8-Gingerol regulates colorectal cancer cell proliferation and migration through the EGFR/STAT/ERK pathway

SU-MIN HU[1*], XU-HUI YAO[2*], YI-HAI HAO[2], AI-HUA PAN[1] and XING-WANG ZHOU[1]

[1]Sun Yat-sen University Zhongshan School of Medicine;
[2]Guangdong Experimental High School, Guangzhou, Guangdong 510080, P.R. China

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Abstract. 8-Gingerol, which is extracted from ginger (*Zingiber officinale Roscoe*), has been shown to possess antioxidant and anti-inflammatory properties. However, the antitumor effect of 8-gingerol has not been fully elucidated. The aim of the present study was to investigate the therapeutic potential of 8-gingerol against colorectal cancer (CRC). The results demonstrated that 8-gingerol significantly inhibited cell proliferation in CRC cell models. Treatment of CRC cells with 8-gingerol resulted in dose-dependent decreases in migration and invasion. The inhibitory effect of 8-gingerol on CRC cell growth was attributed to cell cycle arrest and increased apoptosis. Moreover, to the best of our knowledge, the present study was the first to demonstrate that 8-gingerol acted as an inhibitor of epidermal growth factor receptor (EGFR) signaling. 8-Gingerol inhibited CRC cell proliferation and migration by targeting the EGFR/signal transducer and activator of transcription/extracellular signal-regulated kinase pathway, and the effects of 8-gingerol depended on the expression of EGFR. Moreover, 8-gingerol reduced the effective dosage of 5-fluorouracil and, thereby, the toxicity of drug combination therapy. These data suggest that 8-gingerol may be a promising candidate for the development of novel anticancer agents against CRC.

Introduction

Human colorectal cancer (CRC) is the third most commonly diagnosed malignancy worldwide, with >1.8 million new cases and 881,000 deaths reported in 2018 (1). The global burden of CRC is expected to increase to >2.2 million new cases and 1.1 million deaths by 2030 (2). CRC is caused by a series of genetic changes in key oncogenes, tumor suppressor genes and signaling pathways, among which the epidermal growth factor receptor (EGFR) pathway and its components have been found to be crucial. EGFR overexpression has been observed in several cancers, including CRC, and EGFR expression has been reported to be associated with the survival of CRC patients (3,4). Monoclonal antibodies targeting EGFR, such as cetuximab and panitumumab, have been used in the clinical treatment of metastatic CRC. However, the lack of response in a significant proportion of patients, high cost and side effects compromise the efficacy of these drugs in CRC treatment (5-8). Hence, there is an urgent need for novel anticancer agents against EGFR signaling that exhibit high efficiency and low toxicity.

Chemoprevention by natural dietary phytochemicals or plant-derived compounds appears to be an appealing approach to cancer treatment (9). Ginger (*Zingiber officinale Roscoe*) is widely used as a spice in foods and as an ingredient in traditional herbal medicine (10). Due to its antioxidant and anti-inflammatory properties, ginger has been used to treat various diseases, such as arthritis, rheumatism, indigestion, hypertension, infectious diseases, helminthiasis and cancer (11,12). Ginger contains >400 compounds, with 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol being the major constituents (11); these constituents belong to the pungent compounds of ginger and contain 3-methoxy-4-hydroxyphenyl functional groups (13). Among these compounds, 6-gingerol and 6-shogaol are currently the most extensively investigated in cancer. Previous studies have reported that 6-gingerol exerts suppressive effects on cell proliferation, angiogenesis, or metastasis in various cancers, such as lung, liver, oral, cervical, gastrointestinal and colon cancers (10,14-18). The anticancer effect of 6-gingerol is mainly attributed to its ability to modulate several signaling pathways, including the nuclear factor-κB, AKT, extracellular-signal-regulated kinase (ERK)1/2, c-Jun N-terminal kinase and p53 pathways (10,19). In addition, numerous studies have revealed the antitumor activity of 6-shogaol in colon cancer, head and neck squamous cell carcinoma, pancreatic, breast and lung cancer (20-24). 10-Gingerol has been reported to suppress cell proliferation and migration in breast and colon cancer through manipulating

Correspondence to: Dr Xing-Wang Zhou, Sun Yat-sen University Zhongshan School of Medicine, 74 Zhongshan 2nd Road, Guangzhou, Guangdong 510080, P.R. China
E-mail: zhouxw2@mail.sysu.edu.cn

*Contributed equally

Abbreviations: CRC, colorectal cancer; STAT, signal transducer and activator of transcription; ERK, extracellular-signal-regulated kinase; 5-FU, 5-fluorouracil

Key words: 8-gingerol, epidermal growth factor receptor, colorectal cancer, proliferation, migration

the mitogen-activated protein kinase pathway (25-29). Similar to 6-gingerol, 10-gingerol and 6-shogaol, 8-gingerol has antioxidant and anti-inflammatory properties (30); however, whether 8-gingerol has antitumor properties remains largely unknown.

