(--)-Epigallocatechin-3-gallate promotes pro-matrix metalloproteinase-7 production via activation of the JNK1/2 pathway in HT-29 human colorectal cancer cells

Mihyee Kim, Akira Murakami, Kyuichi Kawabata and Hajime Ohigashi* Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

*To whom correspondence should be addressed. Tel: +81 75 753 6281; Fax: +81 75 753 6284; Email: ohigashi@kais.kyoto-u.ac.jp

Matrix metalloproteinase (MMP)-7 (matrilysin-1) plays significant roles in the growth, invasion, and metastasis of colorectal tumors, while (--)-epigallocatechin-3-gallate (EGCG), a green tea polyphenol with chemopreventive properties, has been shown to be an inhibitor of MMP-2 and MMP-9. In the present study, HT-29 human colorectal cancer cells were treated with EGCG to examine its effects on pro-MMP-7 induction and production using RT–PCR and western blot analyses. Surprisingly, EGCG (10–100 μM) treatment increased both intracellular and extracellular pro-MMP-7 protein levels (2.6–8.4-fold and 1.9–6.4-fold, respectively) in dose- and time-dependent manner, with a significant upregulation of its mRNA expression. EGCG also activated extracellular signal-regulated protein kinase (ERK)1/2, c-JUN NH2-terminal kinase (JNK)1/2 and p38 mitogen-activated protein kinase (MAPK), as previously reported. In addition, the polyphenol triggered the phosphorylation of c-JUN (Ser63 and Ser73) and induced c-JUN/c-FOS, thereby increasing the DNA binding activity of activator protein-1 (AP-1), as shown by an AP-1 luciferase reporter assay. Pharmacological blockade of MAPK activities suggested that pro-MMP-7 expression was induced via JNK1/2 activation, but not in the case of ERK1/2 or p38 MAPK. N-Acetyl-l-cysteine, superoxide (O2-) dismutase and catalase attenuated the EGCG-induced pro-MMP-7 production, suggesting an involvement of oxidative stress in these events. Conversely, EGCG spontaneously generated O2 in a cell-free system that utilized a cytochrome C reduction method. Further, (--)-epicatechin-3-gallate (25 and 100 μM) and green tea polyphenols (33 and 132 μg/ml) induced pro-MMP-7 expression, whereas (--)-epicatechin and (--)-epigallocatechin (100 μM each) did not. Induction of pro-MMP-7 expression by EGCG was also shown in another human colorectal adenocarcinoma cell line, Caco-2. Our results suggest that some green tea catechins induce pro-MMP-7 production via O2 production and the activation of JNK1/2, c-JUN, c-FOS and AP-1 in HT-29 cells.

Introduction

Green tea is a popular beverage in Asian countries, because of its pleasing flavor and aroma and (--)-epigallocatechin-3-gallate (EGCG) is the most abundant catechin. EGCG has been reported to possess marked biological activities, such as antioxidative (1,2), anti-inflammatory (3–5) and anti-mutagenic (6) effects, in a variety of experimental models. EGCG and green tea polyphenols (GTP) have been shown to suppress experimental carcinogenesis in the lungs (7), gastrointestinal tract (8) and colon (9) in rodents. Further, some epidemiologic studies have demonstrated the association of green tea consumption with prevention of cancer development and metastasis (1). For example, subjects in Saitama Prefecture, Japan who consumed >10 cups of green tea per day, showed a reduction of relative risk of cancer in all organs surveyed, which included the lungs, colorectum, liver, and stomach, (10), while the risk of esophageal cancer in subjects in urban Shanghai, China, was reduced following green tea consumption in a case–control study (11).

