

# Synergistic anticancer effect of curcumin and chemotherapy regimen FP in human gastric cancer MGC-803 cells

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**Abstract.** Curcumin is an anticancer compound that exerts anti-proliferative and apoptotic effects via multiple molecular targets. The purpose of the present study was to investigate the anticancer effects of curcumin in combination with 5-fluorouracil plus cisplatin (FP) on the MGC-803 human gastric cancer cell line. Following treatment with curcumin and/or FP for 24, 48 and 72 h, cell viability, cell cycle progression and the apoptosis rate were evaluated using an MTT assay, flow cytometry and dual acridine orange/ethidium bromide staining, respectively. In addition, colony formation, Transwell migration and caspase-3/caspase-8 activity assays were performed. The expression of the apoptosis regulator B-cell lymphoma-2 (Bcl-2) and Bcl-2-associated X protein (Bax) were detected by western blotting analysis. Following treatment with curcumin and/or FP, cell viability, colony formation and cell migration were significantly reduced compared with the untreated control group. The rate of apoptosis, caspase-3/caspase-8 activity and the expression of Bax were significantly increased, whereas Bcl-2 expression was significantly reduced following treatment with curcumin and/or FP, compared with the untreated control group. The efficacy of curcumin combined with low-dose FP was significantly increased, compared with that of curcumin combined with high-dose FP ( $P < 0.05$ ). Therefore, curcumin may enhance the anticancer effects of FP chemotherapy in MGC-803 cells through the promotion of apoptosis via the caspase-3/caspase-8, Bcl-2 and Bax signaling pathways. These results suggest that curcumin may serve as a synergistic drug

with chemotherapy regimen FP for the treatment of gastric cancer.

## Introduction

Gastric cancer (GC) is one of the most invasive and aggressive malignancies and remains a major health problem worldwide due to its high incidence and mortality rate (1). Despite a steady decline in GC incidence, GC is currently the third highest cause of cancer-associated mortality worldwide, with 730,000 patients succumbing to the disease every year (2,3). Patient survival is primarily associated with disease stage, and the cure rate largely depends upon surgical resection. However, <5% of patients with advanced GC may survive  $\leq 5$  years and the role of surgery as a mainstay treatment is limited to ~25% of all patients (4). Chemotherapy has served a major role in the treatment of gastric cancer over the past twenty years. The 5-fluorouracil (5'-FU) plus cisplatin (DDP) regimen (FP) of chemotherapy consists of the continuous infusion of 5-FU with low-dose DDP, which is typically used to treat GC; however, the success rate of this treatment is limited due to the development of chemoresistance and toxic side effects. Therefore, a novel chemotherapy regimen that will improve clinical outcomes is required for patients with GC.

One strategy to improve anticancer treatment regimens may be to combine conventional chemotherapeutics with natural antitumor compounds. Curcumin, also known as 1,7-bis (4-hydroxy-3-methoxyphenol)-1,6-heptadiene-3,5-dione, is obtained and purified from turmeric (*Curcuma longa*), which belongs to the Zingiberaceae plant family indigenous to southern and southeastern tropical Asia (5). It has been widely used as a spice, to color cheese and butter, as a cosmetic and in certain medicinal preparations (6). The safety of *Curcuma* has been investigated in various animal models, and it has been established that turmeric is not toxic even at high doses (7). Previous studies have suggested that curcumin has a number of pharmacological effects, including anti-inflammatory, antioxidant and anticancer properties (8-10).

In the present study, the effects and underlying molecular mechanisms of curcumin combined with the FP regimen of chemotherapy were investigated in the MGC-803 human gastric cancer cell line. The results may aid with developing novel treatment strategies for patients with GC.

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**Key words:** curcumin, 5-fluorouracil plus cisplatin, gastric cancer, MGC-803 cells, synergistic anticancer effect, proliferation, apoptosis

## Materials and methods

**Cell culture and reagents.** MGC-803 cells were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). The cells were maintained in RPMI-1640 medium (HyClone; GE Healthcare Life Sciences, Logan, UT, USA), supplemented with 2 mmol/l L-glutamine, 100 U/ml penicillin, 0.1 µg/ml streptomycin and 10% fetal bovine serum (FBS, Tianhang Biotechnology Co., Ltd., Zhejiang, China) at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. The culture medium was replaced once every two days. Curcumin was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). DDP was purchased from Qilu Pharmaceutical Co., Ltd. (Shandong, China). The 5-FU was purchased from Tianjin Jinyao Amino Acid Co., Ltd. (Tianjin, China).

**Cell groups.** There were six treatment groups used in the present study, as follows: Control (curcumin or FP concentration at 0 µmol/l); CUR (15 µmol/l curcumin); CUR+LD FP [curcumin (15 µmol/l) combined with low dose FP (25 µmol/l 5-FU + 1 µmol/l DDP)]; CUR+MD FP [curcumin (15 µmol/l) combined with medium dose FP (50 µmol/l 5-FU + 2 µmol/l DDP)]; MD FP [medium dose FP (50 µmol/l 5-FU + 2 µmol/l DDP)] and HD FP [high dose FP (100 µmol/l 5-FU + 4 µmol/l DDP)].

