Epigallocatechin-3-gallate inhibits the proliferation and migration of human ovarian carcinoma cells by modulating p38 kinase and matrix metalloproteinase-2

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Abstract. Epigallocatechin-3-gallate (EGCG), a major catechin in green tea, has recently been reported to exhibit anticancer effects on a number of types of cancer cells in vitro; however, the molecular mechanisms of this anticancer effect remain poorly understood. In the current study, the effects of EGCG on the proliferation and migration of the OVCAR-3 human ovarian carcinoma cell line were investigated. Cells were treated with EGCG and their proliferation rates were determined by an MTT assay. In addition, cell migration was detected by transwell assay. The activity of mitogen-activated protein kinases (MAPKs) and the expression of matrix metalloproteinase-2/9 (MMP-2/9) were examined by western blotting. The results showed that EGCG significantly inhibited (P<0.05) the proliferation of OVCAR-3 cells in a time- and concentration-dependent manner. EGCG (100 µM) time-dependently increased (P<0.05) the activity of p38, but not extracellular signal-regulated kinases 1/2. SB203580, a specific p38 MAPK inhibitor, completely diminished EGCG-induced phosphorylation of p38 and partially blocked EGCG-inhibited OVCAR-3 cell proliferation. Furthermore, EGCG (0-100 µM) dose-dependently inhibited (P<0.05) OVCAR-3 cell migration. The protein expression levels of MMP-2, but not MMP-9, were dose-depedently decreased following treatment with EGCG (0-100 µM) for 48 h. These data indicated that EGCG inhibited OVCAR-3 cell proliferation and migration, potentially mediated via the activation of p38 MAPK and downregulation of the protein expression of MMP2. Thus, the therapeutic potential of EGCG for ovarian cancer requires further investigation.

Introduction

Epigallocatechin-3-gallate (EGCG), a major biologically active polyphenol in green tea has long been known to exhibit potential health benefits, including anti-oxidant, anticancer and anti-inflammatory effects (1-3). More recently there have been an increasing number of studies demonstrating the potent anticancer effect of EGCG against various cancer cell lines in vitro, including breast (4), pancreatic (5), colorectal (6) and gastric cancer cell lines (7).

Ovarian cancer is a major cause of mortality among the gynecological malignancies globally. Despite significant improvement in surgical technology and therapy regimens in previous years, the molecular mechanisms underlying the disease progression remain poorly understood (8,9). The development of novel therapeutic agents targeting this potentially fatal gynecological disease is important to improve the prognosis of treatment. However, little is known of the effects of EGCG on human ovarian cancer progression and the associated molecular signaling mechanisms.

It is well demonstrated that a number of cellular, extracellular and cytokine-associated components trigger multiple downstream protein kinase pathways, thus exhibiting a role in the regulation of cell proliferation and migration during cancer development. Among these pathways, the mitogen-activated protein kinases (MAPK) cascades are the most well-studied (10-12). MAPK are proline-directed serine/threonine kinases that have been classified into at least six subfamilies. As the most important member of the MAPKs family, extracellular signal-regulated kinases 1 and 2 (ERK1/2) is required for cell mobility, proliferation and migration (13,14). Another important member of the MAPK family, p38, is essential in regulating a number of cellular processes, including inflammation, cell differentiation, cell growth and cell death (15-17). However, whether these signaling pathways are involved in EGCG-regulated cell proliferation and migration during ovarian cancer growth remains unknown. In addition, the secretion of matrix metalloproteinases (MMPs) is crucial in cancer cell metastasis and is closely associated with the migration behavior of cancer (18,19). The effects of EGCG on MMP expression in ovarian cancer remain to be investigated.

In the current study, the effect of EGCG on the cell proliferation and migration in OVCAR-3 cells was investigated, as well as the signaling pathways involved in these actions.
Materials and methods

Cell line and cell culture. The OVCAR-3 human ovarian adenocarcinoma cell line was obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) and maintained in RPMI-1640 (Gibco-BRL, Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, HyClone, Logan, UT, USA), 100 U/ml penicillin, 100 µg/ml streptomycin and 10 mM L-glutamine. All cells were cultured in a humidified atmosphere of 5% CO2 at 37°C. The cells used in this study were at passages 23-26.

MTT assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) viability assay was performed as previously described with slight modifications (17). Briefly, cells were seeded into 96-well plates with 5x104 cells/well. Following 16 h of attachment, different concentrations of EGCG (0-200 µM, PeproTech, Rocky Hill, NJ, USA) were applied to the cells in RPMI-1640 culture media and incubated for a further 48 h. The cells were washed with phosphate-buffered saline (PBS) and 200 µl MTT (0.5 mg/ml) was added to each well and further incubated for 4 h. The MTT solution was carefully removed by aspiration and the formazan product was dissolved in 150 ml dimethylsulfoxide. Absorbance was measured at 570 nm on a microplate reader (BioTek Instruments, Winooski, VT, USA). The same experiments were performed for time-course (2, 4 and 6 days) treatment with 100 µM EGCG. To determine the role of the p38 MAPK pathway in cell proliferation, additional cells were subjected to the same assay in the presence of 10 µM SB203580 (a specific p38 inhibitor, 1 h pretreatment; CalBiochem, San Diego, CA, USA). Cell proliferation studies were performed in three independent experiments.

