Effects of Tea Polyphenols on the Invasion and Matrix Metalloproteinases Activities of Human Fibrosarcoma HT1080 Cells

Mari Maeda-Yamamoto,*,† Hiroharu Kawahara,† Nobufumi Tahara,† Kenkou Tsuji,† Yukihiko Hara,‡ and Mamoru Isemura§

National Research Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries, 2769 Kanaya, Shizuoka 428-8501, Japan; Food Research Laboratories, Mitsui Norin Company, Ltd., Miyahara 223-1, Fujieda, Shizuoka 426-0133, Japan; and School of Food and Nutritional Sciences, University of Shizuoka, Yada, Shizuoka 422-8526, Japan

The effects of tea polyphenols on the invasion of highly metastatic human fibrosarcoma HT1080 cells through a monolayer of human umbilical vein endothelial cells (HUVECs) and the accompanying basal membrane were investigated. Among the tea polyphenols tested, epicatechin gallate (ECg), epigallocatechin gallate (EGCg), and theaflavin strongly suppressed the invasion of HT1080 cells into the monolayer of HUVECs/gelatin membrane, whereas epicatechin, epigallocatechin, tea flavonols, tea flavones, and gallate derivatives had no effect. Both theaflavin-digallate and theasinensin D showed a weak invasion inhibitory effect. ECg significantly inhibited the invasion without cytotoxicity against cancer cells and HUVECs. Ester-type catechins (ECg and EGCg) and theaflavin strongly suppressed the gelatin degradation mediated by matrix metalloproteinase (MMP) 2 and MMP-9, which were secreted into the conditioned medium of HT1080 cells. In conclusion, among the tea polyphenols tested, ECg was considered to be the agent with the most potential antimetastasis activity because it inhibited invasion in the absence of cytotoxicity.

Keywords: *Invasion; tea polyphenols; epicatechin gallate; human fibrosarcoma HT1080; matrix metalloproteinases*

INTRODUCTION

Cancer metastasis, the spread of cancer cells from the primary neoplasm to distant sites and their growth there, is the major cause of death for cancer patients. An important part of cancer metastasis is the ability of cancer cells to adhere to and invade the endothelial cells and basal membranes in a blood vessel (Nicolson, 1988). Matrix metalloproteinases (MMPs), which are a family of zinc-dependent proteinases, are thought to play an essential role in the facilitation of cancer metastasis (Stetler-Stevenson et al., 1993; Sato et al., 1994). Among MMPs, it has been suggested that gelatinases/type IV collagenases (MMP-2 and -9), which are secreted by invasive cancer cells, are 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 sites distant from the primary tumor (Tryggvason et al., 1987). Therefore, inhibition of the adhesion or invasion mediated by MMP-2 or -9 may be a key feature for the prevention of cancer metastasis.

It has recently been reported that tea (*Camellia sinensis*) has various physiological modulative activities, such as carcinogenesis inhibitory effect (Wang et al., 1992), free radical scavenging activity (Bors et al., 1987), antioxidative activity (Okuda et al., 1983), antihypertensive action (Yokozawa et al., 1994), antihypercholesterolemic action (Chisaka et al., 1988), antidental caries action (Sakanaka et al., 1990), antibacterial action (Fukai et al., 1991), and intestinal flora amelioration action (Okuba et al., 1992). Furthermore, it has been found that green tea inhibits the adhesion of mouse carcinoma cells to the endothelial cell layer (Isemura et al., 1993) or the invasion into the basal membranes (Sazuka et al., 1995). The major effectors were shown to be catechins (Kada et al., 1985), which are a group of polyphenolic compounds. Tea contains many kinds of polyphenols, such as catechins, theaflavins, flavonols, and flavones. Therefore, we examined the effects of tea polyphenols by a developed in vitro invasion assay and using human cell lines. This paper describes the inhibitory effects of ester-type catechins and theaflavins on the invasion of highly metastatic human fibrosarcoma HT1080 cells through a monolayer of human umbilical vein endothelial HUV-EC-C cells and MMP-2 and -9 activities of human fibrosarcoma HT1080 cells.