The aim of the present study was to investigate the anti-tumor activity and mechanisms of action of 8-gingerol in CRC cells, and determine whether 8-gingerol can inhibit CRC cell proliferation, migration and invasion. The mechanism underlying the inhibitory effect of 8-gingerol on CRC cell proliferation and the involvement of the EGFR/signal transducer and activator of transcription (STAT)3/ERK cascades were also investigated.

Materials and methods

Chemicals, cell lines, antibodies and reagents. The compound 8-gingerol (99% purity, verified by high-performance liquid chromatography) was obtained from Shanghai Yuanye Bio-Technology Company. 5-Fluorouracil (5-FU; cat. no. F6627) was purchased from Sigma-Aldrich; Merck KGaA, and gefitinib (ZD1839) was purchased from Selleck Chemicals. The human colon cancer cell lines HCT116 and DLD1 were obtained from the American Type Culture Collection. HCT116 cells were maintained in McCoy's 5A medium (Gibco; Thermo Fisher Scientific, Inc.) and DLD1 cells were maintained in RPMI-1640 at 37˚C in a humidified incubator with an atmosphere of 5% CO_2. All media were supplemented with 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 µg/ml streptomycin. Primary antibodies (1:1,000) against caspase-3 (cat. no. 9662), cleaved caspase-3 (cat. no. 9661), cleaved caspase-8 (cat. no. 9496), EGFR (cat. no. 4267), p-EGFR (cat. no. 3777), STAT3 (cat. no. 9139), p-STAT3 (cat. no. 9145), ERK (cat. no. 9107), p-ERK1/2 (cat. no. 4377), and GAPDH (cat. no. 2118) were purchased from Cell Signaling Technology, Inc. Antibodies (1:500) against cyclin D1 (cat. no. Sc-20044), CDK4 (cat. no. Sc-23896), CDK6 (Sc-7961), caspase-8 (cat. no. Sc-56070) and Bcl-2 (cat. no. Sc-509) were purchased from Santa Cruz Biotechnology, Inc. Anti-Myc (1:1,000, cat. no. R95025) and anti-matrix metallopeptidase (MMP)2 (1:1,000, cat. no. 10373-2-AP) antibodies were purchased from Invitrogen; Thermo Fisher Scientific, Inc. and ProteinTech Group, Inc., respectively. Secondary antibodies (anti-rabbit, cat. no. SA00001-1, 1:2,000; and anti-mouse, cat. no. SA00001-2, 1:2,000) were purchased from ProteinTech Group, Inc. Recombinant human EGF protein was purchased from R&D Systems, Inc.

Cell proliferation assay. The effect of 8-gingerol on CRC cell viability was determined by a Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular Technologies, Inc.). Briefly, cells ($5x10^3$ cells/well) were seeded in 96-well plates and treated with different concentrations of 8-gingerol. After incubation for 24, 48 or 72 h, 10 μl of CCK-8 solution was added to each well. After incubation for another 1 h, the absorbance was measured at 450 nm using a spectrophotometric plate reader (Sunrise; TECAN, Inc.). Results were calculated as percentages of vehicle (DMSO)-treated cells. The cell viability assay of 5-FU and the combination of 5-FU and 8-gingerol was performed using the same protocol as mentioned above, except that cells were treated with 5-FU or 5-FU and 8-gingerol combination for 48 h. The IC_{50} values are expressed as the means ± standard deviation from triplicate experiments.

Colony formation assay. Cells (500 cells/well) were plated in 6-well plates and treated with different concentrations of 8-gingerol (0, 10, 30, 50 and 70 μM). After treatment for 10-14 days, cells were fixed with methanol for 15 min at room temperature. Subsequently, the cells were stained with 0.5% crystal violet solution for 10 min at room temperature and washed with PBS three times. The cells were then photographed (GS-800, x1 magnification; Bio-Rad Laboratorie, Inc.) and the colonies were counted.

Cell cycle analysis. Cell cycle analysis was performed using flow cytometry. Cells in 6-well plates were treated with different concentrations of 8-gingerol for 48 h. Subsequently, the cells were harvested and washed with PBS. After centrifugation at 300 x g for 5 min at room temperature, the cell suspension was fixed with cold 100% ethanol for 30 min at 4˚C. After washing with PBS, cells were stained using a Cell Cycle Staining kit (Multisciences Biotech) according to the manufacturer's instructions. The cell cycle distribution was determined using a Gallios™ flow cytometer (Beckman Coulter, Inc.).

