Garcinol acts as an antineoplastic agent in human gastric cancer by inhibiting the PI3K/AKT signaling pathway

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Abstract. Gastric cancer (GC) is one of the most common malignancies worldwide; however, treatment options other than surgery remain limited. Neoadjuvant chemotherapy has the potential to suppress of gastric tumorigenesis. Garcinol has been reported to exert inhibitory effects on the progression of numerous carcinomas. However, its effects in GC remain unclear. Therefore, the aim of the present study was to investigate the effects of garcinol on the proliferation, invasion and apoptosis of gastric carcinoma cells and then to explore the underlying mechanisms. Garcinol significantly decreased the proliferation and invasion of GC cells and increased apoptosis in a dose-dependent manner. Additionally, the expression of $AKT^{p\text{-}Thr308}$, cyclin D1, Bcl-2, BAX, matrix metalloprotease (MMP-2) and MMP-9 in HGC-27 cells following treatment with garcinol. The results obtained in the present study suggested that garcinol may inhibit gastric tumorigenesis by suppressing the PI3K/AKT signaling pathway.

Introduction

Gastric cancer (GC) is a malignant tumor of epithelial origin and is the third most common cancer worldwide (1). Currently, a combination of surgery and perioperative adjuvant or neoadjuvant therapy are the mainstay of treatment for GC, but depend on the disease stage and pathological type (2,3). Although there has been a decline in the incidence of GC in recent decades due to progress in clinical interventions (4,5), the high morbidity and mortality associated with the disease pose challenges to public health. Studies suggest that multiple genetic and environmental factors impact the risk and progression of GC (4,6). The pathways underlying the pathogenesis of GC have not yet been fully elucidated, and a deeper understanding of the dysregulation involved in the intracellular signaling pathways may aid the development of novel therapeutic strategies.

The activation of protein kinase B (AKT) by phosphatidylinositol-3-kinase (PI3K) is required for cell proliferation, invasion, apoptosis and angiogenesis, and is associated with the progression of neoplasms (7-9). Several components of the PI3K/AKT signaling pathway may serve as potential therapeutic targets in a number of human tumors, including GC and breast and lung cancer (8,10-12). Cell cycle regulator D1 (cyclin D1) is activated by mammalian target of rapamycin (mTOR) via phosphorylation of AKT at either serine 473 or threonine 308 and is important in the G1/S transition of tumor cells (13,14). Upregulation of cyclin D1 may lead to shortening of the cell cycle and increased proliferation of tumor cells, thereby expediting tumor progression. Matrix metalloproteinases (MMPs) promote the degradation of the extracellular matrix and therefore affect the invasion and metastasis of cancer cells. The production of MMPs has been shown to be regulated by the PI3K/AKT signaling pathway (15,16). Activation of the PI3K/AKT signaling pathway inhibits apoptosis via the expression of specific downstream proteins, including B-cell lymphoma-2 (Bcl-2) and Bcl-2 associated X protein (BAX) (7,17). Therefore, noveltherapeutic agents that specifically target the PI3K/AKT signaling pathway may improve GC treatment.

Garcinol (Fig. 1A), a polyisoprenylated benzophenone derived from *Garcinia indica* exhibits anti-inflammatory, acetyltransferase inhibitory, antioxidant, and anticancer effects by regulating several signaling pathways (18-20). Previous studies have shown the therapeutic potential of garcinol for gastric ailments, such as ulcers (18,21). Additionally, the anticancer effects of garcinol have been demonstrated in a number of carcinomas *in vitro* and *in vivo*. Specifically, garcinol exerted inhibitory effects in colon and prostate cancer via the PI3K/AKT signaling pathway (22,23). Additionally, garcinol decreased tumor cell proliferation, angiogenesis and cell cycle progression, and increased apoptosis in oral cancer (24). The results of the aforementioned studies suggest that garcinol may serve as a potential antineoplastic agent (20,24). However,

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the effects of garcinol in GC and its underlying mechanism remain unclear.

The present study aimed to investigate the effects of garcinol on the proliferation, invasion, and apoptosis of the GC cell line HGC-27 and to further explore the associated mechanisms. The results revealed that garcinol decreased colony formation ability, viability, migration and invasion in a dose-dependent manner. Moreover, garcinol promoted apoptosis. Further investigation revealed that, garcinol exerted its inhibitory effects on GC cells by regulating the PI3K/AKT signaling pathway.

Materials and methods

Cell culture. The human GC cell line, HGC-27 (cat no. TCHu 22) was obtained from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences and was tested for mycoplasma and authenticated by STR profiling. The cells were cultured in RPMI-1640 medium (Sigma-Aldrich; Merck KGaA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich; Merck KGaA) and 1% penicillin-streptomycin (PS; Beyotime Institute of Biotechnology) at 37˚C in a humidified atmosphere containing 5% CO_2. Garcinol was obtained from Sigma-Aldrich (Merck KGaA) and SC79 was purchased from MedChemExpress.

Cell viability. HGC-27 ($2x10^4$ cells/ml) cells were seeded in 100 μl medium per well in a 96-well plate. Cells were incubated for 6 h and subsequently treated with increasing concentrations of garcinol [0, 2.5, 5, 10, 20, 40, 80 and 160 μM in RPMI-1640 medium supplemented with 10% FBS, 1% PS and 50 mM dimethyl sulfoxide (DMSO)] for 48 h. A total of 10 μl MTT solution (5 mg/ml in PBS; Beyotime Institute of Biotechnology) was added to each well and the cells were incubated for an additional 4 h at 37˚C. The medium was then discarded and the purple formazan crystals were dissolved using 150 μ1 of DMSO. After 10 min of oscillation in dark, the absorbance was read at a wavelength of 570 nm using a SpectraMax Plus 384 plate reader (Molecular Devices, -LLC). The median lethal concentration (LC50) was calculated using SPSS software (version 24.0; IBM Corp.).

Clone formation assay. HGC-27 cells were seeded at a density of $1x10^3$ cells/well in a 6-well plate. Cells were then treated with 5 μM garcinol in serum-containing medium, or an equal volume of DMSO dissolved in medium as a control, for 14 days. The cells were subsequently fixed using 10% formalin for 20 min and stained with 0.1% crystal violet for 5 min at room temperature. Colonies containing >50 cells were counted under a light microscope (Eclipse TS100; Nikon Corporation).