EXPERIMENTAL PROCEDURES

Tea Polyphenols. Theasinensin D (TS) was kindly provided by Dr. M. Sano and Dr. T. Miyase of the University of Shizuoka, Japan. (−)-Epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECg), (−)-epigallocatechin gallate (EGCg), theaflavin (TF1), and theaflavin 3,3′-digallate (TF3) were purified >98% by Mitsui Norin Co., Ltd. Other polyphenols used were purchased from Extrasynthese (Genay, France). These samples were prepared for use by dissolving them in either phosphate-buffered saline (PBS) or ethanol. The structures of tea polyphenols used are shown in Figure 1.

Reagents. ERDF medium and MCDB107 medium were purchased from Kyokuto Seiyaku (Tokyo, Japan). Lipopolysac-

* Author to whom correspondence should be addressed (e-mail marimy@tea.affrc.go.jp; telephone +81-547-45-4830; fax +81-547-46-2169).

† National Research Institute of Vegetables, Ornamental Plants and Tea.

‡ Mitsui Norin Co. Ltd.

§ University of Shizuoka.

10.1021/jf9811525 CCC: $18.00 © 1999 American Chemical Society
Published on Web 05/18/1999

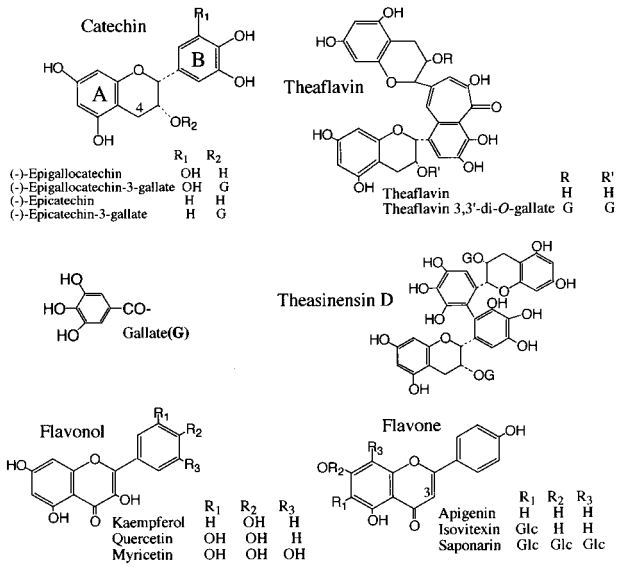

Figure 1. Chemical structures of tea polyphenols.

charide and Triton X-100 were purchased from Sigma Chemical Co. (St. Louis, MO). Gelatin and heparin sodium salt were purchased from Wako Pure Chemical Co. (Osaka, Japan). Fetal bovine serum (FBS), PKH-2, 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate (WST-1) cell counting kit and endothelial cell growth supplement were obtained from Gibco BRL (Grand Island, NY), Zynaxis Inc. (Malvern, PA), Dojindo (Kumamoto, Japan), and Calbiochem (San Diego, CA), respectively.

Cells and Cell Culture. HT1080 (IFO50354) cells and HUV-EC-C (IFO57271) were obtained from the Institute for Fermentation, Osaka Research Communications (Osaka, Japan). HT1080 cells were routinely cultured in ERDF medium supplemented with 5% heat-inactivated FBS, and HUV-EC-C cells were maintained in MCDB107 medium supplemented with 10% heat-inactivated FBS and 1% human umbilical vein endothelial cell (HUVEC) growth factor (0.5 mg of endothelial cell growth supplement and 1 mg of heparin sodium salt/mL of PBS). Both cell lines were incubated at 37 °C under humidified 5% CO_2/95% air. When we tested for the invasion of cancer cells into HUV-EC-C, both cell lines were cultured in MCDB107 medium supplemented with 5% FBS and HUVEC growth factor.

