Ginsenoside Rg3 enhances the anticancer effect of 5-FU in colon cancer cells via the *PI3K/AKT* pathway

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Abstract. Chemotherapy is one of the most commonly used treatments for patients with advanced colon cancer, yet the toxicity of chemotherapy agents, such as 5-fluorouracil (5-FU), limits the effectiveness of chemotherapy. Ginsenoside Rg3 (Rg3) is an active ingredient isolated from ginseng. Rg3 has been shown to display anticancer effects on a variety of malignancies. Yet, whether Rg3 synergizes the effect of 5-FU to inhibit the growth of human colon cancer remains unknown. The present study was designed to ascertain whether Rg3 is able to enhance the anti-colon cancer effect of 5-FU. The results revealed that combined treatment of Rg3 and 5-FU significantly enhanced the inhibition of the proliferation, colony formation, invasion and migration of human colon cancer cells (SW620 and LOVO) *in vitro*. We also found that combined treatment of Rg3 and 5-FU significantly enhanced the apoptosis of colon cancer cells by activating the Apaf1/caspase 9/caspase 3 pathway and arrested the cell cycle of the colon cancer cells in G0/G1 by promoting the expression of *Cyclin D1*, *CDK2* and *CDK4*. In addition, the *PI3K/AKT* signaling pathway in colon cancer cells was suppressed by Rg3 and 5-FU. *In vivo*, Rg3 synergized the effect of 5-FU to inhibit the growth of human colon cancer xenografts in nude mice. Similarly, combined treatment of Rg3 and 5-FU altered the expression of colon cancer protein *in vivo* and *in vitro*. Collectively, the present study demonstrated that ginsenoside Rg3 enhances the anticancer effect of 5-FU in colon cancer cells via the *PI3K/AKT* pathway.

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Introduction

Colon cancer is a common malignant tumor of the digestive tract located in the colon, which mainly occurs at the junction of the rectum and the sigmoid colon. Statistics show that the highest incidence of colon cancer is in the age group of 40-50 years, the ratio of male to female is 2-3:1, and the incidence of colon cancer ranks third among all cases of gastrointestinal tumors (1,2). The 5-year survival rate of patients with colon cancer is approximately 64.9%, yet the 5-year survival rate of patients with advanced stage disease is as low as 12.5%. Since the early symptoms of patients with colon cancer are not obvious, only about 40% of patients can be diagnosed at the early stage of the disease (3,4). Chemotherapy is one of the most important treatments for patients with advanced colon cancer, of which 5-fluorouracil (5-FU) is the most widely used. 5-FU inhibits the proliferation, invasion and migration of tumor cells by interfering with the nucleic acid metabolism of tumor cells, but it is also toxic to normal cells, causing serious adverse reactions, even endangering the life safety of patients, severely limiting its clinical application (5,6). Previous research has shown that 5-FU combined with other agents may reduce the required dosage of 5-FU consequently reducing the adverse reactions caused by 5-FU without affecting or even improving the efficacy of chemotherapy (7,8).

Compared with chemical drugs and biopharmaceuticals, multi-component, multi-target, and less adverse reactions are unique advantages of traditional Chinese medicine for the treatment of diseases. In patients with colon cancer, Chinese medicine can improve patient immunity, reduce the side effects of radiotherapy and chemotherapy or enhance drug sensitivity. Inhibiting the expression of oncogenes helps to inhibit the migration of cancer cells and has a good effect on the treatment of colon cancer (9,10). Ginsenoside Rg3 (Rg3), an active ingredient isolated from ginseng, is a tetracyclic triterpenoid saponin that inhibits neovascularization, induces tumor cell apoptosis, and selectively inhibits tumor cell metastasis and enhances immune function (11,12). Previous studies have shown that Rg3 exhibits an inhibitory effect on proliferation, invasion and migration of human tumor cells, such as lung cancer (13,14), gastric carcinoma (15) and prostate cancer (16). In colon cancer, Rg3 was found to activate the AMPK signaling pathway to accelerate apoptosis in colon

cancer cell line HT-29 *in vitro*, and also to block colon cancer progression by targeting inhibition of cancer stem cells and tumor angiogenesis *in vivo* (17). Although numerous studies have shown that Rg3 increases the efficacy and decreases the toxicity of chemotherapeutic drugs and suppresses the chemotherapeutic resistance in cancer (18,19), its combination with chemotherapeutic agent 5-FU to achieve extra benefits in anti-colon cancer treatment warrants detailed investigation.

In the present study, the effects of a combined treatment of Rg3 and 5-FU on the biological properties of SW620 and LOVO cells were investigated *in vivo* and *in vitro*. We found that a combined treatment of Rg3 and 5-FU not only enhanced the inhibition of colon cancer cell proliferation, migration and invasion, but also promoted apoptosis of colon cancer cells and arrested the cells in the G0/G1 phase. In addition, it was also found that Rg3 could synergize the capacity of 5-FU to inhibit the growth of human colon cancer xenografts in nude mouse, and the combined treatment of Rg3 and 5-FU enhanced the inhibition of the *PI3K/AKT* pathway in colon cancer cells.

Materials and methods

Cell lines and agents. SW620 (CCL-227; ATCC, American Type Culture Collection, Manassas, VA, USA) and LOVO (CCL-229; ATCC) cell lines were cultured with DMEM medium (cat. no. 12491-15; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (FBS; cat. no. 10100-147; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (cat. no. 15640055, Thermo Fisher Scientific, Inc.). The cell lines used in the present study were cultured at 37˚C with 5% CO_2.

Rg3 (cat. no. 64139; Sigma-Aldrich; Merck KGaA) and 5-FU (cat. no. 04541, Sigma-Aldrich; Merck KGaA) were dissolved in DMSO. For the cell experiments, the diluted culture solution of Rg3 or 5-FU was dissolved in DMSO to achieve the experimental concentration and was administered to the cells for 48 h. For animal experiments, PBS diluted Rg3 or 5-FU was dissolved in DMSO to the experimental concentration. The experiments were approved by the Ethics Committee of The Quanzhou First Hospital Affiliated to Fujian Medical University (Quanzhou, Fujian, China).

