Paeonol enhances the sensitivity of human ovarian cancer cells to radiotherapy-induced apoptosis due to downregulation of the phosphatidylinositol-3-kinase/Akt/phosphatase and tensin homolog pathway and inhibition of vascular endothelial growth factor

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Abstract. Radiotherapy is a vital and effective method to treat solid tumors. However, in many tumor types, development of resistance of cancer cells and cytotoxicity in normal tissues presents a major therapeutic problem. It is therefore crucial to identify and develop novel sensitizing agents that may improve the response to radiation therapy without causing any adverse effects. The present study aimed to investigate whether paeonol, a bioactive flavonoid, was able to confer sensitivity to radiation in human ovarian cancer cells. The human ovarian cancer cell lines SKOV-3 and OVCAR-3 were exposed to varying doses of radiation (2, 4 or 6 Gy) in the presence or absence of paeonol (25, 50 or 100 μM). Radiosensitivity was assessed by measuring cell viability using a CCK-8 assay and Annexin V/PI staining. Expression of vascular endothelial growth factor (VEGF), hypoxia inducible factor-1α (HIF-1α), proteins of the phosphatidylinositol-3-kinase (PI3K)/Akt pathway and apoptotic pathway proteins [caspase-3, Bcl-2-associated death promoter, B-cell lymphoma (Bcl)-2, Bcl-2-associated X and Bcl-extra large (Bcl-xL)] were also assessed. Paeonol treatment enhanced apoptosis of SKOV-3 and OVCAR-3 cells that were exposed to radiation. The expression of Bcl-2 and Bcl-xL were markedly upregulated in these cells. Treatment with paeonol concentrations of 50 and 100 μM caused a significant downregulation of VEGF, HIF-1α and PI3K/Akt pathway proteins. Paeonol effectively enhanced the sensitivity of ovarian cancer cells to radiation by significantly altering regulation of the proteins of the PI3K/Akt pathway, in addition to downregulating VEGF and HIF-1α.

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Introduction

Ovarian cancer is the most prevalent cancer amongst women, and is the third most frequent gynecological cancer, representing the most common cause of gynecological cancer-associated mortalities (1). Approximately about 70% of ovarian cancers are diagnosed at advanced stages and, even following surgery and chemotherapy, have high rates of recurrence (42-48%) due to presence of residual disease at microscopic levels (2,3). Radiation therapy may be employed to eliminate this residual disease that is subsequently responsible for recurrence (4). Several reports indicate an increased survival rate following radiation therapy when used in combination with surgery or with surgery and chemotherapy (5,6). Radiotherapy has been demonstrated to induce a cytotoxic response in chemotherapy-resistant ovarian cancers, which increases the possibility of improved tumor control (7). However, cytotoxicity is considered to be a key limiting factor of radiotherapy use; therefore, agents that sensitize ovarian cancer cells to radiation therapy may be of significant clinical value in treatment of chemotherapy-resistant cancers, and in reducing the recurrence of cancer.

Tumor hypoxia is a common feature of malignant tumors, and contributes to resistance to radiotherapy (8). Hypoxia inducible factor-1 (HIF-1) is one of the key regulators of cell response to hypoxic conditions. HIF-1α, the oxygen-sensitive subunit of HIF-1, regulates the expression of numerous downstream target genes, such as vascular endothelial growth factor (VEGF), and is implicated in tumor resistance to radiotherapy and to chemotherapy (9,10).

Furthermore, the phosphatidylinositol-3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway regulates cell growth and proliferation. This pathway exerts crucial roles in apoptosis, tumor generation, tumor development and metastasis, and has been reported to be

involved in resistance to radiotherapy (11,12). In addition, phosphatase and tensin homolog (PTEN), a negative regulator of the PI3K/Akt/mTOR pathway, is a highly effective tumor suppressor (13). PTEN has also been reported to be frequently mutated in multiple human cancers (14,15). Owing to the crucial roles in tumor pathogenesis, the PI3K/Akt/mTOR pathway is a critical target in cancer therapy. Therefore, identification of inhibitors of the pathway could potentially prevent tumor development and also may represent sensitizing agents to radiotherapy in cancer treatment.

Accumulating experimental data have demonstrated the potential of natural products as antitumor drugs (16). Paeonol (4-methoxy-2-hydroxyacetophenone), an active compound from the root bark of *Paeonia suffruticosa*, is used in traditional Chinese medicines (17,18). Paeonol possesses various pharmacological properties, including antioxidant, anti-inflammatory and immunomodulatory effects (19-21). Lee *et al* (22) previously reported that paeonol inhibited cell migration and angiogenesis by downregulating PI3K/Akt signaling. Paeonol was also reported to sensitize lung adenocarcinoma cells to radiotherapy (23). Considering these diverse biological effects, the current study investigated whether paeonol inhibits tumor development in ovarian cancer cells, and whether it sensitizes these cells to radiation.

