were detected using polyclonal antibody and visualized using anti-rabbit IgG conjugated with peroxidase (HRP) and 3,3-diaminobenzidine tetrahydrochloride (DAB) as the HRP substrate.

**Statistical Analysis of the Data**

The data are expressed as means±S.D. Statistical comparisons were made by Student’s t-test. p<0.05 was considered significant.

**RESULTS**

**Cell Cycle Distribution of the Two Types of L929 Cells**

To examine whether the two types of L929 cells have a similar cell growth cycle, flow cytometric analysis was applied. When the L929 cells were treated with 2.5 mM thymidine (an agent that blocks cell cycle transition from G1 to S phase) for

**Cell Cycle Distribution Assay**

L929 cells (1×10⁶) were incubated with 2.5 mM thymidine (cell cycle blocker from G1 to S phase) for 18 h, and collected by centrifugation at 10000 g for 5 min. The cells were suspended in RPMI-1640 medium supplemented with 10% fetal bovine serum and cultured for 15 h. They were treated with 2.5 mM thymidine for 15 h again, centrifuged at 10000 g for 5 min, washed by phosphorylated buffer saline (PBS), then further cultured in RPMI-1640 containing 10% fetal bovine serum and collected at 0, 8, 16 and 24 h. The cells were washed by PBS and stained with PI followed by flow cytometric analysis.

**Flow Cytometric Analysis**

L929 cells, both adherent and floating, were pelleted and washed with PBS. The cells were fixed in 75% ethanol at 4 °C overnight. After washing twice with PBS, the cells were stained with propidium iodine (1.0 ml, 50 mg/l), RNase A (1 g/l) and 0.1% Triton X-100 in sodium citrate (3.8 mM), followed by incubation on ice in the dark for 30 min. Samples were analyzed by a flow cytometer (Becton Dickinson FACScan, Franklin Lakes, CA, U.S.A.) with Cell Quest software, which was used to determine the percentage of cells in different phases of the cell cycle.

**Nuclear Damage Observed by Hoechst 33258 Staining**

Apoptotic nuclear morphology was assessed using Hoechst 33258. Cells were fixed with 3.7% paraformaldehyde for 30 min at room temperature, and then washed and stained with 167 μM Hoechst 33258 at 37 °C for 30 min. Cells were again washed and resuspended in PBS for morphological observation using a fluorescence microscope (Leica, Wetzlar, Germany).

**DNA Fragmentation Assay**

L929 Cells (1×10⁶) were collected by centrifugation at 150×g for 5 min, and washed with PBS. The cells were pelleted and suspended in 10 mM Tris (pH 7.4), 10 mM EDTA (pH 8.0) and 0.5% Triton X-100, then kept at 4 °C for 10 min. The supernatant was incubated with 20 mg/ml RNase A (2 µl) and 20 mg/ml proteinase K (2 µl) at 37 °C for 1 h, then kept in 0.5 M NaCl (20 µl) and isopropanol (120 µl) at −20 °C overnight, and centrifuged at 15000 g for 15 min. DNA was dissolved in TE buffer [10 mM Tris (pH 7.4), 10 mM EDTA (pH 8.0)], subjected to 2% agarose gel electrophoresis at 50 V for 40 min and stained with ethidium bromide.

**Cytotoxicity Assay**

L929 cells were treated with oridonin for different time periods. The data are expressed as means±S.D. Statistical comparisons were made by Student’s t-test. p<0.05 was considered significant.

**RESULTS**

**Cell Cycle Distribution of the Two Types of L929 Cells**

To examine whether the two types of L929 cells have a similar cell growth cycle, flow cytometric analysis was applied. When the L929 cells were treated with 2.5 mM thymidine (an agent that blocks cell cycle transition from G1 to S phase) for
showed that when the two types of the cells were treated with oridonin by Western blot analysis. The results showed that the apoptotic sub-G1 peaks were observed when different doses of oridonin were monitored. The results indicated that p38 signal pathways contributed to the oridonin low sensitivities, specific inhibitors for p38 were employed. After treatment with 2.5 mM thymidine for the indicated time periods, the two types of L929 cells were further cultured in RPMI-1640 (containing 10% fetal bovine serum) and harvested at 0, 8, 16, and 24 h. The DNA contents were detected by fluorescence flow cytometry (Table 1). The results showed that the two types of L929 cells grew at almost the same growth rate.