Matrix metalloproteinases (MMPs) are a family of tightly regulated zinc-dependent proteases that can degrade nearly all components of the extracellular matrix as well as those of the basement membranes (12,13). There is a large body of experimental evidence suggesting that MMPs essentially contribute to the promotion and maintenance of tumor growth in primary and metastatic sites (14–16). However, it should be noted that MMP-7 (matrilysin-1) is distinguished from other MMPs by some unique characteristics (17), such as a broad proteolytic activity against a variety of extracellular matrix substrates, the lowest molecular weight in the family, an ability to trigger activation of an MMP cascade and production by cancer tissues, but not stromal tissues, in many types of tumors ranging from adenomas to carcinomas in several organs (18–20). Further, MMP-7 gene expression has been detected in rectal cancerous tissues taken from experimental animals (21,22) and patients (18). In addition, Hasegawa et al. (22) showed a 70% reduction in the number of metastatic tumors by utilizing antisense oligonucleotides to the MMP-7 gene in spleen injections given to a mouse model of colorectal metastasis. It is also of paramount importance to note that MMP-7 plays roles not only in tumor progression, but also in the early stages of cancer development (12), as over-expression of the MMP-7 gene in transgenic mice led to enhanced tumorigenesis in a breast cancer model (23) and intestinal tumorigenesis was reduced in MMP-7-deficient mice (21).

Recently, EGCG has been shown to inhibit angiogenesis and metastasis by downregulating the expression and activities of membrane type-1 MMP (24), as well as MMP-2 and MMP-9 (25–27). In spite of the important roles of MMP-7 in cancer

Abbreviations: AP-1, activator protein-1; DMEM, Dulbecco’s Modified Eagle Medium; DMSO, dimethylsulfoxide; EGCG, (--)-epigallocatechin-3-gallate; ERK, extracellular signal-regulated protein kinase; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; JNK, c-JUN NH2-terminal kinase; MMP, matrix metalloproteinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; MAPK, mitogen-activated protein kinase; NAC, N-acetyl-l-cysteine; O2-, superoxide; PBS, phosphate-buffered saline; RT-PCR, reverse transcription-polymerase chain reaction; SDS, sodium dodecyl sulfate; SOD, superoxide dismutase.
development and metastasis, the effect of EGCG toward this particular protease remains to be examined. In the present study, the effects of EGCG and other green tea catechins (Figure 1) on pro-MMP-7 expression were examined in a human colorectal cancer cell line, HT-29. Unexpectedly, we found that EGCG and GTP caused a significantly large increase in the expression and production of pro-MMP-7 via free radical generation, c-JUN NH2-terminal kinase (JNK)1/2 activation, c-JUN/c-FOS induction and increased activator protein-1 (AP-1) activity.

Materials and methods

Reagents
GTP, containing 35% EGCG, 70% other catechins and 3% caffeine, was obtained from LKT Laboratories (Minnesota, USA). Dulbecco’s Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA). PD98059, SB203580, and SP600125 came from Calbiochem (La Jolla, CA). Antibodies were purchased from the following sources: anti-h proMMP-7 (F-82), Daiichi Fine Chemical (Toyama, Japan); rabbit anti-phospho-p38 MAPK, rabbit anti-p38 MAPK, rabbit anti-phospho-JNK1/2, rabbit anti-JNK1/2, rabbit anti-phospho-c-JUN (Ser63), rabbit anti-phospho-c-JUN (Ser73), rabbit anti-c-JUN and anti-rabbit antibody horseradish peroxidase-linked IgG antibodies, Cell Signaling Technology (Beverly, MA); mouse anti-c-FOS and goat anti-β-actin antibodies, Santa Cruz Biotechnology (Santa Cruz, CA); horseradish peroxidase-conjugated anti-mouse IgG, anti-rabbit IgG, and anti-goat IgG, Dako (Glostrup, Denmark). Oligonucleotide primers were synthesized by Proligo (Kyoto, Japan). A QIAshredder™ and RNAeasy Mini Kit™ were purchased from Qiagen (Hilden, Germany), and an RNA PCR Kit (ver. 2.1, AMV) came from TaKaRa Bio (Shiga, Japan). All other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan), unless specified otherwise.