Apoptosis analysis. Apoptosis of CRC cells was analyzed by flow cytometry using an Annexin V-FITC/PI Apoptosis kit (Multisciences Biotech) according to the manufacturer's instructions. Briefly, cells were seeded ($2x10^5$ cells/well) in 6-well plates and treated with different concentrations of 8-gingerol for 48 h, harvested, washed with PBS, and resuspended in 1X binding buffer. Subsequently, the cells were incubated with 5 μl Annexin V-FITC and 10 μl PI at room temperature for 10 min in the dark. The samples were analyzed using the Gallios™ flow cytometer.

Transwell migration and invasion assays. Transwell migration assays were performed using Corning chambers with 8.0-μm-pore polycarbonate membranes (Corning, Inc.). Cells cultured in 6-well plates were treated with different concentrations of 8-gingerol for 48 h. Subsequently, $1x10^5$ cells in 100 μl of serum-free medium were seeded in the upper chambers, and 600 μl of basic medium supplemented with 10% FBS was added to the lower chambers. After incubation for 24 h, cells on the upper surface of the membrane were removed with a cotton swab, and the cells that had migrated to the lower surface of the membrane were fixed with methanol for 15 min and stained with 0.5% crystal violet solution for 10 min at room temperature. Cells in five randomly selected fields of the membrane were counted under an inverted microscope (x100 magnification; Leica DMI4000B; Leica, Inc.). Transwell invasion assays were performed using the same protocol as the Transwell migration assays, except that the Transwell membranes were coated with Matrigel (BD Biosciences) for 1 h at 37˚C prior to cell seeding.

Western blot analysis. Cells were harvested and lysed in lysis buffer [50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 0.1% SDS, 1% NP-40] supplemented with protease/phosphatase

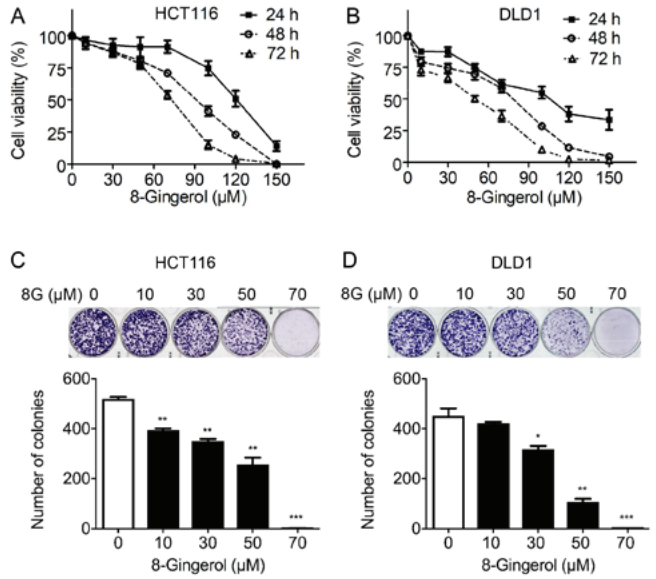

Figure 1. 8-Gingerol suppresses colorectal cancer cell proliferation. (A) HCT116 and (B) DLD1 colorectal cancer cells were treated with the indicated concentrations of 8-gingerol for 24, 48 or 72 h, and cell growth was measured using a Cell Counting Kit-8 assay. (C) HCT116 and (D) DLD1 cells were treated with the indicated concentrations of 8-gingerol for 10-14 days, and cell clonogenic activity was evaluated using a colony formation assay. The values are expressed as the means ± standard deviation of triplicates. *P<0.05, **P<0.01 and ***P<0.001 vs. vehicle (DMSO)-treated cells. DMSO, dimethyl sulfoxide.

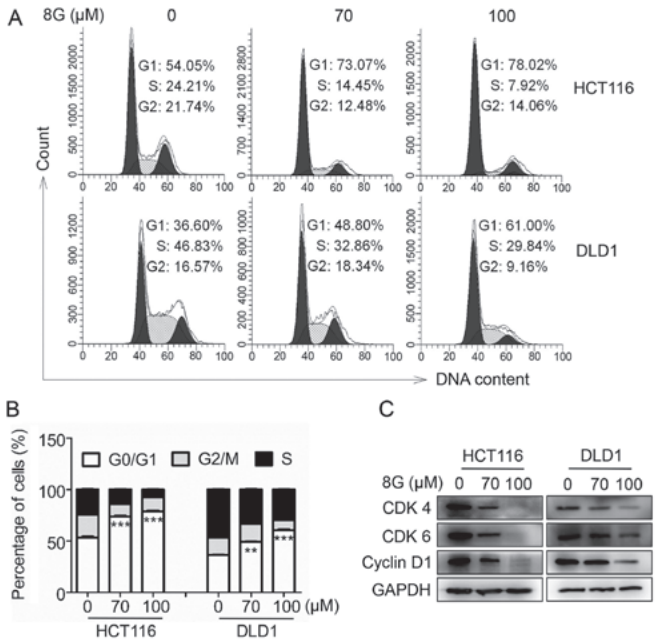

Figure 2. 8-Gingerol induces cell cycle arrest in colorectal cancer cells. (A) HCT116 and DLD1 cells were treated with the indicated concentrations of 8-gingerol for 48 h, and the cell cycle distribution was investigated by FACS analysis of propidium iodide-stained cells. (B) Histogram of the cell cycle distribution in HCT116 and DLD1 cells following 8-gingerol treatment. The values are expressed as the means ± standard deviation of triplicates. **P<0.01 and ***P<0.001 vs. vehicle-treated cells. (C) Western blot analysis of cell cycle-related protein expression. HCT116 and DLD1 cells were treated with the indicated concentrations of 8-gingerol for 48 h, and western blotting was performed with the indicated antibodies.