Invasion Assay. We used an in vitro assay developed for cancer invasion as previously described (Albini et al., 1987; Ohigashi et al., 1989). One hundred microliters of HUV-EC-C (1×10^6 cells/mL) was inoculated into the upper portion of a cell culture insert membrane (8 μm pore size, for 24-well, Falcon 3097) precoated with 0.5% gelatin/PBS solution, while 600 μL of medium was added to the lower chamber. HUV-EC-C cells were precultured for 48 h and then activated by adding lipopolysaccharide (1 μg/well) for 2 h. To efficiently determine the cell number, HT1080 cells were pretreated with PKH-2 reagent, which can be measured quickly and easily using a fluorophotometer. After a washing with PBS, the treated HT1080 cells and tea polyphenols were added to the HUV-EC-C monolayer in the upper chamber. After incubation for 5 h, the surface of each lower chamber was treated with trypsin solution. Trypsinized invasive HT1080 cells were centrifuged, and the pellet was suspended in 250 μL of 1% Triton X-100 solution. After sonication for 5 min, the fluorescence intensity of the cellular extract was measured with a fluorophotometer (excitation at 490 nm and emission at 504 nm). Cell viability was measured using the WST-1 assay according to the method described by Ishiyama et al. (1993, 1996). The results are shown as mean ± SE in triplicated experiments. The significance of the difference between two means was assessed on the basis of a Duncan's multiple-range test.

Gelatinase/Type IV Collagenases (MMP-2 and -9) Activities. The gelatinase activity was examined by gelatin zymography using the protocol described previously (Liu et al., 1995; Kubota et al., 1991). Briefly, supernatant samples of HT1080 cells cultured in FBS-free medium were loaded with a nonreducing sample buffer onto a 10% sodium dodecyl sulfate (SDS)−polyacrylamide gel containing 1 mg/mL gelatin and electrophoresed. After removal of SDS from the gels by washing with 2.5% Triton X-100 solution for 30 min twice and 10 mM Tris-HCl buffer (pH 8.0) for 30 min with shaking, the gels were incubated in 50 mM Tris-HCl (pH 8.0) containing 0.5 mM $CaCl_2$ and 0.1 μM $ZnCl_2$ at 37 °C for 18 h to activate MMPs. The activated gels were stained with 1% Coomassie Blue R-250 in 10% methanol and 5% acetate with shaking and subsequently destained with 10% methanol and 5% acetate solution. The gelatinolytic activity of each gelatinase was detected as a clear band against a blue background.

RESULTS

Effects of Tea Polyphenols on Invasion of HT1080 Cells. To screen for the preventive effectors for cancer metastasis, the inhibitory effects of tea catechins, theaflavins, theasinensin, flavonols, and flavones on invasion of HT1080 cells through a HUV-EC-C/gelatin membrane were examined using the in vitro invasion assay. Figure 2 shows dose-dependent effects of ECg, EGCg, and TF1 on invasion and on HT1080 cell viability. Invasion of human fibrosarcoma HT1080 cell was inhibited by ECg (A) and EGCg (B), both ester-type catechins containing a galloyl moiety, and TF1 (C) in a dose-dependent manner. Because the culture medium was not alkaline, it was surmised that catechins remained almost undegraded (Yoshino et al., 1999). IC_{50} values for ECg and EGCg were ~80 μg/mL, and for TF1 the IC_{50} was ~30 μg/mL. IC_{50} values for ECg (A) and both EGCg (B) and TF1 (C) against cell viability were approximately 1 and 100 mg/mL, respectively. Among these polyphenols, ECg significantly inhibited HT1080 invasion without cytotoxicity against HT1080 cells at 20 μg/mL. TF3 and TS showed a weak invasion inhibitory effect, but EC, EGC, flavonols, and flavones had no effects (data not shown).