Cell culture
Human colorectal cancer cell lines (HT-29, Caco-2, Colo205, Colo320DM, LS174T and LS180) and normal colon fibroblasts (CCD-18Co) were obtained from American Type Culture Collection (Manassas, VA) and maintained in DMEM supplemented with 10% FBS, L-glutamine (330 μg/ml), penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37°C under a humidified atmosphere of 95% air and 5% CO2.

Cell viability
Cell viability was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). After incubation the cells were washed twice with phosphate-buffered saline (PBS), then 50 ml of serum-free medium and 5 ml of MTT stock solution (5 mg/ml) were added to each culture, followed by another incubation at 37°C for 2 h. Next, 1 ml of DMSO was added and the culture
was sonicated for 5 min, after which 500 μl of HCl/2-propanol (3.4 μl/ml) was added to each well. Visible absorbance was measured at 600 and 650 nm using a microplate reader MPR-A4i (Tosho, Japan). Significant cell viability (>90%) was achieved in each experiment.

**Western blotting**

Cells (3.4 x 10⁶ cells/1.7 ml on a 35-mm dish) were incubated for 24 h, washed twice with PBS and cultured in serum-free DMEM. EGCG (0, 1, 10, 25, 50 and 100 μM) was dissolved in dimethylsulfoxide (DMSO) (final 0.05%, v/v) and added to the cells, which were incubated for 1 h and washed twice with PBS. Negative control cells were treated with only 0.05% DMSO, which showed no effect on the assay system (data not shown), and harvested after 23 h in serum-free DMEM. For time-course experiments, cells were treated with 25 μM of EGCG for 0.5-25% DMSO for 1 h and harvested at the indicated times (0, 3, 6, 12 and 24 h). Cells were lysed in a boiling lysis buffer [protease and phosphatase inhibitors cocktail (Takara Bio, Shiga, Japan), 10 mM Tris (pH 7.4), 1% sodium dodecyl sulfate (SDS) and 1 mM sodium vanadate (V)] and boiled for 10 min. Medium from each plate was then collected and centrifuged at 3200 x g for 5 min and the supernatant (750 μl) was incubated with 60% trichloroacetic acid (final 3.75%, v/v) at 4°C overnight. After centrifugation at 9000 x g for 10 min, the pellet was washed with 500 μl of ice-cold diethyl ether. After centrifugation at 9000 x g for 10 min, the pellet was dried in vacuo and then re-suspended in 20 μl of sample buffer (0.125 M Tris (pH 6.8), 4% SDS, 20% glycerol and 10% 2-mercaptoethanol). Protein concentration was determined using a DC protein assay (Bio-Rad Laboratories, Kyoto, Japan), with γ-globulin used as the standard. Denatured proteins (10 μg of pro-MMP-7 and β-actin, 80 μg of other intracellular proteins or 10 μl of extracellular pro-MMP-7) were separated using SDS-PAGE in a 10% polyacrylamide gel and transferred onto Immobilon-P membranes (Millipore, MA). Blots were then developed using ECL Advance western blotting detection system (Berthold Detection System GmbH, Pforzheim, Germany). The luciferase activity was normalized against protein amount and expressed as fold induction over the control cells. Each experiment was performed three times.

**MAPK inhibition assay**

HT-29 cells were inoculated onto a 35-mm dish at a density of 3.4 x 10⁶ cells/1.7 ml and pre-incubated for 24 h. The cells were washed twice with PBS and cultured in serum-free DMEM including a MAPK inhibitor (PD98059, SP600125 or SB203580, 50 μM each) or the vehicle (0.25% DMSO, v/v) for 30 min, followed by exposure to 25 μM of EGCG for 1 h. After washing twice with PBS, the cells were further incubated for 23 h in serum-free DMEM, prior to western blot analysis.

**Effect of exogenous H₂O₂ and anti-oxidants**

HT-29 cells were pretreated with the vehicle (0.25% DMSO, v/v), NAC (10 mM), NAC (5000 U/ml), or catalase (5000 U/ml) for 30 min, then treated with 25 μM of EGCG for 1 h, followed by 23 h of incubation in serum-free DMEM. Cells exposed to the vehicle or 50 μM of SP600125 for 30 min were treated with 1 mM of H₂O₂ for 1 h and then incubated in serum-free DMEM for 23 h. Extracellular pro-MMP-7 protein was detected by western blotting.