Wound healing assay. HGC-27 cells were cultured in 6-well plates to 80% confluence and then serum starved for 24 h. A 100 μl sterile pipet tip was used to create scratches in the confluent cell monolayers and the cells were gently washed with PBS. The cells were incubated with garcinol (0, 10, 20, or 40 μM in RPMI-1640 medium supplemented with 10% FBS and 1% PS) and cells were cultured for an additional 48 h. An optical microscope (Eclipse TS100; Nikon Corporation) was used to image the wound at 0 and 48 h and the width of the wound was measured using Image-Pro Plus software (version 6.0; Media Cybernetics, Inc.).

Matrigel invasion assay. Transwell chambers (8.0-μm pore size; Corning, Inc.) coated with Matrigel (Corning, Inc.) were used to evaluate the effect of garcinol on the invasiveness of HGC-27 cells. HGC-27 cells ($1x10^5$/ml) were seeded into the upper chambers of the inserts in 200 μl serum-free RPMI-1640, while different concentrations of garcinol (0, 10, 20 or 40 μM in RPMI-1640 supplemented with 10% FBS and 1% PS) was added to the lower chambers. Cells were cultured for 48 h at 37˚C in a humidified atmosphere containing 5% CO_2. The invading cells were then fixed and stained using the same conditions described for the clone formation assays. The stained HGC-27 cells were subsequently imaged and counted.

Flow cytometry and cell cycle analysis. HGC-27 cells were seeded in 6-well plates and cultured to 60% confluence. The cells were subsequently treated with garcinol (0, 10, 20 and 40 μM in RPMI-1640 medium supplemented with 10% FBS and 1% PS) for an additional 48 h. The cells were harvested, suspended in cold PBS and centrifuged at 114 x g. The cells were then resuspended and centrifuged two more times. Annexin V-FITC/PI (Beyotime Institute of Biotechnology) double staining was performed according to the manufacturer's instructions. The cells were incubated for 20 min at room temperature in the dark and apoptosis was analyzed using a flow cytometer. For cell cycle analysis, HGC-27 cells were treated as aforementioned, collected and fixed with 70% ethanol at 4˚C overnight. Cells were washed with cold PBS and incubated with propidium iodide for 30 min at room temperature in the dark. ModFit LT software (version 5.0; www.vsh.com) was used to analysis the cell cycle transition of HGC-27 cells treated with increasing concentrations of garcinol.

Hoechst 33258 staining. HGC-27 cells were seeded in 96-well plates and incubated with garcinol (0, 10, 20, and 40 μM) for 48 h. The cells were subsequently fixed with 4% polyoxymethylene for 20 min at room temperature and washed twice with PBS. The cells were stained with 10 μg/ml Hoechst 33258 for 5 min in the dark and washed twice with PBS. The nuclear morphology of the HGC-27 cells was observed using a fluorescence microscope (200x magnification).

Western blotting. HGC-27 cells were treated with garcinol (0, 10, 20 and 40 μM) for 48 h and subsequently harvested. The total cellular protein was extracted using radio-immunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology) at 4˚C. The lysates (10 μ (protein per lane, BCA assay) separated via SDS-PAGE on a 10% gel and transferred onto nitrocellulose membranes. After blocking with 5% non-fat powdered milk for 60 min at room temperature, the membranes were incubated overnight at 4˚C with the following primary antibodies: Anti-PI3K (cat. no. 4249; 1:1,000; Cell Signaling Technology, Inc.), anti-AKT (cat. no. ab32505; 1:1,000; Abcam), anti-$AKT^{p\text{-}Thr308}$ (cat. no. ab38449; 1:1,000, Abcam), anti-$AKT^{p\text{-}Ser473}$ (cat. no. ab81283; 1:1,000, Abcam), anti-mTOR (cat. no. ab2732; 1:1,000; Abcam), anti-$mTOR^{p\text{-}Ser2448}$

