Inhibition of Collagenases from Mouse Lung Carcinoma Cells by Green Tea Catechins and Black Tea Theaflavins

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Theaflavin and theaflavin digallate, which are components of black tea were examined by in vitro invasion assay with mouse Lewis lung carcinoma L1.2-Lu3 cells, which are highly metastatic. The compounds inhibited invasion by the tumor cells. Gelatin zymography showed that the cells secreted matrix metalloproteinases (MMPs), probably including MMP-2 and MMP-9, which may be involved in tumor cell invasion and metastasis. Theaflavin and theaflavin digallate also inhibited MMPs from the culture medium of these tumor cells, as did (−)-epigallocatechin gallate. These results suggest that theaflavin, theaflavin digallate, and (−)-epigallocatechin gallate inhibit tumor cell invasion by inhibiting type IV collagenases of the L1.2-Lu3 cells.

Key words: (−)-epigallocatechin gallate; theaflavin; theaflavin digallate; matrix metalloproteinase; invasion

Orally administered green tea and (−)-epigallocatechin gallate (EGCG), a major constituent of green tea, have antitumor effects. Green tea inhibits in vivo metastasis and in vitro invasion of mouse Lewis lung carcinoma L1.2-Lu3 cells, which are highly metastatic. This inhibition may be related to the inhibition of matrix metalloproteinases (MMPs) from these cells because this family of matrix-degrading zinc-enzymes seem to be important in tumor cell invasion and metastasis.

Theaflavins are constituents of black tea and are derived from catechins by fermentation (Fig. 1). Theaflavins may have effect on tumor cells similar to that of catechins. Here, we examined the effect of theaflavins on invasion by L1.2-Lu3 cells.

Materials and Methods

Materials. Catechins were obtained from Funakoshi Co., Ltd., Tokyo. EGCG, (+)-catechin, theaflavin, and theaflavin digallate each were coupled to CNBr-activated Sepharose 4B (Pharmacia Biotech Inc., Tokyo) at the concentration of 5 mg/ml wet gel as described previously. Theaflavin and theaflavin digallate were prepared as described earlier.

Gelatin (Sigma Chemical Co., St. Louis, MO) was coupled to CNBr-activated Sepharose 4B at the concentration of 10 mg/ml wet gel. Matrigel was purchased from Collaborative Biochemical Products, Bedford, MA. Chemotaxicell chambers with polycarbonate filters (pore size, 8 μm) were obtained from Kurabo Co., Ltd., Osaka.

Cells. Highly metastatic L1.2 cells (L1.2-Lu3 cells) were obtained as described before and maintained in a serum-free culture medium, Cosmedium-001 (Cosmo Bio Co., Ltd., Tokyo).

In vitro Matrigel invasion assay. This assay was done as reported previously. In brief, the upper surface of the chemotaxis cell chamber filter was coated with 10 μg of Matrigel in a volume of 100 μl of Dulbecco’s modified Eagle medium (DMEM) and dried. Filters were washed three times with 0.1% bovine serum albumin in DMEM just before use. L1.2-Lu3 cells were suspended at 4 × 10^6 cells/ml in DMEM with 0.2% bovine serum albumin. Chemotaxis cell chambers were hung on a 24-well microplate. Cell suspensions (100 μl) and 100 μl of DMEM or a test solution were put into upper compartments of the chemotaxis cell chambers and the cell culture medium (500 μl) was put into wells of the microplate. After incubation at 37°C in a CO2 incubator for 6–8 h, the L1.2-Lu3 cells on the upper surface of the filters were wiped away with a cotton swab. Filters were fixed with methanol and stained with hematoxylin and eosin. The numbers of L1.2-Lu3 cells that had penetrated to the lower surface of the

Fig. 1. Structures of EGCG (1), Theaflavin (2), and Theaflavin Digallate (3).
Inhibition of Collagenases by Catechins and Theaflavins

When serum-free conditioned medium of cultured LL2-Lu3 cells was studied by affinity chromatography on gels containing gelatin showed strong gelatinolytic activities of substances with molecular weights of approximately 66,000 and 92,000 together with other positions (Fig. 3). Estimation of molecular mass suggested the presence of 66-kDa gelatinase A (matrix metalloproteinase-2; MMP-2) and 92-kDa gelatinase B (MMP-9).7)

Effects of catechins and theaflavins on type IV collagenase from LL2-Lu3 cells

EGCg, theaflavin, and theaflavin digallate inhibited type IV collagenase activity in a concentration-dependent way (Fig. 4). (+)-Catechin and (−)-epicatechin at concentrations up to 100 μM did not inhibit type IV collagenases.

