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Binding interaction between (−)-epigallocatechin gallate causes impaired spreading of cancer cells on fibrinogen

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ABSTRACT

Green tea and tea catechins, especially (−)-epigallocatechin gallate (EGCG), have been shown to have various health benefits including anti-cancer, anti-metastasis, and anti-cardiovascular disease effects. Our previous studies demonstrated that three plasma proteins, fibronectin, histidine-rich glycoprotein, and fibrinogen were bound by EGCG, and that one specific domain in fibronectin was responsible for its binding interaction with EGCG. Fibrinogen consists of 6 chains linked by the disulfide bonds of two each of the α-, β-, and γ-chains. The present study examined whether fibrinogen had a specific domain interacting with EGCG. The results of affinity chromatography under reducing conditions demonstrated that each of the α-, β-, and γ-subunit chains of fibrinogen was bound by EGCG. We also demonstrated that several peptides generated by treatment with cyanogen bromide or thermolysin were bound by EGCG. The amino acid sequences analyzed revealed that these peptides included those derived from the α-, β-, and γ-chains of fibrinogen. EGCG inhibited the spreading of mouse metastatic LL2-Lu3 lung cancer cells on the fibrinogen substratum, which suggested an impairment in the interaction between cancer cells and fibrinogen. Since the interaction between cancer cells and fibrinogen plays an important role in metastasis, the present results suggest, at least partially, that EGCG inhibited metastasis in the mouse models reported previously by inhibiting such an interaction.

Catechins are a group of polyphenolic compounds that occur naturally in certain species of plants and are found in green tea infusions. Our previous studies demonstrated that three plasma proteins, fibronectin, histidine-rich glycoprotein, and fibrinogen (18) were bound by (−)-epigallocatechin gallate (EGCG), and that one specific domain in fibronectin was responsible for its binding interaction with EGCG (17).

Fibrinogen is a high molecular weight glycoprotein found in plasma and consists of 6 chains linked by the disulfide bonds of two each of the α-, β-, and γ-chains (15). It has been shown to have various biological roles including blood coagulation and metastasis (4, 14). In previous studies, peroral administration of an infusion of green tea and a catechin fraction mainly composed of EGCG was shown to prevent the metastasis of mouse Lewis lung carcinoma cells and melanoma B16F10 cells (19, 23). Therefore, the binding interaction of EGCG with fibrinogen may contribute to its inhibition of metastasis.

In the present study, we examined the interaction between EGCG and fibrinogen in more detail and also the effect of EGCG on the interaction between fibrinogen and tumor cells.

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MATERIALS AND METHODS

Chemicals. EGCG was obtained from Funakoshi Co. Ltd., Tokyo, Japan. EGCG was coupled to CNBr-activated Sepharose 4B (Pharmacia Biotech, Tokyo, Japan) at a concentration of 5 mg/mL of wet gel as described previously (17). Thermolysin was obtained from Peptide Institute (Osaka, Japan), and was coupled to CNBr-activated Sepharose 4B at a concentration of 7 mg/mL of wet gel as described previously (17). Human fibrinogen and bovine fibrinogen were from Aldrich-Sigma. Alamar blue (a product of Alamar Biosciences, Sacramento, CA, USA) was from Asahi Techno Glass Corp., Tokyo, Japan. Serum-free cell culture medium Cosmedium 001 was purchased from Cosmo Bio Co. Ltd., Tokyo, Japan. Dithiothreitol (DTT) was from Nacalai Tesque, Kyoto, Japan. Highly metastatic mouse carcinoma LL2-Lu3 cells were obtained as described previously (16) and were cultured and maintained in a medium of 10 % fetal bovine serum in Dulbecco's modified Eagle's medium (DMEM) with 50 U/mL penicillin, 50 µg/mL streptomycin, 2.5 µg/mL amphotericin B, and 50 µg/mL gentamycin at 37°C under 5% CO_2 as described previously (19).