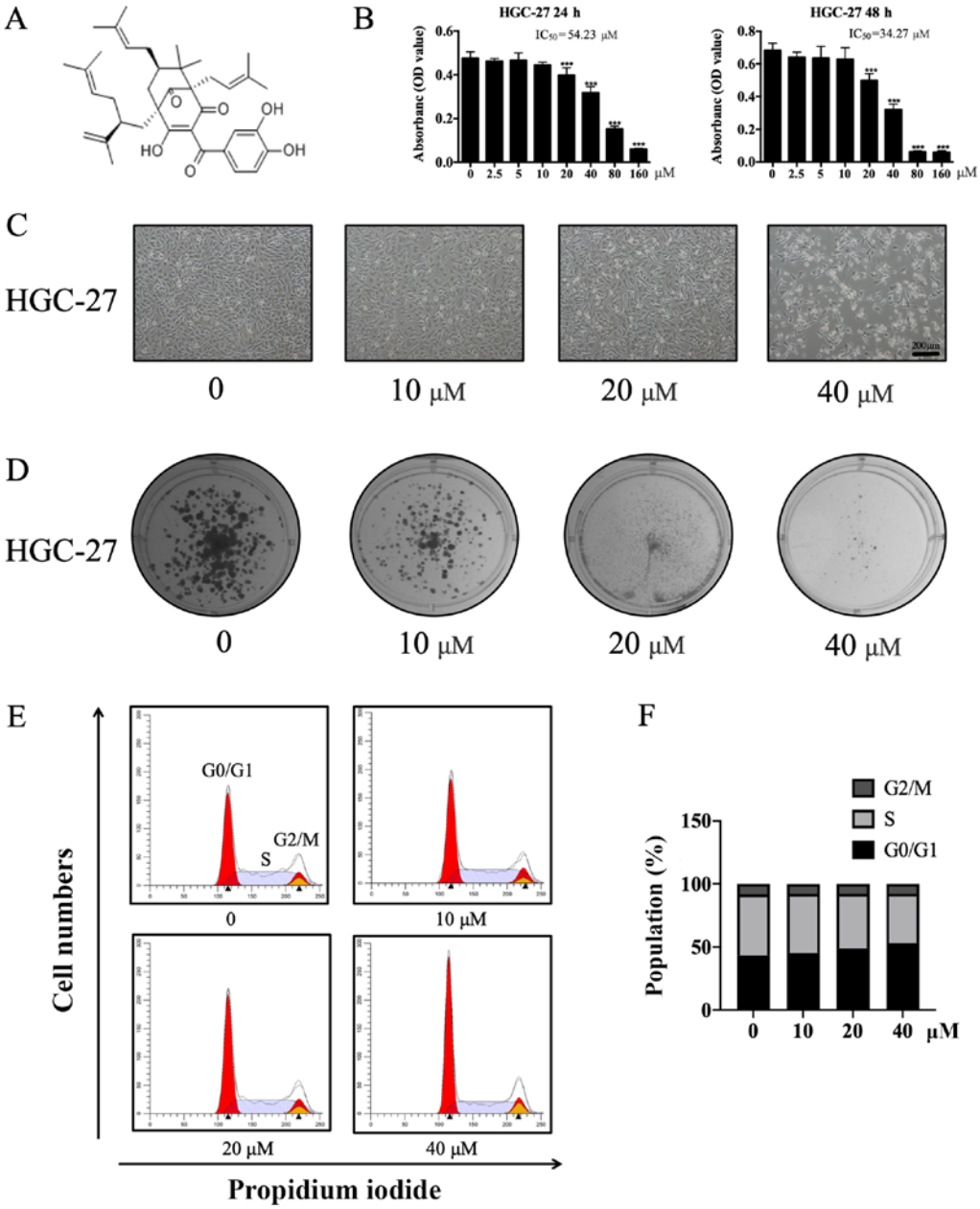

Figure 1. Garcinol decreases the proliferation and viability of gastric cancer cells. (A) Chemical structure of garcinol. (B) Effects of garcinol on the viability of HGC-27 cells at 24 and 48 h as determine by the MTT assay. ***P<0.005 vs. 0 μM. (C) Morphological alterations in HGC-27 cells after treatment with garcinol for 48 h. (D) Clonogenic variation in HGC-27 cells after treatment with garcinol for 48 h. (E) HGC-27 cells treated with garcinol for 48 h exhibited cell cycle arrest. (F) Effect of garcinol on cell cycle arrest at 48 h was analyzed. Each experiment was performed in triplicate. OD, optical density.

(cat. no. 5536; 1:1,000; Cell Signaling Technology, Inc.), anti-cyclin D1 (cat. no. 60186-1-Ig; 1:1,000; ProteinTech, Inc.), anti-Bcl-2 (cat. no. 15071; 1:1,000; Cell Signaling Technology, Inc.), anti-BAX (cat. no. 14796; 1:1,000; Cell Signaling Technology, Inc.), anti-MMP-2 (cat. no. ab97779; 1:1,000; Abcam), anti-MMP-9 (cat. no. ab76003; 1:1,000; Abcam) and anti-β-actin (cat. no. 3700; 1:1,000; Cell Signaling Technology, Inc.). The membranes were then washed three times with 1% Tris-buffered saline/Tween-20 and incubated with the appropriate horseradish peroxidase secondary antibodies (cat. no. A21020 and A21010; 1:1,000; Abbkine Scientific Co., Ltd.) for 4 h at 4˚C. The protein bands were visualized using the BeyoECL kit (Beyotime Institute of Biotechnology). β-actin was used as the loading control.

Statistical analysis. Data are expressed as the mean ± SD of three independent experiments. Statistical analyses were performed using GraphPad Prism software (version 7.0; GraphPad Software, Inc.). One-way analysis of variance with Tukey's post hoc test was used for the statistical analysis of the groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Garcinol decreases the proliferation and viability of GC cells. The proliferation and viability of HGC-27 cells were significantly suppressed by garcinol. HGC-27 cells treated with a low concentration of garcinol exhibited similar optical density

