

Garcinol Alone and in Combination With Cisplatin Affect Cellular Behavior and PI3K/AKT Protein Phosphorylation in Human Ovarian Cancer Cells

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Abstract

Garcinol is a plant-derived compound that has some physiological benefits to human cells. However, the effect of garcinol on ovarian cancer (OC) cell proliferation and apoptosis is unknown. The current study aimed to examine the effects of garcinol alone and in combination with cisplatin (DDP) on cellular behavior and to explore the expression pattern of PI3K/AKT and nuclear factor- κ B (NF- κ B) in human OC cells. We found that OVCAR-3 cell viability was decreased after garcinol treatment. Garcinol alone and in combination with DDP significantly inhibited cell proliferation and had a synergistic effect evaluated by CompuSyn software. The cell cycle analysis showed the S phase arrest by garcinol. Furthermore, garcinol alone and in combination with DDP promoted cell apoptosis. The garcinol-induced apoptosis was further confirmed by the detection of cleavage forms of PARP and caspase 3. An increase in proapoptotic factor Bax expression was also found in garcinol-treated cells. Moreover, garcinol significantly decreased the phosphorylation of PI3K and AKT proteins and downregulated the expression of NF- κ B. Thus, our data demonstrated that garcinol has the potential to be used as an anticancer agent and may synergize the effect of DDP. These actions are most likely through the regulation of the PI3K/AKT and NF- κ B pathways.

Keywords

garcinol, chemoresistance, ovarian tumor, synergistic effect, signaling pathway

Introduction

Ovarian cancer (OC) is one of the fatal malignant tumors because the disease is often diagnosed late due to the lack of effective screening strategies at the early stage and recurrence that about 80% of patients would relapse within 18 months.^{1,2} The first-line therapy for OC is cytoreductive surgery followed by platinum-based chemotherapy.³ Although platinum-based chemotherapy can prolong the survival of patients, about two-thirds of patients eventually develop drug resistance.⁴ Cisplatin (cis-Diamminedichloroplatinum, DDP), as a platinum-based cytotoxicity drug, can form DNA cross-link and inhibit DNA replication, leading to the blockage of protein synthesis. Although DDP is effective initially, the clinical applications of DDP are limited due to the severe side effects.⁵ Therefore, it is necessary to find a suitable sensitizer or synergistic combination therapy in order to reduce the dosage of DDP for patients with OC.

Up to date, several plant-derived small-molecule compounds have been reported as effective anticancer agents,

including garcinol that is a polyisoprenylated benzophenone derivative extracted from the fruiting bodies of *Garcinia indica*.^{6,7} Garcinol has a similar structure to some well-known antitumor compounds such as curcumin and chalcones, both which contain phenolic hydroxyl groups that may endow the garcinol with strong antioxidant activity.⁸ Traditionally,

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garcinol has a wide range of uses from food ingredients and garnish to the cosmetic constituent; therefore, it may have high medicinal value to treat inflammation and other disorders.⁹ However, the effect of garcinol on human OC cell behavior is unknown. Furthermore, whether garcinol affects the expression of PI3K/AKT and nuclear factor- κ B (NF- κ B) signaling proteins is not explored yet.

The current study aimed to examine the effects of garcinol alone and in combination with DDP on cellular behavior and to explore the expression pattern of PI3K/AKT and NF- κ B in human OC cells.

Materials and Methods

Cell Lines and Reagents

Human OC cell line OVCAR-3 was obtained from the American Type Culture Collection (ATCC). The cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (Gibco). Garcinol (C₃₈H₅₀O₆, molecular weight 602.8, purity \geq 95%; Sigma-Aldrich) was dissolved in dimethyl sulfoxide (Sigma-Aldrich). Cisplatin (C₁₂H₆N₂Pt, molecular weight 300.05, purity \geq 99.9%; Sigma-Aldrich) was dissolved in physiological saline.

Cell Viability Assay

The cell viability assay was performed using the Cell Counting Kit-8 (CCK-8). Briefly, OVCAR-3 cells were seeded into 96-well plates at a density of 6×10^3 cells per well. In the time-course and dose-dependent experiments, different concentrations of garcinol (0, 5, 10, 20, 25, 30, and 50 μ M) were applied in individual wells. Empty (blank, without a cell) and cells without a drug were used as controls. Five replicates were applied. After incubation for 24, 48, and 72 hours, 10 μ L of CCK-8 reagent was added into each well. The cell viability was evaluated by reading the absorbance of optical density (OD) at 490 nm using a microplate reader (BioRad).

For the combination of garcinol and DDP experiments, cells were divided into 3 groups: garcinol alone at different doses (0, 5, 10, 20, 25, and 30 μ M), DDP alone at different doses (0, 0.5, 1, 2, 4, and 8 μ M), and the combination of garcinol and DDP. Five replicate wells were set for each concentration. After cells were treated for 48 hours, 10 μ L of CCK-8 reagent was added to each well and incubated at 37°C for 1 hour. The OD was measured at 490 nm using a microplate reader. To calculate the inhibition rate of cell proliferation, the following equation was used:

$$\text{Inhibition Rate (\%)} = (1 - \text{OD value of treated cells} / \text{OD value of untreated cells}) \times 100\%.$$

The half-maximal inhibitory concentration (IC₅₀) of the drug was also calculated and guided to the subsequent experiments.