MTT assay. A total of $2x10^3$ cells/well were inoculated in a 96-well culture plate containing the indicated medium (DMEM plus 10% FBS). We evaluated the viability of the SW620 and LOVO cells by MTT assay. In short, after 4 h of culture, MTT (10 μl, 10 mg/ml), which was dissolved in DMSO, was added to the cells and incubated. The cell supernatant was removed and then 100 μl DMSO was added. After 30 min, the optical density (OD570) was determined using a plate reader (ELx808; Bio-Tek Instruments).

Cell colony formation assay. A total of $2x10^3$ cells/ml were seeded in 6-well plates with 2 ml medium/well, and medium was exchanged once every 3 days. Cells were routinely cultured for about 3 weeks. When visible clones appeared in the well, the culturing was stopped. The supernatant culture medium was drawn, washed with PBS 2 times, and fixed with 4% formaldehyde for 15 min. The supernatant was drawn, stained with 0.25% crystal violet for 25 min, and slowly rinsed with sterile water. Plates were placed in a sterile purification table and images were captured after drying. The relative proliferation was measured by measuring the absorbance at 595 nm using a plate reader (ELx808; Bio-Tek Instruments).

Western blot analysis. RIPA lysate buffer (cat. no. P0013C; Beyotime Institute of Biotechnology, Shanghai, China) was used to extract total cellular protein, and the BCA kit (cat. no. P0009; Beyotime Institute of Biotechnology) was used to determine the protein concentration. Then cell lysates of SW20 and LOVO cells were separated by 10% SDS-page with 50 μg total protein and transferred to a PVDF membrane. The following primary antibodies were selected as follows: Anti-N-cadherin antibody (ab18203, dilution 1:1,000), anti-E-cadherin antibody (ab1416, dilution 1:50), anti-MMP-9 (ab38898, dilution 1:1,000), anti-active-caspase-9 antibody (ab2324, dilution 1:500), anti-active-caspase-3 antibody (ab2302, dilution 1:500), anti-Apaf1 antibody (ab2324, dilution 1:1,000), anti-PI3K-p85 antibody (ab191606, dilution 1:1,000), anti-PI3K-110β antibody (ab32569, dilution 1:1,000), anti-pan-AKT (phospho T308) antibody (ab38449, dilution 1:500), Anti-pan-AKT antibody (ab8805, dilution 1:500), anti-PDK1 antibody (ab52893, dilution 1:1,000) and anti-GAPDH (ab9484, dilution 1:3,000). The secondary antibody was selected as follows: Goat anti-rabbit (ab150077, dilution 1:1,000), or goat anti-rat (ab150117, dilution 1:1,000). The blocking protocol was with 5% skim milk for 1 h at room temperature. The primary antibody was incubated overnight at 4˚C and the secondary antibody was incubated for 1 h at room temperature. The BeyoECL Plus kit (cat. no. P0018S, Beyotime) was used for the chromogenic protein bands with Beckman Coulter Immunoassay System (UniCel DxI 800; Beckman Coulter), and ImageJ (v2.1.4.7; National Institutes of Health) was used for the densitometric analysis of protein bands. All antibodies were purchased from Abcam unless otherwise stated.

Transwell invasion experiment. The cell density was adjusted to $0.5x10^6$ cells/ml and then the cells were added to a 24-well Transwell upper chamber (Corning, Corning, NY, USA). Medium containing 20% FBS (Gibco; Thermo Fisher Scientific, Inc.) was added into the lower Transwell chamber and the Transwell was incubated at 37˚C for 24 h. The Transwell was taken out and the medium was removed. It was washed twice with PBS, methanol was added, and dried after being fixed for 30 min. After the membrane was dried, it was stained with crystal violet for 20 min, and the relative migration was determined by measuring the absorbance at 595 nm using a plate reader (ELx808; Bio-Tek Instruments, Inc.).

Cell scratch test. A total of $5x10^5$ cells were placed in a 6-well plate (2 ml/well). A scratch was made as far as possible perpendicular to the back of a horizontal line by using tips against a ruler (tips should be vertical and cannot be tilted). The cells were washed with PBS for three times and the scratched cells were removed, and serum-free DMEM was added. Cells were cultured at 37˚C in a 5% CO_2 incubator for 24 h, and images were captured in 0 and 24 h using an CKX41 Olympus inverted microscope (magnification, x100; Olympus Corp.).