**Quantification of O₂⁻**

The production of O₂⁻ was measured using a cytochrome C reduction method (28). EGCG (5, 10, 15, 20 and 25 μM) or DMSO (0.05%, v/v) was added to 1 ml of DMEM medium containing cytochrome C (final 1 mg/ml) and O₂⁻ dismutase (SOD) (final 0 or 2000 U/ml). After being incubated at 37°C for 1 min, the mixture thus obtained was put on ice to terminate the reaction. Visible absorption at 550 nm was measured with a UV-spectrophotometer (Shimadzu, Kyoto, Japan). The O₂⁻ concentration was quantified using the following equation: O₂⁻ (nmol/ml) = 47.7 x [(Abs. EGCG – Abs. DMSO) – (Abs. EGCG plus SOD – Abs. DMSO)].

**Statistical analysis**

Each experiment was performed at least 3 times and the data are shown as mean ± SD where applicable. Statistically significant differences between groups in each assay were determined using a Student’s t-test (two-sided).

**Results**

**EGCG promotes the expression of pro-MMP-7 protein and mRNA in HT-29 cells**

We treated HT-29 human colon adenocarcinoma cells with the vehicle alone (0.05% DMSO) or 1–100 μM of EGCG for 1 h, followed by washing with PBS. The cells were incubated for an additional 23 h in serum-free media for estimating the production of extracellular and intracellular pro-MMP-7 proteins by western blot analysis. Treatment with EGCG increased extracellular production, as compared with vehicle-treated cells, in a dose-dependent manner (Figure 2A, upper panel), with a concerted increase in intracellular protein levels in a range of 10–100 μM (Figure 2A, middle panel). Time-course experiments showed that the vehicle-treated cells spontaneously released pro-MMP-7 protein into the media, which was detectable after 12 h (Figure 2B, upper panel), while 25 μM of EGCG enhanced that release at each respective time point. Further, intracellular production was promoted by EGCG in a time-dependent manner (Figure 2B, middle panel). In addition, MMP-7 mRNA expression, which was consistently detected at low levels in vehicle-treated cells by RT–PCR (0–12 h), was upregulated following treatment with 25 μM of EGCG 2.6-fold from 6–12 h, while exposure to 100 μM of EGCG resulted in an earlier upregulation (Figure 2C).

**EGCG induces activation of MAPKs, c-JUN, c-FOS**

Next, we treated cells with 25 or 100 μM of EGCG for 0–6 h, and cell lysates were subjected to Western blot analysis to detect inactive and activated forms of p38 MAPK, JNK1/2 and ERK1/2, using specific antibodies. As shown in Figure 3A, treatment with EGCG (from 15 min to 6 h) at a concentration of 100 μM, but not at 25 μM, significantly and transiently
phosphorylated p38 MAPK and ERK1/2, which was consistent with previous observations (29). It is noted that the activation of JNK1 sustained for 6 h whereas that of other kinases did not and the protein expression levels of their inactive forms remained unchanged with all of the tested treatments. On the other hand, c-JUN was remarkably phosphorylated at serine 63 and 73 in a concerted manner from 1–6 h, which was accompanied by a concentration-dependent upregulation of both c-JUN and c-FOS proteins.

**EGCG induced the AP-1 reporter activity**

These results led us to examine the effect of EGCG on the AP-1 transcription activity using a reporter system. We transfected AP-1 promoter/luciferase and control vectors and treated HT-29 cells with 0, 25 or 100 μM EGCG for 1 h and washed twice with PBS then incubated them for 5, 11 or 23 h in serum-free media (Figure 3B). Treatment with the vehicle alone slightly increased the AP-1 transcription activity, whereas EGCG (25 and 100 μM)-treated cells significantly increased it 2.4- and 4.2-fold, respectively after 24 h (Figure 2C).