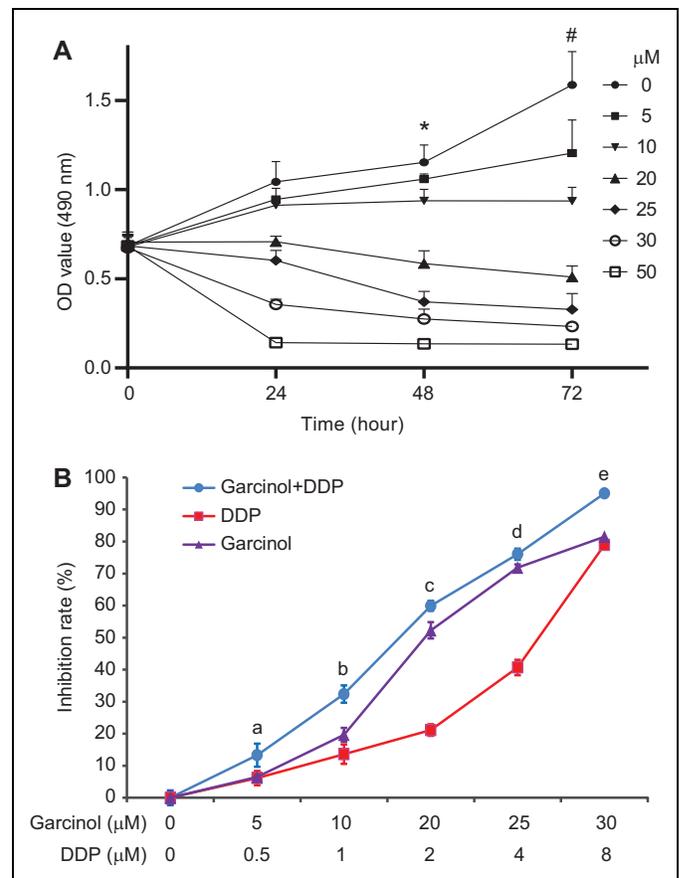


Figure 1. Effect of garcinol alone and in combination with DDP on cell viability in OVCAR-3 cells. Cell viability was evaluated by the CCK-8 assay. (A) Dose-dependent effect of garcinol. Cells were treated with garcinol at different doses (0-50 μ M) for 24, 48, and 72 hours. The OD value was detected by a microplate reader. $n = 5$; * $P < .05$ individual dose (from 10 to 50 μ M) compared to the untreated control at 48 hours; # $P < .05$ all individual dose (from 5 to 50 μ M) compared to the untreated control at 72 hours. (B) Measurement of inhibitory rate. Cells were treated with different concentrations of garcinol alone, DDP alone, or their combination for 48 hours. The inhibition rate was calculated after the detection of the cell viability. The experiment was repeated at least 3 times. Data represent the mean \pm SD. (A-E) $P < .05$ combination group compared to garcinol or DDP alone at the same concentration level. CCK indicates cell counting kit; DDP, cisplatin; OD, optical density; SD, standard deviation.

Evaluation of the Association Between Garcinol and DDP

The association between garcinol and DDP was evaluated using the CompuSyn software (<http://www.combosyn.com/>) according to the principle of the Chou-Talalay combined index method.¹⁰ The calculation of the 2-drug combination index (CI) was followed: $CI = (D_1/D_{X1}) + (D_2/D_{X2}) + \alpha (D_1D_2)/(D_{X1}D_{X2})$, where α is a constant and D and D_X stand for the dose of a drug and a dose in $x\%$ inhibition, respectively.¹¹ Since garcinol and DDP are independent of each other, the constant α equals 0. Thus, the formula becomes $CI = (D_1/D_{X1}) + (D_2/D_{X2})$. The values of $CI < 1$, $CI = 1$, and $CI > 1$, respectively, represent the synergistic, additive, and antagonistic effects of garcinol and DDP.¹⁰