Interaction between fibrinogen subunits and EGCG. Human fibrinogen (10 mg) was dissolved in 10 mL of Tris-HCl buffer (pH 7.5) and reduced with 10 mg of DTT in the presence of 10 mM EDTA at room temperature for 1 h. The reduced fibrinogen was applied to a column of EGCG-agarose (1.2 × 2 cm) equilibrated with Tris-HCl buffer containing 1 M DTT. After washing with 50 mM Tris-HCl buffer, the column was eluted with 4 M urea/1 M NaCl in the same buffer and fractions were collected in each 1 mL portion using a fraction collector. The bound fractions thus obtained were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (7).

Interaction between CNBr fragments of fibrinogen and EGCG. Human fibrinogen (100 mg) was dissolved in 1 mL of 70% formic acid and the fibrinogen solution was treated with 10 mg of CNBr for 24 h at room temperature. After 20 mL of water was added to the mixture, the solution was freeze-dried. The materials obtained were dissolved in 0.15 M ammonium bicarbonate containing 1 M DTT and were centrifuged at 15,000 rpm for 5 min. The precipitates were discarded. The CNBr digest of fibrinogen thus obtained was loaded onto the EGCG-Sepharose column in 0.15 M ammonium bicarbonate containing 1 M DTT, and the bound fractions were eluted with 2 M ammonium bicarbonate. The effluents were analyzed by SDS-PAGE.

Interaction between fibrinogen thermolytic peptides and EGCG. Bovine fibrinogen (50 mg) was dissolved in 5 mL of 0.1 M ammonium bicarbonate and digested with 1 mL of thermolysin immobilized on Sepharose 4B at 37°C as described previously (17). The products generated by 1-h and 24-h digestions were examined by SDS-PAGE. The 1 h-digestion products were incubated with 1 M DTT in the presence of 10 mM EDTA for 1 h, and the mixture was loaded onto the EGCG-Sepharose column in a 50 mM Tris-HCl buffer (pH 7.5) containing 1M DTT and 1 mM EDTA. After washing exhaustively with the buffer, the bound fractions were eluted with 2 M ammonium carbonate and 2-mL fractions were collected. The effluents were analyzed by SDS-PAGE. When necessary, ammonium bicarbonate solutions were freeze-dried repeatedly to remove ammonium bicarbonate and then dissolved in the sample solution for SDS-PAGE under reducing conditions (17).

Determination of amino acid sequence. The partial amino acid sequences of fibrinogen fragments obtained by CNBr digestion or thermolysin digestion were determined after electroblotting on a polyvinylidene difluoride (PVDF) membrane (Boehringer Mannheim, Germany) using an Applied Biosystems model 470 sequencer as described previously (9).

Effect of EGCG on the interaction between carcinoma cells and fibrinogen. The effects of EGCG on cell adhesion to and the spreading of LL2-Lu3 cells on fibrinogen was examined as follows. Plastic 48-well multidishes were coated with fibrinogen at 10 µg/mL in DMEM at 37°C for 30 min. After being washed three times with 0.2 mL DMEM, fibrinogen-coated wells were blocked by incubation with 1% bovine serum albumin in DMEM at 37°C for 30 min. After the wells were washed three times with DMEM, 0.1 mL of DMEM with or without EGCG at various concentrations was added and the wells were kept at 37°C for 30 min. The wells were washed three times with serum-free cell culture medium Cosmedium 001, and freshly trypsinized LL2-Lu3 cells (1×10^4 in 0.1 mL) which had been washed three times with Cosmedium 001 were plated onto each well. The mixture was then incubated at 37°C in a humidified CO_2 incubator. After 1 h, the wells

were washed three times with 0.5 mL of Cosmedium 001 and 0.1 mL of Cosmedium 001 was added. To examine cell adhesion, 10 μL of Alamar blue solution was added to each well, and the mixture was incubated at 37°C in a CO_2 incubator. After a 2-h incubation, fluorescence was measured with excitation at 560 nm and emission at 590 nm as described previously (20). Cells were photographed for the quantitative expression of cell spreading, and cells with a long spindle/short spindle ratio of 2 or more were counted as spread cells.