**Involvement of JNK1/2 activation with pro-MMP-7 protein production**

To determine the MAPK pathway associated with EGCG-induced pro-MMP-7 production, a pharmacological approach
EGCG promotes pro-MMP-7 production in HT-29 cells

was adopted using each MAPK specific inhibitor. Pretreatment with 50 μM of SP600125 (a JNK1/2 inhibitor), but not 50 μM of PD98059 (an ERK1/2 inhibitor) or SB203580 (a p38 MAPK inhibitor), abolished EGCG-induced (25 μM) extracellular pro-MMP-7 production by 80% (Figure 4).

EGCG-generated O$_2^-$ mediates pro-MMP-7 production

It was recently reported that EGCG induces H$_2$O$_2$ generation in culture media (30); therefore, we attempted to ascertain if EGCG induces O$_2^-$ in a cell-free system using a cytochrome C reduction method. EGCG itself was found to reduce cytochrome C (data not shown), while the SOD-inhibited reduction was estimated to cause O$_2^-$ generation. As a result, incubation of EGCG (0–25 μM) at 37°C for 1 min resulted in a linear increase of O$_2^-$ generation (Figure 5A). Next, to investigate whether EGCG induces pro-MMP-7 production via reactive oxygen species generation, HT-29 cells were pretreated with the vehicle, N-acetyl-L-cysteine (NAC), SOD, or catalase for 30 min, followed by exposure to 25 μM of EGCG for 1 h, then incubation in EGCG-free media for 23 h. Treatment with each of the anti-oxidative agents markedly reduced EGCG-induced extracellular pro-MMP-7 production in the media by 63, 52 and 48%, respectively (Figure 5B). Exogenous H$_2$O$_2$ (1 mM) increased pro-MMP-7 production by 67%, while a JNK1/2 inhibitor (SP600125) at 50 μM abrogated it, as expected.

Effects of green tea catechins on pro-MMP-7 production

We also investigated the structure–activity relationships of green tea catechins regarding their ability to induce pro-MMP-7 production in HT-29 cells. The concentrations of commercially available GTP (33 and 132 μg/ml), which contained 90% polyphenols, were produced in such a way as to include 25 and 100 μM of EGCG. Treatment with (−)-epicatechin-3-gallate (ECG) at a concentration of 100 μM, but not (−)-epigallocatechin (EGC) or (−)-epicatechin (EC) (100 μM each), increased extracellular pro-MMP-7 production 2.9-fold (Figure 6A). It was also
Inhibitor  PD  SP  SB
EGCG (25 μM)  -  -  +  +  +
Extracellular pro-MMP-7  28 kDa

Fig. 4. Effects of specific MAPK inhibitors on EGCG-induced pro-MMP-7 protein production. HT-29 cells were pre-incubated with the vehicle, PD98059, SP600125 or SB203580 (50 μM each) for 30 min, then exposed to 25 μM of EGCG for 1 h. PD, PD98059 (ERK1/2 inhibitor); SP, SP600125 (JNK1/2 inhibitor); SB, SB203580 (p38 MAPK inhibitor).

noted that GTP (33 and 132 μg/ml) markedly increased that production 3.7- and 4.4-fold.

Effects of EGCG on pro-MMP-7 production in other colon cancer and normal cell lines

Finally, we attempted to determine which of the tested colon cancer and normal cell lines were susceptible to EGCG-induced pro-MMP-7 production. Marginal and spontaneous pro-MMP-7 expression, which occurs in HT-29 cells, was also observed in Caco-2, but not in the human colon cancer cell lines Colo205, Colo320DM, LS174T and LS180 or in CCD-18Co (human normal colon fibroblasts) (Figure 6B). Treatment of each of those cell lines with EGCG (25 μM) induced both intracellular and extracellular pro-MMP-7 production in Caco-2 cells as well as in HT-29 cells, whereas none of the other 5 cell lines showed a measurable response.

