The antitumoral effect of Paris Saponin I associated with the induction of apoptosis through the mitochondrial pathway

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Abstract
Rhizoma Paridis, a traditional Chinese medicine, has shown promise in cancer prevention and therapy. In the present study, we isolated Paris Saponin I (PSI), an active component of Rhizoma paridis, and evaluated its effects on a panel of human cell lines and in a mouse model of human ovarian cancer to explore the mechanisms of its activity. PSI had more potent and selective cytotoxic effects on tumor cell lines than etoposide had, promoting dramatic G2-M phase arrest and apoptosis in SKOV3 cells in a time- and dose-dependent manner. Furthermore, PSI treatment increased levels of Bax, cytochrome c, activated caspase-3, active caspase-9, and cleaved poly(ADP-ribose) polymerase and decreased both Bcl-2 expression levels and extracellular signal-regulated kinase-1/2 activity. We also assessed the antitumor efficacy of i.p. and p.o. PSI administration in mice bearing SKOV3 tumors; both significantly inhibited the growth of SKOV3 cells in a subcutaneous xenograft mouse model (by 66% and 52%, respectively). These results indicate that PSI mediates its effects via mitochondrial apoptosis, mitogen-activated protein kinase pathways, and G2-M cell cycle arrest. Most important, the efficacy of PSI in xenografts when administered p.o. or i.p. suggests its clinical potential. Thus, PSI is a potent antitumor compound and should be developed as a natural agent for cancer therapy. [Mol Cancer Ther 2009;8(5):1179–88]

Introduction
Ovarian cancer is often diagnosed at an advanced stage wherein the cancer has spread beyond the ovaries. The recommended treatment of choice is surgical debulking followed by a combination treatment with platinum- and taxane-based agents. One systematic review of randomized control trials concluded that i.p. chemotherapy improved the median survival beyond that of i.v. chemotherapy (1). However, i.p. administration of chemotherapy has not been adopted as standard practice, and despite significant clinical improvement in ovarian cancer treatment over the past few decades, this cancer still remains the fourth leading cause of cancer-related deaths among women in both the United States and China. Hence, developing new therapeutic agents that could be effectively administered either i.p. or p.o. could vastly improve both the quality of life and survival duration among ovarian cancer patients.

Rhizoma paridis is the root of either Paris polyphylla Smith var. chinensis (French) Hara or Paris polyphylla Smith var. yunnanensis (French) Hand-Mazz (Trilliaceae family). It has been used in China to treat traumatic bleeding, inflammation, microbial infection, and, over the past decade, cancer (2). Steroidal Paris Saponins are the active components of Rhizoma paridis. These active components are well-known ingredients of the traditional Chinese medicines Yunnan White (a blood coagulant), Gongqunxing (an anti–uterine bleeding agent), and Jidesheng Snake (an antivenom; refs. 3–6). Studies using a hepatic, gastric, or nasal pharyngeal cancer model have shown the inhibitory effects of Rhizoma paridis on tumor growth (7–10). Five Paris Saponins have been identified: Paris Saponin I (PSI), also known as polyphyllin D (10–14), Paris Saponin V (PSV), Paris Saponin VI (PSVI), Paris Saponin VII (PSVII), and Paris Saponin H (PSH). Of the five Paris Saponins, PSI and PSVI have been approved for cancer therapy in China because of their potential involvement in the suppression of tumor growth. Synthetic PSI (12, 13) has also been shown to render cytotoxic effects against non–small-cell lung cancer (14) and hepatocellular carcinoma cell line HepG2 (11). In attempting to further elucidate the biological effects, characteristics, and mechanisms by which PSI works and to improve the effects of this novel anticancer agent, we isolated PSI from Rhizoma paridis and studied the role of PSI in inhibiting tumor cell growth both in vitro and in vivo. Specifically, we evaluated the effects of PSI on cell proliferation, extracellular signal–regulated kinase (ERK1/2) activity, cell cycle arrest, and apoptosis in a panel of human cell lines. We also assessed the antitumor efficacy of i.p. and p.o. PSI administration in mice bearing SKOV3 tumors.