**Cell viability assay.** Cells were seeded in 96-well plates at a concentration of  $4 \times 10^3$  cells/well. Following incubation for 12 h at 37°C, curcumin and/or low, medium or high dose FP at the aforementioned concentrations were added. There were 8 duplicate wells for each group with a total volume of 200 µl/well. Following treatment for 24, 48 and 72 h at 37°C in an atmosphere of 5% CO<sub>2</sub>, 20 µl MTT solution (Sigma-Aldrich; Merck KGaA) at a concentration of 5 g/l was added to each well, then the plates were incubated for 4 h. Dimethyl sulfoxide (DMSO; 150 µl; Sigma-Aldrich; Merck KGaA) was added to each well prior to agitation for 10 min at room temperature. A Model 680 microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to measure the absorbance at 570 nm. The inhibitory rate (%) was calculated using the following equation: Inhibitory rate (%) = [(1-optical density (OD)<sub>E</sub> / OD<sub>C</sub>)] x 100%. OD<sub>E</sub> represents the OD value of the experimental group with drug treatment; OD<sub>C</sub> represents the OD value of the control group without drug treatment. The experiment was repeated ≥3 times.

**Flow cytometry.** Cells were seeded at a density of  $4 \times 10^5$  cells/well in 6-well culture plates (Corning Incorporated, Corning, NY, USA) for 24 h, and treated with/without curcumin and/or FP at various concentrations for 24 h at 37°C in an atmosphere of 5% CO<sub>2</sub>. Apoptosis was then analyzed by flow cytometry using Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI; each purchased from Sungene Biotech Co., Ltd., Tianjin, China) double staining. Prior to flow cytometry analysis, the cells were collected, washed with cold PBS twice and resuspended gently in 400 µl binding buffer. Annexin V-FITC (5 µl) was added to the cells and the samples were gently vortexed prior to incubation for 10 min at 4°C in the dark. PI (10 µl) was added and the

samples were incubated for another 5 min at 4°C in the dark. Flow cytometry was then conducted using a FACSCalibur™ flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA), and the results were analyzed with BD CellQuest™ software (version 5.1; BD Biosciences). The experiment was repeated ≥3 times.

Cell cycle analysis was performed using PI (40 µg/ml) single staining with a flow cytometer. Prior to analysis, cells were washed in PBS containing 2% FBS and the resulting pellet was resuspended in DNase-free RNase (200 µg/ml, 0.5 ml) for 2 h at 37°C. Cells were then stained with PI and analyzed by flow cytometry. The data were analyzed using ModFit LT™ software (version 3.0; Verity Software House, Topsham, ME, USA) and expressed as the percentage of cells in each phase of the cell cycle. The experiment was repeated ≥3 times.

**Dual acridine orange/ethidium bromide (AO/EB) fluorescent staining.** Cells were seeded at a density of  $4 \times 10^5$  cells/well, cultured in 6-well culture plates (Corning Incorporated) for 24 h and treated with/without curcumin and/or FP at various concentrations for 24 h. The medium was removed. Trypsin (0.25%; HyClone; GE Healthcare Life Sciences) was added into each well. When the cells had detached, the suspensions (25 µl) were transferred to glass slides. Dual fluorescent staining solution (1 µl) containing 100 µg/ml AO and 100 µg/ml EB (Sigma-Aldrich; Merck KGaA) was added to each suspension and then covered with a coverslip. The morphology of apoptotic cells was examined and 1,000 cells were counted within 20 min based on randomly chosen fields of view using a fluorescent microscope (Nikon Corporation, Tokyo, Japan). The apoptotic percentage was expressed as a ratio of the number of apoptotic cells in the experimental groups (curcumin and/or FP at low, medium or high dose) compared with that in the untreated control group. The experiment was repeated ≥3 times.

**Colony formation assay.** The 6-well culture plates were seeded with ~500 viable MGC-803 cells and incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> for 24 h. The cells were then incubated with various concentrations of curcumin and FP at 37°C in an atmosphere of 5% CO<sub>2</sub> for 24 h. The curcumin and/or FP containing medium was then removed, and the cells were washed in PBS and incubated for an additional 10 days in complete medium. The colonies obtained were washed with PBS and fixed in methanol-acetic acid (3:1) stationary liquid for 10 min at room temperature, and then washed with PBS followed by staining with Giemsa (10%; Sigma-Aldrich; Merck KGaA). The colonies were counted and compared with in the untreated control group. The inhibitory rate was expressed as a ratio of the number of colonies in the experimental groups (curcumin and/or FP at low, medium or high dose) compared with in the untreated control group.