Effects of Ester-Type Catechins and TF1 on the Secretion of MMPs and Gelatinolytic Activity of HT1080 Cells. It has been found that fibrosarcoma HT1080 cells secrete gelatinases/type IV collagenase (MMP-2 and -9) (Kubota et al., 1991) and that these enzymes play a major role in the facilitation of cancer metastasis (Salo et al., 1983; Nakajima et al., 1990). MMP-2 and -9 are a family of zinc-dependent proteolytic enzymes known to degrade type IV collagen in the basal membrane (Matrisian, 1990), and their activities are regulated at various levels, such as secretion and activation of proMMP to enzymatically active MMP. It was reported that a mixture of hexa-, hepta-, octa-, nona-, and decagalloylglucose strongly inhibited HT1080 invasion through the extracellular matrix and inhibited the activity of gelatinase (Ata et al., 1996). Tea catechins with a galloyl moiety (ECg and EGCg) have been shown to inhibit both eukaryotic and prokaryotic cell-derived collagenases (Makimura et al., 1993). EGCg, TF1, and TF3 have been reported to restrict type IV collagenase activity in a concentration-dependent manner (Sazuka et al., 1997). To clarify whether catechins and TF1 affect secretion of MMPs or gelatinolytic activity of HT1080 cells, we performed gelatin zymography. Subconfluent monolayers of HT1080 cells were cultured for 24 h in serum-free ERDF medium in the presence of various concentrations of catechins or TF1. The conditioned

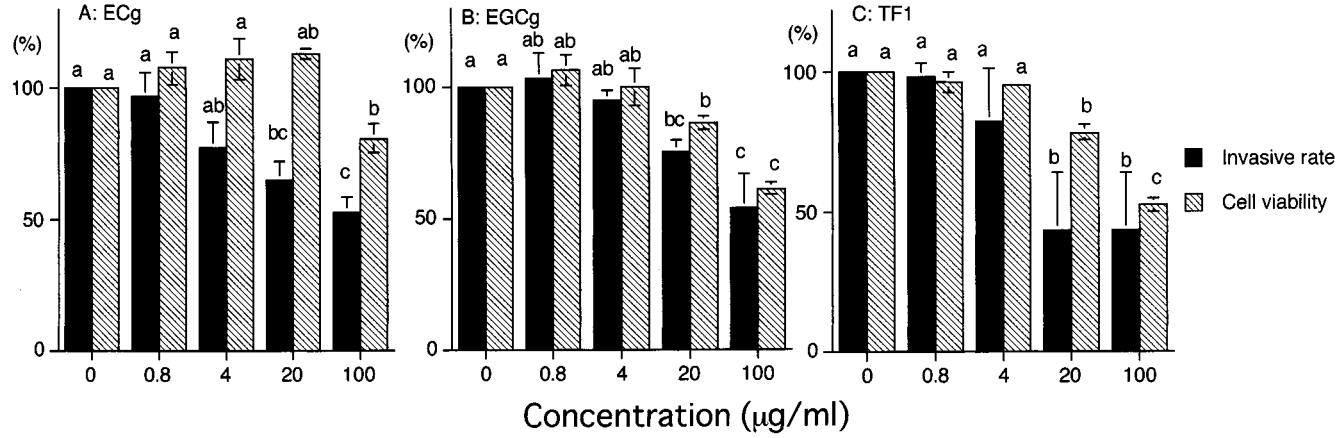

Figure 2. Effects of tea catechins containing a galloyl moiety and theaflavin on invasion of HT1080 through the HUV-EC-C/gelatin membrane and on HT1080 cell viability. An invasive rate of 100% is defined by control experiments of invasive HT1080 cells tested in the absence of catechins. Cell number of HT1080 cells cultured without catechins or TF1 was set as 100% of cell viability. The results are indicated as a mean ± SE in triplicated experiments. Values not sharing a common superscript letter are significantly different between the groups ($p < 0.05$) by a Duncan's multiple range test.

