EGCG Blocks Tumor Promoter-induced MMP-9 Expression *Via* Suppression of MAPK and AP-1 Activation in Human Gastric AGS Cells

HONG S. KIM, MI H. KIM, MIN JEONG, YOUNG S. HWANG, SUN H. LIM,
BOO A. SHIN, BONG W. AHN and YOUNG D. JUNG

*Chonnam University Research Institute of Medical Sciences,
Chonnam National University Medical School, Kwangju, Korea*

Abstract. *Overexpression of matrix metalloproteinases (MMPs) has been known to correlate closely with tumor cell invasion and strategies to down-regulate their expression may ultimately be of clinical utility. In this study, we investigated the effects of (-)-epigallocatechin gallate (EGCG), a major green tea catechin, on the cell invasiveness and MMP-9 induction in human gastric cancer AGS cells. EGCG inhibited the phorbol 12-myristate 13-acetate (PMA)-induced cell invasiveness and MMP-9 expression in a dose-dependent manner. EGCG treatment was found to reduce the MMP-9 transcriptional activity. To further study the mechanisms for the EGCG-mediated regulation of MMP-9, the effects of EGCG on transcription factor AP-1 and mitogen-activated protein kinase (MAPK) activities were examined. The results showed that EGCG suppressed the PMA-induced AP-1 activation. EGCG also abrogated the PMA-induced activation of extracelluar-regulated protein kinase (Erk) and c-jun N-terminal kinase (JNK), which are upstream modulators of AP-1. These results suggest that EGCG may exert at least part of its anti-invasive effect in gastric cancer by controlling MMP expression through the suppression of MAPK and AP-1 activation.*

Although the incidence of gastric cancer has decreased over the last decades, it is still the most frequent digestive tract cancer with a poor prognosis and a high mortality. About 80% of the gastric cancers in Western countries are of an advanced stage at presentation (1). Because of local invasion and metastasis, radiation therapy or chemotherapy do not significantly affect the length or quality of life of

Correspondence to: Young Do Jung, M.D., Ph.D., Department of Biochemistry, Chonnam National University Medical School, 5 Hakdong , Kwangju, Korea 501-190. Tel: 82-62-220-4105, Fax: 82-62-223-8321, e-mail: ydjung@chonnam.ac.kr

patients with advanced gastric cancer. The development of effective anti-invasive strategies for gastric cancer would be helpful in improving treatment.

Epidemiological studies have shown that the consumption of green tea decreases the risk of developing human cancers (2). The anti-carcinogenic and anti-proliferative effects of green tea have been attributed to the biological activities of its polyphenol components. Green tea extract contains (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epicatechin (EC) (3). EGCG, the most abundant polyphenol in green tea, has been shown to inhibit proliferation and induce apoptosis of tumor cells (4). In addition to having a cancer chemopreventive activity, polyphenols have been shown to inhibit tumor invasion, which is a crucial step for the metastasis of all solid tumors.

Cancer invasion and metastasis are multistep processes and require the coordinated action of cell-secreted proteolytic enzymes and their inhibitors. Matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes which are involved in the degradation of different components of the extracellular matrix and there is considerable evidence indicating that individual MMPs have important roles in tumor invasion and spread (5). MMPs are divided into four subgroups according to their substrate specificity and structural homology, and each subgroup can degrade at least one component of the extracellular matrix (5). MMP-9 (gelatinase B, also known as 92 kDa type IV collagenase) has been suggested to play an important role in the cell migration occurring in tumor cell invasion by facilitating the destruction of the type IV collagen-containing basement membrane which separates the epithelial and stromal compartments (6). In gastric cancer, it was recently reported that MMP-9 is related to the initial step of cancer cell invasion and that the plasma level of MMP-9 in patients correlates with the tumors' metastatic potential.

In this study, we examined the effects of EGCG on tumor promoter-induced cell invasiveness and MMP-9 expression in gastric cancer AGS cells, and their underlying mechanisms are discussed.

Materials and Methods

Cell culture and reagents. Human gastric carcinoma AGS cells were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured at 37˚C in a 5% CO_2 atmosphere in RPMI1640 supplemented with 10% FBS and 1% penicillin-streptomycin. EGCG, EC, ECG, PMA and curcumin were obtained from Sigma Chemical Co (St. Louis, MO, USA) and PD98059 and SB203580 were from Calbiochem (San Diego, CA, USA). The rabbit polyclonal anti-phosphospecific p44/42 MAPK (Erk-1/2), JNK and P38 MAPK antibodies were purchased from New England Biolabs Inc. (Beverly, MA, USA).