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Materials and Methods

Materials

The PSI and PSVI were obtained from the Department of Pharmacology of Sichuan University (Chengdu, Sichuan, China). PSI was purified as previously described (15). Briefly, PSI was isolated from *Rhizoma paridis* using silica gel, macroporous adsorption resin, Sephadex LH-20, and RP-C18 column chromatography. Its structure, diosgenin-3-O-α-L-rhamnopyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→4)]-β-D-glucopyranoside, was determined by electrospray ionization-mass spectrometry $^1$H and $^{13}$C nuclear magnetic resonance spectral analysis (Fig. 1; ref. 16). Etoposide (VP16), cyclosporin A, PD98059 (17, 18), ghrelin (17, 18), and β-actin antibody were purchased from Sigma Chemical Co. Primary antibodies against Bcl-2, Bax, ERK1/2, phospho-ERK1/2 (Thr202/Tyr204), Akt, phospho-Akt (Ser473), CCAAT/enhancer binding protein homologous transcription factor (CHOP), cytochrome c, caspase-3, and caspase-9 were obtained from Santa Cruz Biotechnology, Inc.

![A. the structure of PSI, including an α-L-rhamnopyranosyl group at C-2 of the glucosyl moiety that plays an important role in the activity of PSI. The α-L-arabinofuranosyl and β-D-glucopyranoside at C-4 of the inner glucosyl moiety have limited roles (16). B. PSI does not inhibit the survival of non-tumorigenic human meningeal, human vascular smooth muscle, human bronchial, or ovarian surface epithelial (OSE) cells. PSI decreases cell viability and inhibits the growth of HEC-1A A549, HepG2, and SiHa cells. C. dose-effect curves for PSI and the clinical antitumor drug VP16 on the SKOV3 cell line for 1, 3, 5, and 7 d. PSI shows stronger cytotoxicity than VP16. D. PSI induces G2-M phase arrest in SKOV3 cells. The SKOV3 cell line was incubated with and without PSI for up to 24 h. All treated cells were stained with propidium iodide and analyzed by fluorescence-activated cell sorting.](image-url)
Cell Lines and Culture
A549, a human lung adenocarcinoma cell line; HepG2, a hepatocellular carcinoma cell line; CaSkI and SKOV3, ovarian cancer cell lines; SiHa and HeLa, cervical carcinoma cell lines; OSE, an ovarian surface epithelial cell line; and HEC-1A, an endometrial carcinoma cell line, were obtained from the American Type Culture Collection. Vascular smooth muscle, bronchial epithelial, and meningeal cells were isolated from individual tissues obtained from the gynecologic laboratory of Sichuan University. The use of human tissue in the study was approved by the Institutional Review Board of the Institute for Nutritional Sciences (Shanghai, China). Cells were cultured in RPMI 1640 (GIBCO BRL, Life Technologies) supplemented with 10% fetal bovine serum (HyClone) at 37°C with 5% CO2. Treated cells were cultured in fresh medium.

PSI Treatment and Determination of Cell Growth
We evaluated the effects of PSI on cell proliferation in both cell lines and normal primary cultures. Cells were seeded at a density of 5 x 10^3 per well in 96-well tissue culture plates. Cells were treated with 10 μmol/L of PSI, and carrier DMSO (<0.1%) was used as a control. The viability of each tumor cell line was examined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) assay (19). SKOV3 cells were also treated with different concentrations of PSI (1, 5, 10, and 20 μmol/L). Cell viability was assessed every 24 h by MTT assay. The dose- and time-dependent curve of the PSI-treated SKOV3 cell line was generated. The cytotoxic effects of PSI were expressed as the 50% inhibiting concentration (IC50), total growth-inhibiting concentration (TGI), and LC50 values.

Transmission Electron Microscopy
SKOV3 cells were treated with 10 μmol/L PSI for 24 h. Treated cells were fixed in 5% glutaraldehyde and 3% paraformaldehyde, dehydrated in an ascending acetone series, embedded in PolyBed 812 resin, sectioned into ultrathin longitudinal sections, and imaged using a transmission electron microscope (JEOL 1010, Jeol) as previously described (20).