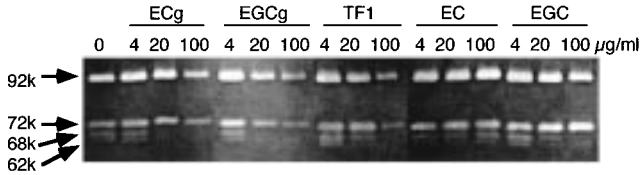

Figure 3. Zymogram showing the effects of catechins and TF1 on MMPs production by HT1080 cells. Subconfluent monolayers of HT1080 cells were cultured for 24 h in serum-free ERDF medium in the presence of various concentrations of catechins and TF1. The conditioned media were analyzed by gelatin zymography. Clear zones of degradation at 92 kDa (MMP-9), 72 kDa, 68 kDa, and 62 kDa (MMP-2) are seen.

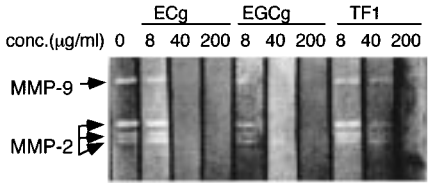

Figure 4. Zymogram showing the effects of ester-type catechins and TF1 on the gelatinolytic activities of MMP-2 and -9. Subconfluent monolayers of HT1080 cells were cultured for 24 h in serum-free ERDF media. Samples of the conditioned media were subjected to electrophoresis in gelatin-containing SDS−polyacrylamide gel. After SDS−PAGE, the gel strips were incubated in the activation buffer in the presence or absence of catechins or TF1 for 18 h.

media were analyzed by gelatin zymography. As shown in Figure 3, ECg, EGCg, and TF1 reduced the secretion of the 72 kDa proMMP-2 and the 62 kDa active-type MMP-2 proteins (gelatinase A) and the 92 kDa MMP-9 protein (gelatinase B) in a dose-dependent manner. Neither EC nor EGC affected the secretion of both MMP-9 and MMP-2. In addition, subconfluent monolayers of HT1080 cells were cultured for 24 h in serum-free ERDF media. The samples of the conditioned media were applied to electrophoresis in gelatin containing SDS−polyacrylamide gel. After SDS−polyacrylamide gel electrophoresis (SDS−PAGE), the gel strips were incubated in an activation buffer in the presence or absence of catechins and TF1 for 18 h. As shown in Figure 4, ECg, EGCg, and TF1 strongly inhibited the gelatinolysis mediated by both MMPs in a dose-dependent manner. Both galloyl catechins almost completely suppressed MMPs activities over 20 μg/mL.

DISCUSSION

The highly metastatic human fibrosarcoma cell line HT1080, secreting several MMPs, is one of the most popular cell lines to screen for the inhibitory factor of invasiveness (Nagase et al., 1998; Cha et al., 1998; Ikeda et al., 1998; Kawada et al., 1995). The in vitro invasion assay has been established first by using this cell line (Albini et al., 1987).

In this paper, we demonstrated that ECg and EGCg, both ester-type catechins with a galloyl moiety, and TF1 could inhibit the invasive ability of HT1080 cells, although EC and EGC, both free catechins, had no effect. IC_{50} of ECg for invasiveness was shown to be ~80 μg/mL, and the IC_{50} for HT1080 cell viability was ~1 mg/mL. At 20 μg/mL, only ECg significantly inhibited cancer cell invasion without affecting cell viability. Plasma concentration of catechins administered orally has not been established well. It was reported that human plasma catechin concentration reached 2 μM after oral ingestion of 400 mg of catechins (Pietta et al., 1998). In the case of 3-*O*-methyl-(+)-catechin, a plasma level of 11−18 μg/mL was observed after a single 2 g oral dose (Hackett et al., 1985). At present it is not known whether the effective concentrations of ECg and EGCg are achievable in serum by drinking tea. Dietary supplements may help for such a purpose. The inhibitory effect of EGCg on invasion was obscured, because EGCg suppressed both invasion and cell viability. These results suggest that the cancer invasive inhibitory effect of ECg is not caused by cytotoxicity and ECg may be useful as an effector for the prevention of cancer metastasis. These findings demonstrate that tea catechins containing a galloyl moiety possess the ability to inhibit cancer invasion. Accordingly, we examined three kinds of gallate derivatives (gallic acid, methyl gallate, and lauryl gallate) by the invasion assay. As far as we investigated, the gallate derivatives had no effects (data not shown). In addition, other tea polyphenols, such as flavonols (kaempferol, quercetin, and myricetin) and flavones (apigenin, isovitexin and saponarin), did not affect the invasive ability of HT1080 cells at all (data not shown). These results suggest that both flavanol skeleton and galloyl moiety are necessary for the inhibitory action.