inhibitors. After incubation on ice for 20 min, the cells were centrifuged at 12,000 x g for 15 min at 4˚C. Protein concentration was determined using a BCA Protein Assay kit (Beyotime Institute of Biotechnology) according to the manufacturer's instructions. Protein samples (50 μg per lane) were separated by 10% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked in TBST [50 mM Tris-HCl, 150 mM NaCl, 0.1% Tween-20 (pH 7.6)] containing 5% non-fat milk. Subsequently, the membranes were incubated with the aforementioned primary antibodies against caspase-3, cleaved caspase-3, cleaved caspase-8, EGFR, p-EGFR, STAT3, p-STAT3, ERK, p-ERK1/2, GAPDH, cyclin D1, CDK4, CDK6, caspase-8, Bcl-2, anti-Myc and MMP2 overnight, followed by horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature. Protein bands were detected using Immobilon™ Western Chemiluminescent HRP Substrate (ECL; EMD Millipore).

Statistical analysis. Statistical analysis was performed using SPSS version 22.0 (IBM Corp.). Data are expressed as the means ± standard deviation. Statistical comparisons were performed using one-way ANOVA. The Bonferroni post hoc test was used for multiple comparisons between groups (Fig. 5), and Dunnett's t-test was used for comparison with the control group. P<0.05 was considered to indicate statistically significant differences.

Results

8-Gingerol suppresses CRC cell proliferation. To gain insight into the role of 8-gingerol in CRC, the effects of increasing concentrations of 8-gingerol on the viability of the CRC cell lines HCT116 and DLD1 for 24, 48 and 72 h were determined via a CCK-8 assay. The results demonstrated that 8-gingerol decreased the viability of HCT116 and DLD1 cells in a time- and dose-dependent manner (Fig. 1A and B). The IC_{50} value (50% inhibition) of 8-gingerol for HCT116 cells was 118.2±7.37 μM at 24 h, 77.4±4.70 μM at 48 h, and 61.8±3.57 μM at 72 h. The IC_{50} value of 8-gingerol for DLD1 cells was 100.3±6.32 μM at 24 h, 53.7±2.24 μM at 48 h, and 34.5±2.33 μM at 72 h. Consistent with the CCK-8 assay results, the colony formation assay results also revealed that 8-gingerol dose-dependently inhibited the clonogenic activity of both the HCT116 and DLD1 cell lines (Fig. 1C and D).

8-Gingerol induces G0/G1 cell cycle arrest in CRC cells. To elucidate the mechanism underlying the inhibitory effect of 8-gingerol on cell proliferation, the cell cycle distribution of CRC cells following 8-gingerol treatment was first investigated by flow cytometry. It was observed that 8-gingerol treatment markedly induced G0/G1 phase cell cycle arrest in both HCT116 and DLD1 cells (Fig. 2A and B). In agreement with the cell cycle arrest pattern, the levels of CDK4, CDK6 and cyclin D1, the key regulators of the G0/G1 phase transition, were markedly decreased in both the HCT116 and DLD1 cell lines following 8-gingerol exposure (Fig. 2C). Taken together, these results suggest that 8-gingerol induces G0/G1 cell cycle arrest in CRC cells.

8-Gingerol enhances apoptosis in HCT116 cells. Apoptosis is another important cause of cell growth inhibition. Next, we