Agarose Gel Electrophoresis for Analysis of DNA Fragmentation
SKOV3 cells were treated with different concentrations of PSI (1, 5, 10, or 20 μmol/L) for 24 h. Cellular DNA was extracted using an apoptotic DNA ladder kit (Promega Corp.). DNA samples were diluted in Tris-EDTA buffer [10 mmol/L Tris (pH 7.5) and 1 mmol/L EDTA] and immediately analyzed by electrophoresis on a 1.5% agarose gel.

Flow Cytometry Analysis for Apoptosis
Flow cytometry was used to analyze the loss of membrane symmetry and membrane integrity using FITC Annexin V and propidium iodide (BD ApoAlert Annexin V-FITC Apoptosis kit, BD Biosciences), respectively, as previously described (21). Briefly, cells (5 x 10^5/ml) were labeled with propidium iodide (0.01 mg/ml; Sigma) for 30 min at room temperature. After filtration, cellular DNA contents were analyzed by FACSort (Becton Dickinson). Data were further analyzed by CellQuest software, version 3.1 (Becton Dickinson) and ModFit LT 3.2 (Verity Software House).

Terminal Deoxynucleotidyl Transferase–Mediated dUTP Nick End Labeling Assay
Cells seeded on cover slides were treated with PSI. Treated and nontreated cells were fixed by 4% paraformaldehyde solution for 15 min at room temperature, washed in PBS, and permeated by 0.1% Triton X-100 solution at 4°C for 2 min. The terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay kit (Phoenix Flow Systems, Inc.) was used according to the manufacturer’s protocol to label DNA fragments at their 3’-hydroxyl ends (Roche Applied Science). Random fields were recorded using a Leica TCS4D confocal scanning laser microscope (Leica; ref. 22). The apoptosis index was determined as the percentage of TUNEL-positive cells of 1,000 4,6-diamidino-2-phenylindole–stained nuclei.

Detection of Protein Expression by Immunohistochemistry
PSI-treated SKOV3 cells were analyzed by immunohistochemistry to determine the apoptotic index (23). PSI-treated cells were fixed by 3-aminopropyltriethoxysilane at 60°C for 60 min. The paraffin sections were treated with 0.3% hydrogen peroxide for 5 min at room temperature to block endogenous peroxidase activities; 5% bovine serum albumin was added to prevent nonspecific binding. Fixed sections were incubated for 1 h with the following antibodies: Bcl-2 (1:100), Bax (1:50), caspase-3 (1:100), and caspase-9 (1:100; Santa Cruz Biotechnology). Treated slides were further incubated with freshly prepared 0.05% 3’-diaminobenzidine tetrahydrochloride for 5 min. Images were digitized (gray values) using a Cool Snap Pro video camera interfaced to an Olympus BX2 microscope with a 20× objective.

The Effect of PSI in a Xenograft Model of Ovarian Cancer
The xenograft tumor model we used in this study has been described previously (24). Briefly, 5 x 10^6 SKOV3 cells were s.c. injected into 4–to 6-week-old female BALB/athymic nude mice (Shanghai Experimental Animal Center, Shanghai, China). All experiments conformed to the animal care and use guidelines of the Institute for Nutritional Sciences (Shanghai, China). The mice were randomly divided into six groups of five mice. One week after SKOV3 implantation, the treatment groups received their first doses of PSI dissolved in a vehicle solution of DMSO (<0.1%) and diluted in saline solution. PSI dosage and administration schedules were based on preliminary toxicologic and pharmacokinetic studies. Briefly, PSI was injected at either 15 or 25 mg/kg i.p. into tumor-bearing mice on 4 consecutive days per week for 4 wk (between days 8 and 35). In parallel, PSI was also given p.o. to tumor-bearing mice at either 20 or 30 mg/kg on the same schedule. The two control groups received the vehicle (DMSO, <0.1%) in saline solution, one group by injection and one by p.o. administration. The same quantity of saline...
solution containing DMSO was used in these groups. General clinical observations of the mice, including determination of body weight and tumor growth, were made twice weekly. To determine tumor size, we measured two perpendicular diameters of the xenograft in centimeters by calipers. Tumor mass was estimated using the formula \((a \times b^2)/2\), where \(a\) is the long diameter and \(b\) is the short diameter (25, 26). All mice were euthanized by carbon dioxide asphyxiation 2 wk after the last injection, and the tumor tissues were removed and processed for evaluation.