Materials and methods

Cell lines, equipment and reagents. The human ovarian carcinoma cell lines SKOV-3 and OVCAR-3 were obtained from ATCC and were cultured according to the instructions provided by ATCC. Cells were incubated with various doses of paeonol (0, 25, 50 or 100 μM) for 12 h and subsequently exposed to X-ray radiation for 24 h, at doses of 0, 2, 4 or 6 Gy. Irradiation was performed at a dose rate of 0.40 Gy/min using a 180-KVp X-ray generator [IXS2050; VJ Technologies China (Suzhou) Co., Ltd., Suzhou, China]. Paeonol was procured from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Antibodies against VEGF (cat. no. 2463), HIF-1α (cat. no. 79233), β-actin (cat. no. 3700), caspase-3 (cat. no. 9662; all Cell Signaling Technology Inc., Danvers, MA, USA), B-cell lymphoma (Bcl)-2 (cat. no. sc-509), Bcl-2-associated death promoter (Bad; cat. no. sc-943), Bcl-2-associated X (Bax; cat. no. sc-4239), Bcl-extra-large (xL; cat. no. sc-136132), Akt (cat. no. sc-24500), p-Akt (cat. no. sc-135650), glycogen synthase kinase (GSK)-3β (cat. no. sc-221692), p-GSK-3β (cat. no. sc-81494), mammalian target of rapamycin complex 1 (mTORc1; cat. no. sc-293089) and phosphatase and tensin homolog (PTEN; cat. no. sc-400103; all Santa Cruz Biotechnology, Inc., Dallas, TX, USA) were used for western blotting. All antibodies were used at 1:1,000.

All other reagents used in the study were of analytical grade and were procured from Sigma-Aldrich unless otherwise specified.

Cell viability assay. The sensitivity of SKOV3 and OVCAR-3 to radiation and/or paeonol was assayed using a Cell Counting kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan). Cells were incubated with paeonol (25, 50 or 100 μM) for 12 h and then exposed to radiation. The control cells were exposed to equal volume of DMSO instead of paeonol. After radiation exposure for 24 h, the cells were seeded in a 96-well plate at a density of $1x10^4$ cells/well, and incubated for 48 h in an atmosphere containing 5% CO_2 at 37˚C. CCK-8 solution (10 μl) was added to each well, and the plate was incubated for 2 h at room temperature. The absorbance at 450 nm was measured using a microplate reader (Synergy HT; Bio-Tek Instruments, Inc., Winooski, VT, USA).

Flow cytometry analysis for cell viability. Following irradiation for 24 h, the cells were collected, resuspended in fresh medium and analyzed for viability using an Annexin V-fluorescein isothiocyanate apoptosis detection kit (BD Biosciences, San Jose, CA, USA), according to the manufacturer's instructions. The apoptotic cells were detected by flow cytometry (FACSCalibur with CellQuest software version 5.1; BD Biosciences).

Colony formation assay. A colony formation assay was performed as previously described (24). Following irradiation, cells ($1x10^3$ cells/plate) were seeded into 60-mm Petri dishes with standard culture medium (RPMI 1640; Sigma-Aldrich; Merck KGaA) and incubated at 37˚C in an atmosphere containing 5% CO_2. After 14 days of incubation, the cells were fixed with 4% formaldehyde and stained with crystal violet. Colonies containing >50 cells were counted and the surviving fraction was calculated by normalization to their respective non-irradiated control. The surviving fraction (SF) for a given dose of paeonol was calculated as follows: SF=number of colonies following irradiation/the number of cells inoculated x cell planting rate (i.e., the number of cells in the appropriate 0 Gy group). The multitarget click model in GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA) was used to determine the cell survival curves.