Matrigel invasion assay. Cell invasion assay was performed using BIOCOAT matrigel invasion chambers (Becton-Dickinson, Bedford, MA, USA) according to the manufacturer's protocol. Briefly, 10^5 cells in 300 μl were added to each chamber and allowed to invade matrigel for 24 h. The noninvading cells on the upper surface of the membrane were removed from the chamber and the invading cells on the lower surface of the membrane were stained with Quick-Diff stain kit (Becton-Dickinson). After two washes with water, the chambers were allowed to air dry. The number of invading cells was counted using a phase-contrast microscope. To determine the effect of EGCG on PMA-induced AGS cell invasion, cells were pretreated with 0-50 μM EGCG for 1 h and and then stimulated with 200 nM PMA for 24 h.

Quantitation of MMP-9. The amounts of MMP-9 in conditioned media from AGS cells were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's instruction.

Zymography. This was performed as previously described (7). In brief, culture supernatants were electrophoresed on a 7.5% polyacrylamide gel containing 0.1% (w/v) gelatin. The gel was incubated at room temperature for 2 h in the presence of 2.5% Triton X-100 and subsequently at 37˚C overnight in a buffer containing 10 mM $CaCl_2$, 0.15 M NaCl and 50 mM Tris-HCl (pH 7.5). The gel was then stained for protein with 0.25% Coomassie Blue and photographed on a light box. Proteolysis was detected as a white zone in a dark field.

Chloramphenicol acetyl transferase (CAT) assay. The transcriptional regulation of MMP-9 was studied using transient transfection with an MMP-9 promoter-CAT reporter construct (8). AGS cells (5 x 10^5) were seeded on a 6-well plate and grown to 60-70% confluence and 1 μg of an expression vector bearing the MMP-9 promoter-CAT reporter construct was transfected into cells using FuGENE[6] (Boehringer Mannheim, Indianapolis, IN, USA) according to the manufacturer's protocol. The CAT activity was subsequently measured by incubating cell lysate at 37˚C for 8 h with 4 μM [^{14}C]chloramphenicol and 1 mg/ml acetyl coenzyme A. After 4-h incubation, the acetyl coenzyme A was replenished. The acetylated products were extracted with ethyl acetate and subjected to thin layer chromatography using chloroform/methanol (95:5) as the mobile phase.

Nuclear extraction and electrophoretic mobility shift assay (EMSA). Nuclear extraction was performed as previously described (9). EMSA was performed with the Gel Shift Assay system (Promega, Madison, WI, USA). Briefly, oligonucleotide with the consensus sequence for AP-1 (spanning nucleotides -91 to -60 relative to the transcriptional start site) (Promega) was end-labeled with [γ-^{32}P]adenosine triphosphate (3 mCi/mmol, Amersham Pharmacia Biotech, Buckinghamshire, UK) using T4 polynucleotide kinase. The labeled oligonucleotide was then purified in Microspin G-25 columns (Sigma) and used as a probe for EMSA. Nuclear extract proteins (6 μg) were pre-incubated with the binding buffer (10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.5 mM EDTA, 1 mM $MgCl_2$, 0.5 mM dithiothreitol, 4% (v/v) glycerol and 0.05 mg/ml poly (deoxyinosine-deoxycytidine)) for 5 min and then incubated with the labeled probe for 15 min at 37˚C. The samples were electrophoresed in a 5% nondenaturing polyacrylamide gel in 0.5 x Tris-borate-EDTA buffer (pH 7.4) at 150 V for 4 h. The gel was dried and subjected to autoradiography. In competition studies, a 50-fold excess of unlabeled oligonucleotide was included in the reaction mixture along with the radiolabeled probe.

Transient transfection of AP-1 reporter. The AP-1 reporter construct was purchased from Clonetech (Palo Alto, CA, USA). At 70-80% confluency, cells were washed with RPMI 1640 and incubated with RPMI 1640 without serum and antibiotics for 5 h. The cells were then transfected with 1μg AP-1 reporter containing pGL3 vector using FuGENE[6] (Boehringer Mannheim) for 24 h. To examine the effect of EGCG on PMA-induced AP-1, the cells were pretreated with 0-50 μM EGCG for 1 h and incubated with 200 nM PMA for 24 h. After incubation, cells were lysed and luciferase activity was measured using a luminometer.

AP-1 decoy oligodeoxynucleotides (ODNs). The phosphorothioate double-stranded ODNs with the sequences against the AP-1 binding site (5'-CAG CTC AGA AGT CAC TTC-3', 3'-GAA GTG ACT TCT GAG CTG-5') of the MMP-9 gene were prepared (Genotech Corp., Yusung, Korea) and annealed (AP-1 decoy). AGS cells (5x10^5) were incubated with 0-8 μg AP-1 decoy in the presence of Lipofectamine Reagent (Gibco BRL, Grand Island, NY, USA) for 18 h. The cells were then stimulated with 200 nM PMA for 24 h and the MMP-9 activity in culture supernatants was determined by zymographic analysis.

Western blot hybridization. Protein extraction and Western blot analysis were performed as previously described (9). The active forms of Erk-1/2, JNK and P38 MAPK were detected using polyclonal anti-phosphospecific antibodies (all at a 1:1000 dilution). Protein bands were visualized using a commercially available chemiluminescence kit (Amersham Corp.).