Ester-type catechins (ECg and EGCg) and TF1 have been demonstrated here to strongly suppress the deg-

radation of gelatin mediated by MMP-2 and -9. ECg, EGCg, and TF1 inhibited also the secretion of MMPs by HT1080, but the effective concentration range did not parallel the inhibitory effects on HT1080 cell invasion. Sazuka et al. (1997) reported that EGCg, TF1, and TF3 inhibited MMP-9 activity from mouse lung carcinoma LL2 cells and matrigel invasion in a concentration-dependent manner. These findings revealed that ester-type catechin and TF1 inhibited MMP activity of both human and mouse tumor cells. Also, Nagase et al. (1998) reported that delphinidine, one of the plant polyphenols, inhibited MMP secretion from HT-1080 cells. Because polyphenols are known to possess the ability to form complexes with various substances including proteins and metal ions, the inhibition of MMP-2 and -9 activities by ester-type catechins and TF1 may be partly related to the ability to chelate zinc metal, which is essential for enzymatic activity. The present results suggest that ester-type catechins with a galloyl moiety and TF1 inhibit the invasion of cancer cells by inhibiting the activation of MMP-2 and -9 enzymes. In conclusion, among the tea polyphenols tested, ECg was considered to be the agent with the most potential antimetastasis activity because it inhibited invasion in the absence of cytotoxicity.

ABBREVIATIONS USED

HUVEC, human umbilical vein endothelial cell; EC, (−)-epicatechin; EGC, (−)-epigallocatechin; ECg, (−)-epicatechin gallate; EGCg, epigallocatechin gallate; TF1, theaflavin; TF3; theaflavin-digallate; PBS, phosphate-buffered saline; FBS, fetal bovine serum; MMP(s), matrix metalloproteinase(s); IC_{50}, 50% inhibitory concentration; SDS, sodium dodecyl sulfate.

ACKNOWLEDGMENT

We thank M.S. Yuichi Yamaguchi and Dr. Kazuhiro Osada for technical advice and Mr. Takashi Aoyama, Ms. Makiko Suzuki, and Ms. Nahomi Matsuda for skillful technical assistance.

LITERATURE CITED

Albini, A.; Iwamoto, Y.; Kleinman, H. K.; Martin, G. R.; Aaronson, S. A.; Kozlowski, J. M.; McEwan, R. N. A rapid in vitro assay for quantitating the invasive potential of tumor cells. *Cancer Res.* **1987**, *47*, 3239−3245.

Ata, N.; Oku, T.; Hattori, M.; Fujii, H.; Nakajima, M.; Saiki, I. Inhibition by galloylglucose (GG6-10) of tumor invasion through extracellular matrix and gelatinase-mediated degradation or type IV collagenase by metastatic tumor cells. *Oncol. Res.* **1996**, *8*, 503−511.

Bors, W.; Saran, M. Radical scavenging by flavonoid antioxidants. *Free Radical Res. Commun.* **1987**, *2*, 289−294.

Cha, H. J.; Park, M. T.; Chung, H. Y.; Kim, N. D.; Sato, H.; Seiki, M.; Kim, K. M. Ursolic acid-induced down-regulation of MMP-9 gene is mediated through the nuclear translocation of glucocorticoid receptor in HT1080 human fibrosarcoma cells. *Oncogene* **1998**, *16*, 771−778.

Chisaka, T.; Matsuda, H.; Kubomura, Y.; Mochizuki, M.; Yamahara, J.; Fujimura, H. The effects of crude drugs on experimental hypercholesteremia: Mode of action of (−)-epigallocatechin gallate in tea leaves. *Chem. Pharm. Bull.* **1988**, *36*, 227−233.