Migration assay. Cell migration was detected using a 24-well transwell chamber with 8.0-µm pore polycarbonate filter inserts (Costar, Cambridge, MA, USA). Cells (5x104 cells/well) suspended in serum-free RPMI-1640 were overlaid in the upper chamber. In each lower chamber, 800 µl RPMI-1640 with 10% FBS in the presence of various concentrations of EGCG (0-100 µM) was added. The inserts were incubated at 37°C in a humidified atmosphere containing 5% CO2 for 16 h. Cells that had migrated to the bottom of the inserts were stained with Calcein AM (0.2 mg/ml; Molecular probes, Eugene, OR, USA) for 30 min, examined and recorded under a microscope (ECLIPSE Ti; Nikon, Tokyo, Japan) mounted with a CCD camera (Nikon). The numbers of migrated cells were counted using the Metamorph image analysis program (Universal Imaging Corporation, West Chester, PA, USA). Cell migration studies were performed in four independent experiments.

Western blotting. Cells were treated with 100 µM EGCG for 0-120 min in RPMI-1640 culture media and in 10 µM SB203580 for 60 min. To determine changes in total and phosphorylated ERK1/2 and p38 protein levels, cells were washed twice with cold PBS, harvested and lysed by sonication (Sonicator 300, Misonix, Inc., Farmingdale, NY, USA) in buffer (4 mM sodium pyrophosphate; 50 mM HEPES, pH 7.5; 100 mM NaCl; 10 mM EDTA; 10 mM sodium fluoride; 2 mM sodium orthovanadate [Na3VO4]; 1 mM PMSF; 1% Triton X-100; 5 mg/ml leupeptin and 5 mg/ml of aprotinin). The protein concentrations in the supernatants of the lysates were determined. Proteins (15-20 µg/lane) were subjected to western blot analysis. Proteins were separated on 10% SDS-PAGE gels and electroblotted onto Immobilon-P membranes (Millipore, Bedford, MA, USA). Proteins on the membranes were probed with an antibody against total or phospho-specific ERK1/2 (1:2,000 dilution; rabbit polyclonal), total (1:2,000 dilution; rabbit polyclonal) or phospho-specific p38 (1:1,000; dilution; rabbit polyclonal; all Cell Signaling Technology, Inc., Danvers, MA, USA). Changes in total and phosphorylated ERK1/2 and p38 protein levels were quantified. Data on phosphorylated ERK1/2 and p38 were normalized to total ERK1/2 and p38. The same assay was performed on the expression of MMP-2/9 in response to EGCG treatment (0-100 µM). Western blotting studies were run in at least three independent experiments.

Statistical analysis. Data were analyzed using one-way analysis of variance (SigmaStat, Jandel Co., San Rafael, CA, USA). When an F-test was significant, data were compared with their respective control by the Bonferroni's multiple comparison test or Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

EGCG inhibits the growth of human ESCC cells in a time- and dose-dependent manner. EGCG inhibits tumor growth in a number of cancer types (4-7). Based on these studies, the effects of EGCG on the proliferation of ESCC cells were determined. The doses of EGCG used were comparable to those used in previous studies (7). OVCAR-3 cells were treated with EGCG (0-200 µM) for 48 h. As shown in Fig. 1A, EGCG dose-dependently (P<0.05) inhibited the proliferation of OVCAR-3 cells. In addition, the cell proliferation following treatment with EGCG for 2, 4 and 6 days, respectively, was analyzed. As shown in Fig. 1B, EGCG time-dependently (P<0.05) inhibited OVCAR-3 cell proliferation at a concentration of 100 µM.

EGCG dose-dependently inhibits OVCAR-3 cell migration. The transwell chamber assay is a commonly used model for analyzing the molecular mechanisms underlying cell migration. In this assay (Fig. 2), EGCG (0-100 µM) dose-dependently inhibited (P<0.05) OVCAR-3 cell migration.

EGCG time-dependently inhibits the phosphorylation of p38, but not ERK1/2. The potential effects of EGCG on the activation of ERK1/2 and p38, which closely associated with carcinoma cell proliferation and migration, were analyzed. As shown in Fig. 3, EGCG time-dependently (P<0.05) increased the phosphorylation of p38 in comparison with the time 0. However, a significant change in the phosphorylation of ERK1/2 was not observed following treatment with 100 µM of EGCG.

SB203580 partially blocks EGCG-inhibited OVCAR-3 cell proliferation. To further determine whether the p38 signaling
pathway is involved in the EGCG-inhibited OVCAR-3 cell proliferation, the effects of SB203580 on EGCG regulated cell proliferation were examined. As shown in Fig. 4A and B, SB203580 completely diminished EGCG-induced p38 activation, but partially blocked EGCG inhibited (P<0.05) OVCAR-3 cell proliferation, suggesting that the p38 signaling pathway may be partially involved in the EGCG-mediated inhibitory effects.