Reverse transcription (RT)-polymerase chain reaction (PCR). Following exposure to paeonol and irradiation, cells were subjected to RT-PCR analysis to assess the expression of VEGF. The cells ($1x10^6$ cells) were lysed and total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol, followed by cDNA synthesis (1 μg RNA was used for cDNA synthesis) as described previously (25). VEGF-A mRNA levels were determined by PCR using a One-Step SYBR PrimeScript RT-PCR kit (Takara, Inc., Otsu, Japan) using a Thermal Cycler Dice Real Time System II (Takara Bio, Shiga, Japan) according to the manufacturer's protocol. The thermocyclying conditions were as follows: Initial denaturation at 95˚C for 8 sec followed by 45 cycles of denaturation at 95˚C for 3-6 sec and annealing and extension at 60˚C for 35-38 sec. The primers used were as follows: VEGF-A: Forward, 5'-CCAGCAGAAAGAGGAAAG AGGTAG-3'; reverse, 5'-CCCCAAAAGCAGGTCACTCAC-3'; GAPDH: Forward, 5'-GAAGGTGAAGGTCGGAGTC-3'; and reverse, 5'-GAAGATGGTGATGGGATTTC-3'. VEGF expression levels were normalized to those of GAPDH expression. The intensity of the PCR products was determined using Image Lab version 4.1 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Western blotting. Cells were harvested after 24 h following irradiation and were lysed as described previously (26). Protein concentration was determined by Bradford assay using a protein assay kit (Bio-Rad Laboratories, Inc.). An equal concentration

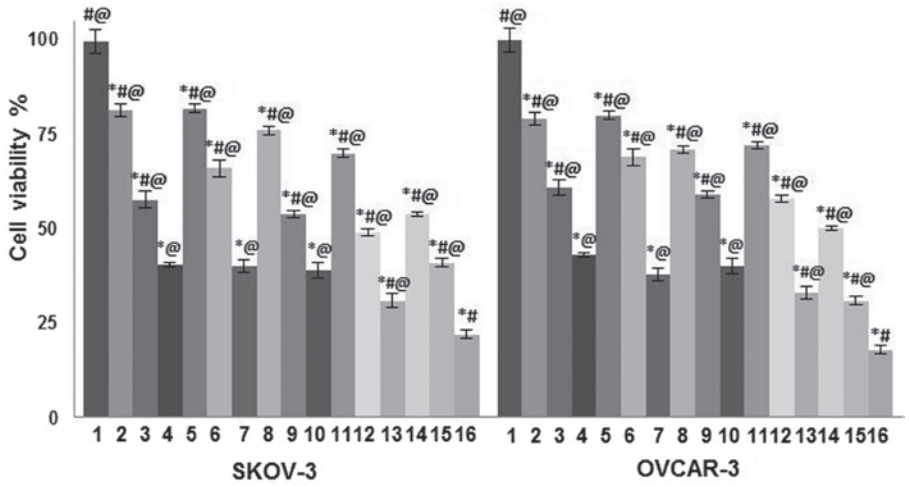

Figure 1. Effect of paeonol on cell viability, determined by a cell counting kit-8. Values are presented as mean ± standard deviation; n=6 experiments. *P<0.05 vs. control, #P<0.05 vs group 7, @P<0.05 vs. group 16. 1, Control; 2, 25 μM paeonol; 3, 50 μM paeonol; 4, 100 μM paeonol; 5, 2 Gy; 6, 4 Gy; 7, 6 Gy; 8, 25 μM paeonol + 2 Gy; 9, 50 μM paeonol + 2 Gy; 10, 100 μM paeonol + 2 Gy; 11, 25 μM paeonol + 4 Gy; 12, 50 μM paeonol + 4 Gy; 13, 100 μM paeonol + 4 Gy; 14, 25 μM paeonol + 6 Gy; 15, 50 μM paeonol + 6 Gy; 16, 100 μM paeonol + 6 Gy.