RESULTS

Human fibrinogen subunits bound by EGCG

We previously demonstrated that fibrinogen was bound by an EGCG column under non-reducing conditions. Since fibrinogen subunits are linked by disulfide bonds, whether each of the 3 subunits of fibrinogen had binding affinity for EGCG independently remained unknown. In the present study, we reduced human fibrinogen with DTT and examined the interaction of products with EGCG-agarose. Three protein bands of 54 kDa (γ-chain), 60 kDa (β-chain) and 68 kDa (α-chain) were detected when the bound fractions were analyzed by SDS-PAGE (Fig. 1). These results indicated that each of the fibrinogen subunit chains (α-, β-, and γ-chains) was bound by EGCG independently.

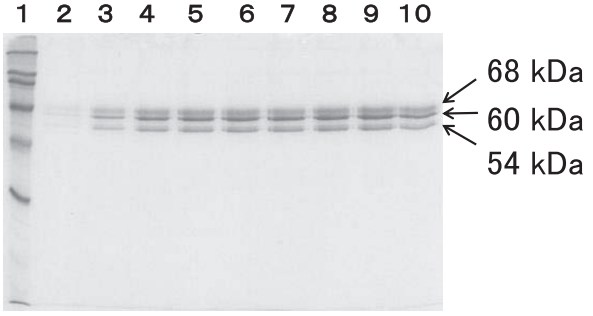

Fig. 1 SDS-PAGE of the EGCG-bound fraction of DTT-treated human fibrinogen subunits. Human fibrinogen reduced with DTT was applied to a column of EGCG-agarose. The bound fractions were eluted with Tris buffer containing 4 M Urea /1 M NaCl and subjected to SDS-PAGE. Molecular marker proteins (lane 1): rabbit muscle myosin (220 kDa), *E coli* β-galactosidase (116 kDa), rabbit muscle phosphorylase b (97 kDa), bovine serum albumin (66 kDa), chicken ovalbumin (45 kDa), and bovine carbonic anhydrase (29 kDa) from top to bottom. Lanes 2–10 represent fractions 1–9 eluted with 4 M urea/1M NaCl, respectively. Proteins were stained with Coomassie brilliant blue R-250.

EGCG-bound fraction of human fibrinogen digested by CNBr

Human fibrinogen was digested by CNBr and the digestion products were applied to an EGCG-agarose column. When the EGCG-bound fraction was analyzed by SDS-PAGE and electroblotting, the results indicated that 26 kDa, 24 kDa, 22 kDa, 18 kDa, 15 kDa, and 12 kDa fragments were bound by EGCG (Fig. 2). The *N*-terminal amino acid sequence of the 12 kDa fragment was determined as FFSTYDRD- (Table 1). This sequence was identical to that of the segment starting from Phe^{374} in the β chain of fibrinogen. The sequence of the 22 kDa fragment was determined as KYEASILTHDSSIRY-, which corresponded to the *N*-terminal sequence of the 15 residues in the segment starting from Lys^{95} in the γ chain (Table 2). These peptides were generated by cleavage at the peptide bonds involving the carboxyl group of methionine, which was consistent with the specificity of the CNBr digestion (Tables 1 and 2).