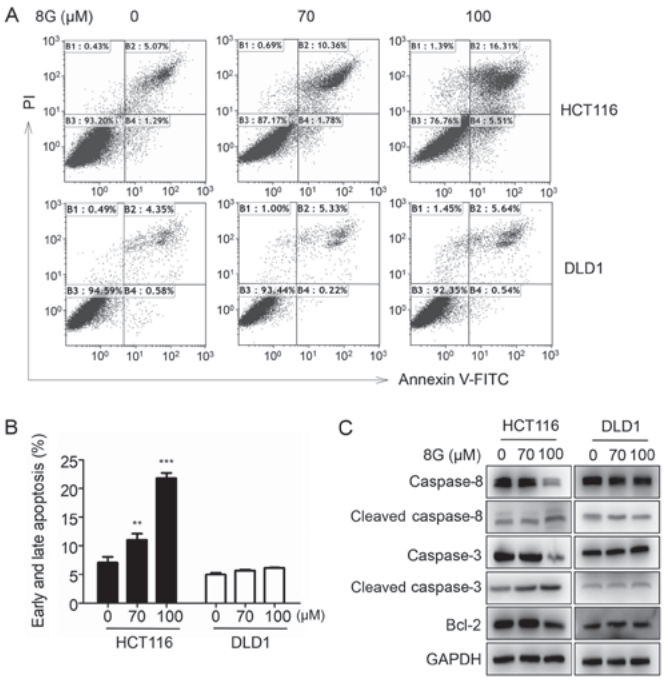

Figure 3. 8-Gingerol induces apoptosis in HCT116 cells. (A) HCT116 and DLD1 cells were treated with the indicated concentrations of 8-gingerol for 48 h, and apoptosis analysis was performed by flow cytometry with Annexin V-FITC/propidium iodide double staining. (B) Histogram of apoptosis rates of HCT116 and DLD1 cells following 8-gingerol treatment. The values are expressed as the means ± standard deviation of triplicates. **P<0.01 and ***P<0.001 vs. vehicle-treated cells. (C) Western blot analysis of apoptosis-related protein expression. HCT116 and DLD1 cells were treated with the indicated concentrations of 8-gingerol for 48 h, and western blotting was performed with the indicated antibodies.

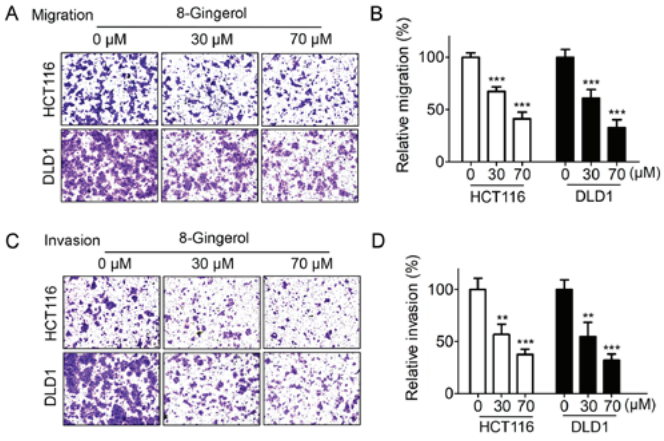

Figure 4. 8-Gingerol suppresses colorectal cancer cell migration and invasion. (A) HCT116 and DLD1 cells were treated with the indicated concentrations of 8-gingerol for 48 h, and a Transwell migration assay was performed. (B) Histogram of migration inhibition rates in HCT116 and DLD1 cells following 8-gingerol treatment. ***P<0.001 vs. vehicle-treated cells. (C) HCT116 and DLD1 cells were treated with the indicated concentrations of 8-gingerol for 48 h, and a Transwell invasion assay was performed. (D) Histogram of invasion inhibition rates in HCT116 and DLD1 cells following 8-gingerol treatment. **P<0.01 and ***P<0.001 vs. vehicle-treated cells.

investigated whether 8-gingerol was also able to induce apoptosis in CRC cells. Indeed, flow cytometric analysis revealed that treatment with 8-gingerol increased the apoptosis rates of HCT116 cells in a dose-dependent manner; however, this phenomenon was not observed in DLD1 cells (Fig. 3A and B). Consistent with these results, the expression levels of the apoptosis markers cleaved caspase-3 and cleaved caspase 8 were significantly increased, and the expression level of the antiapoptotic regulator Bcl-2 was decreased in HCT116 cells following 8-gingerol exposure (Fig. 3C). Taken together, these data suggest that 8-gingerol induces apoptosis in HCT116 cells.

8-Gingerol inhibits CRC cell migration and invasion. It was next investigated whether 8-gingerol affects CRC cell migration and invasion. The Transwell migration assay results revealed that 8-gingerol dose-dependently decreased the migration of both HCT116 and DLD1 cells (Fig. 4A and B). Similarly, 8-gingerol treatment markedly reduced the invasion ability of HCT116 and DLD1 cells, as demonstrated by the Transwell invasion assay (Fig. 4C and D). Collectively, these results suggest that 8-gingerol exerts an inhibitory effect on CRC cell migration and invasion.