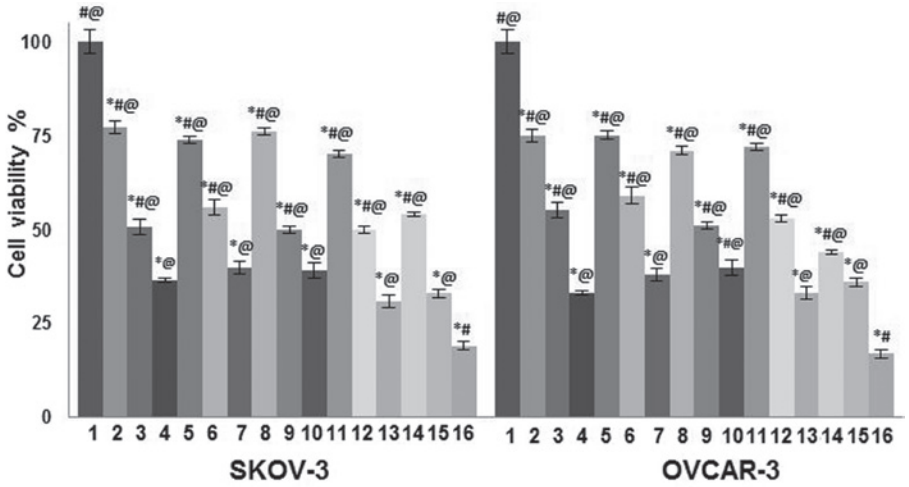

Figure 2. Effect of paeonol on apoptosis in ovarian cancer cells, based on flow cytometric analysis. Values are presented as mean ± standard deviation; n=6 experiments. *P<0.05 vs. control, #P<0.05 vs. group 7, @P<0.05 vs. group 16. 1, Control; 2, 25 μM paeonol; 3, 50 μM paeonol; 4, 100 μM paeonol; 5, 2 Gy; 6, 4 Gy; 7, 6 Gy; 8, 25 μM paeonol + 2 Gy; 9, 50 μM paeonol + 2 Gy; 10, 100 μM paeonol + 2 Gy; 11, 25 μM paeonol + 4 Gy; 12, 50 μM paeonol + 4 Gy; 13, 100 μM paeonol + 4 Gy; 14, 25 μM paeonol + 6 Gy; 15, 50 μM paeonol + 6 Gy; 16-100 μM paeonol + 6 Gy.

of total protein (60 μg) from cells of each treatment group was subjected to electrophoresis by SDS-PAGE, and the separated proteins were blotted and transferred onto nitrocellulose membranes. The membranes were blocked with 5% skim milk and incubated with primary antibodies overnight at 4˚C. This was followed by incubation with horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h. The immunoreactive bands were detected by enhanced chemiluminescence (GE Healthcare Life Sciences, Little Chalfont, UK). The densities of the positive bands were further analysed by ImageQuant TL software (version 7.0; GE Healthcare Life Sciences). The analyzed protein band densities were normalized to those of β-actin using anti-β-actin antibodies.

Statistical analysis. The values obtained are presented as mean ± standard deviation, from six independent experiments. The data were analyzed for statistical significance by one-way analysis of variance and post-hoc analysis by Duncan's Multiple Range Test. P<0.05 was considered to indicate a statistically significant difference.

Results

Paeonol reduces the viability of ovarian cancer cells. The cytotoxic effects of paeonol on non-irradiated and irradiated carcinoma cells were determined using a CCK-8 assay. Paeonol significantly inhibited the proliferation of all cell lines in a dose-dependent manner, irrespective of exposure to radiation (P<0.05; Fig. 1). Paeonol also improved the sensitivity of SKOV-3 and OVCAR-3 cells to radiation, in a dose-dependent manner; when compared with the cells irradiated but not treated with paeonol, viability was significantly reduced following combined exposure (P<0.05) (Fig. 1). Flow cytometric analysis for assessment of apoptotic cell counts by Annexin V/FITC staining revealed comparable results (Fig. 2). In both assays, the highest dose of paeonol (100 μM)