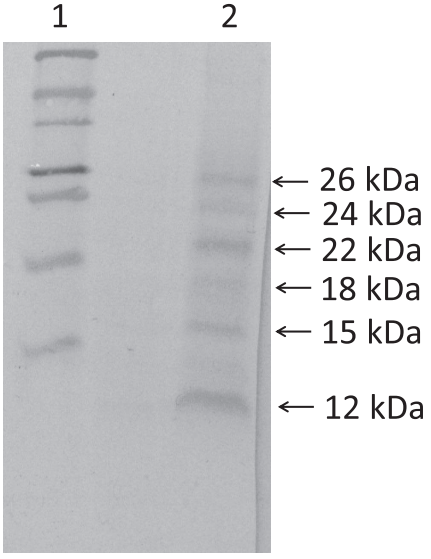

Fig. 2 Analysis of the EGCG-bound fragments derived from the CNBr digests of human fibrinogen. Human fibrinogen was digested with CNBr and the mixture was applied to an EGCG-agarose column. Electrically blotted proteins on a PVDF membrane were visualized by staining with Coomassie brilliant blue R-250. Lane 1, marker proteins; bovine serum albumin (66 kDa), egg albumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), bovine carbonic anhydrase (29 kDa), bovine pancreas trypsinogen (24 kDa), soybean trypsin inhibitor (20 kDa), and bovine milk α-lactoalbumin (14.2 kDa) from top to bottom. Lane 2, the EGCG-bound fraction of human fibrinogen digested by CNBr. Proteins giving bands with molecular masses of 26 kDa, 24 kDa, 22 kDa 18 kDa, 15 kDa, and 12 kDa were subjected to amino acid sequence analysis.

Table 1 *Determined amino acid sequence of the 12 kDa fragment derived from CNBr digestion products and the amino acid sequence of the human fibrinogen β-chain*

12 kDa fragment	FFSTYDRD-
Human fibrinogen β-chain	
1	QGVNDNEEGF FSARGHRPLD KKREEAPSLR PAPPPISGGG
41	YRARPAKAAA TQKKVERKAP DAGGCLHADP DLGVLCPTGC
81	QLQEALLQQE RPIRNSVDEL NNNVEAVSQT SSSSFQYMYL
121	LKDLWQKRQK QVKDNENVVN EYSSELEKHQ LYIDETVNSN
161	IPTNLRVLRS ILENLRSKIQ KLESDVSAQM EYCRTPCTVS
201	CNIPVVSGKE CEEIIRKGGE TSEMYLIQPD SSVKPYRVYC
241	DMNTENGGWT VIQNRQDGSV DFGRKWDPYK QGFGNVATNT
281	DGKNYCGLPG EYWLGNDKIS QLTRMGPTEL LIEMEDWKGD
321	KVKAHYGGFT VQNEANKYQI SVNKYRGTAG NALMDGASQL
361	MGENRTMTIH NGMFFSTYDR DNDGWLTSDP RKQCSKEDGG
401	GWWYNRCHAA NPNGRYYWGG QYTWDMAKHG TDDGVVWMNW
441	KGSWYSMRKM SMKIRPFFPQ Q

Table 2 *Determined amino acid sequence of the 22 kDa fragment and the complete amino acid sequence of the human fibrinogen γ-chain*

22 kDa fragment	KYEASILTHDSSIRY-
Human fibrinogen γ-chain	
1	YVATRDNCCI LDERFGSYCP TTCGIADFLS TYQTKVDKDL
41	QSLEDILHQV ENKTSEVKQL IKAIQLTYNP DESSKPNMID
81	AATLKSRIML EEIMKYEASI LTHDSSIRYL QEIYNSNNQK
121	IVNLKEKVAQ LEAQCQEPCK DTVQIHDITG KDCQDIANKG
161	AKQSGLYFIK PLKANQQFLV YCEIDGSGNG WTVFQKRLDG
201	SVDFKKNWIQ YKEGFGHLSP TGTTEFWLGN EKIHLISTQS
241	AIPYALRVEL EDWNGRTSTA DYAMFKVGPE ADKYRLTYAY
281	FAGGDAGDAF DGFDFGDDPS DKFFTSHNGM QFSTWDNDND
321	KFEGNCAEQD GSGWWMNKCH AGHLNGVYYQ GGTYSKASTP
361	NGYDNGIIWA TWKTRWYSMK KTTMKIIPFN RLTIGEGQQH
401	HLGGAKQAGD V