8-Gingerol affects CRC cell proliferation and migration via EGFR/STAT/ERK cascades. EGFR signaling plays a key role in CRC development and progression (3). Therefore, whether this pathway is involved in the effects of 8-gingerol in CRC was next examined. Western blot analysis revealed that 8-gingerol significantly decreased the level of phosphorylated EGFR and, accordingly, the phosphorylation levels of its downstream effectors, STAT3 and ERK, leading to downregulated expression of the target genes cyclin D1, c-Myc and MMP2, in both the HCT116 and DLD1 cell lines; however, a decrease in Bcl-2 protein expression was only observed in HCT116 cells, whereas Bcl-2 protein expression was unchanged in DLD1 cells (Fig. 5A). By contrast, addition of EGF restored the phosphorylation of EGFR, STAT3 and ERK and the expression of cyclin D1, c-Myc, Bcl-2 and MMP2 (Fig. 5B). Moreover, in the colony formation assay, administration of EGF partially restored the proliferation of HCT116 and DLD1 cells suppressed by 8-gingerol (Fig. 5C and D). Similarly, in the Transwell migration assay, administration of EGF partially restored the migration of HCT116 and DLD1 cells inhibited by 8-gingerol (Fig. 5E and F). These results suggest that EGFR/STAT/ERK signaling contributes to the inhibitory effects of 8-gingerol on CRC cell proliferation and migration.

The chemotherapeutic effects of 8-gingerol are dependent on EGFR protein expression. To investigate whether the efficacy of 8-gingerol depends on EGFR protein expression levels in CRC cells, the levels of EGFR and EGFR phosphorylation were examined in HCT116 and DLD1 cell lines following exposure to 8-gingerol or the positive drug control gefitinib. The results demonstrated that the endogenous EGFR level of DLD1 cells was higher compared with that of HCT116 cells. Following treatment with 8-gingerol or gefitinib, the EGFR levels decreased in both HCT116 and DLD1 cells, but the degree of EGFR reduction in DLD1 cells was greater compared with that in HCT116 cells (Fig. 6A). Consistently with the EGFR protein expression levels, the CCK-8 results revealed that the effect of 8-gingerol and gefitinib on DLD1 cells was more prominent compared with

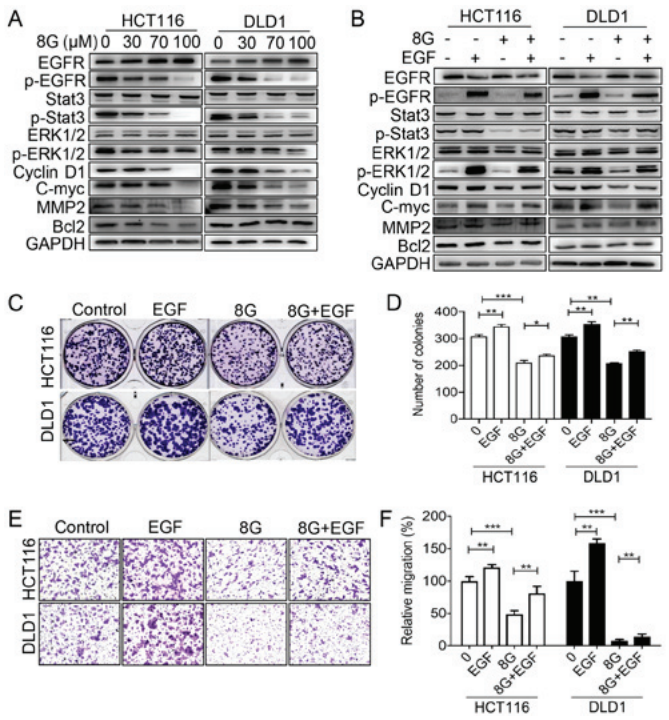

Figure 5. 8-Gingerol affects colorectal cancer cell proliferation and migration via the EGFR pathway. (A) HCT116 and DLD1 cells were treated with the indicated concentrations of 8-gingerol and western blot analysis was performed with the indicated antibodies. (B) HCT116 and DLD1 cells were treated with 8-gingerol (100 μM) and/or EGF (100 ng/ml), and western blot analysis was performed with the indicated antibodies. (C) Treatment with EGF restored the proliferation of HCT116 and DLD1 cells inhibited by 8-gingerol. HCT116 and DLD1 cells were treated with 8-gingerol (100 μM) and/or EGF (100 ng/ml), and a colony formation assay was performed. (D) Histogram of the colony numbers in HCT116 and DLD1 cells following 8-gingerol and/or EGF treatment, *P<0.05, **P<0.01 and ***P<0.001 vs. vehicle (DMSO)-treated cells. (E) Treatment with EGF restored the migration abilities of HCT116 and DLD1 cells inhibited by 8-gingerol. HCT116 and DLD1 cells were treated with 8-gingerol (100 μM) and/or EGF (100 ng/ml), and a Transwell migration assay was performed. (F) Histogram of migration inhibition rates in HCT116 and DLD1 cells following 8-gingerol and/or EGF treatment, **P<0.01 and ***P<0.001 vs. vehicle-treated cells. EGFR, epidermal growth factor receptor; DMSO, dimethyl sulfoxide.

that on HCT116 cells (Fig. 6B). Moreover, the inhibitory effects of 8-gingerol (100 μM) on HCT116 and DLD1 cells were stronger compared with those of gefitinib (20 μM). These data indicate that the effects of 8-gingerol on CRC cells depends on the expression level of EGFR in the two cell lines.