Results

Effect of EGCG on PMA-induced MMP-9 expression. To examine whether EGCG could inhibit the PMA-induced MMP-9 expression in gastric cancer AGS cells, cells were treated with 0-50 μM EGCG for 1 h prior to PMA
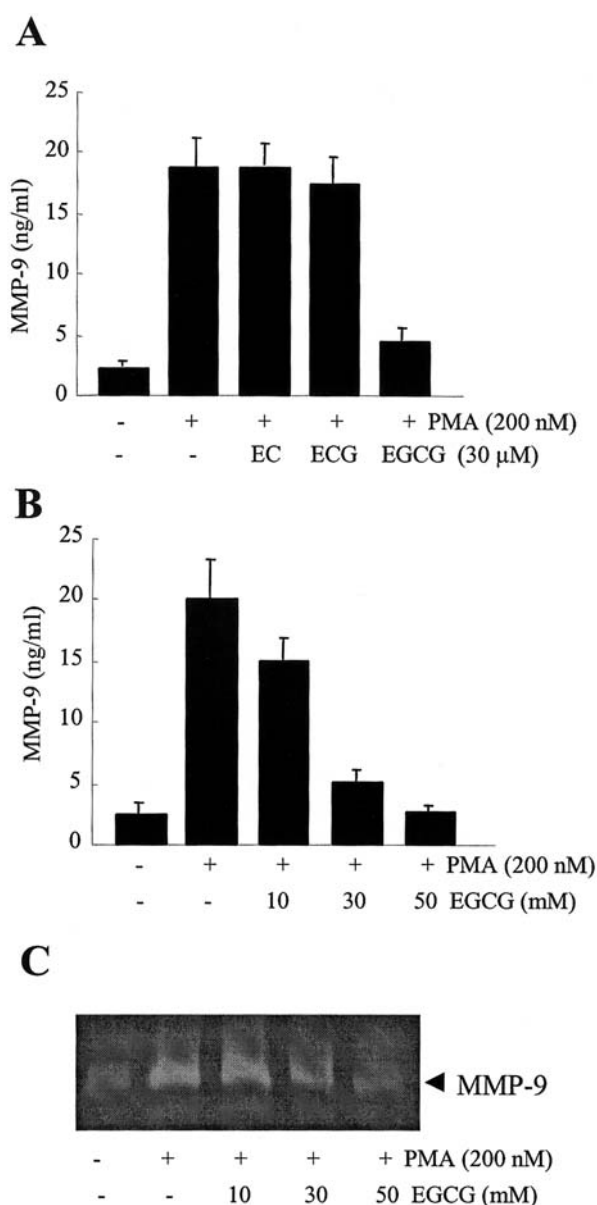
A

B

C

Figure 1. *Effects of EGCG on PMA-induced MMP-9 expression in AGS cells. A. AGS cells, after being pretreated with 30 μM EC, ECG and EGCG for 1 h, were exposed to 200 nM PMA for 24 h and the levels of MMP-9 were determined by ELISA in culture supernatants. B. AGS cells, after being pretreated with 0-50 μM EGCG for 1 h, were exposed to 200 nM PMA for 24 h and the MMP-9 levels were determined by ELISA in culture supernatants. C. AGS cells, after being pretreated with 0-50 μM EGCG for 1 h, were exposed to 200 nM PMA for 24 h and zymographic analysis was performed for the MMP-9 activity in culture supernatants.*

Figure 2. *Effect of EGCG on PMA-induced AGS cell invasiveness. AGS cells were plated on the modified Boyden chambers. The cells were then pretreated with 0-50 μM EGCG for 1 h and exposed to 200 nM PMA for 24 h. The invading cells on the undersurface of the chambers were counted using a phase contrast light microscope after staining with Diff-Quick Stain Kit. Data represent the means±SD from triplicate measurements.*

treatment and the levels of MMP-9 in culture supernatants were determined by ELISA. EGCG at 30 μM strongly inhibited the MMP-9 induction. However, other te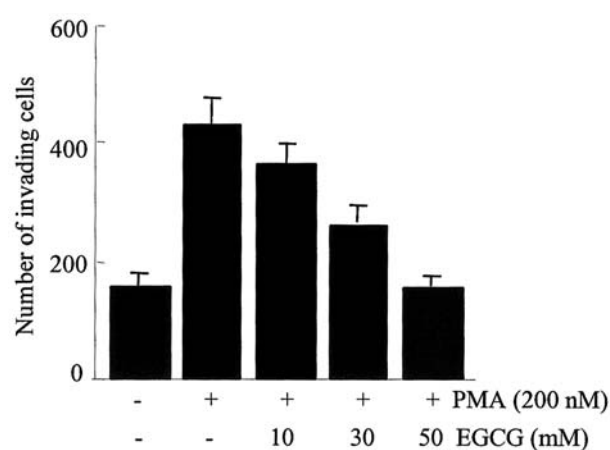a catechins such as EC and ECG had no significant effect on the MMP-9 induction at the same concentration (Figure 1A). EGCG inhibited the MMP-9 induction in a dose-dependent manner (Figure 1B). The inhibitory effects of EGCG on MMP-9 induction could also be confirmed by zymographic analysis (Figure 1C). EGCG at the concentrations used did not affect cell viability.