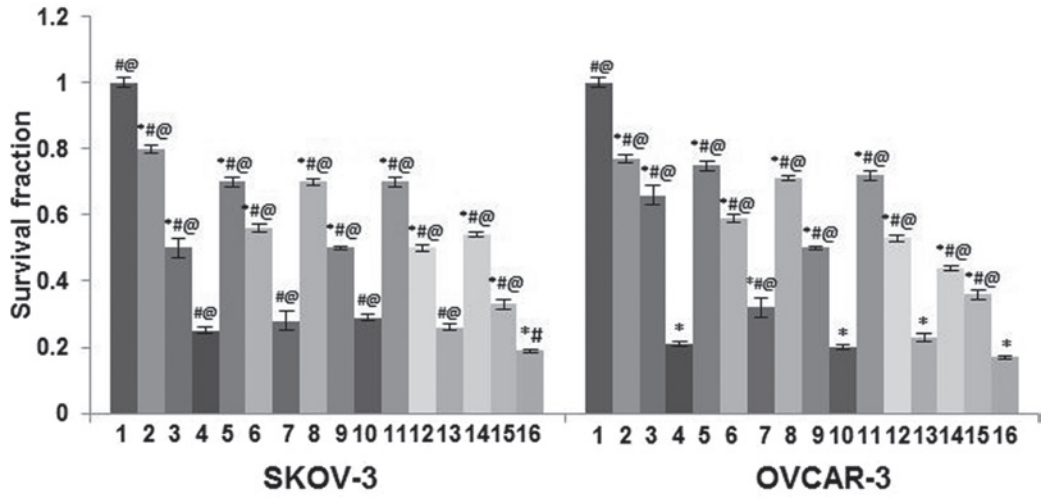

Figure 3. Effect of paeonol on survival fraction in ovarian cancer cells, based on a clonogenic assay. Values are presented as mean ± standard deviation; n=6 experiments. *P<0.05 vs. control, #P<0.05 vs. group 7, @P<0.05 vs. group 16. 1, Control; 2, 25 μM paeonol; 3, 50 μM paeonol; 4, 100 μM paeonol; 5, 2 Gy; 6, 4 Gy; 7, 6 Gy; 8, 25 μM paeonol + 2 Gy; 9, 50 μM paeonol + 2 Gy; 10, 100 μM paeonol + 2 Gy; 11, 25 μM paeonol + 4 Gy; 12, 50 μM paeonol + 4 Gy; 13, 100 μM paeonol + 4 Gy; 14, 25 μM paeonol + 6 Gy; 15, 50 μM paeonol + 6 Gy; 16-100 μM paeonol + 6 Gy.

exhibited the maximum cytotoxic effects. These results indicate that paeonol effectively inhibited viability of cancer cells and induced apoptosis, in addition to enhancing sensitivity of the two cell lines to radiation.

Paeonol sensitizes ovarian cancer cells to radiation. Treatment with paeonol (25, 50 or 100 μM) significantly enhanced the intrinsic sensitivity of both SKOV-3 and OVCAR-3 cells to radiation, as assessed by clonogenic survival assays as compared with cells exposed to radiation alone (P<0.05; Fig. 3). The surviving fraction of the cells was significantly reduced with an increase in radiation dose from 4 to 6 Gy (P<0.05). The cells exposed to paeonol and radiation presented a much lower SF than the cells exposed to either radiation or paeonol. The cells exposed to 6 Gy radiation and 100 μM paeonol were observed to have a significantly lower SF compared with cells exposed to 4 and 2 Gy radiation, irrespective of paeonol concentration. Nevertheless, paeonol at all doses was demonstrated to be more effective in sensitizing SKOV-3 cells to radiation than OVCAR-3 cells.

Paeonol modulates the expression of apoptotic pathway proteins. To assess the molecular events associated with reduced cell viability of the SKOV-3 and OVCAR-3 cells following exposure to paeonol, the expression of caspase-3 and pro-apoptotic (Bad and Bax) and anti-apoptotic (Bcl-2 and Bcl-xL) proteins were determined. Paeonol treatment was revealed to enhance the expression of caspase-3 in a dose-dependent manner (Fig. 4). Furthermore, corresponding with the apoptotic cell counts observed through Annexin V/PI staining, the expression of Bad and Bax proteins was significantly upregulated in cells exposed to paeonol and/or radiation. However, combined exposure revealed strikingly increased levels of apoptotic proteins, with markedly downregulated Bcl-2 and Bcl-xL proteins. This expression analysis suggests the possible involvement of apoptotic proteins in paeonol-mediated enhanced sensitivity to radiation in the SKOV-3 and OVCAR3 cells.

Paeonol downregulates HIF-1α and VEGF expression. HIF-1, the expression of which is induced by hypoxic tumor conditions, has been implicated in radiation-resistant tumor cells (27), and HIF-1 regulates the expression of VEGF (28). PCR and Western blot analyses to determine VEGF and HIF-1α expression revealed that expression of these mRNA sequences and corresponding proteins may be modulated by paeonol (Fig. 5). Notably, downregulated expression was observed in irradiated cells with no exposure to paeonol. However, paeonol at doses of 50 and 100 μM caused significantly greater reductions in expression levels of both HIF-1α and VEGF, and the expression level of VEGF mRNA corresponded to this, suggesting that paeonol affected the expression at the gene level. These expression analyses revealed the involvement of HIF-1 and VEGF in the paeonol-induced response.

Effects of paeonol on the proteins of PI3K/Akt signaling cascade. The PI3K/Akt signaling pathway serves an important role in the regulation of cell survival, proliferation and apoptosis (29). Previous studies have indicated that inhibition of the pathway effectively enhances the sensitivity of lung cancer cells and cervical cancer cells to radiation (30,31). The present study noted markedly higher expression of Akt, p-Akt and mTORc1 in ovarian cancer cells that were not exposed to paeonol or radiation (Fig. 6). Irradiated cells in all paeonol treatment groups exhibited significantly downregulated expression of Akt and p-Akt, GSK-3β, p-GSK-3β and mTORc1, and markedly increased PTEN expression. Furthermore, this downregulation was more pronounced with 100 μM paeonol concentration at all radiation dose levels. These observations suggest that the inhibition of the PI3K/Akt pathway by paeonol may enhance the sensitivity of ovarian cancer cells to radiation.