**Transwell migration assay.** A Transwell migration assay (Corning Incorporated) was performed according to the manufacturer's protocol in 24-well plates. MGC-803 cells were cultured in RPMI-1640 medium for 5 days, then the cells ( $1 \times 10^5$ ) in 100 µl serum-free medium with/without curcumin and/or FP at various concentrations were seeded into the upper

chamber of an 8-mm pore size Transwell insert. The lower chambers in the system were filled with Dulbecco's modified Eagle's medium (HyClone; GE Healthcare Life Sciences, Logan, UT, USA) supplemented with 10% FBS. After 10 h of incubation, non-migratory cells in the upper chamber were removed. MTT solution (100  $\mu$ l; 5 g/l) was added to each well, then the 24-well plate was incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> for 4 h. DMSO (150  $\mu$ l) was added to each well prior to agitation for 10 min at room temperature. The absorbance was measured using a microplate reader at 570 nm. The migration rate (%) was calculated using the following equation: Migration rate (%) = (OD<sub>E</sub> / OD<sub>C</sub>) x 100%. The experiment was repeated  $\geq 3$  times.

**Determination of caspase-3 and caspase-8 activity.** MGC-803 cells were seeded in a 6-well culture plate at a density of 4x10<sup>6</sup> cells/well, and treated with or without curcumin and/or FP at the aforementioned concentrations for 24 h. The medium was removed, and the MGC-803 cells were washed three times with PBS for 1 min each time, digested with Trypsin (0.25%; HyClone; GE Healthcare Life Sciences), and collected by centrifuging at 600 x g for 5 min at 4°C. The activities of caspase-3 and caspase-8 were measured using a caspase-3 activity assay kit and caspase-8 activity assay kit (Appligen Technologies Inc., Beijing, China) according to the manufacturer's protocol. Each sample was measured in triplicate. Relative caspase-3 activity was expressed as the absorbance compared with the untreated control group based on the following equation: Relative activity=OD of experimental group/OD of control group.

**Western blot analysis.** Cells were seeded at a density of 4x10<sup>6</sup> cells/well in a 6-well culture plate and treated with or without curcumin and/or FP at various concentrations for 24 h. A total of 1x10<sup>6</sup> cells/well were acquired by cell scraper, washed with PBS and then suspended in 250  $\mu$ l lysis buffer (pH 7.5, 1% Triton-X-100, 40 mmol/l Tris-HCl, 150 mmol/l KCl, 1 mmol/l EDTA, 100 mmol/l NaVO<sub>3</sub> and 1 mmol/l phenylmethylsulfonyl fluoride). Following protein extraction and concentration detection (BCA Protein Assay Kit; Beyotime Biotechnology Co., Ltd., Haimen, China), proteins were separated using SDS-PAGE and then transferred to polyvinylidene fluoride membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked with 5% skimmed milk. The membranes were then incubated with antibodies against the apoptosis regulator B-cell lymphoma-2 (Bcl-2; cat. no. 13CM357; dilution 1:1,000; Boster Biological Technology Co., Ltd., Wuhan, China), Bcl-2-associated X protein (Bax; cat. no. 196841; dilution 1:1000; Boster Biological Technology Co., Ltd.) and  $\beta$ -actin (cat. no. E0610; dilution 1:1000; Kerui Biotechnology Co., Ltd., Wuhan, China) in TBS-Tween containing 5% skimmed milk at 4°C for 12 h. Following washing, the membranes were incubated with a horseradish peroxidase immunoglobulin G antibody (cat. no. BST10F01A; dilution 1:10,000; Sungene Biotech Co., Ltd., Tianjin, China) at room temperature for 1 h. The blots were developed using an enhanced chemiluminescence kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Quantification of immunoblot intensity was performed using ImageJ software (version 2.1.4.7; National Institutes of

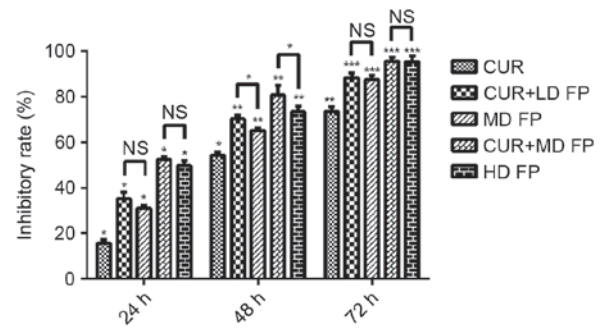


Figure 1. Effect of curcumin and/or FP treatment on the viability of MGC-803 cells. Cell viability was assessed using an MTT assay following treatment with curcumin and/or FP for 24, 48 and 72 h. The inhibitory rate was calculated compared with the viability of the untreated control cells. Data are presented as the mean  $\pm$  standard deviation and are representative of  $\geq 3$  independent experiments. Compared with control: \*P<0.05, \*\*P<0.01, \*\*\*P<0.005. FP, 5'-fluorouracil plus cisplatin; CUR, curcumin; LD, low-dose; MD, low-dose; HD, high-dose; NS, not significant.

Health, Bethesda, MD, USA). The experiment was repeated  $\geq 3$  times.

**Statistical analysis.** Data analyses were performed using SPSS software (version 16.0; SPSS Inc., Chicago, IL, United States). Data are presented as the mean  $\pm$  standard deviation. Group comparisons were evaluated using a one-way analysis of variance. Two-sided tests were used to evaluate comparisons between two groups. P<0.05 was considered to indicate a statistically significant difference.