Discussion

The results of epidemiological studies suggest that green tea constituents show anticarcinogenic properties in several types of populations (10,11), while tea polyphenols and other catechins inhibited experimental carcinogenesis in animal models (8,9,31). However, some important insights into the molecular mechanisms of green tea catechins in the prevention of carcinogenesis have been derived from studies that utilized cell lines. For example, Fang et al. (32) reported that EGCG inhibits 5-cytosine DNA methyltransferase activity in KYSE 150 esophageal cancer cells, thereby reactivating some methylation-silenced genes and EGCG was shown to trigger apoptotic cell death in HT-29 cells by inducing caspase-3 and -9 activities (29). EGCG was also shown to abrogate the constitutive activation of phosphatidylinositol 3-kinase and Akt (protein kinase B) in human prostate cancer cells (33). These findings provide the rationale for the administration of green tea catechins to reduce the potential risk of onset and progression of certain cancers.

In contrast to the above observations, we found that EGCG (10–100 μM) increased the intracellular and extracellular production of pro-MMP-7 protein in a dose- and time-dependent manner in HT-29 human colon cancer cells. The present results may have had relevance in a previous report by Hirose et al. (34), who demonstrated that feeding of green tea catechins enhanced the development of colon carcinogenesis in a rodent model. However, Watanabe et al. (35) reported no association of green tea ingestion with colorectal cancers and, along a similar line, green tea consumption failed to show a significant association with the risk reduction of gastric cancer in a prospective cohort study in Japan (36). In contrast to these, Mizuno et al. (37) indicated an increased risk of pancreatic cancer with high green tea consumption (>5 cups/day), which was determined through a questionnaire. In addition, in vitro studies have shown that catechins increased the formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine, a footprint of free radical-damaged DNA, in HL-60 human promyelocytic leukemia cells (38), while another study demonstrated that EGCG increases the expression of cyclooxygenase-2 (39), which plays a central role in carcinogenesis by suppressing apoptosis and inducing angiogenesis in multiple organs in rodents and humans (40). In a clinical study, green tea toxicity was manifest in patients with androgen-independent prostate carcinoma (41). These results from cellular and animal experiments as well as epidemiologic surveys, in conjunction with our present data, show that the beneficial effects of EGCG and related catechins for cancer prevention are not definitive, and that their efficacy may be dependent on the laboratory experimental conditions or physiological nature of the target population.

EGCG, ECG and GTP, though not EC or EGC, notably induced pro-MMP-7 production in the present study (Figure 6A), which is consistent with a previous report that indicated the importance of the galloyl moiety in catechins for their interactions with lipid bilayers (42). This moiety is also required for the induction of apoptosis in prostate cancer cells (43) and inhibiting 5-cytosine DNA methyltransferase activity in esophageal cancer cells (32). Further, a lack of biological or biochemical activity by EC in cellular systems is in accordance with other previous findings (44,45). Furthermore, Akagawa et al. (46) have recently reported that tea polyphenols generate H2O2 and the order of H2O2 production levels by the tea catechins was found to be EGCG > ECG ≡ EGC > EC > catechin, which has partial correlation with our data (Figure 6A). While the concentrations of EGCG and other catechins in normal green tea beverages can rise as high as 300 μM (1), those in the gastrointestinal tract and their
bioconversion should be examined to state the relevance of our data on MMP-7 production in HT-29 cells to in vivo situations (Figure 2). Although green tea catechins have been shown to be highly glucuronidated when absorbed in the small intestine (47), significant amounts of EGCG were detected in the small intestine and colon, and the levels of EGCG in feces were greater than those in urine. This followed intravenous and intragastric administrations (48), implying the relevance of the present results to in vivo situations. However, because EGCG pro-MMP-7 induction was not seen in all of the colon cancer cell lines examined (Figure 6B), it is of great interest and importance to discern which cellular phenotype(s) determine susceptibility to pro-MMP-7 induction. In this context, we speculated that a slight but constitutive pro-MMP-7 expression may be the determinant (Figure 6B), which is now under investigation in detail in our laboratory.