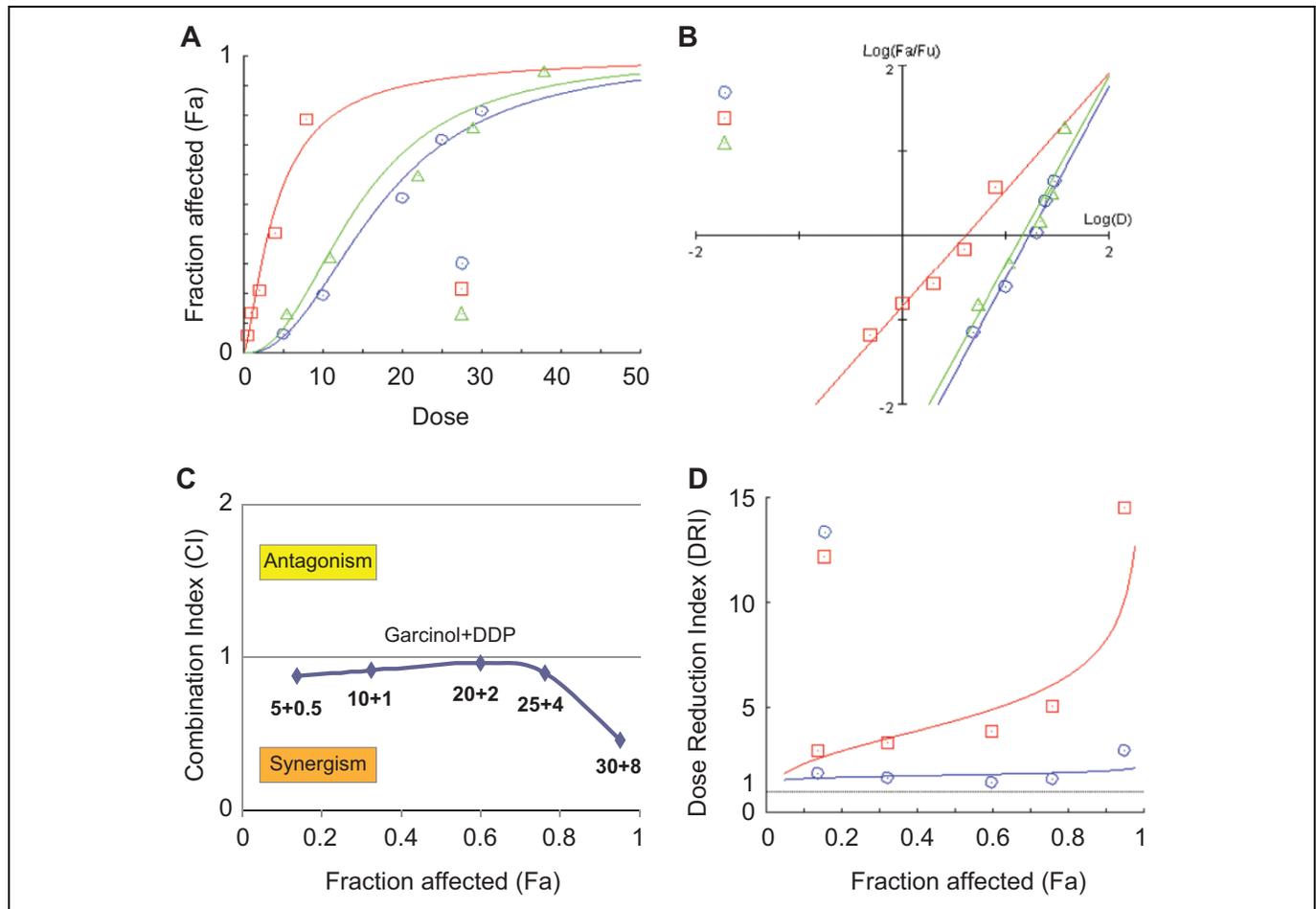


Figure 2. Dose effectiveness of garcinol and DDP on cancer cell growth was evaluated by the CompuSyn software. Cells were treated with garcinol or DDP or both drugs at different doses. (A) Dose–effect curves. The inhibitory effect of garcinol and DDP on cancer cell growth inhibition was shown. (B) Median-effect plot. The half-maximal inhibitory concentration (IC_{50}) of the drug was calculated. The x-axis intercept ($\log D$) represents the medium-effect dose of IC_{50} . (C) Combination index plot. The inhibition index was calculated after cells were treated with different concentrations of garcinol (5, 10, 20, 25, and 30 μM) in combination with DDP (0.5, 1, 2, 4, and 8 μM). The effect of garcinol and DDP was synergistic in OVCAR-3 cells. (D) DRI plot. Dose reduction was evaluated after cells treated with garcinol and DDP. CI indicates combination index; DDP, cisplatin; DRI, Dose-Reduction Index; Fa, fraction affected; IC_{50} , the half-maximal inhibitory concentration of a drug.

Cell Cycle Analysis

OVCAR-3 cells were seeded into 6-well plates at 1×10^6 /well and incubated with different concentrations of garcinol (0, 10, 20, and 25 μM). After cultivation for 48 hours, cells were collected by centrifugation, washed with cold phosphate-buffered saline (PBS), and then fixed with 70% ice-cold ethanol at $4^\circ C$ overnight. Subsequently, cells were incubated with 500 μL PBS containing 50 $\mu g/mL$ propidium iodide (PI) and 0.1 mg/mL RNase A (Sigma-Aldrich) in the dark at room temperature for 30 minutes. Cell cycle distribution was determined by a FACS Calibur Flow Cytometry (BD Biosciences). The experiment was repeated 3 times.

Detection of Apoptotic Cells by Flow Cytometry

Apoptotic cells were detected using an Annexin V-FITC Apoptosis Detection kit (BD Biosciences). Briefly, OVCAR-3 cells

were suspended at a density of 2×10^6 /well in a 6-well plate. After incubation for 24 hours, garcinol and DDP were added into each well. Experiments were divided into 3 groups: garcinol alone group (20 or 25 μM), DDP alone group (2 or 4 μM), and combined group (20 μM garcinol plus 2 μM DDP or 25 μM garcinol plus 4 μM DDP). Triplicates were set at each concentration. After incubation with a drug for 48 hours, the cells were collected and washed once with PBS, followed by adding 200 μL of a binding buffer which contains 5 μL of Annexin V-FITC (fluorescein isothiocyanate) and 10 μL of PI staining solution. After incubation at room temperature for 15 minutes, apoptotic cells were detected by flow cytometry analysis. The experiment was repeated 3 times.

Western Blot Analysis

After treatment with garcinol (0, 10, 20, and 25 μM) for 48 hours, OVCAR-3 cells were collected and lysed with RIPA