Effect of EGCG on PMA-induced gastric cancer cell invasiveness. As shown in Figure 2, PMA treatment increased the AGS cell invasiveness up to about 300%. The PMA-induced cell invasiveness was strongly inhibited by EGCG pretreatment. However, other tea catechins such as EC and ECG did not show any significant effect on the PMA-induced AGS cell invasiveness. (data not shown).

Effect of EGCG on PMA-induced MMP-9 transcriptional activity. Next, we investigated whether the EGCG treatment inhibited the MMP-9 transcriptional activity induced by PMA. To this end, AGS cells were transiently transfected with MMP-9 promoter-reporter (CAT) construct and the promoter-mediated CAT activities were determined. As shown in Figure 3, cells pretreated with 30-50 μM EGCG lost most of the PMA-induced MMP-9 transcriptional activity.

Role of AP-1 in EGCG-mediated MMP-9 regulation. The above results indicated that EGCG could inhibit the PMA-induced MMP-9 expression at the transcriptional level. Several tentative transcription factors have been suggested to control the MMP-9 expression (8) and AP-1 has been
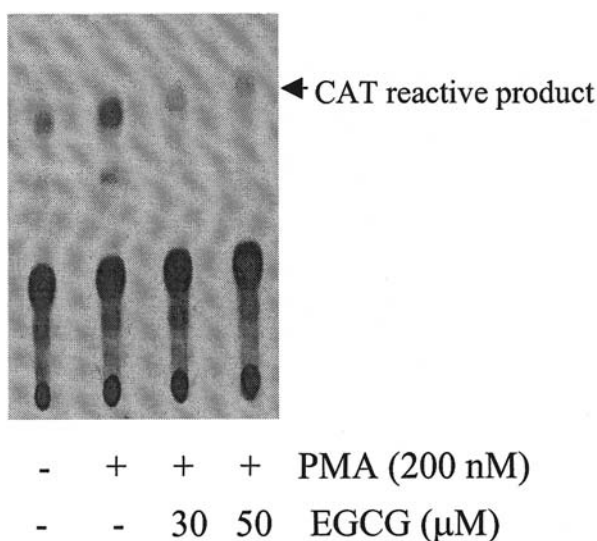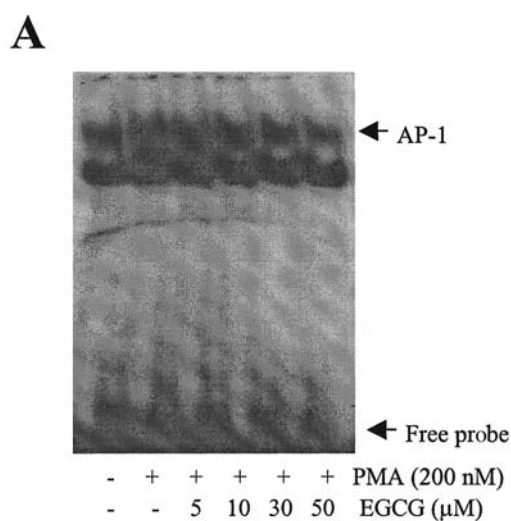
◄ CAT reactive product

| - | + | + | + | PMA (200 nM) |
| - | - | 30 | 50 | EGCG (µM) |

Figure 3. *Effect of EGCG on PMA-induced MMP-9 promoter activity. AGS cells were transiently transfected with MMP-9 promoter-CAT reporter construct. The transfected AGS cells, after being pretreated with 0-50 µM EGCG for 1 h, were exposed to 200 nM PMA for 8 h and the MMP-9 promoter activity was determined by thin layer chromatography.*

reported as an essential transcription factor involved in MMP-9 induction by PMA. Accordingly, we performed EMSA to examine if the EGCG treatment caused a decrease in the quantity of active AP-1 in the nucleus. The results showed that the amount of AP-1 which could form a complex with the radiolabeled oligonucleotide probe increased in the cells treated with PMA, as compared with the non-treated control cells (Figure 4A). Further, the PMA-induced increase in the amount of active AP-1 was strongly inhibited by pretreatment of cells with EGCG (Figure 4A). The AP-1-probe complex formation could be totally inhibited by a 50-fold excess of unlabeled oligonucleotide (data not shown). Furthermore, EGCG treatment caused a decrease in the AP-1-dependent transcriptional activity, as revealed by the transient transfection study using pAP-1-Luc reporter construct (Figure 4B). To confirm that AP-1 plays an important role in the MMP-9 expression in AGS cells, the cells were transiently transfected with AP-1 decoy oligonucleotides and change in the MMP-9 activity was examined. As shown in Figure 5, the MMP-9 activity in culture supernatants was significantly decreased by AP-1 decoy transfection. The above results suggest that the transcription factor AP-1 is a decisive factor in PMA-induced MMP-9 expression and that AP-1 may be a molecular target in EGCG-mediated MMP-9 regulation.