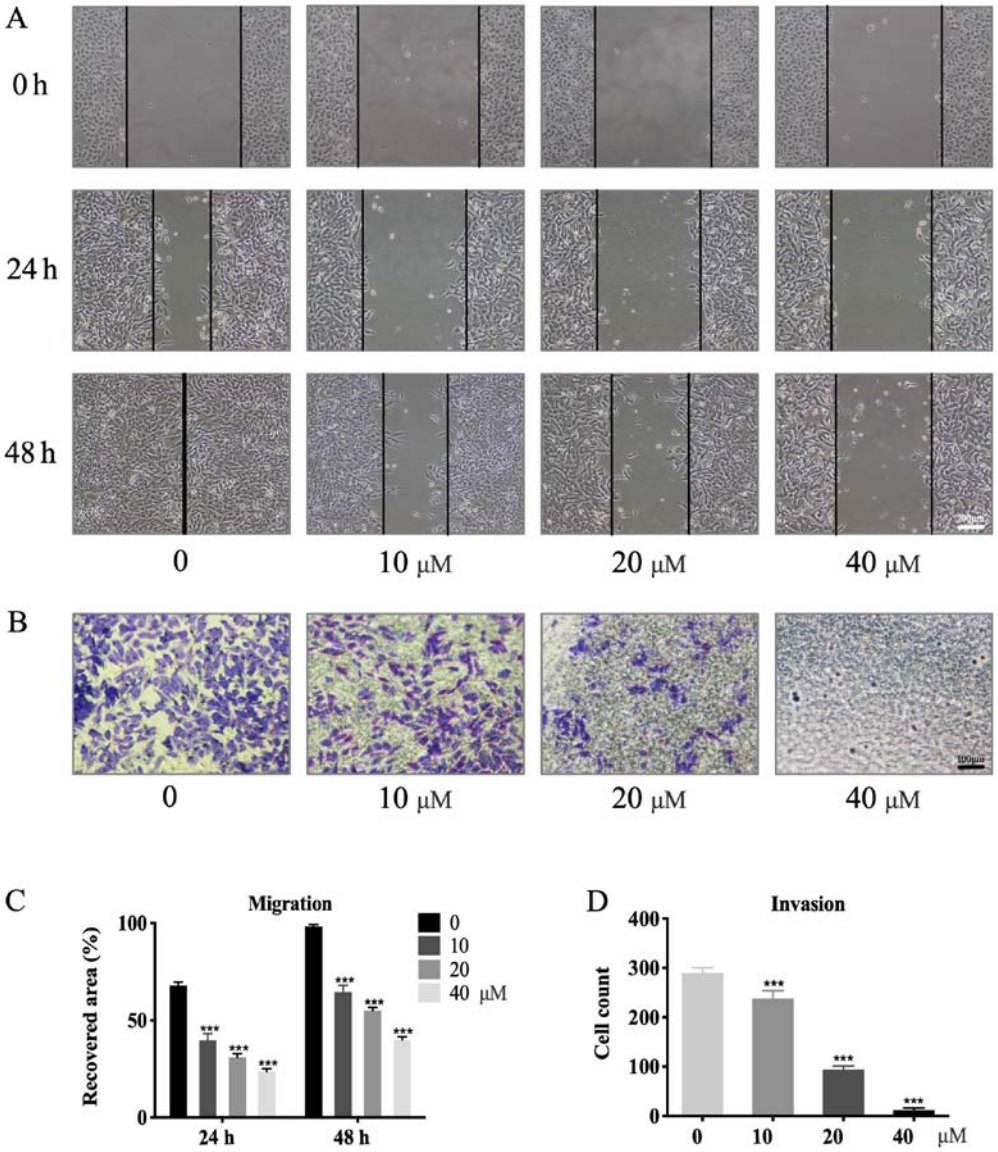

Figure 2. Garcinol inhibits the migration and invasion of gastric cancer cells. (A) HGC-27 cells were treated with garcinol at a concentration of 0, 10, 20 and 40 μM. Images were captured at 0, 24 and 48 h using an optical microscope. Scale bar, 200 μm. (B) Matrigel-coated Transwell chambers were used to examine the invasiveness capability of HGC-27 cells following garcinol treatment (0, 10, 20 and 40 μM) for 48 h. Scale bar, 100 μm. (C) Width of the wound was measured using Image-Pro Plus software (version 6.0). ***P<0.005 0 μM. (D) Invading cells were imaged and counted. Each experiment was performed in triplicate and results were analyzed by one-way analysis of variance with Tukey's post hoc test. ***P<0.005 vs. 0 μM. Data are presented as the mean ± SD.

values to their respective negative controls (Fig. 1B and C). However, the optical density values of HGC-27 cells treated with higher doses of garcinol were significantly reduced (T-test: 0.50±0.042, 20 μM, P<0.001; 0.32±0.034, 40 μM, P<0.001; 0.06±0.005, 80 μM, P<0.001; 0.06±0.009, 160 μM, P<0.001). The median lethal concentration (LC50) of garcinol treatment in HGC-27 cells was 34.27 μM. HGC-27 cells treated with garcinol (0, 10, 20 and 40 μM) for 48 h displayed a marked decrease in cell growth (Fig. 1B and C). Furthermore, garcinol significantly decreased the colony formation ability of HGC-27 cells in a dose-dependent manner (Fig. 1D). In order to further investigate the effects of garcinol on the cell cycle transition of HGC-27 cells, cell cycle analysis was performed. The percentage of cells in the G0/G1 phase was significantly increased (ANOVA: 45.33±0.182, 10 μM, P<0.05; 48.86±1.148, 20 μM, P<0.001; 53.11±0.769, 40 μM, P<0.001) while the percentage of cells in the S phase was decreased (ANOVA: 46.69±0.201, 10 μM, P<0.05; 43.15±1.151, 20 μM, P<0.001; 38.91±0.757, 40 μM, P<0.001) in garcinol-treated cells compared with controls (43.39±0.350, 0 μM, G0/G1 phase; 48.66±0.424, 0 μM, S phase; Fig. 1E and F).

Garcinol inhibits the migration and invasion of GC cells. Garcinol exerted a dose-dependent inhibitory effect on migration and invasion in HGC-27 cells (Fig. 2A and B). Wound-healing assays were performed to investigate the effects of garcinol on the migration of HGC-27 cells. Compared with the control group (98.3±0.9%; Fig. 2C) the percentage wound closure exhibited a significant decrease with increasing garcinol concentrations after 48 h of treatment: 64.6±2.75% (10 μM; P<0.001), 55.1±1.3% (20 μM; P<0.001), 40.0±1.2% (40 μM; P<0.001; Fig. 2C). A similar trend was observed at the 24-h time point (P<0.001; Fig. 2C). Matrigel-coated Transwell chambers were used to evaluate the