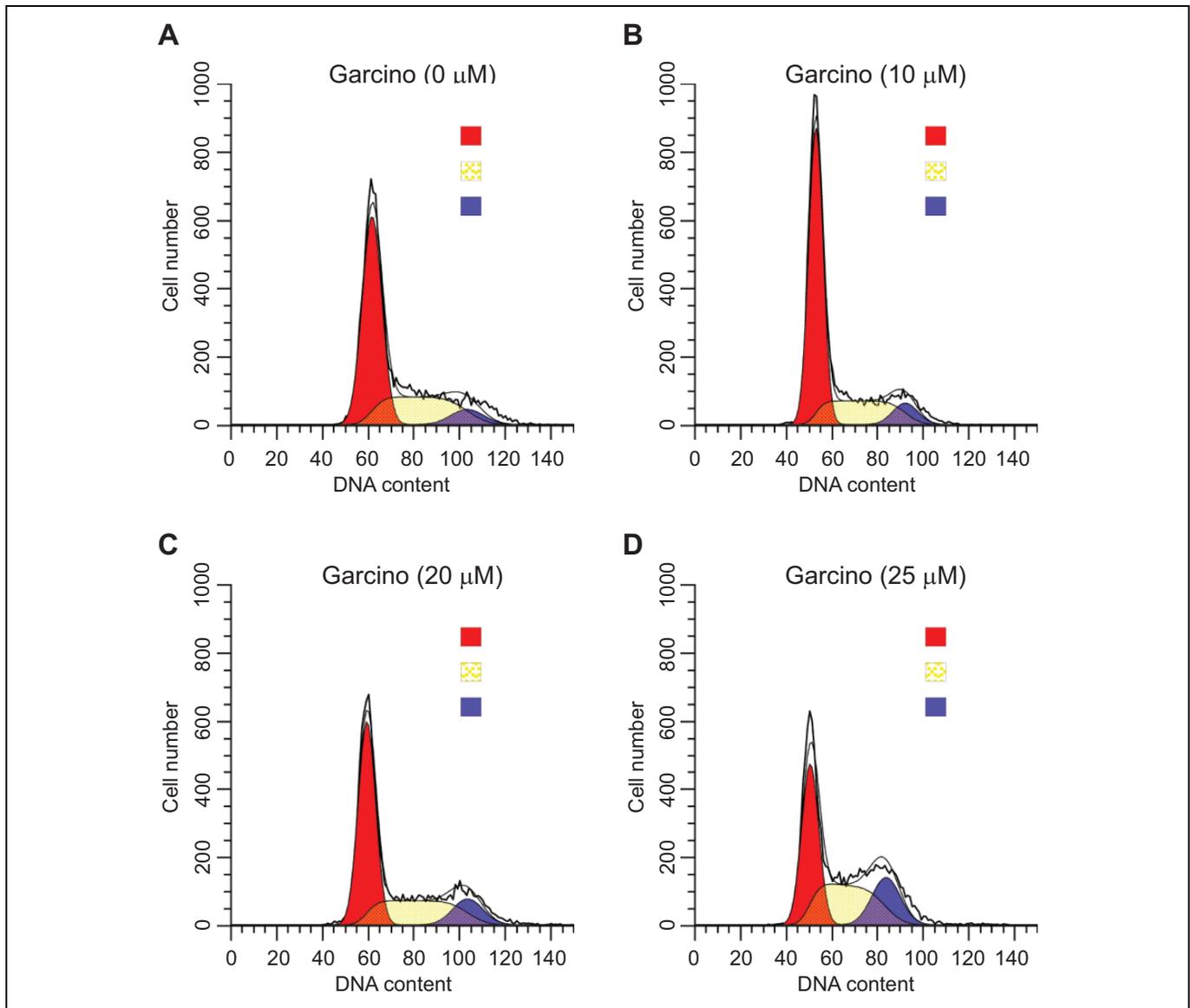


Figure 3. Effect of garcinol on cell cycle. OVCAR-3 cells were treated with different concentrations (0, 10, 20, and 25 μM) of garcinol for 48 hours. The cell number at different phases of the cell cycle was determined by flow cytometry. (A) 0 μM garcinol treatment, (B) 10 μM garcinol treatment, (C) 20 μM garcinol treatment, and (D) 25 μM garcinol treatment.

lysis buffer (Millipore). The protein concentration was determined using the BCA Protein Assay Kit (Millipore) according to the manufacturer's instruction. An equal amount of protein was separated by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membrane (Cell Signaling Technology). After blocking with 5% nonfat milk for 1 hour at room temperature, the membrane was incubated with primary antibody against PARP, caspase 3, Bax, Bcl-xL, PI3K, phosphorylated (p)-PI3K (Tyr458, p85 subunit), AKT, phosphorylated (p)-AKT (Ser473), NF- κB , and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Cell Signaling Technology), respectively, overnight at 4°C. After incubation of the HRP-conjugated secondary

antibody (Cell Signaling Technology) for 2 hours, the membrane was rinsed with TBS-T and incubated with ECL reagent. The signals were then detected using an Enhanced Chemiluminescence Detection kit (Pierce) and analyzed by ImageJ software (NIH). GAPDH was used as an internal control. The experiment was repeated 3 times.

Statistical Analysis

Data were analyzed using SPSS 19.0 software (SPSS Inc). The experimental data were expressed as mean \pm the standard deviation (mean \pm SD). All statistical analyses were performed using GraphPad Prism version 6.0 software (GraphPad). The difference between the 2 groups was evaluated by

the Student *t* test, and the difference among 3 or more groups was examined by 1-way analysis of variance, followed by Bonferroni post hoc test. Statistical significance was considered when $P < .05$. All experiments were repeated at least 3 times.

Results

Garcinol Alone and in Combination With DDP Inhibit OVCAR-3 Cell Viability

OVCAR-3 cells were treated with different concentrations (0, 5, 10, 20, 25, 30, and 50 μM) of garcinol for 24, 48, and 72 hours. Using a CCK-8 assay, the dose-dependent and time-course studies showed that the cell viability was decreased in OVCAR-3 cells after garcinol treatment compared to the control group (Figure 1A). The cell growth was significantly inhibited after garcinol treatment at the doses from 10 to 50 μM for 48 ($P < .05$). After treatment for 72 hours, all doses (5–50 μM) of garcinol treatment resulted in an inhibition of cell viability compared to the control group ($P < .05$), indicating a time- and dose-dependent manner.

Next, we examined the effect of garcinol in combination with DDP on cell viability. OVCAR-3 cells were treated with different concentrations of garcinol and DDP for 48 hours. The inhibition rate was significantly increased after 5, 10, 20, 25, and 30 μM garcinol alone and 0.5, 1, 2, 4, and 8 μM DDP alone treatment ($P < .05$; Figure 1B), respectively. The inhibition rate was further increased in the combination group after both garcinol and DDP treatment ($P < .05$).

Subsequently, IC_{50} was calculated in these 3 groups. The IC_{50} of garcinol and DDP was 17.93 and 4.34 μM , respectively, after treatment for 48 hours. Using the CompuSyn software, the dose effectiveness of garcinol and DDP was calculated. The inhibitory effect of garcinol and DDP on cancer cell growth was increased when the concentration of drugs was risen (Figure 2A), again indicating a dose-dependent manner. The analysis of the median-effect using IC_{50} evaluation showed that the combination of garcinol and DDP had a more inhibitory effect compared to a single agent (Figure 2B). In accordance with the fraction affected (Fa)-CI curve, the combination of the 2 drugs could have synergistic inhibition if $\text{CI} < 1$. After the treatment of combined 2 drugs at different concentrations, the $\text{CI} < 1$ was observed in all 5 pairs of combinations (Figure 2C), indicating that the effect of garcinol in combination with DDP was synergistic in OVCAR-3 cells. The analysis of the Dose-Reduction Index (DRI) was calculated after garcinol and DDP combined treatment. The DRI plot showed that both drugs had a DRI value above 1 (Figure 2D), indicating that both garcinol and DDP had an inhibitory effect, and the combined treatment was better than a single-drug treatment. The degree of dosage reduction was greater in DDP than garcinol, suggesting that garcinol may be beneficial to reduce the side effects of DDP in combination therapy.

Table 1. Distribution of Cell Population in the Cell Cycle.^a

Garcinol, μM	G0/G1 phase	S phase	G2/M phase
0	65.22 \pm 0.34	27.31 \pm 0.72	7.47 \pm 0.56
10	58.13 \pm 1.56 ^b	32.47 \pm 0.58 ^b	9.4 \pm 0.98 ^c
20	55.71 \pm 0.97 ^b	33.94 \pm 0.54 ^b	11.06 \pm 1.12 ^b
25	43.20 \pm 0.63 ^b	37.04 \pm 0.31 ^b	19.75 \pm 0.53 ^b

^aOVCAR-3 cells were treated with different doses of garcinol for 48 hours. n = 3, mean \pm standard deviation (%).

^b $P < .01$, compared with the untreated group.

^c $P < .05$, compared with the untreated group.