EGCG dose-dependently inhibits the protein expression of MMP2, but not MMP9. Given MMP involvement in the cancer migration process, the effects of EGCG on MMP expression were further investigated by western blotting. As shown in Fig. 5A, following 48 h treatment, EGCG significantly (P<0.05) decreased protein expression of MMP-2 at 10, 50 and 100 µM, respectively. However, EGCG did not alter MMP9 and GAPDH protein levels at any time point of the EGCG treatments observed (Fig. 5B).

Discussion

EGCG is one of the most widely characterized polyphenols in green tea and has been extensively investigated for its chemopreventive effects on tumor formation and development in several types of cancer (4-7). In the present study, significant evidence is provided that EGCG is capable of inhibiting proliferation and migration of human ovarian carcinoma cells, partially through the regulation of the activation of p38 kinase and reduction of MMP2 expression, suggesting that EGCG may possess anticancer potential in human ovarian cancer.

An increasing number of studies have indicated that EGCG is capable of inhibiting the growth of various types of cancer cells, including lung (4), colon (6) and gastric (7) cancer cells in culture. This inhibitory effect was also demonstrated in the ovarian cancer cells in the current study. The current data showed that EGCG significantly inhibited ovarian cancer cell growth in a dose- and time-dependent manner. However, little is known of the signaling pathway involved in this inhibitory effect on ovarian cancer growth.

Depending on the cell type, various signaling kinases have been shown to be involved in cell proliferation during tumor development. It is well established that the MAPK family...
Figure 3. Effects of EGCG on ERK1/2 and p38 phosphorylation in OVCAR-3 cells. Cells were cultured in culture dishes until reaching 60-70% confluency. Cells were treated with 100 µM of EGCG or DMSO for 0-120 min. Proteins were subjected to western blotting and detected using antibodies against phospho-specific and total ERK1/2 or p38. Data normalized to total ERK1/2 or p38 are expressed as means ± SEM fold of the control from three individual experiments. *P<0.05, vs. control (DMSO treated group). EGCG, epigallocatechin-3-gallate; ERK 1/2, extracellular signal-regulated kinases 1/2.

Figure 4. Effects of SB203580 on EGCG-inhibited OVCAR-3 cell proliferation. Cells were treated with 100 µM EGCG in the presence or absence of SB for 2 days, respectively. (A) Cell proliferation was determined by an MTT assay and the graphs show the results of four independent experiments. Quantified data are expressed as the mean ± standard error of the mean from four independent experiments. aP<0.05 and bP<0.05, compared with the DMSO control. (B) Phosphorylation of p38 was detected with western blotting. EGCG, epigallocatechin-3-gallate; SB: SB203580; DMSO, dimethylsulfoxide.

Figure 5. Effects of EGCG on MMP-2/9 protein expression. Cells were cultured in culture dishes until reaching 60-70% confluency. Cells were treated with EGCG (0-100 µM) for 48 h. Proteins were subjected to western blot analysis and detected using antibodies against MMP-2/9 and GAPDH. Data normalized to GAPDH are expressed as the mean ± standard error of the mean fold of the control from three individual experiments. *P<0.05, vs. control. EGCG, epigallocatechin-3-gallate. MMP-2/9, matrix metalloproteinase-2/9.
are actively involved in the regulation of cellular functions, particularly of cell proliferation (20,21). A previous study by Shankar et al (5) showed that EGCG significantly reduced ERK activity and enhanced p38 and JNK activities in a human pancreatic tumor xenograft model. In accordance with this report, the current study showed that EGCG significantly activated p38 in a time-dependent manner. SB203580, the specific inhibitor of p38, completely inhibited the EGCG-induced increased phosphorylation of p38. However, significant changes in the activity of ERK1/2 in response to EGCG were not observed in the present data, which may be attributable to the different cell types.

Cancer metastasis is a highly coordinated multistep process involving cell invasion, cell-cell and cell-matrix adhesion and remodeling of the extracellular matrix (22,23). The current study indicated that EGCG dose-dependently inhibits OVCAR-3 cell migration. However, the molecular mechanisms underlying this effect remain unknown. It is well observed that a number of proteinases are involved in the degradation of the ECM by cancer cells, including MMPs, serine proteinase, and in particular, members of the uPA-plasmin system (24). The secretion of MMPs is crucial in cancer cell metastasis and is involved in cancer cell migration and adhesion. Among human MMPs, MMP-2/9 are abundantly expressed in various malignant tumors, including ovarian cancer (25-27). The present data demonstrated that EGCG significantly decreased the expression of MMP-2, but not MMP-9, which is also supported by previous studies where EGCG reduced MMP-2 expression in human lung and breast cancer cells (28,29).

In conclusion, this preliminary investigation has shown that the anticancer effect of EGCG in an ovarian cancer cell model may be mediated via the activation of p38 MAPK signaling pathway as well as the decreased expression of MMP-2. These findings reveal EGCG as a potential novel therapeutic anticancer therapy.

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References