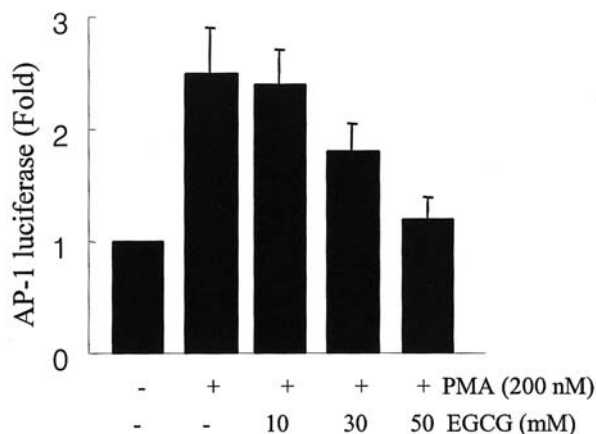**A**

◄ AP-1

◄ Free probe

| - | + | + | + | + | + | PMA (200 nM) |
| - | - | 5 | 10 | 30 | 50 | EGCG (µM) |

B

| - | + | + | + | + | PMA (200 nM) |
| - | - | 10 | 30 | 50 | EGCG (mM) |

Figure 4. *Effect of EGCG on PMA-induced AP-1 activation. A. AGS cells, after being pretreated with 0-50 µM EGCG for 1 h, were incubated with 200 nM PMA for 8 h. After incubation, nuclear extracts from the cells were analyzed by EMSA for active AP-1 using a radiolabeled oligonucleotide probe. B. AGS cells were transiently transfected with pAP-1-Luc reporter construct. The transfected AGS cells, after being pretreated with 0-50 µM EGCG for 1 h, were exposed to 200 nM PMA for 8 h and the luciferase activities were determined using a luminometer. Data represent the means±SD from triplicate measurements.*

Role of MAPKs in EGCG-mediated MMP-9 regulation. It has been reported that Erk and JNK subsets of MAPK play an important role in various gene expressions since these kinases regulate the synthesis and/or activity of transcription factors which bind to the AP-1 motif (10). Thus, we
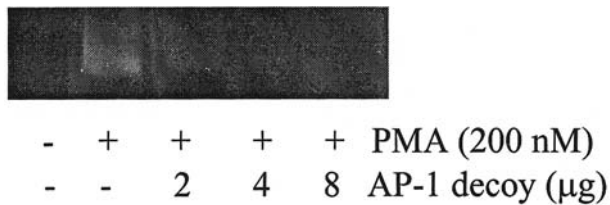
Figure 5. *Effect of AP-1 decoy on PMA-induced MMP-9 expression. AGS cells (5x10^6) were transiently transfected with 0-8 μg AP-1 decoy oligonucleotide in the presence of Lipofectiamine reagents for 18 h and exposed to 200 nM PMA for 24 h. The MMP-9 activities in culture supernatants were then assayed by zymographic analysis.*

Figure 6. *Effect of EGCG on PMA-induced Erk and JNK activation. A. AGS cells were incubated with 200 nM PMA for the indicated time and the levels of phosphorylated Erk and JNK were determined in cell lysates by Western blot analysis. B. AGS cells, after being pretreated with 0-50 μM EGCG for 30 min, were incubated with 200 nM PMA for 15 min and Western blot analysis was performed for the phosphorylated Erk and JNK.*

considered the possibility that the inhibitory effect of EGCG on PMA-dependent AP-1 activation might result from inhibition of Erk and JNK. To clarify this possibility, changes in Erk- and JNK-phosphorylation in AGS cells treated with EGCG were examined by Western blot analysis. As shown in Figure 6A, Erk- and JNK - phosphorylation in AGS cells was increased by PMA treatment. The phosphorylation was increased to a peak at 5-30 min after PMA treatment. However, the PMA-induced Erk-and JNK-phosphorylation was inhibited by EGCG in a dose-dependent manner (Figure 6B). The P38 MAPK activity was not significantly altered by PMA and EGCG treatments (data not shown). To further study the mechanism by which MAPKs regulate the PMA-induced MMP-9 expression, the effects of Erk, JNK and P38 MAPK inhibitors on the MMP-9 induction were examined. As shown in Figure 7, PD98059 (50 μM, a specific inhibitor of MEK-1, an upstream effector of Erk-1/2) and curcumin (10 μM, a JNK inhibitor) significantly suppressed the MMP-9 induction, whereas SB203580 (25 μM, a specific P38 MAPK inhibitor) showed only a negligible effect. These results suggest that inhibition of AP-1 activation by EGCG may be caused by inhibition of Erk- and JNK-phosphorylation.