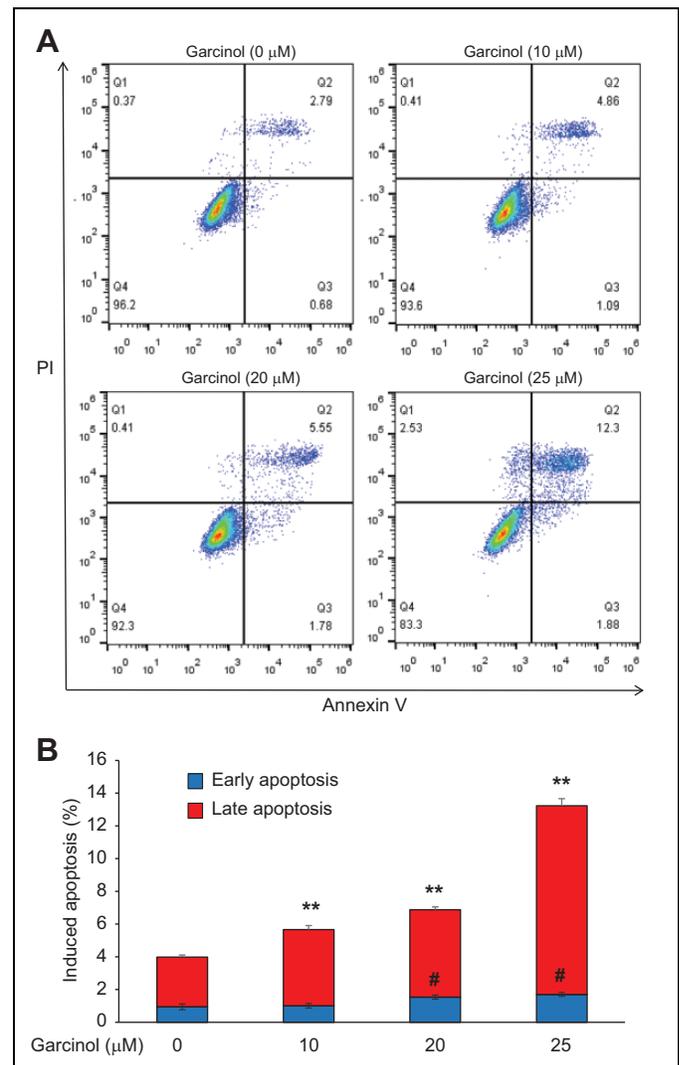


Figure 4. Effect of garcinol on cell apoptosis. OVCAR-3 cells were treated with different concentrations (0, 10, 20, and 25 μM) of garcinol for 48 hours. (A) Flow cytometry analysis of cell apoptosis. (B) The percentage of apoptotic cells in the histogram. Blue indicates the early apoptosis and red indicates the late apoptosis. Data represent the mean \pm standard deviation. n = 3; ** $P < .01$ compared to the untreated control for total apoptotic cells; # $P < .05$ compared to the untreated control for early apoptotic cells.