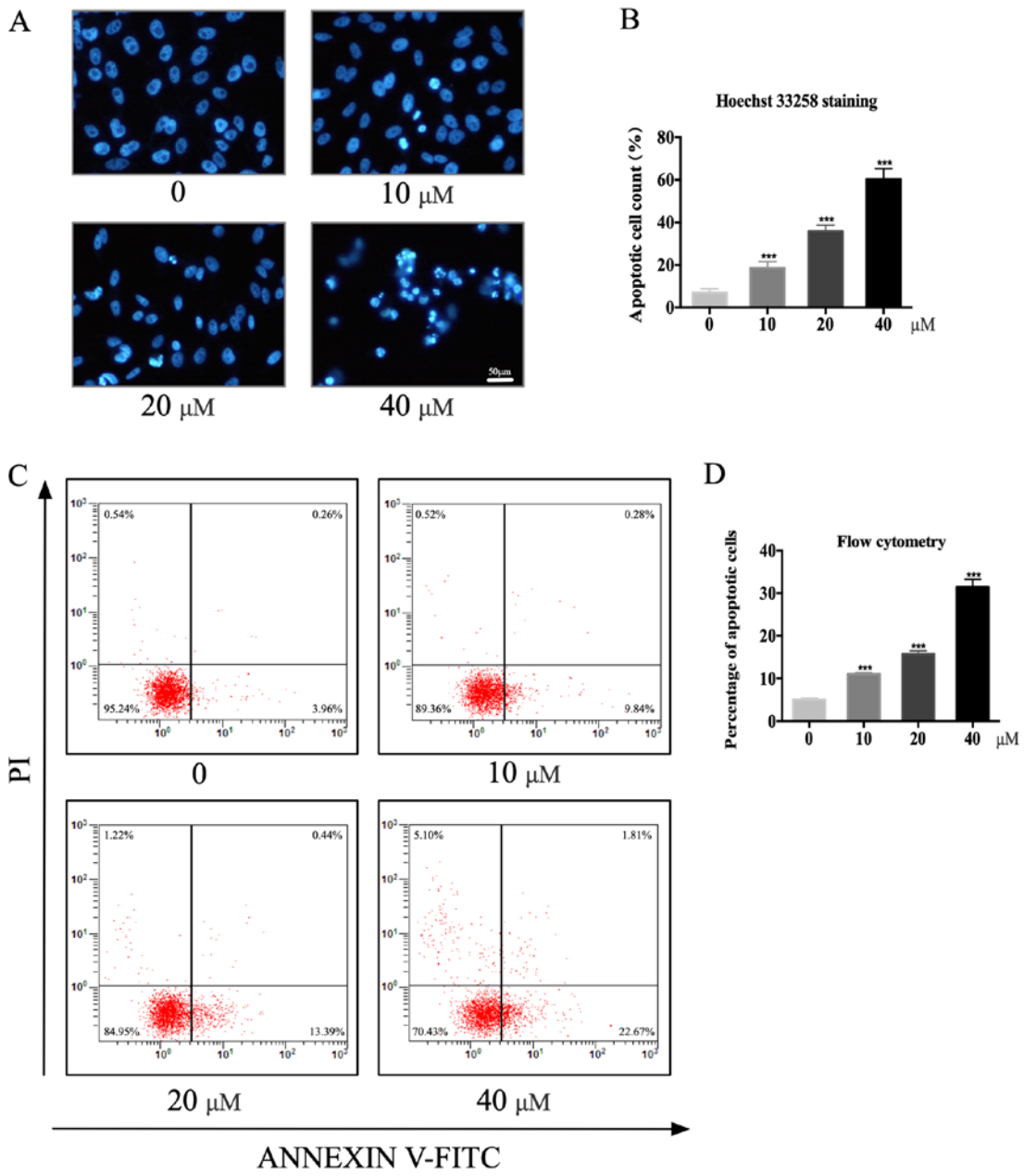

Figure 3. Garcinol induces apoptosis of gastric cancer cells. (A) HGC-27 cells were treated with garcinol (0, 10, 20 and 40 μM) for 48 h and stained with Hoechst 33258. The cells were subsequently imaged using a fluorescence microscope. (B) Cells with condensed and fragmented cell nuclei were counted to assess the impact of garcinol on cell apoptosis. ***P<0.005 vs. 0 μM. (C) Annexin V-FITC/PI staining and flow cytometry analysis were used to assess the apoptotic ratio of HGC-27 cells treated with garcinol (0, 10, 20 and 40 μM). (D) Quantitative analysis for flow cytometry. ***P<0.005 vs. 0 μM. Each experiment was performed in triplicate. PI, propidium iodide.

inhibitory effects of garcinol on the invasion of HGC-27 cells. The control group exhibited the highest number of invading cells (255.3±13.0). This number significantly decreased with increasing concentrations of garcinol at 48 h: 196.7±13.3 (10 μM; P<0.001), 83.3±6.3 (20 μM; P<0.001) and 12.0±3.2 (40 μM; P<0.001; Fig. 2D).

Garcinol induces apoptosis of GC cells. Garcinol was found to induce the programmed cell death of HGC-27 cells. Hoechst 33258 staining was used to observe nuclear changes in HGC-27 cells. Cells with condensed and fragmented nuclei were considered apoptotic (Fig. 3A). The number of apoptotic HGC-27 cells increased from 6.9±1.52 in the control group to 18.6±2.46 (P<0.001), 35.9±2.34 (P<0.001) and 60.3±4.10 (P<0.001) in cells treated with 10, 20 and 40 μM garcinol for 48 h, respectively (Fig. 3B). The annexin V-FITC/PI staining assay revealed a similar trend. In particular, the number of early apoptotic HGC-27 cells (annexin V^+/PI^-) significantly increased with garcinol concentrations as follows: 11.1±0.32% (10 μM; P<0.001), 15.8±0.67% (20 μM; P<0.001), 31.5±1.81% (40 μM; P<0.001; Fig. 3C and D).

Garcinol down-regulates the activation of the PI3K/AKT signaling pathway and its downstream effectors. Western blotting was used to determine the concentration-dependent effects of garcinol on the expression of several key proteins in