Statistical Analysis and Reproducibility
The results are given as the SE. Statistical analysis was done using Student's t test. \(P < 0.05\) was considered significant.

Results
PSI Treatment Inhibits the Growth of Human Tumor Cell Lines
The chemical structure of PSI is shown in Fig. 1A. To investigate the cytotoxic effects of PSI on human tumor cells, we treated a panel of human tumor cell lines that included

Figure 2. PSI induces dose- and time-dependent apoptosis in SKOV3 human ovarian cancer cells. A, time-dependent apoptosis. Representative flow cytometric analysis of PSI-treated SKOV3 cells. SKOV3 cells were treated with 10 \(\mu\)mol/L PSI for 0, 12, 24, and 48 h and harvested for apoptosis analysis by flow cytometry at indicated times. B, columns, mean \((n = 3)\); bars, SE. C, dose-dependent apoptosis. Representative flow cytometric analysis data. SKOV3 cells were treated with various doses of PSI (1, 5, 10, and 20 \(\mu\)mol/L). PSI-treated cells exhibited special Ap peaks, which were characteristic of apoptosis. D, columns, mean \((n = 3)\); bars, SE. E, TUNEL data presented as the percentage of apoptotic cells (green fluorescence) per the total number of treated cells. Random fields \((n = 10)\) were selected per slide \((n = 3; P < 0.05)\). Representative figures of treated cells labeled for TUNEL and counterstained with 4,6-diamidino-2-phenylindole (DAPI) are shown. Columns, mean; bars, SE.
HEC-1A, A549, HepG2, SiHa, CaSki, and SKOV3 with PSI. As shown in Fig. 1B, 10 μmol/L PSI treatment resulted in <50% cell survival after 4 days of treatment compared with that of the nontreated groups. The IC₅₀ of PSI for all of the tested cancer cell lines was <15 μmol/L, albeit with much variation below that number, except for the case of HeLa cells (Supplementary Fig. S1—Table 1). In addition, prolonged
exposure of cancer cells to PSI vastly increased its inhibitory effect. To determine the selectivity of PSI on tumor cells, human ovarian surface epithelial, non–cancer-derived airway epithelial, vascular smooth muscle, and human meningal cells were treated with PSI. PSI treatment resulted in only a marginal toxic effect on noncancer cells (Fig. 1B).

Because our primary interest was ovarian cancer, further studies were conducted using only SKOV3 cells. Kinetic studies (Fig. 1C) showed that PSI treatment inhibited SKOV3 cell growth in a concentration-dependent manner. Interestingly, at the same concentration, PSI killed more cells than did our positive control, VP16 (Fig. 2C). For example, the maximum inhibition ratio attained with 10 μmol/L PSI treatment was 81.3%, compared with 70.6% inhibition produced by 10 μmol/L VP16. PSI treatment also had lower IC50, TGI, and LC50 values (3.1, 9.3, and 9.0 μmol/L, respectively) than control treatments had (3.2, 9.7, and 15.9 μmol/L, respectively). Flow cytometric analysis further suggested that PSI treatment increased the population of cells trapped in the G2 phase and reduced the population of cells in the G0/G1 and S phases in a concentration-dependent manner (Fig. 1D).