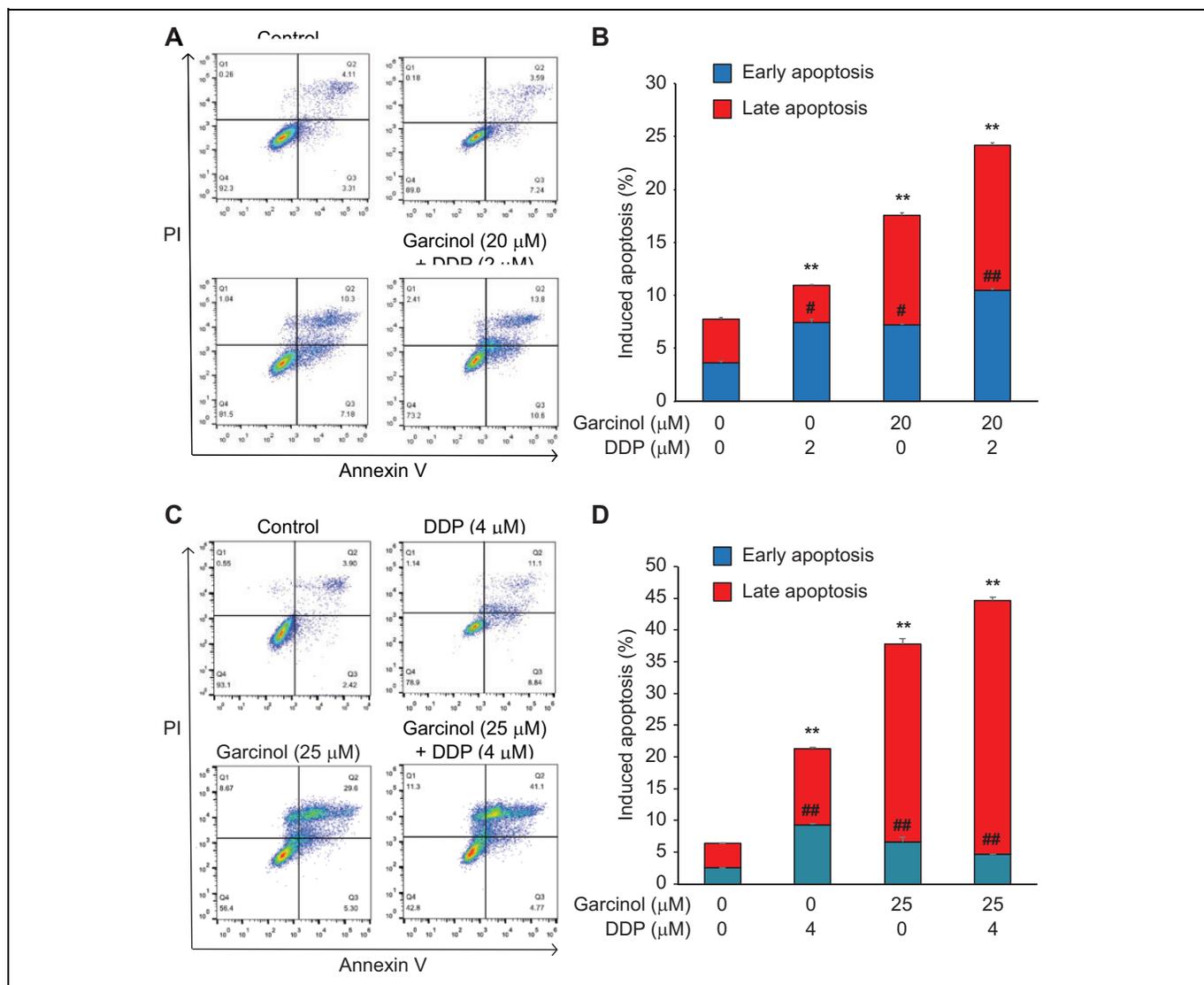


Figure 5. Effect of garcinol in combination with cisplatin (DDP) on cell apoptosis in OVCAR-3 cells. (A) Flow cytometry analysis of cell apoptosis after the treatment of 20 μM garcinol alone, 2 μM DDP alone, or both for 48 hours. (B) The histogram showed the percentage of apoptotic cells from (A). (C) Flow cytometry analysis of cell apoptosis after the treatment of 25 μM garcinol alone, 4 μM DDP alone, or both for 48 hours. (D) The histogram showed the percentage of apoptotic cells from (C). Blue indicates the early apoptosis and red indicates the late apoptosis. Data represent the mean \pm standard deviation. $n = 3$; $**P < .01$ compared to the untreated control for total apoptotic cells; $\#P < .05$ and $###P < .01$ compared to the untreated control for early apoptotic cells.

Garcinol Induces Cell Cycle Arrest in OVCAR-3 Cells

The OVCAR-3 cells were treated with different concentrations of garcinol for 48 hours, followed by flow cytometry for cell cycle analyses. The number of cells was gradually decreased at the G0/G1 phase as the dose of garcinol increased, and there was a significant difference between garcinol treatment (10, 20, and 25 μM) and untreated control groups ($P < .01$; Figure 3A-D). The number of cells at the S and G2/M phases was significantly increased in the treatment groups compared to the untreated control group (Table 1). These results demonstrated that garcinol arresting cell cycle starts at the S phase.

Garcinol Alone and in Combination With DDP Induce OC Cell Apoptosis

Next, we examined the effect of garcinol alone and in combination with DDP on cell apoptosis. The flow cytometry analysis showed that garcinol alone induced OVCAR-3 cell apoptosis in a dose-dependent manner (Figure 4A). The number of total apoptotic cells was significantly increased, and the percentage of apoptotic cells was $5.66\% \pm 0.17\%$, $6.88\% \pm 0.28\%$, and $13.23\% \pm 0.55\%$ in the groups of 10, 20, and 25 μM garcinol treatment, respectively. Compared to the untreated control, the administration of 20 and 25 μM garcinol

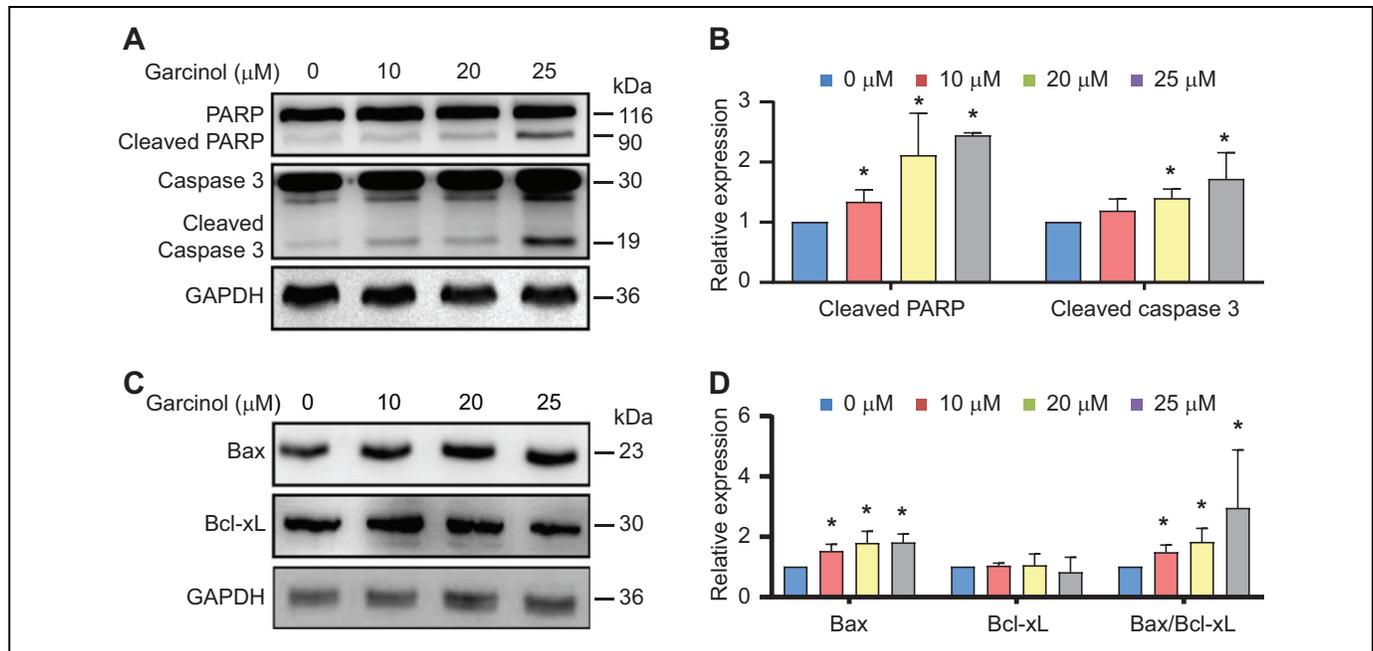


Figure 6. Effect of garcinol on apoptotic protein expression. OVCAR-3 cells were treated with different concentrations (0, 10, 20, and 25 μM) of Garcinol for 48 hours. (A) PARP, cleaved PARP, caspase 3, and cleaved caspase 3 were detected by Western blot. (B) Densitometric analysis of the cleaved PARP and caspase 3 from (A). (C) Effect of Garcinol on Bax and Bcl-xL protein detected by Western blot. (D) Densitometric analysis of the expression of Bax and Bcl-xL and the ratio between Bax and Bcl-xL protein from (C). Data represent the mean \pm standard deviation. $n = 3$; * $P < .05$ compared to the untreated control group.

for 48 hours significantly induced the early apoptosis of OVCAR-3 cells ($P < .05$; Figure 4B).