Thermolysin peptides from bovine fibrinogen bound by EGCG

When bovine fibrinogen was digested with thermolysin, digestion for 1 h gave several bands on SDS-PAGE (Fig. 3). These fragments were also detected in the 24-h digestion products, which suggested that these thermolysin peptides were strongly resistant to further digestion, presumably representing the domain structures in fibrinogen. When the 1-h digestion products were examined for the binding interaction with EGCG, the results of SDS-PAGE indicated that 51 kDa, 46 kDa, 36 kDa, and 28 kDa fragments were bound by EGCG (Fig. 4). After blotting onto a PVDF membrane, these peptides were subjected to amino acid sequence analysis. The results indicated that the 28 kDa fragment was derived from the segment starting from Leu^{23} in the α-chain (Table 3). Similarly, the 36 kDa fragment was considered to have been derived from the segment starting from Tyr^{1} in the γ-chain (Table 4). These results indicated that the α- and γ-chains of bovine fibrinogen possess respective binding sites for EGCG.

Cell adhesion to and cell spreading on EGCG-treated fibrinogen

To examine whether the binding association of

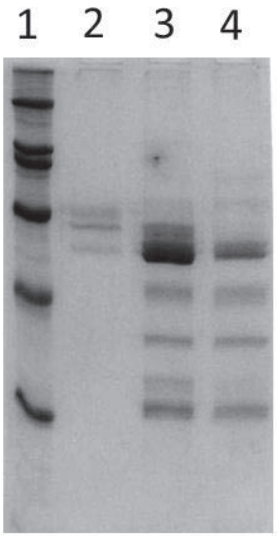

Fig. 3 SDS-PAGE of the thermolytic peptides derived from bovine fibrinogen. Bovine fibrinogen was digested with thermolysin for different periods of time. The products were analyzed by SDS-PAGE. Lane 1, marker proteins as used in Fig. 1; lane 2, bovine fibrinogen; lane 3, 1-h digestion products; lane 4, 24-h digestion products.

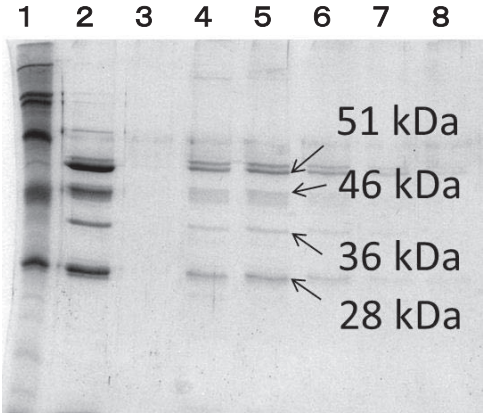

Fig. 4 SDS-PAGE of EGCG-unbound and EGCG-bound peptides in the thermolysin digestion products of bovine fibrinogen. Products obtained by the 1-h digestion were applied to the EGCG-agarose column. Proteins were visualized by staining with Coomassie brilliant blue R-250. Lane 1, marker proteins as used in Fig. 1. Lane 2, EGCG-unbound fraction. Lanes 3-8, EGCG-bound fractions 1-6, respectively.

EGCG with fibrinogen affected the interaction between fibrinogen and tumor cells, we investigated the effects of EGCG on cell adhesion and spreading of LL2-Lu3 cells on the fibrinogen substratum. These cells attached to and spread on plastic dishes coated with fibrinogen. Although EGCG did not interfered with cell attachment to the fibrinogen-coated dishes, the spreading of these cells on fibrinogen-treated dishes was inhibited by prior treatment with EGCG (Fig. 5).

DISCUSSION

Fibrinogen is a major plasma protein of 350 kDa and consists of 6 S-S-linked subunit chains of 2 α-chains, 2 β-chains, and 2 γ-chains (15). It has been shown to play an important role in blood clotting, cellular and matrix interactions, inflammation, wound healing, and neoplasia (4, 14). Fibrinogen was also shown to induce proliferative signals by serving as a scaffold to support the binding of growth factor and promote the cellular responses of adhesion, proliferation, and migration during wound healing, angiogenesis, and tumor growth (1, 2, 4, 22). We previously reported that three plasma proteins including fibrinogen were bound by EGCG (18). Hung *et al.* also demonstrated the binding interaction of EGCG with fibrinogen, which inhibited the adhesion of human foreskin fibroblasts to the substratum (5). However, it remains unclear whether the binding of EGCG to fibrinogen causes an impairment in tumor cell adhesion and/or spreading, which are important for tumor cell invasion and metastasis. In the present study, we examined whether EGCG caused any impairment in the interaction between tumor cells and fibrinogen.