Fukai, K.; Ishigami, T.; Hara, Y. Antibacterial activity of tea polyphenols against phytopathogenic bacteria. *Agric. Biol. Chem.* **1991**, *55*, 1895−1897.

Hackett, A. M.; Griffiths, L. A. The quantitative disposition of 3-*O*-methyl-(+)-[U-^{14}C]catechin in man following oral administration. *Xenobiotics* **1985**, *15*, 907−914.

Ikeda, T.; Murakami, K.; Hayakawa, Y.; Fujii, H.; Ohkoshi, M.; Saiki, I. Anti-invasive activity of synthetic serine protease inhibitors and its combines effect with a matrix metalloproteinase inhibitor. *Anticancer Res.* **1998**, *18*, 4259−4265.

Isemura, M.; Suzuki, Y.; Satoh, K.; Narumi, K.; Motomiya, M. Effects of catechins on the mouse lung carcinoma cell adhesion to the endothelial cells. *Cell Biol. Int.* **1993**, *17*, 559−564.

Ishiyama, M.; Shiga, M.; Sasamoto, K.; Mizoguchi, M.; He, P. G. A new sulfonated tetrazolium salt that produces a highly water-soluble formazan dye. *Chem. Pharm. Bull.* **1993**, *41*, 1118−1122.

Ishiyama, M.; Tominaga, H.; Shiga, M.; Sasamoto, K.; Ohkura, Y.; Ueno, K. A combined assay of cell viability and in vitro cytotoxicity with a highly water-soluble tetrazolium salt, neutral red and crystal violet. *Biol. Pharm. Bull.* **1996**, *19*, 1518−1520.

Kada, T.; Kaneko, K.; Matsuzaki, S.; Hara, Y. Detection and chemical identification of natural bio-antimutagens; A case of the green tea factor. *Mutat. Res.* **1985**, *150*, 127−132.

Kawada, M.; Umezawa, K. Suppression of in vitro invasion of human fibrosarcoma cells by a leupeptin analogue inhibiting the urokinase-plasmin system. *Biochem. Biophys. Res. Commun.* **1995**, *209*, 25−30.

Kubota, S.; Fridman, R.; Yamada, Y. Transforming growth factor-beta suppresses the invasiveness of human fibrosarcoma cells in vitro by increasing expression of tissue inhibitor of metalloproteinase. *Biochem. Biophys. Res. Commun.* **1991**, *176*, 129−136.

Liu, X.-H.; Rose, D. P. Suppression of type IV collagenase in MDA-MB-435 human breast cancer cells by eicosapentaenoic acid in vitro and in vivo. *Cancer Lett.* **1995**, *92*, 21−26.

Makimura, M.; Hirasawa, M.; Kobayashi, K.; Indo, J.; Sakanaka, S.; Taguchi, T.; Otake, S. Inhibitory effect of tea catechins on collagenase activity. *J. Periodontol.* **1993**, *64*, 630−636.

Matrisian, L. A. M. Metalloproteinases and their inhibitors in matrix remodeling. *Trends Genet.* **1990**, *6*, 121−125.

Nagase, H.; Sasaki, K.; Kito, H.; Haga, A.; Sato, T. Inhibitory effect of delphinidin from *Solamun melongena* on human fibrosarcoma HT-1080 invasiveness in vitro. *Planta Med.* **1998**, *64*, 216−219.

Nakajima, M.; Morikawa, K.; Fabra, A.; Bucana, C.-D.; Fidler, I.-J. Influence of organ environment on extracellular matrix degradative activity and metastasis of human colon carcinoma cells. *J. Natl. Cancer Inst.* **1990**, *82*, 1890−1898.

Nicolson, G. L. Organ specificity of tumor metastasis: role of preferential adhesion, invasion and growth of malignant cells at specific sites. *Cancer Metastasis Rev.* **1988**, *7*, 143−188.