Discussion

Ovarian cancer is one of the prevalent cancers in women (1) and radiotherapy is often employed in treatment following surgery (4). However, development of resistance to radiation

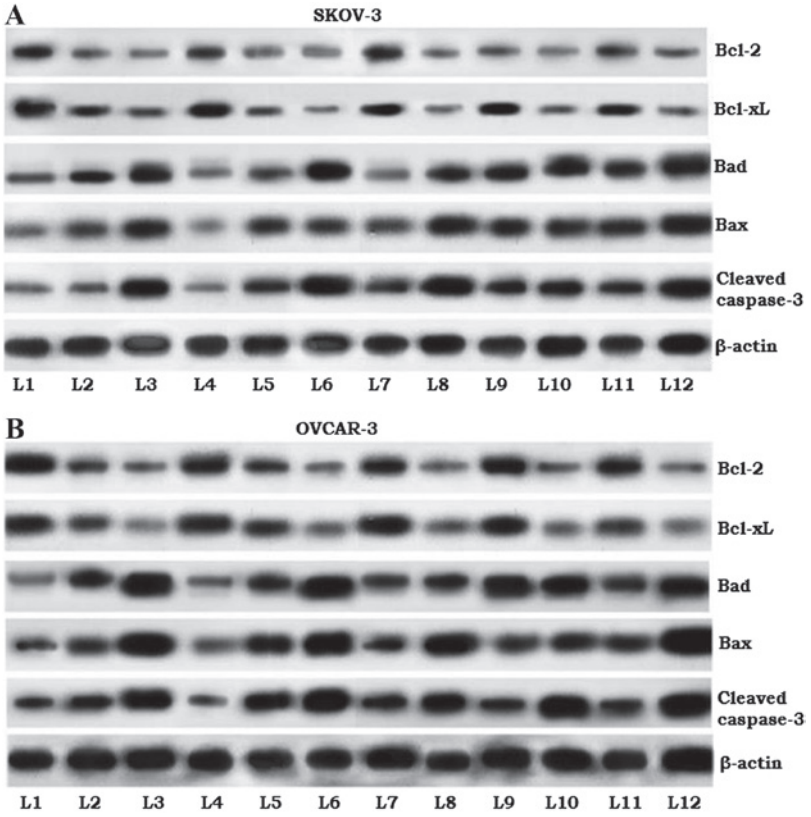

Figure 4. Effects of paeonol on the expression of apoptosis pathway proteins in (A) SKOV3 and (B) OVCAR-3 cells. L1, control; L2, 50 μM paeonol only; L3, 100 μM paeonol only; L4, 2 Gy radiation only; L5, 4 Gy radiation only; L6, 6 Gy radiation only; L7, 50 μM paeonol + 2 Gy radiation; L8, 100 μM paeonol + 2 Gy radiation; L9, 50 μM paeonol + 4 Gy radiation; L10, 100 μM paeonol + 4 Gy radiation; L11, 50 μM paeonol + 6 Gy radiation; L12, 100 μM paeonol + 6 Gy radiation. Bcl-2, B-cell lymphoma-2; Bcl-xL, Bcl-extra large; Bad, Bcl-2-associated death promoter; Bax, Bcl-2-associated X.