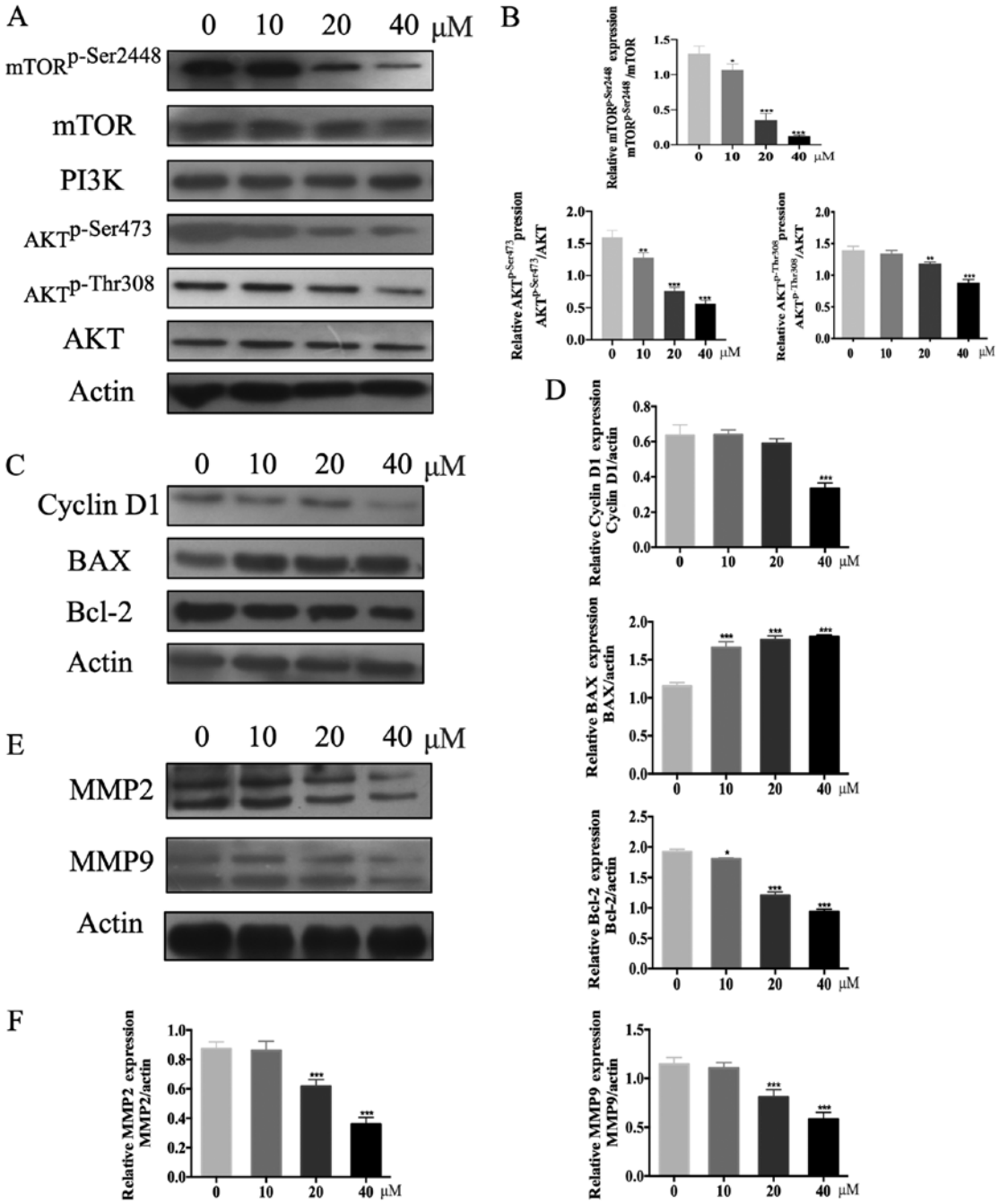

Figure 4. Garcinol downregulateds PI3K/AKT and its downstream signaling pathway. (A) HGC-27 cells were treated with garcinol (0, 10, 20 and 40 μM) for 48 h and western blotting was used to determine the protein expression levels of PI3K, AKT, AKT$^{p\text{-}Thr308}$, AKT^{Ser473}, mTOR, mTOR$^{p\text{-}Ser2448}$ and β-actin. (B) Quantitative analysis for the western blotting. The protein expression levels of PI3K, AKT, AKT$^{p\text{-}Thr308}$, AKT^{Ser473}, mTOR, mTOR$^{p\text{-}Ser2448}$ and β-actin in each dose group (10, 20 and 40 μM) were compared with those in the no drug treatment group (0 μM). *P<0.05, **P<0.01 and ***P<0.005 vs. 0 μM. (C) HGC-27 cells were treated with garcinol (0, 10, 20 and 40 μM) for 48 h and the protein expression levels of cyclin D1, BAX, Bcl-2 and β-actin were determined by western blotting. (D) Semi-quantitative analysis was performed to compare the protein expression levels of cyclin D1, BAX, Bcl-2 and β-actin in each dose group (10, 20 and 40 μM) with those in the no drug treatment group (0 μM). *P<0.05 and ***P<0.005 vs. 0 μM. (E) Western blotting was used to determine the protein expression levels of MMP-2, MMP-9 and β-actin in HGC-27 cells under the treatment of garcinol (0, 10, 20 and 40 μM) for 48 h. (F) Protein expression levels of MMP-2, MMP-9 and β-actin in each dose group (10, 20 and 40 μM) were quantitatively analyzed and compared with those in the no drug treatment group (0 μM). ***P<0.005 vs. 0 μM. Each experiment was performed in triplicate. MMP, matrix metalloprotease.

the PI3K/AKT signaling pathway in HGC-27 cells. Garcinol significantly inhibited the levels of AKT$^{p\text{-}Thr308}$ and AKT$^{p\text{-}ser473}$ in HGC-27 cells, in a dose-dependent manner, while PI3K and total AKT levels were not affected (Fig. 4A and B). Additionally, garcinol significantly reduced the phosphorylation of mTOR, while the expression of total mTOR remained stable (P<0.05; Fig. 4A and B). Cyclin D1 levels were then examined to further evaluate the impact of garcinol on the G1/S transition of GC cells. Garcinol treatment was found to decrease cyclin D1 levels in HGC-27 cells in a dose-dependent manner (P<0.05; Fig. 4C and D). Garcinol also significantly reduced the expression of the proteolytic enzymes MMP-2 and MMP-9 (P<0.05; Fig. 4E and F), which are considered to be crucial for the malignant invasion and metastasis of