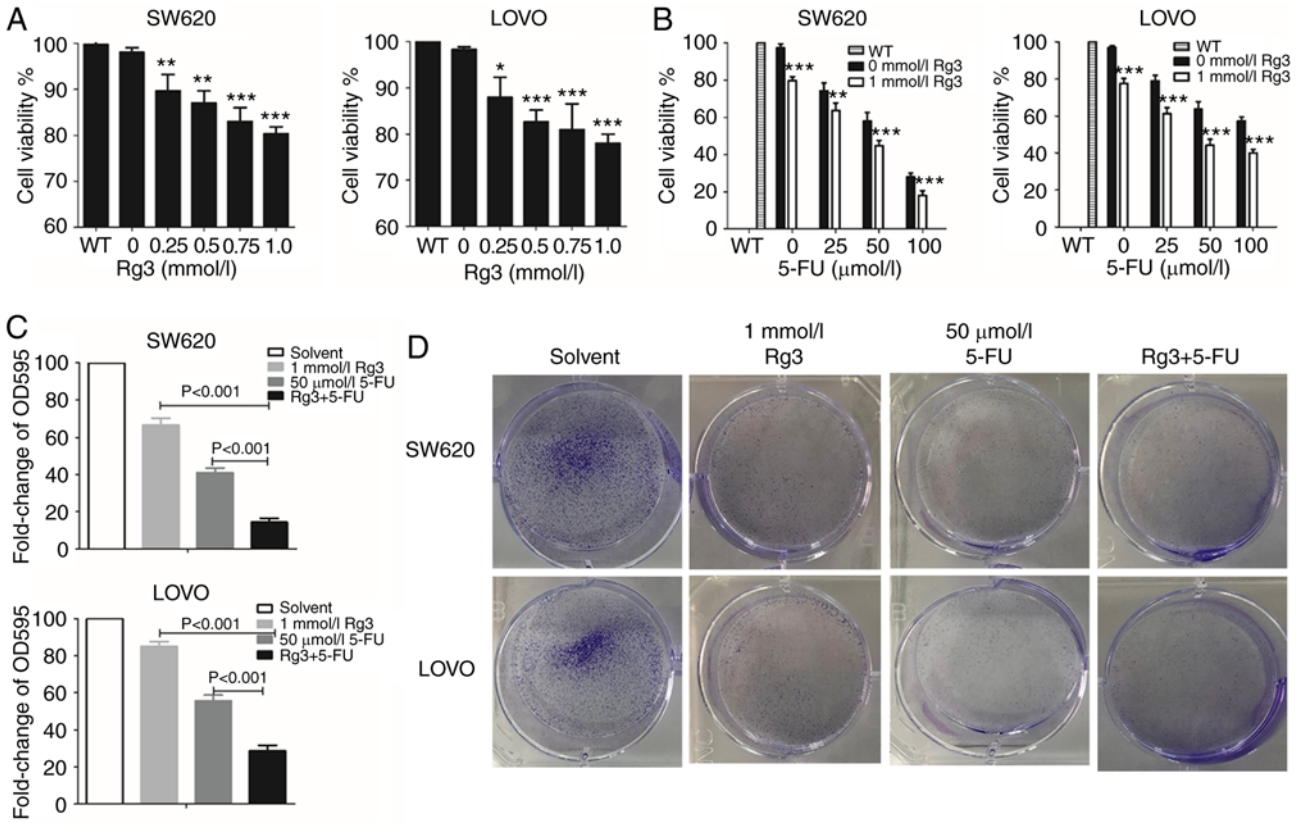

Figure 1. Effect of the combined treatment of Rg3 and 5-FU on proliferation of colon cancer cells *in vitro*. SW620 and LOVO cells were treated with different doses of (A) Rg3 (mmol/l) or (B) 5-FU (μmol/l), and then the MTT assay was used to detect cell viability. (C and D) After treatment with Rg3 (1 mmol/l) or 5-FU (50 μmol/l) or their combination, the colony formation of the colon cancer cells was photographed. WT group was used as a baseline for cell viability and cell colony formation. Three independent repetitions were performed for each experiment. *P<0.05, **P<0.01 and ***P<0.001, compared with the WT group. 5-FU, 5-fluorouracil; Rg3, ginsenoside Rg3.

Flow cytometric analysis. Cells that had been treated in different manners were collected and 70% pre-cooled ethanol (pre-chilled PBS and water-free configuration) was added at 4˚C overnight. Then the cells were washed with PBS and stained with propidium iodine (PI) (for cell cycle). MACSQuant® Analyzer 10 Flow cytometer (Miltenyi Biotec=) was used to detect the cell cycle, and the Annexin V FITC/PI kit (Invitrogen; Thermo Fisher Scientific, Inc.) was used for flow cytometry to detect apoptosis.

Animal experiment. Human colon cancer cells ($5x10^6$/0.2 ml) in the logarithmic phase were selected. A total of 20 female nude mice (5-6 weeks of age, 18-25 g; Shanghai Lingchang Biological Technology Co., Ltd.) that were adaptive for feeding [room temperature of 20-24˚C half day (light) and night (dark) cycle, air humidity of 60%] for one week were selected. Mice were anesthetized [3% sodium pentobarbital, 50 mg/kg, intraperitoneal (i.p.)], and then the lateral skin of the nude mice was selected as a cell inoculation site to inoculate $5x10^6$/0.2 ml human colon cancer cells (at the logarithmic phase of growth). When the tumor tissue grew to a volume of approximately 50 mm^3, then the mouse were randomly assigned to the Solvent group (equal amount of PBS + DMSO), Rg3 group (200 mg/kg, gavage administration once every two days), 5-FU group (20 mg/kg, i.p. injection once every two days) and Rg3+5-FU group (combined Rg3 and 5-FU group administration). After 3 weeks of treatment, the mice were sacrificed using cervical dislocation and breathing and heartbeat for 3 min were observed to determine death, and tumor tissues were extracted and weighed with an analytical balance (BSA124S; Beijing Sartorius Instruments Ltd., Beijing, China). All animal experiments were approved by the Ethics Committee of Quanzhou First Hospital Affiliated to Fujian Medical University.

Statistical analysis. All data are expressed as mean ± standard deviation, and SPSS 20.0 (IBM Corp.) was used to analyze the data. Student's t-test was used to compare differences between two groups, and multiple groups were compared with one-way ANOVA followed by Duncan test as a post hoc test. P<0.05 was assigned to indicate that a difference was statistically significant.

Results

Combined treatment of Rg3 and 5-FU enhances inhibition of cell proliferation. After treatment with different doses of Rg3 or 5-FU, MTT assay was used to measure the cell viability. The results revealed that the cell viability of SW620 and LOVO cells was significantly and gradually decreased with an increasing dose of Rg3. Thus, we chose 1.0 mmol/l Rg3 for subsequent experiments (Fig. 1A). As shown in Fig. 1B, the proliferative activity of the colon cancer cells in the combined treatment group of Rg3 and 5-FU was significantly lower than