## Results

**The effect of curcumin and/or FP on the viability of MGC-803 cells.** An MTT assay was used to examine the effect of curcumin and FP on MGC-803 cell viability following drug treatment for 24, 48 and 72 h. All treatments significantly decreased cell viability compared with the untreated control (\*P<0.05, \*\*P<0.01, \*\*\*P<0.005, Fig. 1). Following drug treatment for 24 and 72 h, no significant differences were observed between the MD FP and CUR+LD FP groups, or between the HD FP and CUR+MD FP groups. However, treatment with CUR+LD FP for 48 h significantly decreased cell viability compared with the MD FP treatment group (P<0.05), and treatment with CUR+MD FP for 48 h significantly decreased cell viability compared with the HD FP group (P<0.05). Therefore, curcumin enhanced the effects of FP on MGC-408 cell viability.

**Effect of curcumin and/or FP on the apoptosis and cell cycle of MGC-803 cells.** Flow cytometry was performed to investigate the effect of curcumin and/or FP on the apoptosis and cell cycle of MGC-803 cells. Compared with the controls, all drug treatments significantly increased the percentage of apoptotic cells (Fig. 2A and B). The apoptotic percentages of the combined groups were significantly higher than for the FP treatment groups 24 h following treatment (P<0.01; CUR+LD FP vs. MD FP; CUR+MD FP vs. HD FP). The apoptotic percentage was examined using two staining methods



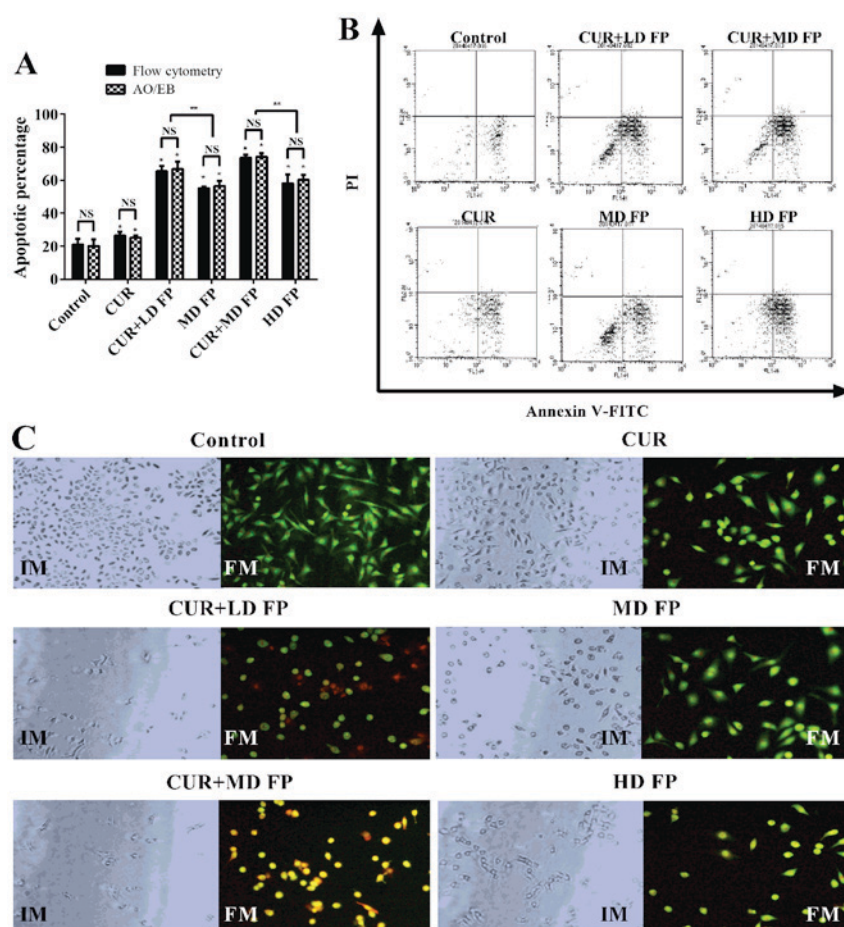


Figure 2. Effect of curcumin and/or FP treatment on MGC-803 cell apoptosis. (A) The percentage of apoptotic MGC-803 cells following treatment with curcumin and/or FP for 24 h, measured using Annexin V-FITC/PI staining and dual AO/EB staining. (B) Representative flow cytometry charts of Annexin V-FITC/PI staining in untreated (control) MGC-803 cells and cells treated with curcumin and/or FP. (C) Morphological changes, imaged with an IM, and dual AO/EB staining, imaged with a FM, of MGC-803 cells following treatment with curcumin and/or FP. Magnification,  $\times 200$ . Data are representative of  $\geq 3$  independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ . FP, 5'-fluorouracil plus cisplatin; FITC, fluorescein isothiocyanate; PI, propidium iodide; AO, acridine orange; EB, ethidium bromide; CUR, curcumin; LD, low-dose; MD, low-dose; HD, high-dose; IM, inverted microscope; FM, fluorescence microscope.