To obtain further evidence to indicate the interaction between fibrinogen and EGCG, we examined whether each of the 3 subunit chains (α-, β-, and γ-chains) of fibrinogen had an EGCG-binding site. The results of affinity chromatography under reducing conditions suggested that each of the subunit chains possessed at least one specific site bound by EGCG. Consistent with this finding, it was further demonstrated that the CNBr fragments derived from the β- and γ-chains of human fibrinogen and those derived from the α- and γ-chains of bovine fibrinogen generated by thermolysin digestion were bound by EGCG as discussed below.

The 12 kDa human fibrinogen fragment derived from CNBr-digestion products was found to be bound by the EGCG column. Its *N*-terminal 8 amino acid sequence corresponded to that of the segment starting from Phe^{374} in the β-chain. The 22 kDa human fibrinogen fragment derived from the γ-chain was also shown to be bound by EGCG. The amino acid sequences of other fragments could not be determined. Although the reason for this remains unclear, one possible explanation may be the blocking of their *N*-terminals by the formation of a pyroglutamyl residue after the non-specific cleavage of pep-

Table 3 *The amino acid sequence of the 28 kDa fragment and the complete amino acid sequence of the bovine fibrinogen α-chain*

28 kDa fragment	LVERQQSACKETGWPF-
Bovine fibrinogen α-chain	
1	SDPPSGDFLT EGGGVRGPRL VERQQSACKE TGWPFCSDED
41	WNTKCPSGCR MKGLIDEVDQ DFTSRINKLR DSLFNYQKNS
81	KDSNTLTKNI VELMRGDFAK ANNNDNTFKQ ISEDLRSRIE
121	ILRRKVIEQV QRIKVLQKNV RDQLVDMKRL EVDIDIKIRS
161	CKGSCSRALE HKVDLEDYKN QQKQLEQVIA INLLPSRDIQ
201	YLPLIKMSTI TGPVPREFKS QLQEAPLEWK ALLEMQQTKM
241	VLETFGGDGH ARGDSVSQGT GLAPGSPRKP GTSSIGNVNP
281	GSYGPGSSGT WNPGRPEPGS AGTWNPGRPE PGSAGTWNPG
321	RPEPGSAGTW NPGRPEPGSA GTWNPGRPEP GSAGTWNTGS
361	SGSSSFRPDS SGHGNIRPSS PDWGTFREEG SVSSGTKQEF
401	HTGKLVTTKG DKELLIDNEK VTSGHTTTTR RSCSKVITKT
441	VTNADGRTET TKEVVKSEDG SDCGDADFDW HHTFPSRGNL
481	DDFFHRDKDD FFTRSSHEFD GRTGLAPEFA ALGESGSSSS
521	KTSTHSKQFV SSSTTVNRGG SAIESKHFKM EDEAESLEDL
561	GFKGAHGTQK GHTKARPARG IHTSPLGEPS LTP

Table 4 *The amino acid sequence of the 36 kDa fragment and the complete amino acid sequence of the bovine fibrinogen γ -chain*