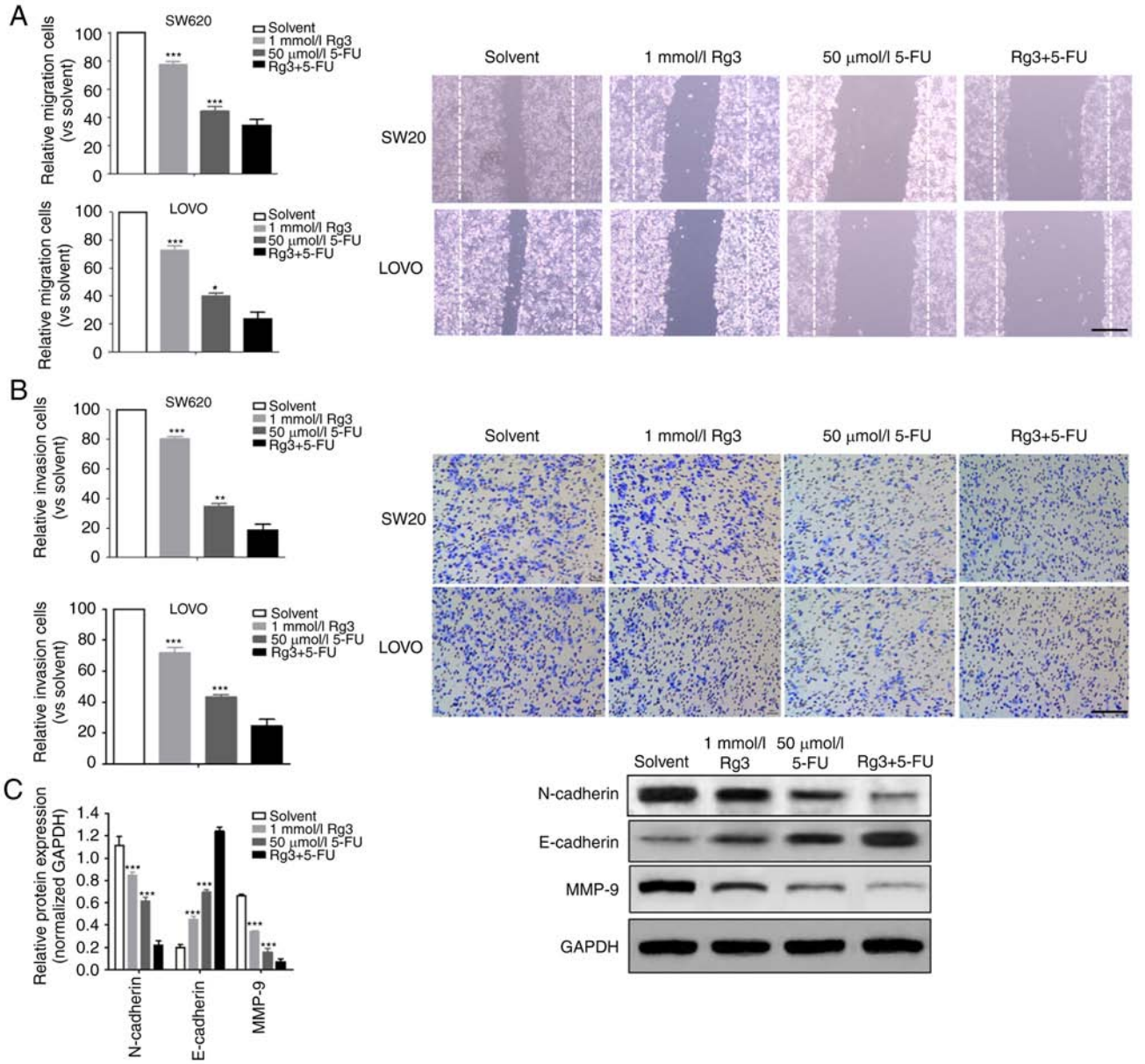

Figure 2. Effect of the combined treatment of Rg3 and 5-FU on migration and invasion of colon cancer cells *in vitro*. Treatment of colon cancer cells with combined treatment of Rg3 (1 mmol/l) and 5-FU (50 μmol/l) inhibited the migration (A) and invasion (B) abilities of the SW620 and LOVO cells. Scale bar, 100 μm. (C) Western blot analysis was used to detect the expression of EMT-related protein (N-cadherin, E-cadherin and MMP-9). The Solvent group was used as a baseline for the migration and invasion of cells. Three independent repetitions for each experiment were performed. *P<0.05, **P<0.01 and ***P<0.001 compared with the Rg3+5-FU group. 5-FU, 5-fluorouracil; Rg3, ginsenoside Rg3; EMT, epithelial-mesenchymal transition; MMP, matrix metalloproteinase.

that of the 5-FU treatment alone group. In addition, the cell viability of SW620 and LOVO cells gradually decreased with the increasing dose of 5-FU. However, after treatment with the combination of 1 mmol/l Rg3 and 100 μmol/l 5-FU for 48 h, the cell viability of SW620 cells was only 10-20% which was not conducive to subsequent protein detection experiments. Thus, 1 mmol/l Rg3 and 50 μmol/l 5-FU were chosen for subsequent experimentation.

Cell clone formation assays were also used to detect *in vitro* proliferation of colon cancer cells. As shown in Fig. 1C and D, the number of colonies formed by the colon cancer cells treated with Rg3 and 5-FU was significantly lower than that of Rg3 or 5-FU alone. These findings indicated that combined treatment of Rg3 and 5-FU enhanced the inhibition of colon cancer cell proliferation *in vitro*.

Combined treatment of Rg3 and 5-FU enhances the inhibition of cell migration and invasion. The ability of tumor cells to invade and migrate is the key to tumor progression. In the present study, we compared the effects of different treatment conditions on the invasion and migration of colon cancer cells. It was demonstrated that the invasion and migration ability of the colon cancer cells treated with Rg3 combined with 5-FU was significantly lower than that of Rg3 or 5-FU alone (Fig. 2A and B). Epithelial-mesenchymal transition (EMT) is the source of tumor cell ability to acquire higher invasion and migration capacity. Thus, we determined the levels of three EMT-related proteins (N-cadherin, E-cadherin and MMP-9) and found that the expression of N-cadherin and MMP-9 protein in the Rg3+5-FU group was significantly lower than that of Rg3 or 5-FU alone group, but