Fig. 3. Zymography of NMPs from LL2-Lu3 Cells.

Standard proteins (lane 1): rabbit muscle myosin (205,000), E. coli β-galactosidase (116,000), rabbit muscle phosphofructokinase (97,000), bovine serum albumin (66,000), chicken ovalbumin (45,000), and bovine carbonic anhydrase (29,000) from top to bottom. Lane 2, conditioned culture medium of LL2-Lu3 cells. Lane 3, gelatin-agarose bound fraction obtained by elution with 1 M NaCl. Lane 4, EGCg-agarose bound fraction obtained by elution with 1 M NaCl. Lane 5, theaflavin-agarose bound fraction obtained by elution with 1 M NaCl. Lane 6, theaflavin digallate-agarose bound fraction obtained by elution with 1 M NaCl. Bands with collagenase activity are still unstained after staining with Coomassie brilliant blue R-250.

Fig. 4. Effects of Theaflavins and EGCg on Type IV Collagenases from LL2-Lu3 Cells.

The activity of type IV collagenases with no theaflavins present was taken as 100%. The results are expressed as the mean plus or minus SD for triplicate experiments. ○, theaflavin; [], theaflavin digallate; □, EGCg. Results for EGCg are cited from a previous paper.41
Interaction between type IV collagenases and EGCg, (+)-catechin, theaflavin, or theafavin digallate

When serum-free conditioned medium of cultured LL2-Lu3 cells was studied by affinity chromatography with EGCg-agarose, zymography showed that collagenases were bound to and eluted from the column (Fig. 3). The collagenases seemed to include MMP-2 and MMP-9. When similar experiments were done with theaflavin-agarose and theafavin digallate-agarose, bands of MMPs were also detected from the bound fraction (Fig. 3). When plain (unsubstituted) Sepharose 4B was used, MMP bands were not detected from the bound fraction. MMP bands were not detected in the fraction bound by (+)-catechin-agarose, either.

Discussion

Here, we found that invasion by lung carcinoma LL2-Lu3 cells was inhibited by theaflavin and theafavin digallate. Two catechins that contain gallate, (+)-epicatechin gallate and EGCg, inhibit such invasion, but (+)-catechin and (-)-epicatechin do not; we have proposed that the mechanism of inhibition includes the inhibition of collagenase by these catechins.5)

LL2-Lu3 cells produced MMPs, probably including MMP-2 and MMP-9. That the type IV collagenase activity of these matrix metalloproteinases was inhibited by EGCg, theaflavin, and theafavin digallate, but was not inhibited by (+)-catechin which did not inhibit invasion by cells either, suggests that invasion of such invasion and inhibition of these collagenases are related. In other words, EGCg, theaflavin, and theafavin digallate inhibited the Matrigel invasion of LL2-Lu3 cells by inhibiting their matrix metalloproteinases.

Inhibition of cell invasion and collagenase activities by theaflavin, which lacks a gallate group, is interesting. Perhaps EGCg and theaflavin have similar steric configurations of phenolic hydroxyl groups.

Affinity chromatography showed that LL2-Lu3 cell collagenases, presumably including MMP-2 and MMP-9, were bound to EGCg, theaflavin, and theafavin digallate, although the MMP-2-like collagenase seems to have less affinity than MMP-9 (Fig. 3). This finding, together with the finding that the collagenases were not bound by (+)-catechin-agarose, suggests that the mechanism of the inhibition of collagenases by EGCg and these theaflavins involves direct binding between them.

Wang et al. found that orally administered black tea could inhibit the formation of ultraviolet-light-induced skin tumors, and decreased tumor size in SKH-1 mice treated with 7,12-dimethylbenz[a]anthracene.5) Their results may be explained, at least in part, by our finding that theaflavins, components of black tea, inhibit collagenases thought to be involved in tumor cell invasion and endothelial growth.6) It is possible that oral administration of black tea might also prevent cancer metastasis.

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References