Discussion

Localized gastric cancer can be cured with surgery. However, the high mortality rate in advanced gastric cancer is associated with a propensity for the tumor to metastasize. The process of metastasis requires the ability of cancer cells to invade the endothelial cells and basal membranes in a blood vessel. MMPs are thought to play an essential role in facilitating cancer metastasis. Among MMPs, MMP-9 (type IV collagenase) is important in cancer cell invasion and metastasis because tumor cells must cross the type IV collagen-rich basement membrane of the vessel wall to spread to remote sites from the primary tumor (11).
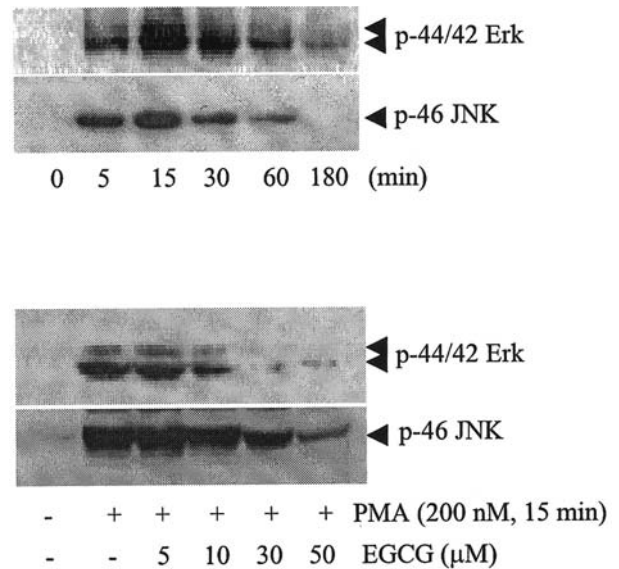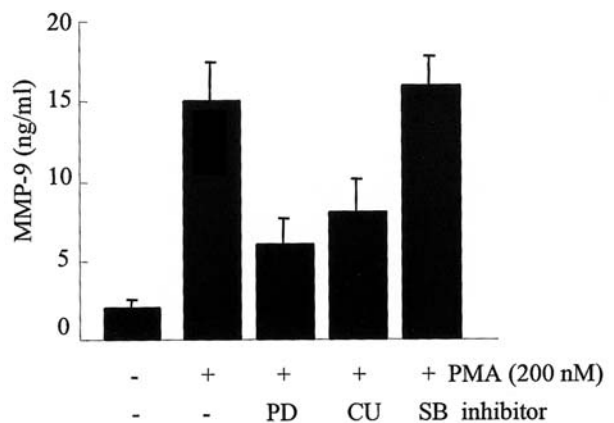

Figure 7. *Effect of MAPK inhibitors on PMA-induced MMP-9 expression. AGS cells, after being pretreated with 50 μM PD98059 (PD), 10 μM curcumin (CU) and 20 μM SB203580 (SB), were exposed to 200 nM PMA for 24 h. The MMP-9 levels were then determined by ELISA in culture supernatants. Data represent the means±SD from triplicate measurements.*

Recently, EGCG, a major green tea flavanol, has been shown to inhibit tumor invasion and angiogenesis. Interest in green tea as a cancer chemopreventive agent in humans has intensified for several reasons. First, epidemiological

evidence suggests that people who consume a large amount of green tea have a lower risk of various cancers (12). Second, green tea has been shown in animal models to protect against the development and progression of skin, lung, mammary gland and gastrointestinal tract tumors (13). Third, green tea extracts have been shown *in vitro* to stimulate apoptosis of various cancer cell lines, including prostate, lymphoma, colon and lung (14). Finally, green tea consumption is associated with few adverse events and it is readily available at low cost (15).

In this study, we showed that EGCG could suppress the PMA-induced MMP-9 expression in human gastric cancer AGS cells. This suppression of MMP-9 expression occurred at the transcriptional level as revealed by a transient transfection study using the MMP-9 promoter-reporter construct. A portion of the 5'-flanking region of the MMP-9 gene has been cloned. The promoter of the MMP-9 gene contains AP-1(-533, -79), NF-Î B (-600), PEA3 (-540) and Sp1 (-558) sites for stimuli that can induce MMP-9 expression (8). Our results showed that the level of active AP-1 induced by PMA was decreased by EGCG treatment, indicating that inhibition of AP-1 activation is crucial in the suppression of MMP-9 expression by EGCG in human gastric AGS cells. A similar finding was made by Dong *et al.* (16), who observed that EGCG inhibited EGF- or tumor promoter-AP-1-dependent transcription activity and cell transformation in JB6 mouse epidermal cell. They suggested that the inhibition of AP-1 activation by EGCG occurs through the inhibition of the JNK- but not Erk-dependent pathway.