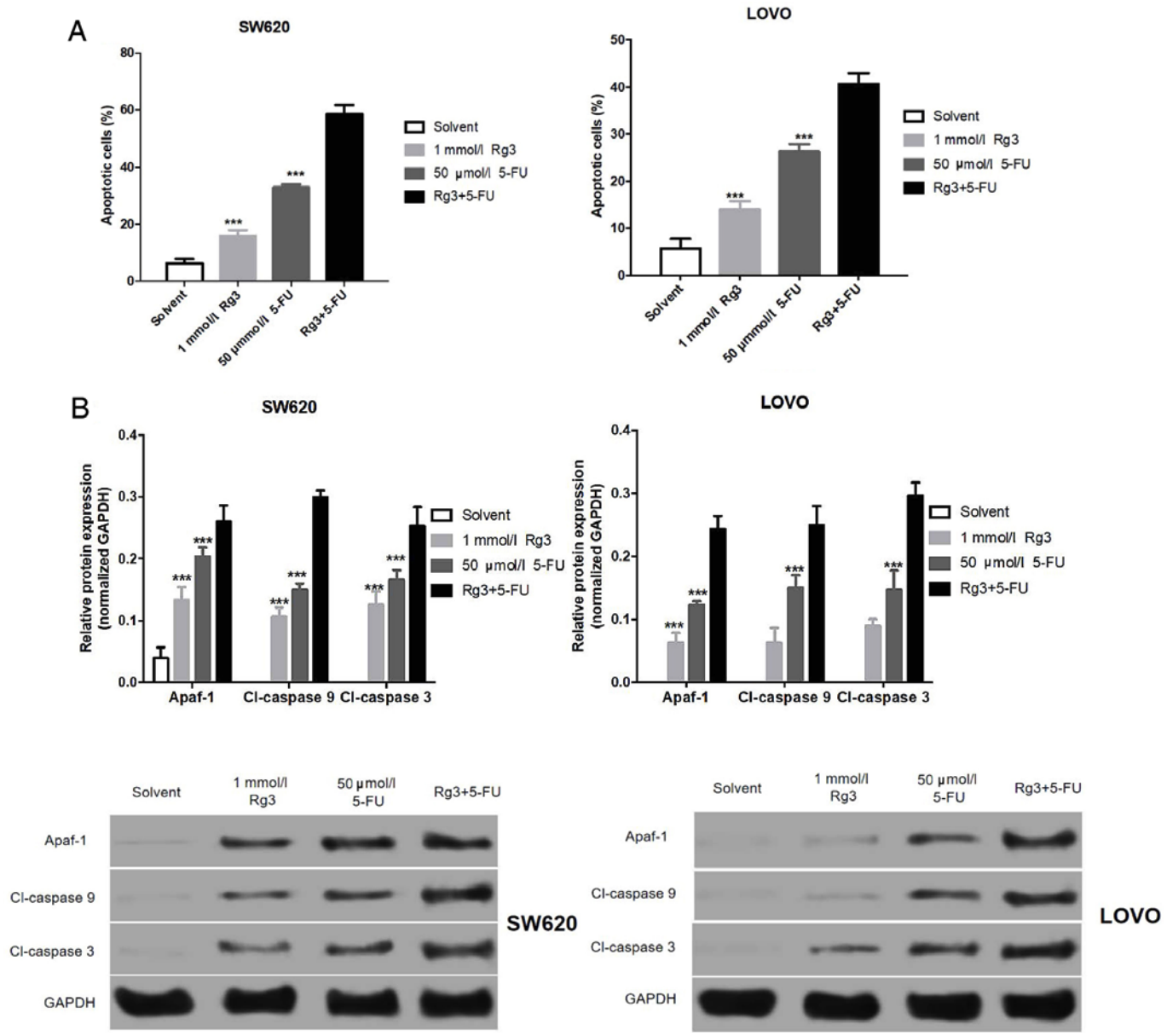

Figure 3. Effect of the combined treatment of Rg3 and 5-FU on the apoptosis of colon cancer cells *in vitro*. (A) The percentage of apoptotic SW620 and LOVO cells in the different groups. (B) Apoptosis-related proteins [(cleaved (Cl)-caspase 9, Cl-caspase 3 and Apaf-1] were assessed by western blot analysis in SW620 and LOVO cells. Three independent repetitions for each experiment were carried out. ***P<0.001, compared with the Rg3+5-FU group. 5-FU, 5-fluorouracil; Rg3, ginsenoside Rg3; Apaf-1, Apoptotic protease activating factor 1.

the expression of E-cadherin protein was significantly higher (Fig. 2C).

Combined treatment of Rg3 and 5-FU promotes apoptosis of colon cancer cells. Fist, we found that the apoptosis of the colon cancer cells treated with Rg3 combined with 5-FU was significantly higher than that of Rg3 or 5-FU alone (Fig. 3A). The levels of apoptosis-related proteins in the SW620 and LOVO cells were assessed by western blot analysis. As shown in Fig. 3B, expression levels of Apaf-1, cleaved (Cl)-caspase 9 and Cl-caspase 3 protein in colon cancer cells (SW620 and LOVO) treated with Rg3 were significantly increased, and the expression of these apoptosis-related protein in colon cancer cells following 5-FU treatment was significantly higher than that treated with Rg3. More importantly, expression levels of these apoptosis-related proteins in colon cancer cells treated with the combination of Rg2 and 5-FU were significantly higher than levels treated with Rg3 or 5-FU alone.

We analyzed the cell cycle distribution of the colon cancer cells after treatment with the different agents. As shown in Fig. 4A, the percentages of colon cancer cells in the G0/G1 phase treated with the Rg3 and 5-FU combination were significantly higher than the percentages following Rg3 or 5-FU alone. Similarly, we also detected cell cycle-associated protein by western blot analysis. As shown in Fig. 4B, the expression levels of cyclin D1, CDK2 and CDK4 protein in colon cancer cells which were treated with the Rg3 and 5-FU combination were significantly lower than levels following treatment with Rg3 or 5-FU alone.

Combined treatment of Rg3 and 5-FU suppresses PI3K/AKT signaling in colon cancer cells. The *PI3K/AKT* signaling