The effect of garcinol in combination with DDP on the induction of apoptosis was also performed in OVCAR-3 cells that were divided into 4 groups: untreated control, DDP alone, garcinol alone, and DDP plus garcinol. The flow cytometry analysis showed that the percentage of total apoptotic cells was $24.13\% \pm 0.13\%$ in 2-drug combined group (2 μM DDP and 20 μM garcinol), which was significantly higher than the single-drug group ($10.94\% \pm 0.29\%$ in the DDP group and $17.56\% \pm 0.12\%$ in the garcinol group; $P < .01$; Figure 5A and B). When the doses of drugs increased to 4 μM of DDP and 25 μM of garcinol, more apoptotic cells at the late phase were observed (Figure 5C-D), implying the cytotoxicity was increased. In addition, the induction rate of apoptosis by a single-drug analysis was found to be higher in the garcinol alone group than the DDP alone group.

Subsequently, we examined apoptotic proteins by Western blot. The levels of cleaved PARP and cleaved caspase 3, 2 important proteins represented the active apoptosis, were significantly increased after garcinol treatment for 48 hours (Figure 6A). Garcinol at 10, 20, and 25 μM significantly increased the cleavage form of PARP, whereas it is at 20 and 25 μM significantly increased the cleavage form of caspase 3 (Figure 6B). Furthermore, garcinol increased the proapoptotic protein Bax expression but no antiapoptotic protein Bcl-xL expression (Figure 6C). Statistical analysis showed that garcinol at 10, 20, and 25 μM significantly upregulated the

expression of Bax and increased the ratio of Bax–Bcl-xL compared to the untreated control group ($P < .05$; Figure 6D).

Garcinol Suppressed the Phosphorylation of AKT and PI3K and the Expression of NF- κ B

Since the PI3K/AKT and NF- κ B signaling pathways are involved in cell proliferation and apoptosis, we next examined the effect of garcinol on protein expression by Western blot. We found that garcinol (10, 20, and 25 μM) treatment for 48 hours significantly decreased the phosphorylation of PI3K and AKT in OVCAR-3 cells ($P < .05$; Figure 7A-D). The expression of NF- κ B was also downregulated by garcinol treatment ($P < .05$; Figure 7E and F).

Discussion

The present study investigated the effect of garcinol alone and in combination with DDP on cancer cell behavior and explored the potential mechanism underlying the regulation of the expression of PI3K, AKT, and NF- κ B signaling proteins in OC cells. We found that garcinol decreased OVCAR-3 cell viability, arrested cell cycle at the S phase, induced cell apoptosis, inhibited the phosphorylation of PI3K/AKT, and downregulated the expression of NF- κ B.

Ovarian cancer is the most lethal cancer in women, and the conventional treatment of OC is debulking surgery plus chemotherapy.¹² Cisplatin is a first-line anti-OC drug that is