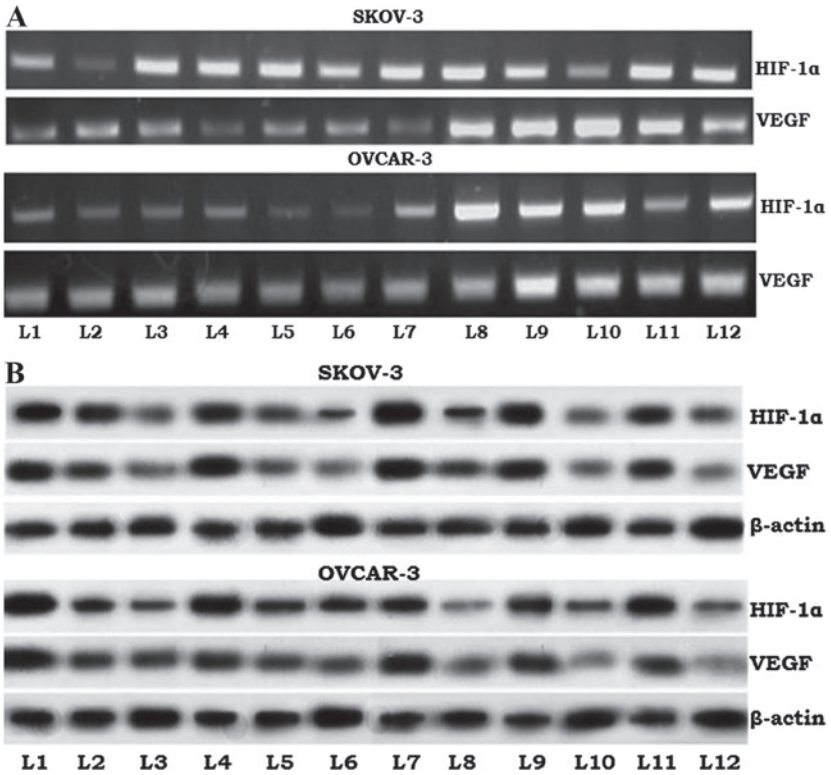

Figure 5. Effects of paeonol on the apoptosis pathway components HIF-1α and VEGF in the ovarian cancer cell lines SKOV-3 and OVCAR-3, as observed by (A) reverse transcription-polymerase chain reaction and (B) western blotting. L1, control; L2, 50 μM paeonol only; L3, 100 μM paeonol only; L4, 2 Gy radiation only; L5, 4 Gy radiation only; L6, 6 Gy radiation only; L7, 50 μM paeonol + 2 Gy radiation; L8, 100 μM paeonol + 2 Gy radiation; L9, 50 μM paeonol + 4 Gy radiation; L10, 100 μM paeonol + 4 Gy radiation; L11, 50 μM paeonol + 6 Gy radiation; L12, 100 μM paeonol + 6 Gy radiation. HIF, hypoxia inducible factor; VEGF, vascular endothelial growth factor.

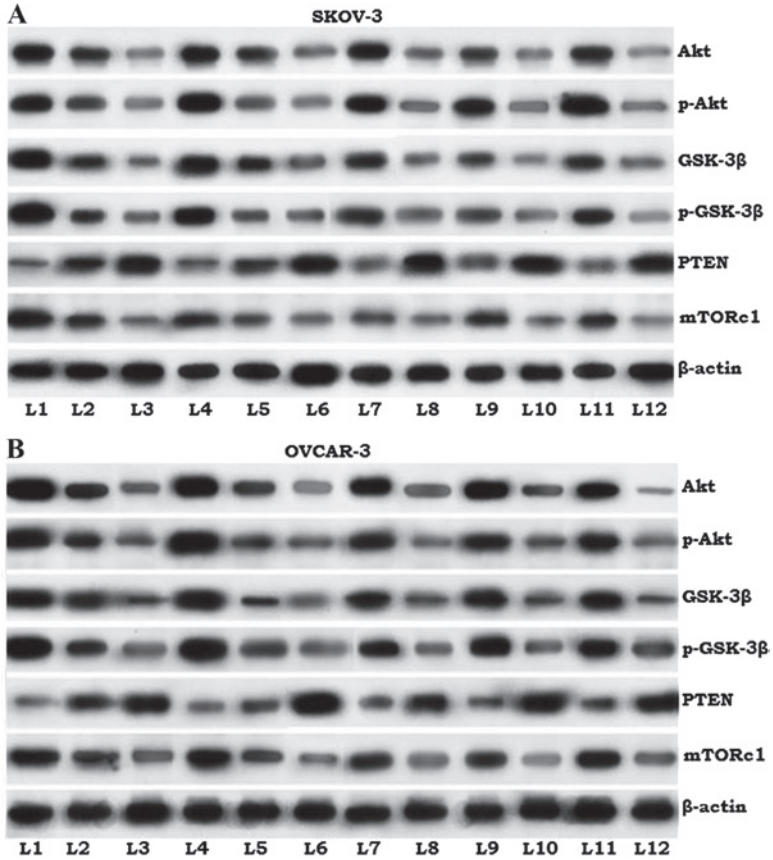

Figure 6. Effects of paeonol on proteins of the PI3K/Akt/mTOR pathway in (A) SKOV3 and (B) OVCAR-3 cells. L1, control; L2, 50 μM paeonol only; L3, 100 μM paeonol only; L4, 2 Gy radiation only; L5, 4 Gy radiation only; L6, 6 Gy radiation only; L7, 50 μM paeonol + 2 Gy radiation; L8, 100 μM paeonol + 2 Gy radiation; L9, 50 μM paeonol + 4 Gy radiation; L10, 100 μM paeonol + 4 Gy radiation; L11, 50 μM paeonol + 6 Gy radiation; L12, 100 μM paeonol + 6 Gy radiation. GSK, glycogen synthase kinase; PTEN, phosphatase and tensin homolog; mTORc1, mammalian target of rapamycin complex 1.

is currently a major obstacle. Understanding the mechanisms and the signaling pathways associated with regulation of resistance to radiation is crucial in development of approaches to overcome this. Previous research has focused on identifying tumor-specific sensitizing agents to enhance radiotherapy, and multiple studies have demonstrated the effect of many natural compounds, including curcumin and berberine, in improving sensitivity of cancer cells to radiation (31-33). A previous study has also reported the antitumor effects of paeonol on human lung adenocarcinoma cells (34).