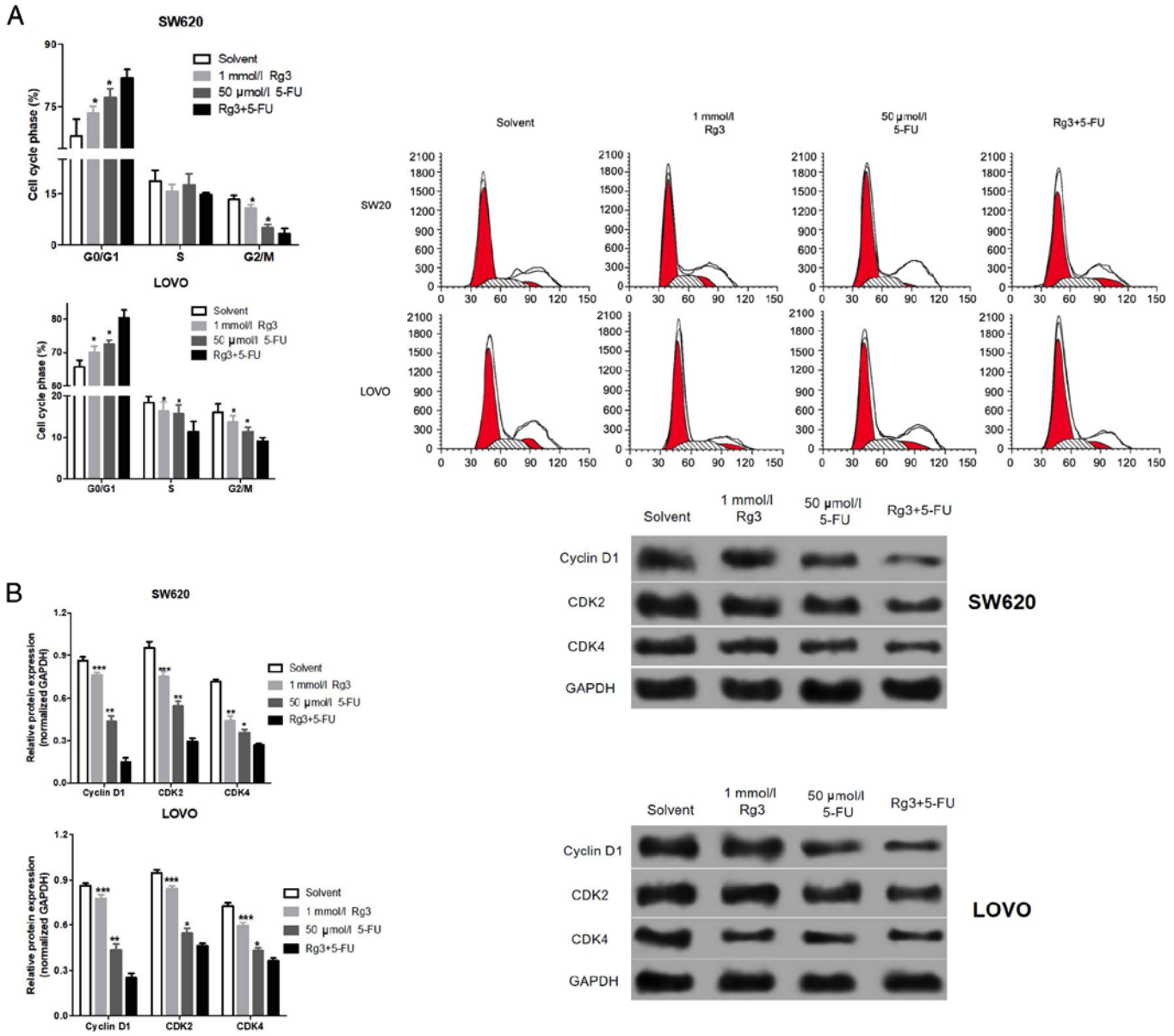

Figure 4. Effect of the combined treatment of Rg3 and 5-FU on cell cycle progression of colon cancer cells *in vitro*. (A) Flow cytometry was used to analysis the cell cycle in colon cancer cells after treatment with Rg3 (1 mmol/l) or 5-FU (50 μmol/l) or the combination. (B) Cell cycle-associated protein (cyclin D1, CDK2 and CDK4) were assessed by western blot analysis. Three independent repetitions were performed for each experiment. *P<0.05, **P<0.01 and ***P<0.001, compared with the Rg3+5-FU group. 5-FU, 5-fluorouracil; Rg3, ginsenoside Rg3; CDK, cyclin-dependent kinase.

pathway is a signaling pathway involved in cancer cell proliferation, invasion and migration, and its abnormal activation can confer high proliferation, invasion and migration ability of cancer cells. In the present study, we found that the expression levels of p-p85, p-110β, p-PDK1 and p-AKT protein in the colon cancer cells which was treated with Rg3 and 5-FU combination were significantly lower than levels in the cells treated with Rg3 or 5-FU alone (Fig. 5). These results indicated that the combined treatment of Rg3 and 5-FU enhanced the inhibition of the *PI3K/AKT* signaling pathway in colon cancer cells *in vitro*.

Combined treatment of Rg3 and 5-FU suppresses tumor growth in nude mice. Based on the results of *in vitro* studies, we further investigated the effects of the Rg3 and 5-FU combination on colon cancer cell proliferation and protein expression in nude mice. SW620 cells were injected into the armpits of nude mice. After 3 weeks of treatment, the mice were sacrificed, and the weight and volume of tumor tissues were measured. It was found that the weight and volume of tumor tissues in the Rg3+5-FU group were significantly lower than these parameters in the groups treated with Rg3 or 5-FU alone (Fig. 6A and B).

Moreover, western blot analysis was used to detect the expression of EMT-related proteins, cell cycle-related proteins and key proteins in the *PI3K/AKT* signaling pathway. It was found that although the effects of the Rg3 and 5-FU combination were not as obvious as the *in vitro* results compared with Rg3 of 5-FU alone, the overall trend in protein expression was consistent (Fig. 6C-E). These results demonstrated that Rg3