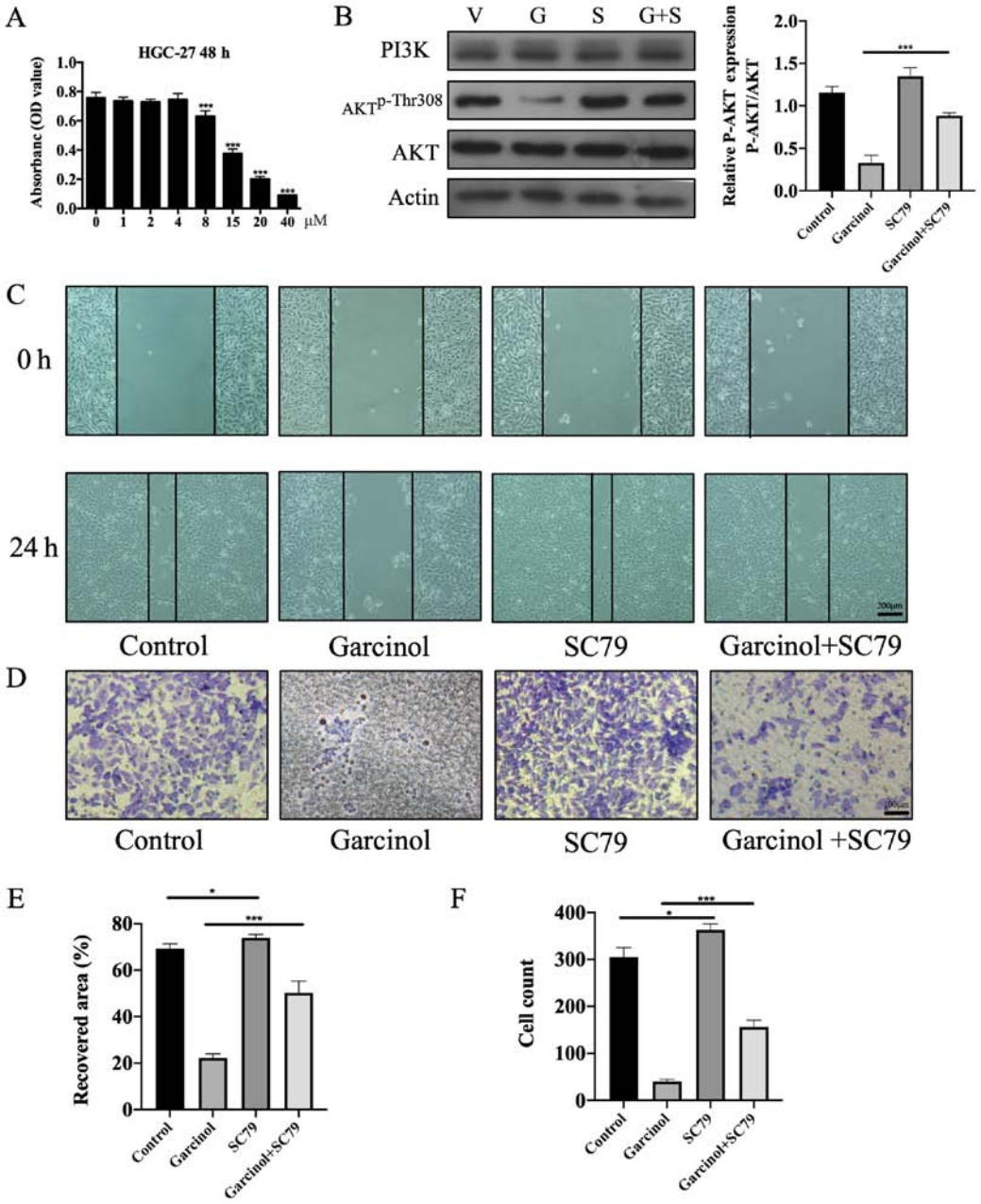

Figure 5. SC79, a specific AKT agonist, rescues the inhibitory effect of garcinol on the proliferation and invasion of HGC-27 cells. (A) Safe effective dose of SC79 on HGC-27 cells at 48 h was measured by MTT assay. The absorbance of each dose group (1, 2, 4, 8, 15, 20 and 40 μM) was compared with no SC79 treatment group (0 μM). ***P<0.005 vs. 0 μM. (B) Western blotting was performed to estimate the protein expression levels of PI3K, AKT, AKTp-Thr308 and β-actin in HGC-27 cells at 48 h. ***P<0.005, as indicated. (C) Width of the wound was measured at 0 and 24 h. Scale bar, 200 μm. (D) A Transwell invasion assay was performed to investigate the invasion of HGC-27 cells in the different groups at 48 h. Scale bar, 100 μm. (E) Recovered area of each group was compared with vacant control group at 24 h. *P<0.05 and ***P<0.005, as indicated. (F) Invaded cell counts of each intervention group were compared with the vacant control group at 48 h. *P<0.05 and ***P<0.005, as indicated. V, vacant control; G, garcinol; S, SC79; G+S, garcinol+SC79.

carcinomas (15,16). Furthermore, an increase in expression of the pro-apoptotic BAX protein, together with a decrease in expression of the anti-apoptotic Bcl-2 protein, was observed (P<0.05; Fig. 4C and D).

Specific AKT agonist SC79 rescues garcinol-induced inhibitory effects in HGC-27 cells. The specific AKT agonist SC79 (25) was used to rescue the garcinol-induced inhibitory effects on HGC-27 cell proliferation, invasion and apoptosis. The MTT assay was performed to identify the most effective dose of SC79 (Fig. 5A). Western blotting was used to detect the expression levels of key proteins in the PI3K/AKT signaling pathway. Garcinol significantly decreased the expression level of AKTp-Thr308 in HGC-27 cells, an effect that was abrogated with SC79 treatment (Fig. 5B). The inhibitory effect of garcinol on cell migration (Fig. 5C and E) and invasion (Fig. 5D and F) was also abrogated following treatment with SC79. The effects of SC79 on garcinol-induced apoptosis of HGC-27 cells were investigated using Hoechst 33258 staining and flow cytometry analysis. The results revealed that SC79 significantly decreased the number of apoptotic cells compared with the garcinol only-treated group (Fig. 6A-D).

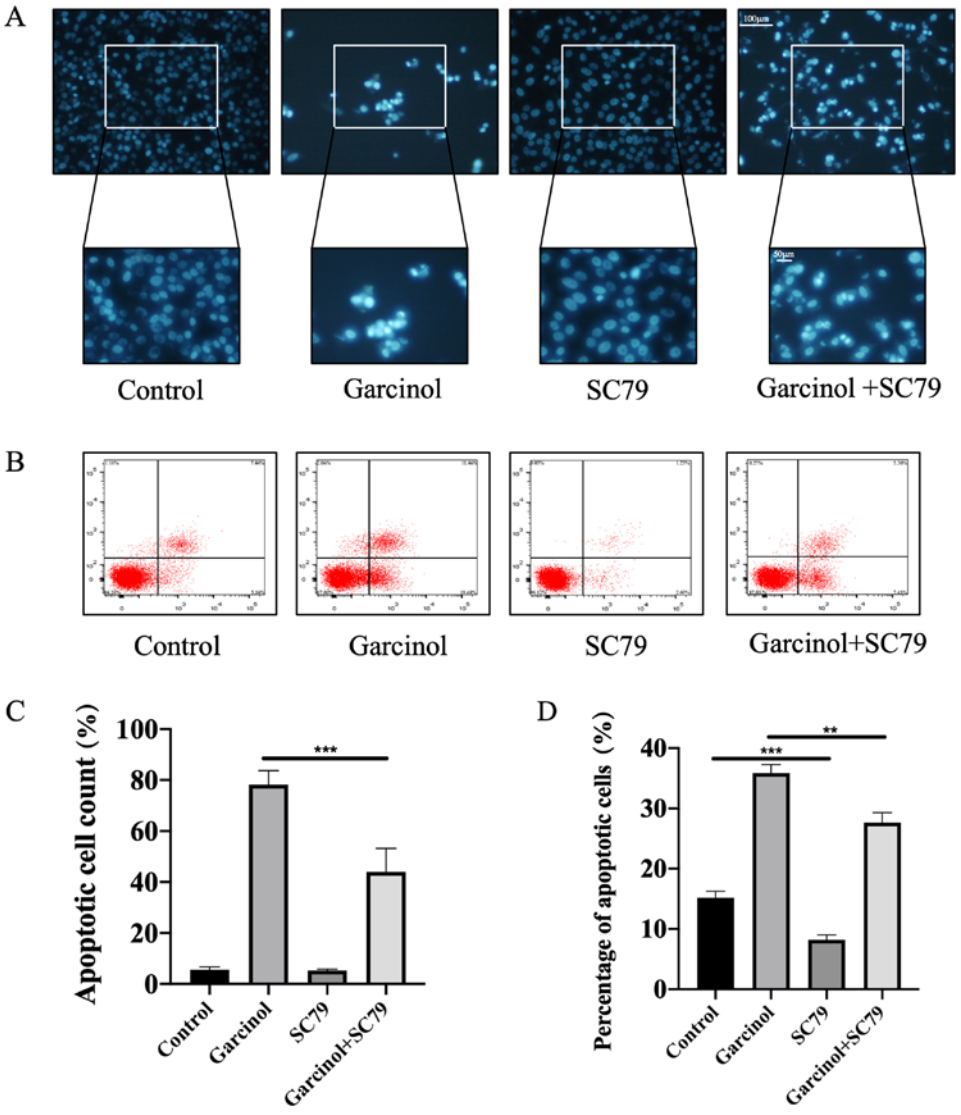

Figure 6. SC79, a specific AKT agonist, rescues garcinol-induced apoptosis in HGC-27 cells. (A) Hoechst 33258 staining was performed to analyze apoptosis. (B) Annexin V-FITC/PI staining was used to assess the apoptotic ratio of HGC-27 cells in the different groups after 48 h. (C) Apoptotic cell counts of each intervention group were compared with the vacant control group. ***P<0.005. (D) Percentages of apoptotic cells of each group were compared with those of the vacant control group at 48 h. **P<0.01, ***P<0.005.