(Annexin V-FITC/PI and dual AO/EB staining). The results of dual AO/EB staining also demonstrated that curcumin significantly increased FP-induced cell apoptosis ( $P < 0.05$ ) and no significant differences were observed between the apoptotic percentage measured by Annexin V-FITC/PI flow cytometry and by AO/EB ( $P > 0.05$ ; Fig. 1A). These results demonstrated that curcumin increases the level of FP-induced cell apoptosis.

Following treatment with curcumin and/or FP for 24 h, there were marked changes in cellular morphology, including cell shrinkage and nuclear fragmentation in the drug treatment groups, compared with in the control group (Fig. 2C). For the non-apoptotic cells, the nucleus was circular and uniformly distributed in the center of the cell. For early apoptotic cells, the nucleus exhibited yellow-green fluorescence by AO staining and concentrated into a crescent or granular nucleus that was located on one side of the cell. For late apoptotic cells, the nucleus exhibited orange fluorescence by EB staining, and, the chromatin condensed and distributed around the nuclear membrane. For necrotic cells, the cell volume was increased, and the nucleus exhibited uneven orange-red fluorescence and an unapparent outline, indicating it was disintegrating.

Cell cycle analysis demonstrated that there was a change in S phase arrest in response to treatment with curcumin and/or FP compared with the control group (Fig. 3). Treatment with FP alone resulted in cell arrest at S phase (MD FP 30.32%, HD FP 47.77%), whereas combined treatment resulted in a significant increase in the number of cells arrested at the S phase, compared with the control group and the FP treatment groups (CUR+LD FP 38.23%, CUR+MD FP 76.38%;  $P < 0.05$ ; Fig. 3B).

**Impact of curcumin and/or FP on the colony formation and migration ability of MGC-803 cells.** A colony formation assay demonstrated that the proliferation rate and colony numbers of the MGC-803 cells treated with curcumin and/or FP were significantly decreased compared with the control group ( $P < 0.05$ ; Fig. 4A and B). The inhibitory rate of the combined treatment group was significantly higher compared with that of the FP treatment group (CUR+LD FP 38.1% vs. MD FP 31.9%, CUR+MD FP 68.1% vs. HD FP 40%;  $P < 0.01$ ; Fig. 4B). In addition, a significantly lower number of cells migrated through the Transwell filter when curcumin and/or FP were added into the migration chamber, as compared with the untreated control group without curcumin and FP ( $P < 0.05$ ;