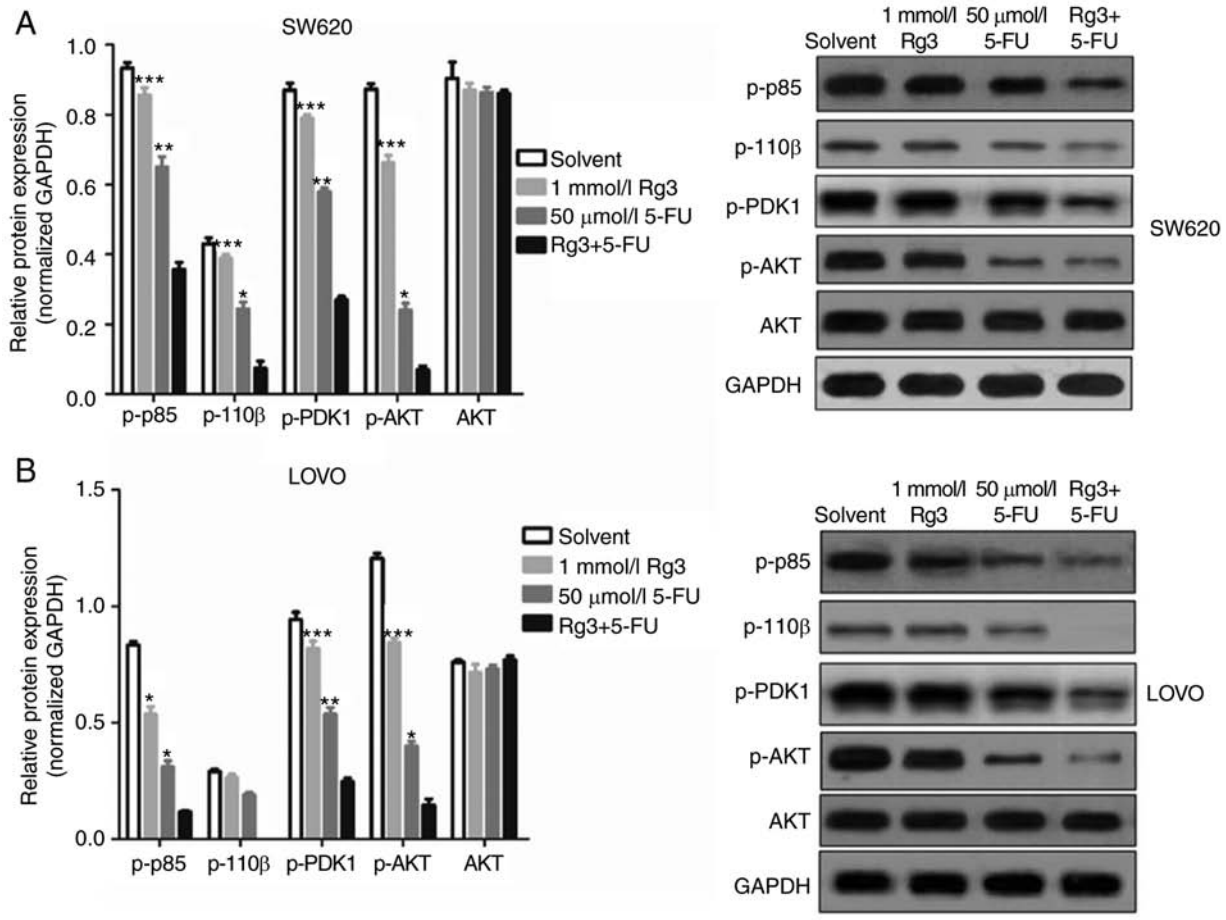

Figure 5. Effect of the combined treatment of Rg3 and 5-FU on PI3K/AKT signaling in colon cancer cells *in vitro*. (A and B) Western blot analysis was used to detect the expression of key proteins in the PI3K/AKT signaling pathway after treatment with Rg3 (1 mmol/l) or 5-FU (50 μmol/l) or the combination. Three independent repetitions were performed for each experiment. *P<0.05, **P<0.01 and ***P<0.001, compared with the Rg3+5-FU group. 5-FU, 5-fluorouracil; Rg3, ginsenoside Rg3.

synergizes the effect of 5-FU to inhibit the growth of human colon cancer xenografts in nude mice.

Discussion

The anticancer effect of 5-fluorouracil (5-FU) is exerted mainly by interfering with tumor cell DNA replication, and it is a commonly used antitumor agent for the treatment of advanced colon cancer (20,21). However, since 5-FU displays non-specific cytotoxicity, it also causes damage to normal cells, causing irreversible renal dysfunction and severe gastrointestinal reactions. These adverse effects limit its clinical application and further improvements in the efficacy of chemotherapy are needed (20,22). Therefore, it is urgent to discover a drug that can enhance the chemotherapeutic effects of 5-FU and reduce the 5-FU toxicity when used in combination with 5-FU.

Ginsenoside Rg3 (Rg3) is one of the main active ingredients extracted from ginseng. Research has shown that ginsenoside Rg3 has certain inhibitory effects on lung cancer, breast and prostate cancer. The antitumor mechanism of Rg3 was that Rg3 reduced the neovascularization, probability of tumor recurrence, proliferation and metastasis in tumors by inhibiting KDR/VEGF protein expression and blocking HIF-1α/COX2/VEGF pathway (12). In the present study, we found that the combined treatment of Rg3 and 5-FU promoted the inhibition of colon cancer cell proliferation *in vivo* and *in vitro*. Tumor growth, development and metastasis are closely related to cell proliferation. The previous study found that Rg3 inhibits the proliferation of tumor cells, such as Rg3-induced *EGFR/MAPK* pathway deactivation was found to inhibit melanoma cell proliferation by decreasing *FUT4/LeY* expression (23). Rg3 was found to inhibit the proliferation of multiple myeloma cells by inducing the secretion of IGF-1 (24).

Promoting tumor cell apoptosis is also a method of inhibiting tumor cell proliferation. In the present study, we found that the combined treatment of Rg3 and 5-FU significantly enhanced the apoptosis of colon cancer cells by activating the *Apaf1/caspase 9/caspase* 3 pathway. In the mitochondrial pathway of apoptosis, apoptosis-related signals release cytochrome *c* by stimulating the mitochondrial outer membrane. Cytochrome *c* enters the cytoplasm which activates caspase-9 by binding with apaf-1. Activation of caspase-9 further activates caspase-3, while the activated caspase-3 can activate caspase-6/7/8 leading to apoptosis (25,26). In addition, we also found that the Rg3 and 5-FU combination enhanced the number of G0/G1 phase colon cancer cells and decreased expression of Cyclin D1, CDK2 and CDK4. The cell cycle refers to the whole process that the cell undergoes from the completion of one division to the end of the next division, and