Cytotoxic Effects of Oridonin on Two Types of L929 Cells The cytotoxic effects of oridonin on the two types of L929 cells were detected by culturing the cells with 2—12 μM oridonin for 24 h; we found that oridonin induced sensitive L929 cell death in a dose-dependent manner with an IC_{50} value of 9.1 μM, but those doses of oridonin did not kill most of the low sensitive cells (Fig. 2A). Higher doses of oridonin, 50—100 μM, however, exerted potent cytotoxic effects on the low sensitive cells with an IC_{50} value of 81.2 μM (Fig. 2B).

Atypical Apoptosis in the Two Types of L929 Cells Induced by Oridonin To investigate whether oridonin-induced cell death in the two types of L929 cells was caused by apoptosis, changes in DNA content during treatment with different doses of oridonin were monitored. The results showed that the apoptotic sub-G1 peaks were observed when the two types of cells were cultured with 9.1 and 81.2 μM oridonin, respectively, for 12 h (Figs. 3B, D). To further confirm this, we also observed the morphologic changes by Hoechst 33258 staining. In the control group, nuclei of L929 cells were round and homogeneously stained (Figs. 3E, G), however, oridonin-treated sensitive and low sensitive L929 cells showed granular apoptotic bodies (Figs. 3F, H). Nucleosomal DNA fragmentation is another hallmark of typical apoptosis. However, in our study, DNA ladders were not observed in the two types of cells treated with oridonin (data not shown).

Up-Regulation of Bcl-2 and Down-Regulation of Bax in Oridonin Low Sensitive L929 Cells It is reported that the Bcl-2 family proteins play important roles in either inhibition or promotion of apoptotic cell death.11) The higher expression of Bcl-2 in human melanoma and other tumors has been correlated with resistance to chemotherapy.12) We also examined the expression of Bcl-2 family proteins, Bcl-2 and Bax, in the two types of cells treated with oridonin by Western blot analysis. The results showed that when the two types of the cells were treated with 9.1 μM oridonin for different time periods, the expression of Bax protein was up-regulated in the sensitive cells and that of Bcl-2 was down-regulated, however, in the low sensitive cells, Bax and Bcl-2 expressions did not change. The elevated Bcl-2/Bax ratio might be characteristic of the low sensitivities to oridonin (Fig. 4).
Vega et al. reported that treatment of 2F7 cells with the 
p38 inhibitor SB203580 inhibited the constitutive p38 
MAPK activity and resulted in inhibition of STAT3 activity 
and Bcl-2 expression, consequently sensitizing the drug-in-
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regulation of Bcl-2 expression in an oridonin dose-indepen-
dent manner.

Contribution of p53 to the Low Sensitivity to Oridonin

p53 plays an important role in induction cell cycle arrest and

**Fig. 3. Oridonin Induced Apoptosis in Both Types of L929 Cells**

Flow cytometric analysis of cell cycle after the sensitive cells were treated with 9.1 \(M\) oridonin, and low sensitive cells with 81.2 \(M\) oridonin for 12 h. Morphological changes of nuclei were observed with Hoechst 33258 staining after the cells were cultured with oridonin for 18 h (×200 magnification E, F, G, H; Sen., oridonin sensitive cell; L. sen., ori-
donin low sensitive cell; Or., oridonin).

**Fig. 4. Westernblot Analysis of Bcl-2 and Bax Protein Expression in the Two Types of L929 Cells Treated with Oridonin**

The oridonin sensitive and oridonin low sensitive cells were treated with 9.1 \(M\) oridonin for different time periods. Cell lysates were separated by 12% SDS-PAGE electrophoresis, and the protein expression was detected by Westernblot analysis. Sen., oridonin sensitive cell; L. sen., oridonin low sensitive cell.

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**Fig. 5. Effects of MAPK Inhibitors on the Two L929 Cell Types**

The oridonin sensitive (A: Sen) and the oridonin low sensitive cells (L: sen) were 
treated with 9.1 \(M\) oridonin and MAPK inhibitors including 5 \(M\) SP600125 (JNK), 
5 \(M\) SB203580 (p38) and 5 \(M\) PD98059 (ERK) for various time periods. Cell death 
was measured by MTT assay. \(n=3\), mean±S.D., **p<0.01 vs. control group (9.1 \(M\) 
oridonin).
apoptosis and it has been reported that melanoma cell lines expressing wild type p53 exhibit a higher response to anticancer agents than melanoma with mutant p53. Western-blot analysis was carried out to compare p53 expression in the two types of L929 cells treated with 9.1 μM oridonin. The p53 phosphorylation increased in the oridonin sensitive L929 cells, however, in the oridonin low sensitive cells, p53 phosphorylation was at low levels (Fig. 7A).