In the present study, CCK-8 assays revealed that paeonol markedly reduced the viability of SKOV-3 and OVCAR-3 cells that were subjected to radiation. The antitumor effects of paeonol have previously been demonstrated to be associated with induction of apoptosis, cell cycle arrest, activation of interleukin-2 and tumor necrosis factor-α, and modulation of Bcl-2 and Bax expression in tumor cells have been reported (35-38).

A decrease in the survival fraction (clonogenic assay) and paeonol-enhanced apoptosis (AnnexinV/PI staining assay) were observed in the current study. Furthermore, paeonol was also revealed to modulate the expression of apoptotic proteins in support of these apoptotic counts. A significant increase in caspase-3, Bad and Bax levels, and downregulation of Bcl-2 and Bcl-xL were observed. Paeonol was also reported, in the present study, to enhance the expression of pro-apoptotic proteins in irradiated cells, suggesting an induction of apoptosis. These observations indicate that paeonol markedly increased the sensitivity of ovarian cancer cells to radiation.

Previous studies have suggested that hypoxia is one of the most influential factors in resistance of solid tumors to radiation (27,39-41). Hypoxia in tumors results from the imbalance between the increased oxygen consumption caused by extensive growth of tumor cells and poor oxygen delivery by disorganized tumor blood vessels (42). Drugs that target the hypoxia associated with resistance to radiation have had promising results (43). In the current study, paeonol caused marked downregulation of HIF-1α and VEGF in a dose-dependent manner with all tested radiation doses. HIF-1α is the oxygen-sensitive subunit of HIF and is induced by hypoxia, but the expression decreases under normoxic conditions (44). However, HIF-1α is upregulated in hypoxic tumour tissues, which may contribute to resistance to radiation (45). Previous studies have demonstrated a positive correlation between the expression levels of HIF-1 and resistance of many solid tumors to radiation (31,46). Hypoxia-induced HIF-1α expression activates VEGF, stimulates angiogenesis and promotes the resistance of cancer cells to chemotherapy and radiotherapy (47,48). Given the critical impact of HIF-1α induced by hypoxia and radiation, effective inhibition of HIF-1α may aid sensitization of tumor cells to chemotherapy and radiotherapy. Therefore, the marked downregulation of HIF-1α and VEGF observed following paeonol treatment may have been

responsible for the raised apoptotic cell counts observed in the irradiated ovarian cancer cell lines.

To better assess the molecular events associated with paeonol-induced sensitivity to radiation, the influence of paeonol on PI3K/Akt pathway proteins was examined, as several prior studies have reported associations between the PI3K pathway and resistance to radiation (49,50). The PI3K/Akt/mTOR pathway is closely associated with pathways such as the androgen receptor pathway (51), the Ras/Raf/mitogen-activated protein kinase kinase/extracellular signal-regulated kinase pathway (52) eventually contributing to cancer cell proliferation, metastasis and resistance (52). Akt, a focal regulator of this pathway is considered to be a potential target in addressing sensitization to radiation. Upon activation, Akt phosphorylates many other downstream proteins such as mTOR, GSK3 and insulin receptor substrate-1 (53). Palomid 529, a novel and potent Akt inhibitor was also previously observed to increase sensitivity to radiation (54). In the current study, reduced phosphorylation of Akt and GSK-3β, and suppressed expression of mTORc1 were observed, indicating inhibition of the PI3K/Akt/mTOR pathway. mTORC1 regulates cell growth through phosphorylation of S6 kinase (55) and induces increased expression of VEGF (56). Therefore, downregulation of VEGF may have been associated with suppression of mTORc1.

Furthermore, PTEN that acts at the molecular level to counteract the functions of PI3K was upregulated by paeonol, acting to inhibit the activated PI3K pathways. This suppression may be responsible for the enhanced apoptosis and sensitivity to radiation.

The observations of the present study suggest that paeonol induces apoptosis of irradiated ovarian cancer cells via modulation of the critical pathways involved in resistance to radiation, namely the HIF-1α/VEGF pathway and PI3K/Akt/mTOR signalling cascades. Therefore, the present study indicates that paeonol may represent a potent sensitizing agent to radiation, which may be of further assistance in cancer therapy.

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