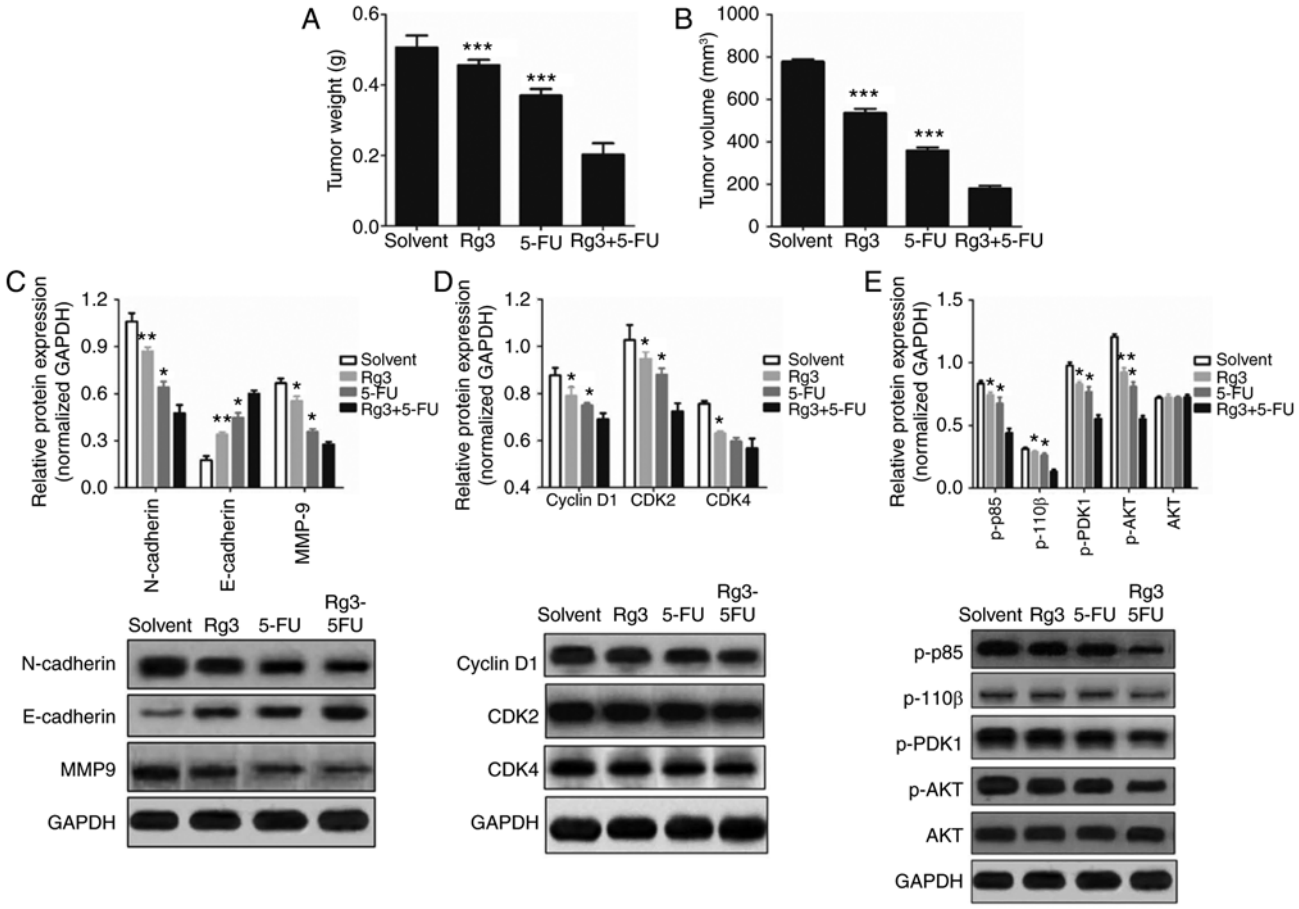

Figure 6. Effects of the combined treatment of Rg3 and 5-FU on tumor growth and protein expression of colon cancer cells *in vivo*. After 3 weeks of treatment, the mice were sacrificed, tumor tissues were excised, and the weight (A) and volume (B) of tumor tissues were measured. (C-E) Total protein was extracted from the colon cancer tumor tissues, and the expression of proteins was detected by western blot analysis. Five nude mice in each group, and at least 3 tumor tissues were used to evaluate protein expression., *P<0.05, **P<0.01 and ***P<0.001, compared with the Rg3+5-FU group. 5-FU, 5-fluorouracil; Rg3, ginsenoside Rg3.

the regulation of the cell cycle is mainly achieved by the retention of the G1 phase. When a cell is in the G1 phase, there is an important node regulating the cell cycle, the R point. When the cell cycle is before the R point, the cell needs the external growth factor to achieve the normal operation of the cell cycle. After the cell cycle crosses the R point, the cell cycle becomes a process that is controlled autonomously by the cell and no longer depends on the presence of external cytokines (27,28). Cyclin D1 is a G1/S-specific cyclin, and its main function is to promote the cell cycle from G1 to S by binding and activating the cyclin-dependent kinase *CDK2/4*, a unique cyclin-dependent kinase of G1, so as to promote cell proliferation (29).

Invasion and migration of tumor cells are the most important features of malignant tumors and the important causes of death in patients with malignant tumors. N-cadherin, E-cadherin and MMP-9 are three proteins that play important roles in cell epithelial-mesenchymal transition (EMT), whereas EMT provides cells the ability to transfer and invade. Promoting tumor cell EMT can inhibit the expression of intercellular junction protein, resulting in decreased intercellular connectivity, which is beneficial to the invasion and migration of tumor cells to surrounding healthy tissues (30,31). Previous studies have found that Rg3 not only inhibits metastasis and invasion of lung cancer cells by inhibiting EMT induced by transforming factor β1 (32), but also inhibited the metastasis of prostate PC-3M cells by downregulating the expression of AQP1 (33). By downregulating *MMP-13*, Rg3 affected the metastasis and invasion ability of melanoma cells (34). The present study demonstrated that the combined treatment of Rg3 and 5-FU significantly suppressed the invasion and migration ability of human colon cancer cell *in vitro* by altering EMT-related protein.

Furthermore, we also found that Rg3 and 5-FU combination inhibited the conduction of the *PI3K/AKT* signaling pathway *in vivo* and *in vitro*. Many studies have shown that the occurrence and development of tumors are the result of multi-factor, multi-gene, and multi-pathway processes, and the cell signal transduction pathway is crucial in the process of tumor development, invasion and metastasis. The phosphatidylinositol 3-kinase/serine/threonine kinase B (*PI3K/Akt*) signaling pathway plays an important role in the regulation of solid tumors [e.g., liver cancer (35), breast cancer (36), colon cancer (37), gastric cancer (38), neuroblastoma (39)] and blood tumors [e.g., leukemia (40)]. *PI3K* acts as a bridge molecule for the relationship between extracellular signals and cellular responses, under the influence of a series of upstream or bypass signaling molecules. It acts on the downstream of the effects of a variety of molecules, thus promotes cell migration,

inhibits cell apoptosis, accelerates the process of the cell cycle and promotes cell proliferation (41). Many previous studies have shown that traditional Chinese medicine or traditional Chinese medicine monomers can play an antitumor role by inhibiting the *PI3K/Akt* signaling pathway (42,43).

In conclusion, Rg3 enhances 5-FU inhibiting proliferation, invasion and migration of colorectal cancer cells, and helps 5-FU block G1 phase induced apoptosis in more colorectal cells. All in all, our study found that Rg3 enhanced the anti-cancer effect of 5-FU on colon cancer cell via *PI3K/Akt* pathway.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

XC made substantial contributions to the conception and design of the study and critically revised it for important intellectual content. SH contributed to the acquisition of the data. WC, ZH, YW, XM, YH and ZL analyzed and interpreted the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal and cell experiments were approved by the Ethics Committee of The Quanzhou First Hospital Affiliated to Fujian Medical University (Quanzhou, Fujian, China).

Patient consent for publication

Not applicable.

Competing interests

The authors state that they have no competing interests.

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