It has been reported that MAPKs play an important role in the expression of various genes since these kinases regulate the transcription factors which bind to the AP-1 motif (17). In mammalian cells, three subgroups of MAPK have been detected: the Erk, JNK and P38 MAPK (18). The active AP-1 complex may comprise a homodimer of c-Jun or heterodimers between c-Fos, c-Jun and ATF2 (19). c-Jun is activated by N-terminal phosphorylation of specific serine residues (ser63/73) and it appears to be exclusively activated by JNK (20). Further, c-Fos activation can be regulated by JNK and Erk signal pathways (19). Gum *et al.* (10) suggested that regulation of MMP-9 expression in UM-SCC-1 cells is regulated by JNK- and Erk-dependent signaling pathways. According to Reddy *et al.* (21), when SKBR-3 cells were exposed to MAPK inhibitors, MMP-9 induction and cell invasion through the reconstituted basement membrane were significantly reduced. Simon *et al.* (22) demonstrated that phorbol ester-enhanced MMP-9 secretion and *in vitro* cell invasiveness were associated with activation of P38 MAPK.

In this study, the PMA-mediated phosphorylation of Erk and JNK were strongly inhibited by EGCG. Earlier studies have suggested that MAPK is one of the target molecules in the signaling cascades regulated by EGCG. We have found

that EGCG treatment suppresses the phosphorylation of Erk, resulting in down-regulation of VEGF in human colon HT 29 cells (23). Katiyar *et al.* (24) demonstrated that pretreatment of human epidermal keratinocytes with EGCG inhibited the ultraviolet-B-induced hydrogen peroxide production and hydrogen peroxide-mediated phosphorylation of the MAPK signaling pathway. Dong *et al.* (16) reported that EGCG inhibited JNK but not Erk activation in JB6 mouse epidermal cells.

The exact mechanism by which EGCG inhibits the activation of MAPK is not known. EGCG is a strong metal chelator. Since some receptor kinases depend on divalent cations for their activity, EGCG may inhibit the activity of receptor kinases by chelating the divalent cations (25). Recently, it was proposed that EGCG may directly impair the activity of MMPs (26). Because EGCG possesses the ability to form complexes with various substances including proteins and metal ions, the inhibition of MMP activities by EGCG may be due to the ability to chelate zinc ion which is essential for MMP activities. However, this hypothesis was refuted by the study in which the addition of excess zinc to EGCG failed to restore full MMP activity and, in fact, augmented the MMP inhibition by EGCG (27).

Understanding the molecular mechanisms by which EGCG inhibits the MAPK and AP-1 activation and MMP-9 expression will serve as a basis for designing even more effective anti-invasion drugs. Further studies are needed to clarify these mechanisms.

Acknowledgements

References

1 Ganesh S, Sier CF, Heerding MM, van Krieken JH, Griffioen G, Welvaart K, van de Velde CJ, Verheijen JH, Lamers CB and Verspaget HW: Prognostic value of the plasminogen activation system in patients with gastric carcinoma. Cancer *77*: 1035-43, 1996.

2 Ji BT, Chow WH, Hsing AW, McLaughlin JK, Dai Q, Gao YT, Blot WJ and Fraumeni JF Jr: Green tea consumption and the risk of pancreatic and colorectal cancers. Int J Cancer *70*: 255-8, 1997.

3 Stoner GD and Mukhtar H: Polyphenols as cancer chemopreventive agents. J Cell Biochem *22*: 169-180, 1995.

4 Paschka AG, Butler R and Young CY: Induction of apoptosis in prostate cancer cell lines by the green tea component, (-)-epigallocatechin-3-gallate. Cancer Lett *130*: 1-7, 1998.

5 Parsons SL, Watson SA, Brown PD, Collins HM and Steele RJ: Matrix metalloproteinases. Br J Surg *84*: 160-6, 1997.

6   McMillan JI, Weeks R, West JW, Bursten S, Rice GC and Lovett DH: Pharmacological inhibition of gelatinase B induction and tumor cell invasion. Int J Cancer *67*: 523-31, 1996.

7   Choi EY, Kim D, Hong BK, Kwon HM, Song YG, Byun KH, Park HY, Whang KC and Kim HS: Upregulation of extracellular matrix metalloproteinase inducer (EMMPRIN) and gelatinases in human atherosclerosis infected with *Chlamydia pneumoniae*: The potential role of *Chlamydia pneumoniae* infection in the progression of atherosclerosis. Exp Mol Med *34*: 391-400, 2002.

8   Sato H and Seiki M: Regulatory mechanism of 92 kDa type IV collagenase gene expression which is associated with invasiveness of tumor cells. Oncogene *8*: 395-405, 1993.