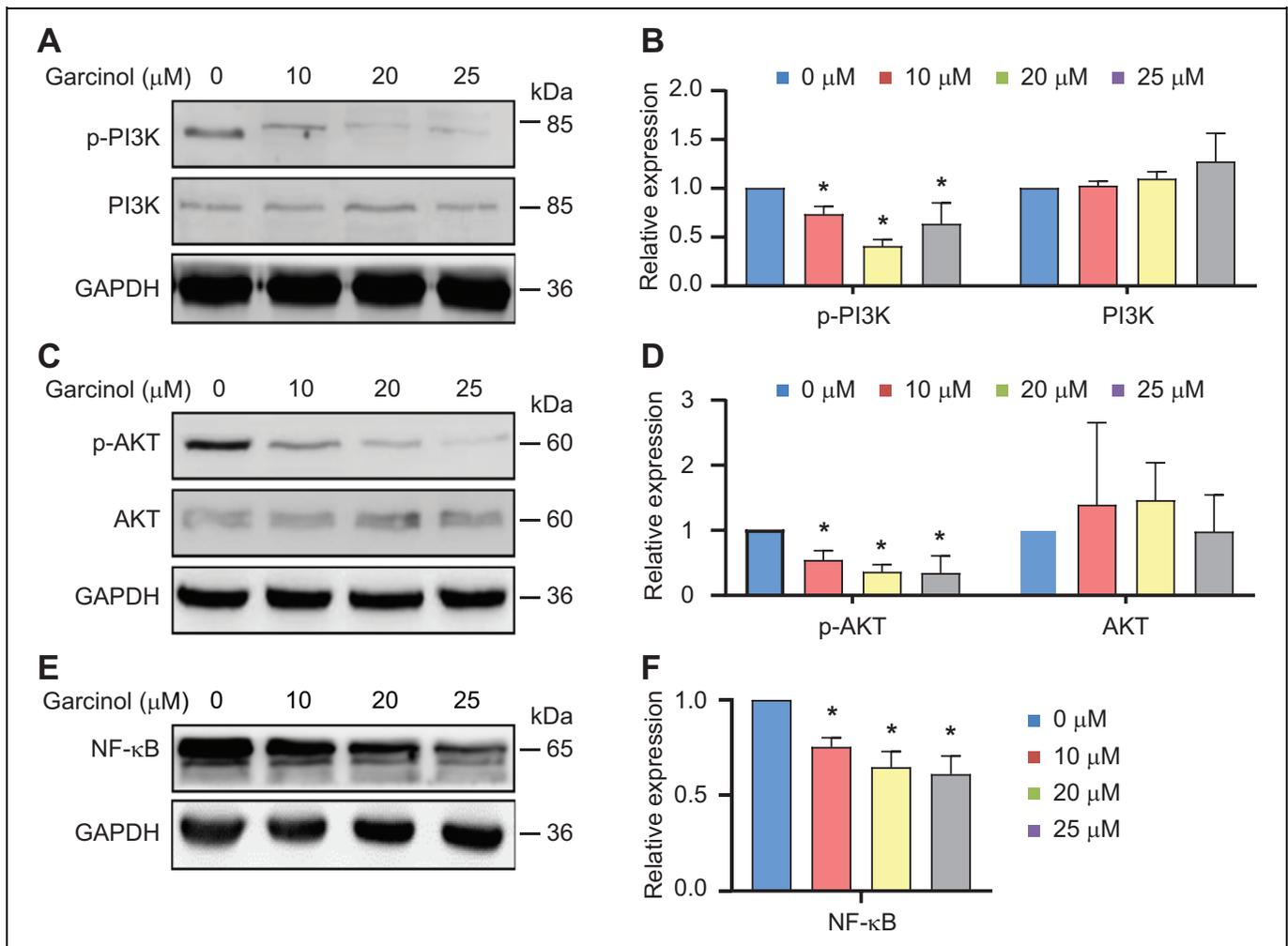


Figure 7. Effect of Garcinol on PI3K/AKT signaling proteins and nuclear factor- κ B (NF- κ B) expression. OVCAR-3 cells were treated with different concentrations (0, 10, 20, and 25 μ M) of Garcinol for 48 hours. (A) The phosphorylation of PI3K and total PI3K were detected by Western blot. (B) Densitometric analysis of the phosphorylation of PI3K and total PI3K protein expression from (A). (C) The phosphorylation of AKT and total AKT were detected by Western blot. (D) Densitometric analysis of the phosphorylation of AKT and total AKT protein from (C). (E) The expression of NF- κ B protein was detected by Western blot. (F) Densitometric analysis of NF- κ B protein from (E). Data represent the mean \pm standard deviation. $n = 3$; * $P < .05$ compared to the untreated control group.

efficient initially in patients with OC but has a side effect and often leads to resistance.¹³ Therefore, finding a synergic or sensitized agent to reduce the dose of DDP usage may be important. In recent years, some plant-derived small-molecule compounds have been identified as potential candidates to be used as adjuvants for patients with cancer. These compounds such as curcumin, quercetin, resveratrol, and garcinol are relatively not susceptible to drug resistance with tiny toxicity.^{14,15} It has been shown that garcinol is a potential drug that has anticancer properties¹⁶ and affected on cell proliferation in several cancer cells such as breast, colon, esophageal, prostatic, pancreatic, cervical, oral, and lung cancer cells.¹⁷⁻²³ However, the effect of garcinol on human OC cells has not been explored. The current study showed for the first time that garcinol also had the function of anticancer cell proliferation, and this effect was time- and dose dependent in vitro.

Our cell cycle analysis showed that the suppression of OVCAR-3 cell growth by garcinol treatment was mostly due to the arrest of the cell cycle at the S/G2 phases. A similar S/G2 arrested by garcinol was also observed in HL-60 promyelocytic leukemia cells by another group.²⁴ However, garcinol can also lead to cell cycle arrest at the G1 phase in BxPC-3 and Panc-1 pancreatic cancer cells and H1299 lung cancer cells^{25,26} and the G2/M phase in 3T3-L1 preadipocytes.²⁷ These results suggest that the effect of garcinol on the cell cycle progression may be cell-type specific.

The combination of garcinol and DDP may have a synergistic effect on OVCAR-3 cell growth inhibition in DDP-sensitive and nonsensitive cells. Using computerized data analyses,²⁸ we obtained that the combination of 2 drugs was effective based on the median-effect principle. The selected method allows us to estimate the potencies of each drug and the combination of 2

drugs. The CI is widely used to judge the synergy ($CI < 1$), additive ($CI = 1$), and antagonistic ($CI > 1$) association between drugs in combination therapy. Cisplatin plays a role in inhibiting duplication through forming DDP-DNA because of a cross-link. The inhibition of cell growth by 2 drugs further suggested that garcinol may synergize the action of DDP and help to reduce its dose usage. However, the optimal concentration ratios are required to further verify. Our data may just provide a reference for future animal experiments and even clinical trials.

Consistent with the cell proliferation experiment, garcinol alone induced OVCAR-3 cell apoptosis, which was similar to the result found in p53-deficient H1299 lung cancer cells in the previous report.²⁵ In addition, we also observed that garcinol synergistically enhanced DDP-induced cell apoptosis. After garcinol and DDP were combined, more early apoptosis was observed in the 20+2 concentration group, whereas more late apoptosis was observed in the 25+4 concentration group. These data suggest that the dose ratio of 2 agents is important. The induction of apoptosis by garcinol was further confirmed by the detection of cleaved PARP and cleaved caspase 3 elevation and the ratio of proapoptotic and antiapoptotic factors such as Bax and Bcl-xL. These results were similar to the previous works in non-OC cells by other groups.^{26,29-31} Cleaved PARP and cleaved caspase 3 have their function during apoptotic processes and are also considered as apoptotic biomarkers,^{32,33} whereas the specific value of Bax/Bcl-xL indicates the intrinsic pathway of apoptosis.^{34,35} In hepatocellular carcinoma Hep3B cells, garcinol administration results in the accumulation of reactive oxygen species (ROS) and activates the ROS-dependent apoptosis pathway.³⁶ Although the caspase-independent apoptosis has not been ruled out in the current study, the outcome from this work may at least in part imply the regulatory mechanism underlying garcinol-induced, caspase-dependent apoptosis in OC cells.

However, how garcinol triggers OC cell cycle arrest and induces apoptosis was not clear. Several potential signaling molecules such as PI3K/AKT and NF- κ B were under the investigation in the current study. It has been shown that the PI3K/AKT signal pathway participates in cellular processes, cell growth, and death,³⁷ and the dysregulation of this pathway is implicated in cancer development and progression.³⁸ NF- κ B is a nuclear factor also involved in the cell proliferation and apoptosis. The alteration of NF- κ B expression has been found in various cancers.³⁹⁻⁴¹ Indeed, garcinol inhibits the activation of the PI3K/AKT signaling pathway in HT-29 colorectal cancer cells³⁰ and downregulates NF- κ B expression in MDA-MB-231 breast cancer cells.⁴² Our data also demonstrated that garcinol decreased the phosphorylation of PI3K and AKT and downregulated NF- κ B expression in OC cells, suggesting that garcinol-induced dephosphorylation of PI3K/AKT and decrease of NF- κ B expression may weaken their signaling pathways and in the end arrest OVCAR-3 cell cycle and promote cell apoptosis. However, the specific mechanism remains unclear and needs to be further explored.

Nevertheless, inhibition of these pathways by garcinol may enhance the sensitivity of tumor cells to chemotherapy drugs such as DDP.

Conclusions

The current study demonstrated that garcinol has an anticancer potential and in combination with DDP it synergizes the effectiveness of DDP in OC cells. The cellular behavior of what garcinol inhibited cell growth, arrested cell cycle, and induced cell apoptosis may be altered and regulated through the PI3K/AKT and NF- κ B signaling pathways. These data commendably provide the base of using DDP plus garcinol as combination therapy for the treatment of OC.

Declaration of Conflicting Interests

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