36 kDa fragment	YVATRDNCCILDERFG-
Bovine fibrinogen γ-chain	
1	YVATRDNCCI LDERFGSYCP TTCGIADFLN NYQTSVDKDL
41	RTLEGILYQV ENKTSEAREL VKAIQISYNP DQPSKPNNIE
81	SATKNSKSMM EEIMKYETLI STHESTIRFL QEVYNSNSQK
121	IVNLRDKVVQ LEANCQEPCQ DTVKIHDVTG RDCQDVANKG
161	AKESGLYFIR PLKAKQFLVY CEIDGSGNGW TVFQKRLDGS
201	LDFKKNWIQY KEGFGHLSPT GTGNTEFWLG NEKIHLISTQ
241	SSIPYVLRIQ LEDWNGRTST ADYASFKVTG ENDKYRLTYA
281	YFIGGDAGDA FDGYDFGDDS SDKFFTSHNG MQFSTWDSDN
321	DKYDGNCAEQ VGIGWWMNKC HAGHLNGVYY QGGTYSKTST
361	PNGYDNGIIW ATWKSRWYSM KKTTMKIIPL NRLAIGEGQQ
401	HQLGGAKQVG VEHHVEIEYD

tide bonds in which the amino group of the glutamic acid residue was involved. Since evidence for the binding interaction of a fragment derived from the α-chain was not obtained by the experiment using CNBr-peptides derived from human fibrinogen, we next examined the fragments obtained by the thermolysin digestion of bovine fibrinogen.

Among the thermolytic fragments obtained, the 28 kDa and 36 kDa fragments were bound by EGCG. The determined amino acid sequence indicated that the 28 kDa fragment was derived from the α-chain, and the 36 kDa fragment was considered to be derived from the γ-chain. These results indicated that each of three fibrinogen subunits had at least one EGCG-binding site independent of the other subunits.

EGCG has been shown to interact with several proteins at specific molecular sites. For example, EGCG was proposed to inhibit urokinase by fitting into the catalytic cavity formed with His^{57}, Asp^{102},