From our results, we deduced that EGCG-induced pro-MMP-7 production is mediated by spontaneous O2− generation, subsequent H2O2 production, JNK1/2 activation, c-JUN phosphorylation (Ser63 and Ser73), c-JUN/c-FOS induction and the resultant increase in AP-1 transcription activity (Figure 7). In spite of the fact that a great number of studies have shown the anti-oxidative properties of EGCG, its inverse profiles, including spontaneous H2O2 generation (30), are also well documented (49). We concluded that the portions of EGCG responsible for O2− generation are, at least in part, the catechol and pyrogallol moieties that are readily converted into the ortho quinone, thereby liberating O2−. This mechanism has been shown for catechol estrogens that increase the activities of MMP-2 and -9 via free radical generation (50). Further, permeation of H2O2 may lead to the phosphorylation and activation of JNK1/2, as there is ample evidence showing that oxidative stress, in general, triggers it (51), whereas the identities of EGCG-derived reactive oxygen species directly responsible for the activation of JNK1/2 remain unclear. In addition, it is not conceivable that SOD attenuated EGCG-induced MMP-7 production (Figure 5B) since exogenous SOD would convert O2− into H2O2 for enhancing it.

Although EGCG at a concentration of 100 µM, but not 25 µM, also activated ERK1/2 and p38 MAPK, the involvement of those protein kinases in the pro-MMP-7 production pathway can be ruled out, based on our experimental data for the effects of MAPK inhibitors (Figure 4A). On the other hand, it is intriguing to note that EGCG induced ERK1/2 and JNK1/2, while p38 MAPK activation may be associated with the apoptotic cell death process in HT-29 cells (29). In addition, EGCG-triggered JNK1/2 and p38 MAPK activation have been proposed as pro-apoptotic events in both human leukemia U937 and OCI-AML1a cells (52). Even if EGCG-derived MAPK activation simultaneously initiates the transduction of signals for both apoptosis and pro-MMP-7 induction, it is evident that HT-29 cells release considerable amounts of pro-MMP-7 protein long before apoptotic cell death, as shown by our time-course experiments (Figure 2B), in which no detectable cytotoxicity was observed during the production of this protease (data not shown).

Our results of the EGCG-induced AP-1 activation (Figure 3C) support the recent data by Jeong et al. (53), who reported that EGCG potentiated to AP-1 transcription activity in a similar cell line. Extensive analyses of the MMP-7 promoter sequence have revealed that PEA3, TCF-4 and AP-1 are critical transcription factors and their contribution to MMP-7 expression is dependent upon the cell types and stimuli used (54,55). For example, MMP-7 mRNA expression was dominantly induced by AP-1 activation in SW1417 colon cancer cells (56). Further, Escherichia coli DNA (57), human femail water (58) and tumor necrosis factor-α (59) have each been reported to activate the transcriptional activity of AP-1 in HT-29 cells. AP-1 transcription factor, a homo- and heterodimer comprising the JUN, FOS, ATF and MAF protein superfamily, has been known to be activated by endogenous and exogenous oxidative stress (60), which supports our present data (Figures 3B and 5A). In addition, we found that treatment of HT-29 cells with EGCG led to the induction of c-JUN/c-FOS proteins and phosphorylation of c-JUN (Ser63 and Ser73), suggesting that the EGCG-formed AP-1 complex consists of, at least in part, these activated partner proteins, though additional studies are needed to elucidate the identity of the AP-1 components.
In conclusion, we showed that EGCG enhances the production of pro-MMP-7 via the generation of reactive oxygen species and activation of JNK1/2 and c-JUN/c-FOS induction, as well as AP-1 transactivation, in HT-29 cells. Further studies are required to establish the rationale for drinking green tea for cancer prevention, as well as to determine the populations that receive more benefit and less toxicity by that strategy.

Acknowledgement

This research was supported by a Grants-in-Aid from the The Ministry of Agriculture, Forestry and Fisheries of Japan.

Conflict of Interest Statement: None declared.

References

EGCG promotes pro-MMP-7 production in HT-29 cells


Received September 16, 2004; revised February 2, 2005; accepted April 16, 2005