9   Jung YD, Fan F, McConkey DJ, Jean ME, Liu W, Reinmuth N, Stoeltzing O, Ahmad SA, Parikh AA, Mukaida N and Ellis LM: Role of P38 MAPK, AP-1, and NF-kappaB in interleukin-1beta-induced IL-8 expression in human vascular smooth muscle cells. Cytokine *18*: 206-13, 2002.

10 Gum R, Wang H, Lengyel E, Juarez J and Boyd D: Regulation of 92 kDa type IV collagenase expression by the jun aminoterminal kinase- and the extracellular signal-regulated kinase-dependent signaling cascades. Oncogene *14*: 1481-93, 1997.

11 Rolli M, Fransvea E, Pilch J, Saven A and Felding-Habermann B: Activated integrin alphavbeta3 cooperates with metalloproteinase MMP-9 in regulating migration of metastatic breast cancer cells. Proc Natl Acad Sci *100*: 9482-7, 2003.

12 Kono S, Ikeda M, Tokudome S and Kuratsune M: A case-control study of gastric cancer and diet in northern Kyushu, Japan. Jpn J Cancer Res *179*: 1067-74, 1998.

13 Rogers AE, Hafer LJ, Iskander YS and Yang S: Black tea and mammary gland carcinogenesis by 7,12-dimethylbenz[a]anthracene in rats fed control or high fat diets. Carcinogenesis *19*: 1269-73, 1998.

14 Yoo HG, Shin BA, Park JC, Kim HS, Kim WJ, Chay KO, Ahn BW, Park RK, Ellis LM and Jung YD: Induction of apoptosis by the green tea flavonol (-)-epigallocatechin-3-gallate in human endothelial ECV 304 cells. Anticancer Res *22*: 3373-8, 2002.

15 Fujiki H, Suganuma M, Okabe S, Sueoka N, Komori A, Sueoka E, Kozu T, Tada Y, Suga K, Imai K and Nakachi K: Cancer inhibition by green tea. Mutat Res *402*: 307-10, 1998.

16 Dong Z, Ma W, Huang C and Yang CS: Inhibition of tumor promoter-induced activator protein 1 activation and cell transformation by tea polyphenols, (-)-epigallocatechin gallate, and theaflavins. Cancer Res *57*: 4414-9, 1997.

17 Gum R, Lengyel E, Juarez J, Chen JH, Sato H, Seiki M and Boyd D: Stimulation of 92-kDa gelatinase B promoter activity by ras is mitogen-activated protein kinase kinase 1-independent and requires multiple transcription factor binding sites including closely spaced PEA3/ets and AP-1 sequences. J Biol Chem *271*: 10672-80, 1996.

18 Cobb MH and Goldsmith EJ: How MAP kinases are regulated. J Biol Chem *270*: 14843-6, 1995.

19 Ramirez-Carrozzi V and Kerppola T: Asymmetric recognition of nonconsensus AP-1 sites by Fos-Jun and Jun-Jun influences transcriptional cooperativity with NFAT1. Mol Cell Biol *23*: 1737-49, 2003.

20 Wulf GM, Ryo A, Wulf GG, Lee SW, Niu T, Petkova V and Lu KP: Pin1 is overexpressed in breast cancer and cooperates with Ras signaling in increasing the transcriptional activity of c-Jun towards cyclin D1. EMBO J *20*: 3459-72, 2001.

21 Reddy KB, Krueger JS, Kondapaka SB and Diglio CA: Mitogen-activated protein kinase (MAPK) regulates the expression of progelatinase B (MMP-9) in breast epithelial cells. Int J Cancer *82*: 268-73, 1999.

22 Simon C, Simon M, Vucelic G, Hicks MJ, Plinkert PK, Koitschev A and Zenner HP: The p38 SAPK pathway regulates the expression of the MMP-9 collagenase *via* AP-1-dependent promoter activation. Exp Cell Res *271*: 344-55, 2001.

23 Jung YD, Kim MS, Shin BA, Chay KO, Ahn BW, Liu W, Bucana CD, Gallick GE and Ellis LM: EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells. Br J Cancer *84*: 844-50, 2001.

24 Katiyar SK, Afaq F, Azizuddin K and Mukhtar H: Inhibition of UVB-induced oxidative stress-mediated phosphorylation of mitogen-activated protein kinase signaling pathways in cultured human epidermal keratinocytes by green tea polyphenol (-)-epigallocatechin-3-gallate. Toxicol Appl Pharmacol *176*: 110-7, 2001.

25 Yang CS and Wang ZY: Tea and cancer. J Natl Cancer Inst *85*: 1038-49, 1993.

26 Maeda-Yamamoto M, Kawahara H, Tahara N, Tsuji K, Hara Y and Isemura M: Effects of tea polyphenols on the invasion and matrix metalloproteinases activities of human fibrosarcoma HT1080 cells. J Agric Food Chem *47*: 2350-4, 1999.

27 Garbisa S, Sartor L, Biggin S, Salvato B, Benelli R and Albini A: Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. Cancer *91*: 822-32, 2001.