Discussion

GC is one of the most common malignant diseases (1) and is responsible for the second largest number of cancer-associated mortalities. There are major challenges in developing effective therapeutic strategies for advanced GC, and novel therapies are urgently required.

Garcinol is a bioactive phytochemical with anti-carcinogenic properties. Previous studies have demonstrated that garcinol decreases proliferation and induces apoptosis in numerous tumor cells (18,19,26,27). The anti-neoplastic activities of garcinol are reported to occur via a variety of tumor-associated signaling pathways. Garcinol inhibited autophagy and increased apoptosis of prostate cancer cells by regulating the PI3K/AKT signaling pathway (23). Furthermore, garcinol suppressed the progression of pancreatic (28) and breast (29) carcinomas by mediating NF-κB signaling and down-regulated the growth of hepatocellular carcinoma cells by modulating STAT3 (30). However, to the best of our knowledge, the effects and underlying mechanisms of garcinol in GC cells have not been previously reported. The results obtained in the present study revealed that garcinol significantly reduced the viability and colony formation ability of GC cell line HGC-27 in a dose-dependent manner. Garcinol resulted in a significant, dose-dependent decrease in the migration and invasion of HGC-27 cells, which was accompanied by an increase in apoptosis. Additionally, the percentage of HGC-27 cells in the G0/G1 phase was significantly increased while the percentage in the S phase was significantly decreased following treatment with garcinol, implying significant cell cycle arrest. The G0/G1 phase is essential for DNA replication and cell division, while a sustained G1 block may result in apoptosis in HGC-27 cells (31-33). Therefore, garcinol may serve as a potential therapeutic agent for GC.

Tumor growth and metastasis are crucial steps in tumorigenesis. Previous studies have indicated that mTOR is expressed in 60-80% of gastric adenocarcinomas (34,35). The regulation

of cyclin D1 production by mTOR is the primary mechanism by which mTOR mediates cell proliferation (36). Moreover, overexpression of cyclin D1 promotes the G1-S cell cycle transition and accelerates the GC tumorigenesis (37). Therefore, mTOR/cyclin D1 inhibitors may suppress tumor proliferation. The present study revealed that garcinol decreased the protein expression of mTOR, $mTOR^{p\text{-}Ser2448}$ and cyclin D1 in HGC-27 cells in a dose-dependent manner, suggesting that it suppresses the viability and proliferation of GC cells. Moreover, garcinol significantly reduced the expression of MMP-2/9 proteins in a dose-dependent manner. As proteolytic enzymes, the MMP family participates in the degradation of the extracellular matrix and MMP-2/9 proteins play important roles in invasion and angiogenesis in malignant tumors (38). The present study revealed that garcinol may inhibit the proliferation and extravasation of gastric carcinoma cells, thereby exerting anti-neoplastic effects and decreasing the rate of malignant progression.

A dynamic balance between cell proliferation and death is necessary to maintain homeostasis. Cancer cells are characterized by their ability to evade tumor suppressors and cell death and to sustain proliferative signaling and replication, thus activating invasion and metastasis (39). Apoptosis appears to be attenuated in numerous malignancies, thus enabling tumor cells to resist cell death and to proliferate. Apoptosis is regulated by the proapoptotic- and anti-apoptotic Bcl-2 family members (40). The upregulation of BAX and downregulation of Bcl-2 increases apoptosis. In agreement with this concept, the results obtained in the present study revealed that garcinol increased HGC-27 cell apoptosis by down-regulating Bcl-2 and an up-regulating BAX in a dose-dependent manner. These results indicated that garcinol may induce programed cell death in GC and may serve as a promising therapeutic agent.

The mechanism underlying the anti-tumor effects of garcinol may be attributed to the modulation of the PI3K/AKT signaling pathway. The PI3K/AKT signaling pathway is closely associated with neoplastic transformation, since the activation of AKT is known to drive cellular growth, differentiation and survival (41-43). Phosphorylated AKT enters the nucleus and activates mTOR and downstream signaling, which subsequently accelerates neoplasm progression (44). Previous studies suggested that the PI3K/AKT/mTOR pathway is activated in gastric tumor tissues, compared with non-tumor tissues (14,35,45), indicating that targeted blocking of this pathway may be able to suppress gastric tumorigenesis. Additionally, activated AKT promotes the expression of MMPs, and reduces the binding of the Bcl-2/XL-associated death (BAD) gene promoter to Bcl-2/XL (46,47), to increase the invasion and decrease the apoptosis of gastric carcinoma cells. The present study revealed that the protein levels of $AKT^{p\text{-}Thr308}$, $AKT^{p\text{-}Ser473}$ and $mTOR^{p\text{-}Ser2448}$ in HGC-27 cells decreased following treatment with garcinol in a dose-dependent manner. These aforementioned results suggested that garcinol exerts its anti-tumor effects in HGC-27 cells by inhibiting the PI3K/AKT signaling pathway. In order to further validate these findings, rescue experiments using SC79, a specific agonist of AKT, were performed. The results revealed that SC79 abrogated the garcinol-induced inhibitory effects on HGC-27 cells, which further consolidated the evidence garcinol inhibits the PI3K/AKT signaling pathway.

In conclusion, the present study demonstrated that garcinol suppresses tumorigenesis in GC by decreasing cell proliferation, inhibiting cell invasion and migration and promoting cell apoptosis. Further investigation revealed that garcinol is likely to exhibit these effects by inhibiting the PI3K/AKT signaling pathway and downregulating the expression of Cylin D1, MMP2, MMP9 in HGC-27 cells, which can be rescued by SC79, a specific agonist of AKT, at the cellular level (Fig. S1). Therefore, garcinol may play crucial roles in GC and may serve a novel therapeutic agent.

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Availability of data and materials

All data generated and/or analyzed during this study are included in this published article.

Authors' contributions

AM, CG and XW conceived and designed the research. YZ, CG, XZ performed the experiments and analyzed the data. YZ wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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