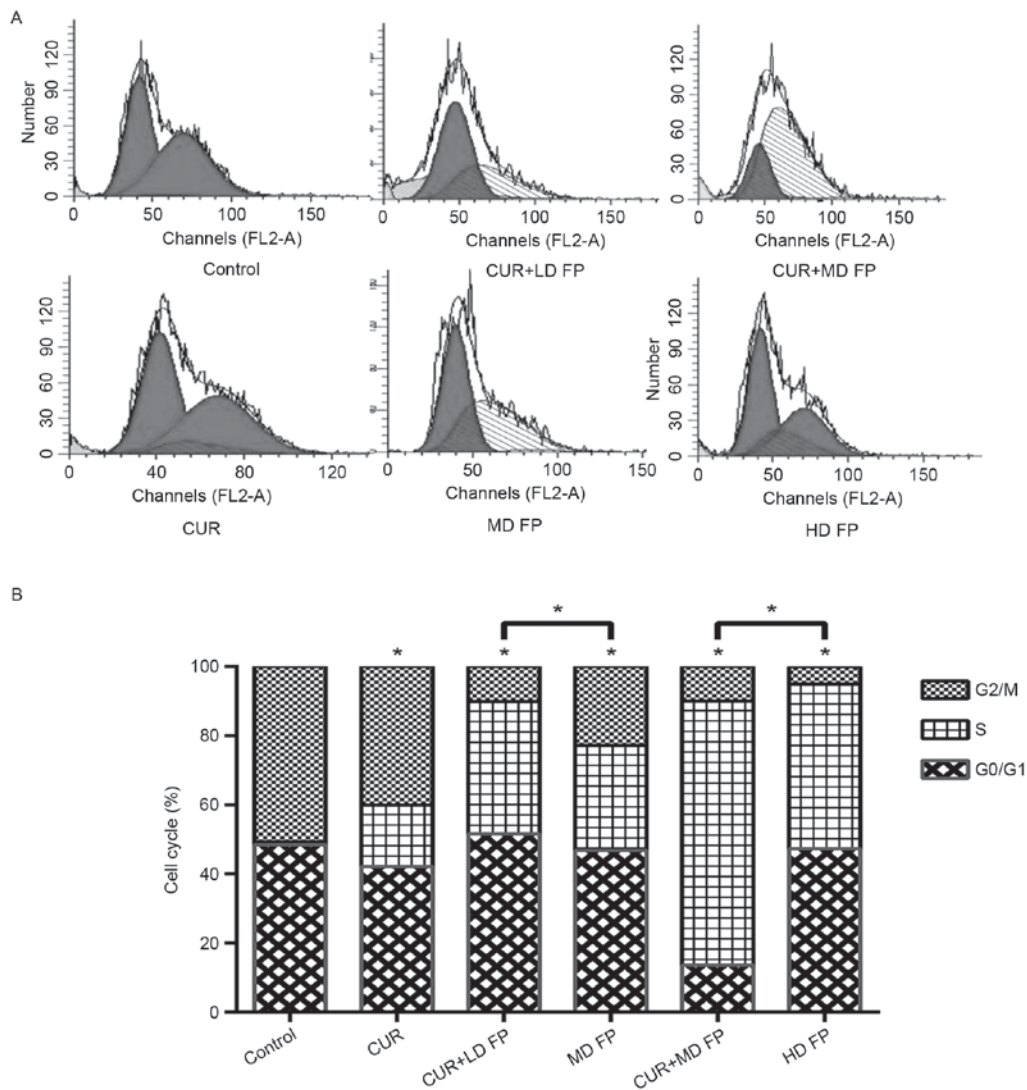


Figure 3. Effect of curcumin and/or FP on the cell cycle of MGC-803 cells. (A) Flow cytometry charts of propidium iodide staining in untreated (control) MGC-803 cells or MGC-308 cells treated with curcumin and/or FP. (B) Quantification of flow cytometry analysis of cell cycle distribution. Data are representative of  $\geq 3$  independent experiments. \* $P < 0.05$ . FP, 5'-fluorouracil plus cisplatin; CUR, curcumin; LD, low-dose; MD, low-dose; HD, high-dose.

Fig. 4C). No significant differences were observed in the migration rate of the combined treatment group compared with that of the FP treatment group ( $P > 0.05$ ; Fig. 4C). Overall, curcumin enhanced the effects of FP treatment on cell viability, and the colony formation and migration abilities of MGC-803 cells.

**Effect of curcumin and/or FP on the expression and activity of apoptosis-associated proteins in MGC-803 cells.** To investigate the underlying molecular mechanisms of the effects of combined curcumin and FP treatment on MGC-803 apoptosis the expression and activity of apoptosis-associated proteins was investigated using western blot analysis and activity assay kits, respectively, including Bcl-2, Bax, caspase-3 and caspase-8. Treatment with curcumin and/or FP significantly decreased the expression of Bcl-2 and increased the expression of Bax, compared with in the untreated control group ( $P < 0.05$ ; Fig. 5A and B). Bcl-2 expression was significantly higher in the combined treatment groups compared with in the FP treatment groups (CUR+LD FP 0.19 vs. MD FP

0.52, CUR+MD FP 0.09 vs. HD FP 0.37;  $P < 0.01$ ; Fig. 5B). However, no significant differences were observed in Bax expression between the combined treatment groups and the FP treatment groups.

The relative caspase-3 and caspase-8 activities of the drug treatment groups with curcumin and/or FP were significantly elevated compared with the untreated control group without curcumin and FP ( $P < 0.05$ , or  $P < 0.01$  for the CUR+MD FP group; Fig. 5C). The relative caspase-3 and caspase-8 activities of the CUR+MD FP group were significantly higher compared with those of the HD FP group (caspase-3: 22.1 vs. 12.5; caspase-8: 20.1 vs. 11.3;  $P < 0.05$ ; Fig. 5C), whereas no significant differences were observed between the caspase-3/caspase-8 activities of the CUR+LD FP group and the MD FP group (caspase-3: 11.6 vs. 7.86; caspase-8: 10.4 vs. 6.54;  $P > 0.05$ ; Fig. 5C). These data suggest that curcumin enhances the apoptotic effects of FP treatment in MGC-803 cells via the promotion of Bcl-2 and the inhibition of Bax, followed by elevating the activation of caspase-3 and caspase-8.