Effects of treatment with 5-FU and 8-gingerol on the CRC cell lines HCT116 and DLD1. To determine the potential use of 8-gingerol in future drug combination therapy, cell proliferation experiments were conducted with of 5-FU and 8-gingerol treatment. The results of the CCK-8 assay revealed that the presence of 8-gingerol reduced the IC_{50} value of 5-FU from 12.2±2.42 to 9.1±0.75 μM in HCT116 cells, and from 11.2±0.85 to 3.6±0.37 μM in DLD1 cells; however, the IC_{50} value of 8-gingerol alone was only 77.4±4.70 μM in HCT116 cells and 53.7±2.24 μM in DLD1 cells (Table I). These results indicate that 8-gingerol may reduce the effective concentration of 5-FU, thereby decreasing the toxicity of 5-FU in drug combination therapy.

Table I. Effects of treatment with 5-FU and 8-gingerol on the colorectal cancer cell lines HCT116 and DLD1.

	IC_{50} (μM)$_{48\ h}$	
Compound	HCT116	DLD1
8-Gingerol	77.4±4.70	53.7±2.24
5-FU	12.2±2.42	11.2±0.85
5-FU + 8-Gingerol[a]	9.1±0.75	3.6±0.37

[a]5-FU and 8-gingerol were used at the same concentration. Tumor cells were treated with 8-gingerol or 5-FU for 48 h. Cell viability was measured by a Cell Counting Kit-8 assay, and the IC_{50} values are shown as the means ± standard deviation of triplicates. 5-FU, 5-fluorouracil.

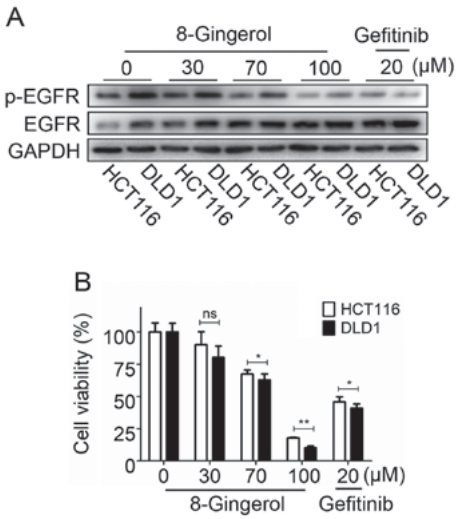

Figure 6. The chemotherapeutic effects of 8-gingerol are dependent on EGFR protein expression in HCT116 and DLD1 cells. (A) HCT116 and DLD1 cells were treated with 8-gingerol or gefitinib (an EGFR tyrosine kinase inhibitor) for 48 h, western blot analysis was performed with the indicated antibodies. (B) Colorectal cancer cells were treated with the indicated concentrations of 8-gingerol or gefitinib for 48 h, and cell growth was measured using a Cell Counting Kit-8 assay. *P<0.05 and **P<0.01 represented significant differences. EGFR, epidermal growth factor receptor; ns, not significant.

Discussion

Chemotherapy is one of the most common types of treatment for metastatic CRC (33); however, resistance and serious side effects are major obstacles to effective treatment (31). Novel strategies to enhance chemotherapeutic effectiveness and reduce resistance and side effects are urgently needed. Natural products are a good source of novel anticancer drugs. Ginger is a major ingredient in traditional herbal medicine used for treating a number of diseases, including cancer (10). 8-Gingerol is one of the major non-volatile components of ginger (11). Apart from its antioxidant and anti-inflammatory properties, the activity of 8-gingerol against cancer is unclear. To the best of our knowledge, the present study is the first to report the suppressive effects of 8-gingerol on CRC cells *in vitro*.

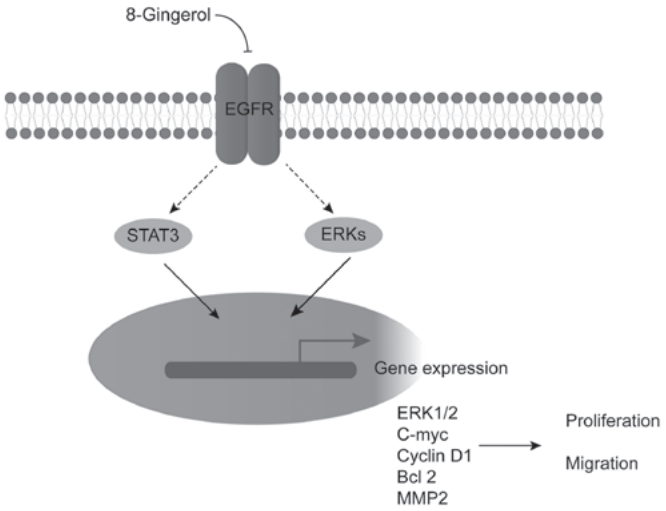

Figure 7. A proposed action mechanism of 8-gingerol in CRC cells. 8-Gingerol decreases the EGFR phosphorylation level, resulting in inhibition of STAT3 and ERK signaling pathway activity, leading to blockade of cyclin D1, c-Myc, Bcl-2 and MMP2 expression, thus suppressing cell proliferation and migration. CRC, colorectal cancer; EGFR, epidermal growth factor receptor; STAT, signal transducer and activator of transcription; ERK, extracellular signal-regulated kinase; MMP, matrix metallopeptidase; Bcl-2, B-cell lymphoma 2.