Wild type p53 promotes cell cycle arrest and apoptosis in response to DNA damage most likely by regulation of target genes Bcl-2 and Bax. After the low sensitive cells were treated with a high dose of 81.2 μM oridonin for 12 h, p53 phosphorylation and Bax expression increased, but Bcl-2 expression did not change, compared with 9.1 μM oridonin-treated low sensitive cells (Fig. 7B). These results suggested that a low expression of p53 phosphorylation might result in Bax expression at low levels and that this, in part, contributed to the low sensitivity to oridonin.

Up-Regulation of Bcl-2 Expression and Down-Regulation of Bax Expression Suppressed PARP Cleavage, But Did Not Inhibit Caspase Activation in the Oridonin Low Sensitive L929 Cells

In mitochondria, when apoptosis occurs the cytochrome c is released into cytoplasm, and then cytochrome c, Apaf-1 and procaspase-9 form a complex. Activated caspase-9 cleaves caspase-3 proenzyme. Poly (ADP-ribose) polymerase (PARP) is the substrate of caspase-3, and is cleaved to an 85-kDa fragment during apoptosis.

In our study, we used fluorometric assay to measure caspase-9 and -3 activities after treatment with 9.1 μM oridonin in the two types of L929 cells for different time periods. The results showed that caspase-9 or -3 activation was not suppressed in the oridonin low sensitive cells, but changed in almost the same degree to the oridonin sensitive cells (Fig. 8A). In order to further confirm this result, the two types of cells were pretreated with caspase-9 inhibitor z-LEHD-fmk, and caspase-3 inhibitor z-DEVD-fmk, and then cultured with 9.1 μM oridonin for 15 h. z-LEHD-fmk and z-DEVD-fmk made the two types of cells even more sensitive to 9.1 μM oridonin. z-LEHD-fmk augmented the cell death by almost 12%, and z-DEVD-fmk increased the cell death by almost 22% in both cell types (Fig. 8B), indicating that caspase-9 and -3 played protective roles in both cells, but did not mediate the oridonin-induced apoptosis.

To examine whether Bcl-2 up-regulation and Bax down-regulation affected downstream PARP cleavage in the oridonin low sensitive L929 cells, Western-blot analysis was carried out to detect PARP fragmentation. After treatment with 9.1 μM oridonin for different time periods in the oridonin sensitive cells PARP was cleaved to an 85 kDa fragment (Fig. 9A). However, in oridonin low sensitive cells, PARP was not cleaved, but p38 inhibitor SB203580 and a high dose of 81.2 μM oridonin promoted PARP fragmentation (Fig. 9B). PARP inhibitor DPQ 20 μM partially inhibited the cell death in the oridonin sensitive and low sensitive L929 cells treated, respectively, with 9.1 or 81.2 μM oridonin (Fig. 9C). It is reported that apoptosis-inducing factor (AIF) is confined to the mitochondrial intermembrane space. When apoptosis occurred AIF from the mitochondria translocated to the cytoplasm and nucleus and resulted in apoptosis in a caspase-independent manner. Western-blot analysis performed to examine AIF expression in the oridonin sensitive and low sensitive L929 cells showed that in the 9.1 μM oridonin-treated sensitive cells, AIF significantly increased at 6 h, but in oridonin low sensitive cells, AIF was low but p38 inhibitor SB203580 or 81.2 μM oridonin promoted this expression (Fig. 10). These results demonstrated that although in oridonin-treated L929 cells, caspase-9 and -3 protected against...
the cell death, the suppression of PARP cleavage in oridonin low sensitive cells was still the result of a high ratio of upstream Bcl-2/Bax. PARP fragmentation and AIF expression were observed in 9.1 μM oridonin-treated sensitive cells and in a high dose of 81.2 μM oridonin-treated low sensitive L929 cells, therefore AIF might be responsible for PARP cleavage in a caspase-independent manner.

**DISCUSSION**

Resistance to chemotherapy or radiotherapy is a major obstacle for the treatment of malignant tumors that are surgically incurable because of their diffusely infiltrative nature. In addition to the conventional drug resistance mechanisms including drug intra/extracellular transport, mutation of the epidermal growth factor receptor (EGFR), secretion of anti-apoptotic factor and DNA repair (1), suppression of apoptosis is another means of acquiring drug resistance and could be mediated by specific genetic alterations in tumor cells, such as inactivating mutations of p53 and/or overexpression of Bcl-2.12,14 In our study, subcloned oridonin-sensitive and low sensitive L929 cells were employed. The two types of L929 cells grew at almost the same growth rate and oridonin caused apoptosis in both.