**PSI Induces Apoptosis in Ovarian Cancer Cells**

To determine the mechanism involved in PSI inhibition of tumor cell growth, we examined the effects of PSI on SKOV3 cells. Kinetic studies with fluorescence-activated cell sorting analysis indicated that prolonged exposure of SKOV3 to PSI led to an increase in the incidence of apoptosis (Fig. 2A and B) in a time-dependent manner. For example, compared with the untreated SKOV3 cells, PSI treatment resulted by up to 97.9% cell death after a 48-hour treatment. Interestingly, 12-hour PSI treatment induced anoikis, a phenomenon in which cells shrink and detach from the culture surface (data not shown). DNA fragmentation assays, however, indicated that PSI-treated SKOV3 cells produced oligonucleosomal DNA ladders (Supplementary Fig. S2A), a typical characteristic of cells undergoing apoptosis. Using transmission electron microscopy, we further showed that PSI-treated SKOV3 cells displayed morphologic changes characteristic of apoptosis (Supplementary Fig. S2B). Specifically, ultrastructural images showed complete membrane blebbing, fragmentation with apoptotic bodies, swelling of organelles (e.g., mitochondrial and Golgi bodies), and nuclear condensation.

The presence of integral cell membranes in treated cells also suggested that PSI induced apoptosis but not necrosis. Together, these data indicate that PSI treatment led to the induction of apoptosis in SKOV3 cells. Indeed, PSI-induced cancer cell death in a concentration-dependent manner. Figure 2C shows that the percentage of apoptotic cells increased with increased PSI concentrations. TUNEL staining for PSI-treated SKOV3 showed a large percentage of TUNEL-positive cells (Fig. 2E). In contrast, the control group showed minimal fluorescence, signifying a low percentage of TUNEL-positive cells. Together, these results indicate that PSI inhibits the proliferation of cancer cells by inducing apoptosis or cell cycle arrest or both.

**PSI Activates the Mitochondrial Apoptosis Pathway and Down-Regulates Proteins in the Mitogen-Activated Protein Kinase Pathway**

To further determine the apoptotic pathway involved in the response of cells to PSI treatment, we examined the components of the apoptotic pathways. The initial observation that a 4-hour PSI exposure resulted in significantly increased levels of cytochrome c prompted us to examine the mitochondrial apoptotic pathway. The 24-hour PSI treatment led to the activation of several apoptotic proteins [such as caspase-9, caspase-3, and its substrate, poly(ADP-ribose) polymerase] by cleavage (Fig. 3A). Increased concentrations of PSI resulted in the disappearance of the intact proteins and the appearance of proteolytic cleavage bands in a concentration-dependent manner. Furthermore, increased levels of the proapoptotic protein Bax and a dramatic reduction of Bcl-2 levels were also associated with increased PSI concentrations (Fig. 3A). The reduction of Bcl-2 levels is known to correlate with endoplasmic reticulum (ER) stress and the induction of CCAAT/enhancer binding protein homologous transcription factor (CHOP; refs. 27, 28). A recent study also showed that synthetic PSI treatment promoted ER-stress-mediated apoptosis, elevated levels of CHOP, and reduced levels of Bcl-2 (14). For confirmation, we also examined these findings on PSI-treated SKOV3 cells. Consistent with these studies, reduced Bcl-2 levels were accompanied with increased levels of CHOP in treated SKOV3 cells (Fig. 3A).

Immunochemical studies also showed that in PSI-treated cells, Bcl-2 levels were lower than in the controls. In contrast, the treatment promoted higher levels of Bax, caspase-3, and caspase-9 in a concentration-dependent manner (Fig. 3B and C). Increased concentrations of PSI in treated SKOV3 cells resulted in elevated levels of cytosolic cytochrome c and reduced levels of mitochondrial cytochrome c (Fig. 3A). Because cytochrome c is often involved in changes in mitochondrial membrane permeabilization, we further exposed PSI-treated cells to cyclosporine A, which is likewise known to block the pores in intact mitochondria and to suppress apoptosis (29–31). Figure 3D shows that cyclosporine A treatment indeed led to a dramatic (50%) decrease in cell death. Collectively, these data suggest that PSI induces cancer cell death through the mitochondrial apoptotic pathway.