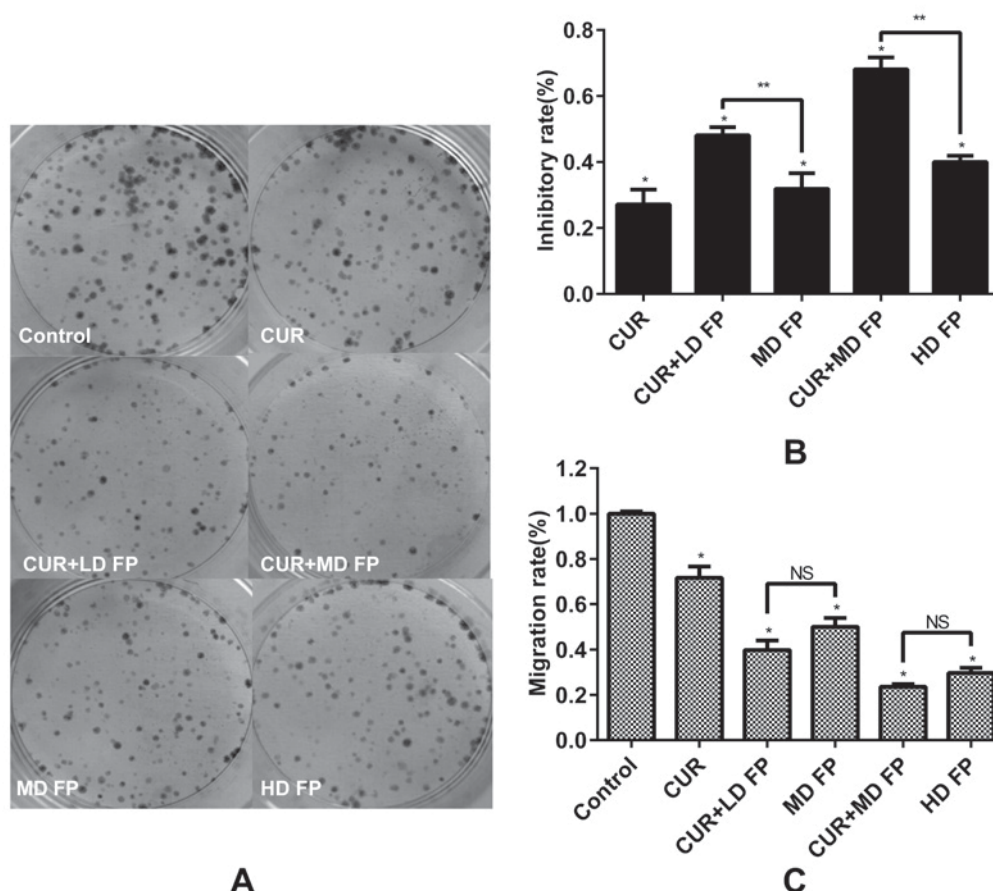


Figure 4. Effect of curcumin and/or FP on the colony formation and migration abilities of MGC-803 cells. (A) Representative images of the colony formation assay of MGC-803 cells following treatment with curcumin and/or FP for 24 h (magnification,  $\times 1.5$ ). (B) Inhibitory rate of MGC-803 cells calculated from the colony formation assay. (C) Transwell migration assay with MGC-803 cells following treatment with curcumin and/or FP. Data are presented as the mean  $\pm$  standard deviation and is representative of  $\geq 3$  independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ . FP, 5'-fluorouracil plus cisplatin; CUR, curcumin; LD, low-dose; MD, low-dose; HD, high-dose; NS, not significant.

## Discussion

GC is one of the most common types of malignant tumor (1). It has previously been reported that, although  $>50\%$  of patients diagnosed with GC successfully undergo surgical tumor resection, 60% of those patients subsequently present with local recurrence or distant metastasis (11). Chemotherapy remains an indispensable form of treatment, particularly for patients with advanced stage GC. Regimens based on 5-FU and DDP treatments have been a typical approach for patients with GC. The synergistic effect between 5-FU and DDP was reported as early as the end of the 1970s (12). FP as a combination chemotherapy regimen was established for the treatment of cancer, particularly for patients with advanced-stage cancer (13,14). FP primarily exerts its cytotoxic effects by inhibiting enzyme activity, preventing the synthesis of DNA, blocking cell cycle progression and promoting apoptosis (15). The effect of 5-FU is enhanced by low-dose DDP. However, chemotherapy resistance and side effects have become challenges for the treatment of cancer using this method. Thus, it is essential to explore alternative effective anticancer chemotherapy regimens.

Curcumin is a plant polyphenol extracted from the spice turmeric (*Curcuma longa*), which is used as an herbal remedy

in traditional Chinese and Indian medicine (5). The efficacy, pharmacological safety and cost effectiveness of curcumin and no observed toxicity make it an ideal compound to investigate for its anticancer properties (16,17). Curcumin has previously been reported to possess anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antiarthritic, antibacterial, antifungal, antiprotozoal, antiviral, anti-Alzheimer, anti-psoriatic and neuroprotective activities (18-20). Curcumin exerts anti-proliferative and apoptotic effects in various types of cancer, including lung, ovarian, esophageal and liver cancer, in addition to glioma (21-26). The molecular mechanisms underlying the anticancer effects of curcumin are complex. Previous studies have demonstrated that curcumin can mediate apoptosis through the upregulation of caspase-8 and caspase-3 (27). Curcumin may also be able to attenuate the incidence of cancer via the reduction of phospho-I $\kappa$ B $\alpha$  and 8-OHdG expression during tumor initiation (28). In addition, curcumin may reduce the invasive ability of A431 cells by inhibiting the activation of the STAT3 signaling pathway and the expression of STAT3 as a target gene in the pathway (29).