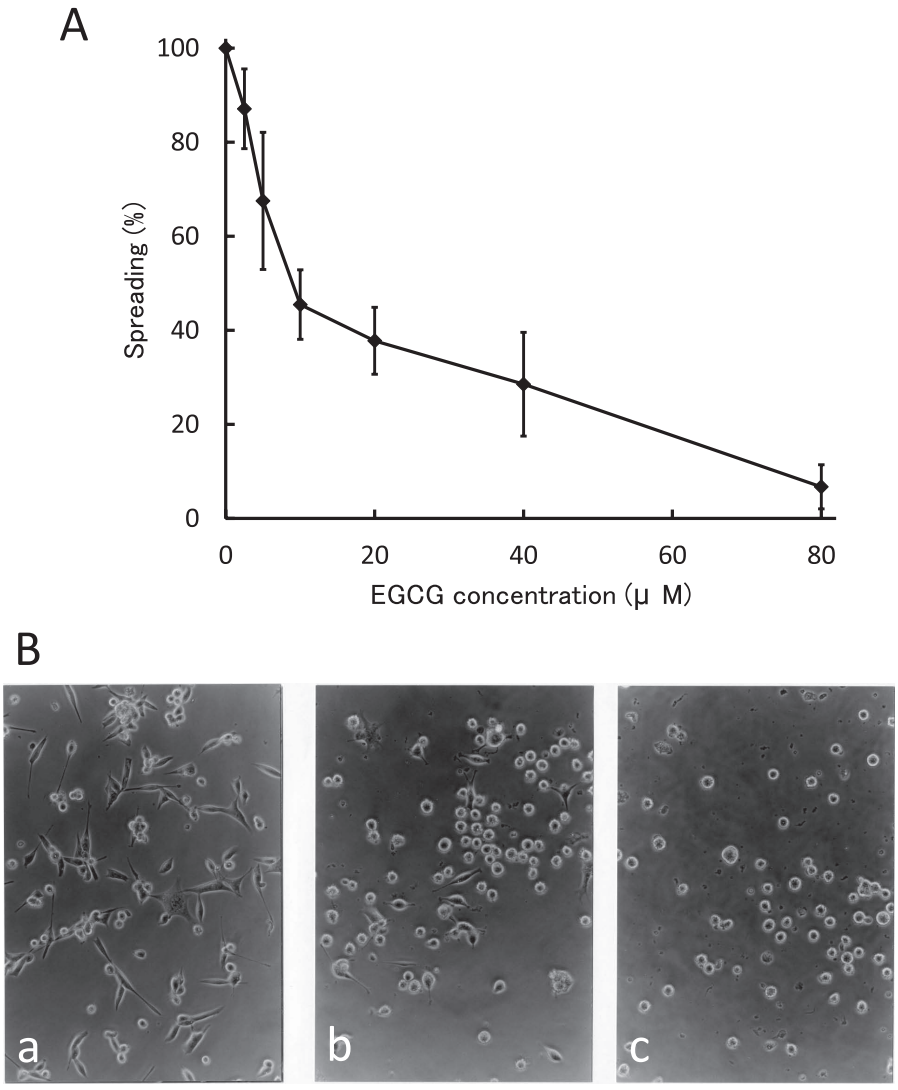

Fig. 5 Effects of EGCG on spreading of LL2-Lu3 cells on fibrinogen. **A**, plastic multidishes with 48 wells coated with fibrinogen were treated with EGCG at various concentrations. The wells were washed to remove unbound EGCG and freshly trypsinized LL2-Lu3 cells were plated to an each well. After a 60-min incubation, cells were photographed and spreading cells with a long spindle/short spindle ratio of 2 or more were counted. Data presented are averages ± S.D. from triplicate cultures. **B**, a representative photograph taken after the 60-min incubation showing cell spreading on fibrinogen pre-treated with EGCG at 20 μM (b) and 80 μM (c) was less than that on the fibrinogen substratum without the EGCG treatment (a).

Ser^{195}, and Arg^{35} (6) and DNA methyltransferase by formation of hydrogen bonds with the residues Pro^{1223}, Glu^{1265}, Cys^{1225}, Ser^{1229}, and Arg^{1309} (3). Fibrinogen appears to have at least one such binding pocket in each of the three subunit chains, although it is not known what amino acid residues of fibrinogen subunits are involved in the interaction with EGCG. Future studies are needed to reveal the nature of their binding affinity.

In experiments to examine whether EGCG affected the cellular activity such as cell adhesion and spreading on fibrinogen substrata, EGCG was observed to inhibit the spreading of mouse lung carcinoma LL2-Lu2 cells, but not cell adhesion. The various roles of fibrinogen or fibrin on tumor progression and metastasis have been demonstrated previously. Palumbo *et al*. reported that a genetic fibrinogen deficiency strongly diminished the development of lung metastasis in both Lewis lung carcinoma and B16-BL6 melanoma lung metastatic models (14). Ma *et al.* demonstrated that plasma fibrinogen levels were associated with peritoneal carcinomatosis of ovarian carcinoma (10). Takeuchi *et al.* also revealed that higher levels of fibrinogen in

the plasma correlated with tumor progression and metastasis in patients with esophageal squamous cell carcinoma (22). More recently, Lee *et al.* reported an association between pre-operative plasma fibrinogen levels and adjacent organ involvement in advanced gastric cancer (8). These findings suggest that therapeutic strategies directed at fibrinogen may be useful for suppressing metastatic disease (13) and that EGCG, which causes an impairment in fibrinogen activity leading to the inhibition of cancer cell spreading on it, may be a candidate for an inhibitor of tumor cell metastasis, as has been reported in the previous studies (19, 23).

The concentration of EGCG in human plasma was estimated to be 2–4 μM after its oral administration (11, 24). The required concentration for EGCG to inhibit cell spreading on fibrinogen was at least one order higher than this level. Our previous studies showed that EGCG inhibited cell adhesion and spreading on fibronectin and laminin at higher doses than that of the physiologically available dose (12, 21). Thus, these and the present findings suggest that previously observed *in vivo* inhibition of metastasis elicited by EGCG (23) and the green tea infusion (19) may have resulted from a combination of the effects of EGCG caused by its binding interaction with fibronectin, laminin, and fibrinogen.

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