**PSI Inhibits ERK1/2 Activation**

The inhibition of either the mitogen-activated protein kinase (MAPK) pathway or Akt activation has been shown to induce apoptosis in tumor cells (32–34). Hence, to determine whether PSI inhibits tumor cell growth, particularly in SKOV3 cells, and simultaneously promotes apoptosis by modulating the ERK1/2 pathway, we analyzed the ERK/MAPK pathway involved in PSI-induced apoptosis. We found that PSI decreased the levels of phosphorylated ERK1/2 without significantly altering total ERK levels. However, in the presence of PSI, ERK activity may have been partially maintained by the ERK activator ghrelin (ref. 35; Fig. 4A and B). A TUNEL assay was used to verify this effect (Fig. 4C). Using an ERK inhibitor, PD98059, as a
control, we found that PSI and PD98059 had similar effects. The apoptotic incidences increased in both the PSI-treated and PD98059-treated groups. However, this proapoptotic effect was reversed in the presence of ghrelin. These data suggest that PSI treatment reduces ERK activity. PSI treatment also caused a noticeable reduction in the phosphorylated levels of Akt in a concentration-dependent manner, suggesting that Akt may be another target of PSI (Fig. 4D).

**PSI Inhibits SKOV3-Derived Tumor Growth**

Having shown that PSI suppresses tumor cell growth, we sought to investigate its antitumor effects in a xenograft mouse model of ovarian cancer. As shown in Fig. 5A, PSI treatment caused a significant delay in tumor growth. At the terminus of the experiments, the i.p. administration of PSI at 15 and 25 mg/kg also led to reductions in tumor growth of 38% and 66%, respectively, compared with that in control mice treated with vehicle solution of DMSO (< 0.1%) diluted in saline solution \( (P < 0.05) \). No toxic effects were observed in PSI-treated mice. Most intriguing, as shown in Fig. 5B, the data also suggest that p.o. administration of PSI had effects similar to those of i.p. administration; at the terminus of the study, the p.o. administration of PSI at 30 mg/kg reduced the tumor weight by 52%.

To further validate the mechanism by which PSI exerts its effects, we analyzed the in vivo expression of the crucial apoptosis-related proteins. Western blot analyses of tumor samples indicated PSI dose–dependent decreases in Bcl-2 expression levels. In contrast, CHOP, Bax, and activated caspase-3 levels were increased in a dose-dependent manner (Fig. 5C). Altogether, PSI seems to exert its effects via the mitochondrial apoptotic pathway.

**Discussion**

For thousands of years, *Rhizoma Paridis* and its components have been used extensively as anti-inflammatory, hemostatic regulatory, antibacterial, antifungal, and antimicrobial medications in China (36, 37). Whereas PSI has been approved for cancer therapy in China, its biology and mechanism of action in treating cancer are not well understood. In the present study, we have investigated the effects of PSI isolated from *Rhizoma paridis* on the biology of tumor cells. PSI exerted growth inhibition on several cancer cell lines but not on nonneoplastic derived cells. Specifically, PSI inhibited the viability of SKOV3 cells at low IC\(_{50}\) TGI, and LC\(_{50}\) suggesting that it has a powerful cytotoxic effect relative to VP16, a widely used chemotherapeutic medicine. The inhibitory effects of PSI were associated with increased
levels of proapoptotic Bax, cytochrome c, active caspase-9, active caspase-3, and cleaved poly(ADP-ribose) polymerase. PSI also decreased antiapoptotic Bcl-2 expression levels and phosphorylated ERK1/2 in treated cells. We further showed that i.p. and p.o. PSI treatment inhibited the tumor growth of SKOV3 cells in an athymic xenograft mouse model. These results suggest that PSI mediates biological inhibition in cancer cells through ERK/MAPK and the mitochondrial apoptosis pathway.

Signals from distinct signaling cascades of the MAPK family such as stress-activated protein kinase/c-jun NH2-terminal kinase, p38 MAPK, and ERK 1/2 are known to dictate cell fate during DNA damage, mitogenic stimuli, and survival (38). c-jun NH2-terminal kinase was reported to mediate Fas-induced apoptosis in neuronal cells (39), and p38 MAPK has certain cytoprotective effects in nonneuronal cells (40). The activation of the ERK pathway by growth factors often stimulates cell differentiation, mitosis, and hypertrophy (41); ERK MAPK also phosphorylates caspase-9 at Thr125 resulting in the inhibition of caspase-9 processing and caspase-3 activation (42). In the present study, the reduction of ERK MAPK activation in a dose-dependent manner, accompanied by the activation of caspase-9 and caspase-3, supports the notion that PSI elicits its inhibitory effects through multiple targets: impeding the supporting cell-survival function of ERK and activating apoptotic signals.