Ohigashi, H.; Shinkai, K.; Mukai, M.; Ishikawa, O.; Iwanaga, T.; Akedo, H. In vitro invasion of endothelial cell monolayer by rat ascites hepatoma cells. *Jpn. J. Cancer Res.* **1989**, *80*, 818−821.

Okubo, T.; Ishihara, N.; Okura, A.; Serit, M.; Kim, M.; Yamamoto, T.; Mitsuoka, T. In vivo effects of tea polyphenol intake on human intestinal microflora and metabolism. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 558−591.

Okuda, T.; Kimura, Y.; Yoshida, T.; Hatano T.; Okuda, H.; Arichi, S. Studies on the activities of tannins and related compounds from medicinal plants and drugs. I. Inhibitory effects on lipid peroxidation in mitochondria and microsomes of liver. *Chem. Pharm. Bull.* **1983**, *32*, 1625−1631.

Piatta, P. G.; Simonetti, P.; Garnada, C.; Brusamolino, A.; Morazzoni, P.; Bombardelli, E. Catechin metabolites after intake of green tea infusions. *Biofactors* **1998**, *8*, 111−118.

Sakanaka, S.; Sato, T.; Kim, M.; Taniguchi, M.; Yamamoto, T. Inhibitory effects of green tea polyphenols on glucan synthesis and cellular adherence of cariogenic streptococci. *Agric. Biol. Chem.* **1990**, *54*, 2925−2929.

Salo, T.; Liotta, L.-A.; Tryggvason, K. Purification and characterization of a murine basement membrane collagen-degrading enzyme secreted by metastatic tumor cells. *J. Biol. Chem.* **1983**, *258*, 3058−3063.

Sato, H.; Takino, T.; Okada, Y.; Cao, J.; Shinagawa, A.; Yamamoto, E.; Seiki M. A matrix metalloproteinase expressed on the surface of invasive tumour cells. *Nature* **1994**, *370*, 61−65.

Sazuka, M.; Murakami, S.; Isemura, M.; Satoh, K.; Nukiwa, T. Inhibitory effects of green tea infusion on in vitro invasion and in vivo metastasis of mouse lung carcinoma cells. *Cancer Lett.* **1995**, *98*, 27−31.

Sazuka, M.; Imazawa, H.; Shoji, Y.; Mita, T.; Hara, Y.; Isemura, M. Inhibition of collagenases from mouse lung carcinoma cells by green tea catechins and black tea theaflavins. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1504−1506.

Stetler-Stevenson, W. G.; Aznavoorian, S.; Liotta, L. A. Tumor cell interactions with the extracellular the extracellular matrix during invasion and metastasis. *Annu. Rev. Cell Biol.* **1993**, *9*, 54−573.

Tryggvason, K.; Hoyhtya, M.; Salo, T. Proteolytic degradation of extracellular matrix in tumor invasion. *Biochim. Biophys. Acta* **1987**, *907*, 191−217.

Wang, Z.-N.; Hong, J.-Y.; Huang, M.-T.; Reuhl, K. R.; Conney, A. H.; Yang, C. S. Inhibition of *N*-nitrosodiethylamine- and 4-(methyl-nitrosoamino)-1-(3-pyridyl)-1-butanone-induced tumorigensis in A/J mice by green tea. *Cancer Res.* **1992**, *52*, 1943−1947.

Yokozawa, T.; Okura, H.; Sakanaka, S.; Ishigaki, S.; Kim, M. Depressor effect of tannin in green tea on rats with renal hypertension. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 855−858.

Yoshino, K.; Suzuki, M.; Sasaki, K.; Miyase, T.; Sano, M. Formation of antioxidants from (−)-epigallocatechin gallate in mild alkaline fluids, such as authentic intestinal juice and mouse plasma. *J. Nutr. Biochem.* **1999**, *10*, in press.

Received for review October 19, 1998. Revised manuscript received March 29, 1999. Accepted March 31, 1999. This work was supported in part by a grant-in-aid (Bio Renaissance Program) from the Japanese Ministry of Agriculture, Forestry and Fisheries (BRP 98-VII-B-1).

JF9811525