Bcl-2 family members are characterized by containing at least one of four Bcl-2 homology domains (BH1—BH4), and play important roles in regulating apoptosis.11 Bcl-2 seems to influence response to chemotherapy by inhibiting apoptosis...
sis induced by many cytostatic drugs including alkylating agents, topoisomerase inhibitors and antimetabolites. A recent study has shown that the simultaneous down-regulation of Bcl-2 expression and induction of apoptosis by antisense oligonucleotides in melanoma cells of different clinical stages might provide an additional clinical benefit. In our experiments the results showed that Bcl-2 expressed at high levels in oridonin low sensitive L929 cells compared with the sensitive ones. It has been reported that treatment of 2F7 and 10C9 B Non-Hodgkin’s lymphoma (NHL) with the p38 inhibitor SB203580 sensitized the cell lines to various chemotherapeutic drugs by down-regulation of Bcl-2 expression through inhibition of STAT3 transcriptional activity. Endogenous nitric oxide down-regulates the Bcl-2 expression of eosinophils through mitogen-activated protein kinase p38 or ERK pathways in bronchial asthma. The present study showed that p38 inhibitor SB203580 obviously inhibited Bcl-2 expression and augmented low sensitive L929 cell death at the dose of 9.1 μM oridonin indicating that p38 was involved in L929 cell low sensitivity to oridonin through up-regulation of Bcl-2 expression. Whether p38 regulated Bcl-2 expression through STAT3 pathways remains to be investigated. Ras gene mutation is very common in malignant tumor. Ras proteins, upstream of p38, are regulators of multiple signal pathways that control cell growth and apoptosis. Ras oncogene is also involved in drug resistance, and the assumed mechanism is an up-regulation of Bcl-2 expression. According to this, Ras gene might participate in L929 cells low sensitivity to oridonin and this also remains to be elucidated. Bax, a proapoptotic protein, functions to promote apoptosis through suppressing antiapoptotic proteins Bcl-2 and Bcl-X<sub>l</sub>. Raisova et al. reported that a low Bax/Bcl-2 ratio might be characteristic for drug-resistant melanoma cells. Bax expression is regulated by p53, and wild p53 mediates cell cycle arrest and apoptosis in response to DNA damage, and p53 mutation is associated with drug resistance in malignant tumor. Our study showed that in oridonin low sensitive L929 cells, Bax and p53 phosphorylation expressed low and these could be reversed by high dose of 81.2 μM oridonin. Based on those results, we assumed that higher doses of oridonin might cause more serious DNA damage and this initiated multiple p53 activation, consequently increasing Bax expression. Bax/Bcl-2 acts in mitochondria to cause the cytochrome c release into cytoplasm, and then cytochrome c, Apaf-1 and procaspase-9 form a complex which leads to caspase-9 activation; subsequently, activated caspase-9 cleaves caspase-3 proenzyme. PARP is a nuclear enzyme that responds to DNA damage, facilitates DNA repair and contributes to cell survival. It is cleaved to an 85-kDa fragment by caspase-3 when apoptosis occurred. In this study, caspase-9 and -3 did not execute apoptosis, but protected L929 cells against death, and their protease activities were not suppressed by the high ratio of Bcl-2/Bax in oridonin low sensitive cells. However, in the oridonin low sensitive cells, PARP degradation was inhibited in comparison with the sensitive cells, and p38 inhibitor SB203580 and a high dose of 81.2 μM oridonin promoted PARP fragmentation indicating that the suppression of PARP cleavage was still the result of Bcl-2 overexpression and the reduction of Bax expression.

In summary, we demonstrated that the L929 cells low sensitivity to oridonin was correlated with inhibition of drug-induced apoptosis, which was mediated by increased expression of Bcl-2 and decreased p53 phosphorylation which might result in down-regulation of Bax expression. The high ratio of Bcl-2/Bax was responsible for suppression of PARP fragmentation and subsequent cell survival. In the low sensitive L929 cells, p38 phosphorylated at high levels, and p38 inhibitor SB203580 partially suppressed the expression of Bcl-2 and augmented the cell death induced by oridonin.

REFERENCES