PSI contains both a glycon and a rhamnose structure known to bind to a special agglutinin receptor before being internalized. The uptake allows the glycon structure to target the mitochondria and other organelles, resulting in apoptosis (43). In our study, we found that PSI treatment altered the expression levels of several components of the intrinsic mitochondrial apoptotic pathway [i.e., Bax, cytochrome c, caspase-9, caspase-3, and poly(ADP-ribose) polymerase]. The release of cytochrome c can be attributed to ER stress, although not exclusively. ER stress triggers the release of Ca2+, leading to a series of events that include the collapse of mitochondrial potential, the permeabilization of the inner membrane, the release of cytochrome c, and the
activation of procaspase-9 (44). ER stress also induces CHOP (28), which plays a proapoptotic role in severe irre- mediably ER stress (45) by down-regulating Bcl-2 expres- sion levels (27). Bcl-2 is among the important regulators of cytochrome c release from the mitochondria, and the reduc- tion of Bcl-2 levels promotes cytochrome c release, leading to the activation of programmed cell death (46, 47). In the current study, we observed significant increases in the levels of Bax and CHOP in parallel with the dramatic reduction of Bcl-2 expression in PSI-treated tumor cells. Altogether, these data suggest that PSI treatment elicits ER stress and medi- ates intrinsic apoptotic pathways by altering the expression levels of several regulators of these pathways, such as Bcl-2, Bax, and CHOP. Whether PSI activates the mitochondrial apoptotic pathway via ER stress–mediated apoptosis pathways (or simultaneously activates both) is a question for future studies. Here, we present a foundation for the mechanism of action of PSI in cancer cells as illustrated in Fig. 5D. PSI treatment modulates Bcl-2/(Bcl-xL) levels, result- ing in the increase of mitochondrial membrane permea- bilization. The decrease in Bcl-2 and the increase in Bax lead to the release of cytochrome c, which subsequently activates caspase-3 and caspase-9.

In this pilot study, using a currently available model of ovarian cancer, SKOV3 ovarian carcinoma, we have shown that PSI is a potent anticancer agent that can be adminis- tered p.o. or i.p. Previously, a randomized controlled trial of ovarian cancer treatments showed that a combination of i.p. and i.v. chemotherapy yields better survival outcomes than i.v. treatment alone (48). Because early-stage ovarian cancer is mostly restricted to the abdominal cavity, i.p. drug delivery may allow clinicians to administer higher doses of therapy to the tumor site than are allowed under current standards of care, which have been set to limit the toxic effects and the damage to surrounding healthy tissue.

We have shown in our current study that the i.p. adminis- tration of PSI dramatically decreased tumor sizes by 66% after a 4-week treatment period. Most interesting, p.o. ad- ministration of PSI also yielded inhibitory effects compara- ble with those resulting from i.p. administration (52–66%). This attribute confers advantages to PSI that other i.v. agents lack, including convenience, reduction of complica- tions associated with long-term venous access, and cost sav- ings. Nevertheless, because the etiology of ovarian cancer remains elusive (49), additional studies using different ovar- ian cancer model systems should be used to fully establish the therapeutic potential of PSI for ovarian cancer patients at all stages.

In conclusion, after isolating PSI from Rhizoma paridis and systematically showing for the first time, to our knowledge, the antitumor effects of PSI, we have shown that PSI can inhibit ERK activation, induce apoptosis via the mitochon- drial-mediated caspase activation pathway, and inhibit tu- mor growth in a xenograft model of ovarian cancer. Of greatest interest to us was that PSI displayed salient antitu- mor activity, oral availability, and antitumor selectivity, in- dicating that PSI may have great therapeutic potential in clinical settings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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