Cell cycle arrest and apoptosis are the main causes of the cell growth inhibition induced by chemopreventive agents. It has been reported that 6-gingerol and 10-gingerol inhibit cell growth by inducing cell cycle arrest and apoptosis in several cancers, including CRC (25,28,32,33). Consistently with these reports, we herein demonstrated that 8-gingerol induced G0/G1 cell cycle arrest in both the HCT116 and DLD1 cell lines. Notably, 8-gingerol induced apoptosis in HCT116 cells, which express wild-type p53, but not in DLD1 cells, which express mutant p53, consistently with the concept that p53 mutation in cancers usually confers resistance to apoptosis-inducing chemotherapeutic agents (34,35). Although 8-gingerol did not affect the apoptosis of DLD1 cells, it exerted an even greater antiproliferative effect on DLD1 cells compared with HCT116 cells, indicating that this compound may be a promising agent targeting cancer cells, particularly those with mutant p53.

EGFR signaling is crucial for driving the transition from healthy colonic epithelium to malignant tumors, and also for controlling tumor metastasis (36). EGFR is a member of the ERBB family of cell surface receptor tyrosine kinases (37). Upon binding to its ligands, such as EGF and transforming growth factor-α, EGFR is autophosphorylated and activates downstream signaling to promote cell proliferation and metastasis (38). Overexpression of EGFR is frequently observed in CRC tumor tissues; hence, targeting EGFR signaling appears to be a promising strategy for CRC treatment (3). Monoclonal anti-EGFR antibodies, such as cetuximab, have induced a good response in patients with metastatic CRC; however, resistance to these EGFR-targeted therapies eventually develops (39). Moreover, cetuximab is expensive and is associated with a number of side effects (5). Therefore, great efforts are still needed to develop novel chemopreventive agents targeting EGFR signaling. In the present study, 8-gingerol was identified as a novel inhibitor of EGFR signaling. Treatment with 8-gingerol significantly decreased EGFR phosphorylation, and the therapeutic effects of 8-gingerol largely depended on the EGFR expression in CRC cells. The STAT and ERK pathways are two downstream effector pathways of EGFR (40,41). Correspondingly, upon 8-gingerol exposure, the levels of phosphorylated STAT3 and ERK1/2 were significantly decreased, leading to decreased expression of their downstream target genes, such as cyclin D1, c-Myc and MMP2 (42,43), in turn leading to the suppression of cell proliferation and migration. By contrast, addition of EGF partially restricted the inhibitory effect induced by 8-gingerol. However, downregulation of Bcl-2 protein expression only occurred in HCT116 cells, and not in DLD1 cells. Bcl-2 is an antiapoptotic regulator that enhances cell survival and inhibits apoptosis triggered by several different apoptotic pathways (44,45). Englert *et al* demonstrated that suppression of EGFR could promote cell apoptosis (46). The effect of apoptosis induced by EGFR withdrawal may counteract the protective effect of Bcl-2 on cell growth, which ultimately resulted in the unchanged Bcl-2 expression seen in DLD1 cells. Taken together, these data suggest that 8-gingerol inhibits CRC cell proliferation and migration by targeting the EGFR/STAT3/ERK pathway (Fig. 7).

In the present study, the antitumor effects of the natural product 8-gingerol on CRC cells were first verified. Further experiments indicated that 8-gingerol inhibits CRC cell proliferation and migration by targeting EGFR signaling. However, these results require validation in an *in vivo* animal model and confirmation by clinical evidence in the future.

In summary, 8-gingerol was shown to have antitumor activity against CRC cell proliferation, migration and invasion. Additionally, 8-gingerol was found to be a novel inhibitor of EGFR signaling in CRC cells, and its effects depended on the EGFR expression in the two CRC cell types. In addition, the addition of 8-gingerol to 5-FU therapy may reduce the effective concentration of 5-FU, thereby decreasing the toxicity of 5-FU in drug combination therapy. These data suggest that 8-gingerol may be a promising candidate for the development of antitumor agents against CRC.

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Availability of materials and data

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

XWZ initiated and designed the study, revised the manuscript, and participated in data interpretation. SMH and XHY

performed the experiments, analyzed the data and wrote the manuscript. YHH contributed to data analysis. AHP was responsible for data interpretation. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

All the authors declare that they have no competing interests.

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