Curcumin has been reported to inhibit the activation of myeloid-derived suppressor cells (MDSCs), promote the



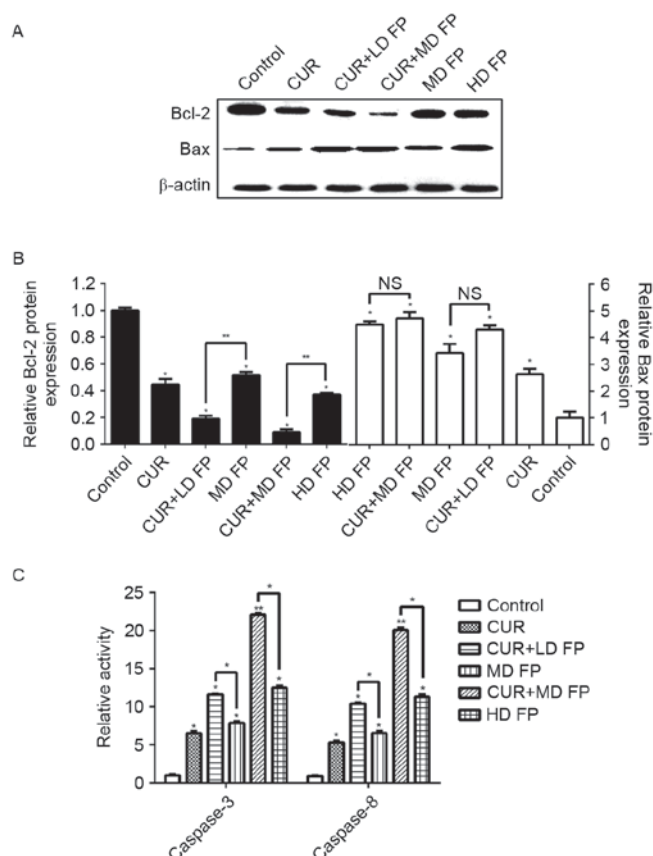


Figure 5. Effect of curcumin and/or FP on the expression and activity of apoptosis-associated proteins in MGC-803 cells. (A) Western blot analysis of Bcl-2 and Bax expression in MGC-803 cells treated with curcumin and/or FP for 24 h.  $\beta$ -actin was used as the internal reference. (B) Quantification of the expression level of Bcl-2 and Bax in MGC-803 cells following treatment with curcumin and/or FP for 24 h. (C) Activities of caspase-3 and caspase-8 in MGC-803 cells treated with curcumin and/or FP for 24 h. Data are presented as the mean  $\pm$  standard deviation and are representative of  $\geq 3$  independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ . FP, 5'-fluorouracil plus cisplatin; CUR, curcumin; LD, low-dose; MD, low-dose; HD, high-dose; NS, not significant; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein.

differentiation of MDSCs, and interfere with the interaction between MDSCs and cancer cells and to suppress tumor growth (30). In addition, curcumin has been demonstrated to protect against chemoresistance in human gastric cancer cells by downregulating nuclear factor  $\kappa$ -light chain enhancer of activated B cells (NF- $\kappa$ B) and subsequent NF- $\kappa$ B-mediated anti-apoptotic genes, including Bcl-2 and Bcl-extra large in the SGC 7901 human gastric cancer cell line (31). Curcumin suppresses proliferation and invasion in human gastric cancer cells by downregulating serine/threonine-protein kinase PAK1 activity and cyclin D1 expression (32). A previous study identified that the anti-metastatic effect of curcumin on endometrial carcinoma is associated with inhibition of the expression and activity of MMP-2 and -9 via downregulation of the ERK signaling pathway (33).

Previous studies have investigated the effects of combination chemotherapy with curcumin. Curcumin and its analogues (PGV-0 and PGV-1) enhance the cytotoxicity of doxorubicin in MCF-7 cells via the inhibition of human epidermal growth factor activity and NF- $\kappa$ B activation (34). Curcumin may enhance the antitumor activity of docetaxel in ATC

cells by interfering with NF- $\kappa$ B and cyclooxygenase-2 (35). However, the anticancer effects of curcumin combined with FP in gastric cancer cells have not yet been reported, to the best of our knowledge. The present study investigated the synergistic effects of curcumin and FP chemotherapy on the MGC-803 human gastric cancer cell line using an MTT assay, flow cytometry, double AO/EB fluorescent staining, a colony formation assay and a Transwell migration assay. The results demonstrated that curcumin combined with low-dose FP or medium-dose FP enhances the effects of FP alone on cell viability and apoptosis. Therefore, the side effects of FP may be reduced via the co-administration of curcumin and low-dose FP chemotherapy, rather than the administration of FP chemotherapy alone.

The present study investigated the molecular mechanisms underlying the anticancer effects of curcumin combined with FP using western blot analyses, and the results suggested that the combination of curcumin and FP can effectively increase Bax expression whilst also decreasing Bcl-2 expression. The Bcl-2, Bax, caspase-3 and caspase-8-associated signaling pathways have been reported to be associated with GC (36,37). In addition, the results from the caspase-3 and caspase-8 activity assay kits demonstrated that the combination of curcumin and FP could significantly promote the activity of caspase-3 and caspase-8.

In conclusion, curcumin enhances the anticancer effects of FP in MGC-803 cells by decreasing cell viability, inhibiting colony formation, inhibiting cell migration and inducing apoptosis via the activation of caspase-3/-8, downregulation of Bcl-2 and upregulation of Bax. These results suggest that curcumin may be used in synergy with chemotherapy regimen FP to treat patients with GC. Further studies are required in order to evaluate the efficacy of this combined treatment *in vivo*.

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