Biochemical and Biophysical Research Communications 371 (2008) 172–176

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Involvement of 67-kDa laminin receptor-mediated myosin phosphatase activation in antiproliferative effect of epigallocatechin-3-*O*-gallate at a physiological concentration on Caco-2 colon cancer cells

Daisuke Umeda [a], Satomi Yano [a], Koji Yamada [a], Hirofumi Tachibana [a,b,*]

[a] *Laboratory of Food Chemistry, Division of Applied Biological Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan*

[b] *Laboratory of Functional Food Design, Department of Functional Metabolic Design, Bio-Architecture Center, Kyushu University, Fukuoka 812-8581, Japan*

ARTICLE INFO

Article history:
Received 8 April 2008
Available online 25 April 2008

Keywords:
Epigallocatechin-3-*O*-gallate
67-kDa laminin receptor
Cell cycle
Myosin regulatory light chain
Myosin phosphatase targeting subunit 1

ABSTRACT

Previously we reported that 67-kDa laminin receptor (67LR) mediates epigallocatechin-3-*O*-gallate (EGCG)-induced cell growth inhibition and reduction of myosin regulatory light chain (MRLC) phosphorylation at Thr-18/Ser-19, which is important for cytokinesis. Here, we found that human colon adenocarcinoma Caco-2 cells exhibited higher expression level of 67LR and EGCG at a physiologically achievable concentration (1 μM) significantly accumulated the cells in G_2/M phase without affecting expression of Wnt-signaling components. We also found that myosin phosphatase targeting subunit 1 (MYPT1) phosphorylation at Thr-696, which inhibits myosin phosphatase and promotes MRLC phosphorylation, was reduced in response to 1 μM EGCG. 67LR knockdown by RNA interference abolished the inhibitory effects of 1 μM EGCG on cell cycle progression and the phosphorylation of MRLC and MYPT1. These results suggest that through 67LR, EGCG at a physiological concentration can activate myosin phosphatase by reducing MYPT1 phosphorylation and that may be involved in EGCG-induced cell growth inhibition.

Green tea polyphenols have been shown to have cancer preventive activity in a variety of organ sites in animal models [1,2] and humans [3]. Among the green tea polyphenols, epigallocatechin-3-*O*-gallate (EGCG) is the most abundant and most active polyphenol in inhibiting experimental carcinogenesis and related reactions [2,4]. However, it is still not clear which EGCG-induced molecular events are responsible for the inhibition of carcinogenesis because the concentrations of EGCG shown to have an effect (20–100 μM) in most previous studies are much higher than the concentrations observed in the plasma or tissues of animals or in human plasma (usually lower than 1 μM) after tea ingestion [1]. Recently we have identified 67-kDa laminin receptor (67LR) as a cell surface EGCG receptor that mediates the anticancer action of physiologically achievable concentrations of EGCG (0.1–1 μM) [5]. Further others showed that RNA interference (RNAi)-mediated silencing of 67LR results in abrogation of EGCG-induced apoptosis in myeloma cells [6].

Myosin II, the conventional two-headed myosin first identified in muscle, is the primary motor protein responsible for cytokinesis [7]. It has been demonstrated that the contractile force of the contractile ring in dividing cells is generated by the association of myosin II with actin [7]. In higher eukaryotes, phosphorylation of the myosin regulatory light chain (MRLC) at Thr-18/Ser-19 increases the actin-activated Mg-ATPase activity of myosin II and the assembly of myosin II filaments, regulating the association between myosin II and actin [8]. Therefore, it is suggested that MRLC phosphorylation is crucial for cell division. Phosphorylation of MRLC is regulated by two classes of enzymes: MRLC kinases and myosin phosphatase [8]. Myosin phosphatase is composed with three subunits: a 37-kDa catalytic subunit, a 20-kDa subunit of unknown function, and a 110- to 130-kDa myosin phosphatase targeting subunit (MYPT1) [9]. The activity of myosin phosphatase is known to be regulated by phosphorylation of MYPT1 and two major sites, Thr-696 and Thr-853, have been extensively investigated and identified as an inhibitory site [9]. We previously reported that EGCG induced reduction of the phosphorylation of MRLC at Thr-18/Ser-19 through 67LR in HeLa cells [10], human basophilic KU812 cells [11] and mouse melanoma B16 cells [12], suggesting involvement of MRLC dephosphorylation in EGCG-induced cell growth inhibition. Further, we have reported that EGCG induced decrease in MYPT1 phosphorylation at Thr-696 through 67LR in HeLa cells and B16 cells, leading to activation of myosin phosphatase and reduction of MRLC phosphorylation [12].

* Corresponding author. Address: Laboratory of Food Chemistry, Division of Applied Biological Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan. Fax: +81 92 642 3008.
E-mail address: tatibana@agr.kyushu-u.ac.jp (H. Tachibana).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.04.041

Colorectal cancer is one of the most prevalent types of cancer in the Western world [13]. Epidemiological studies have suggested that the consumption of green tea may decrease colon cancer risk [14]. The Wnt-pathway appears to play an important role particularly in colon carcinogenesis [15]. The essential event in Wnt-signaling is the stabilization of β-catenin. The resulting accumulation of β-catenin increases the pool of nuclear β-catenin bound to transcription factor TCF/LEF in complexes that can activate certain genes, including oncogene c-Myc. As the main binding partner of β-catenin at cell–cell junctions, E-cadherin plays a pivotal role in β-catenin stabilization and function. E-cadherin commonly repressed in epithelial carcinogenesis and its binding with β-catenin suppressed Wnt-signaling [16]. Previous studies have reported that EGCG-treatment downregulated the β-catenin protein expression [17] or upregulated E-cadherin protein expression [18], suggesting that EGCG suppressed Wnt-signaling. However, it is still not known how a physiological concentration of EGCG induces cell growth inhibition in colorectal cancer cells. In this study, we found for the first time that a physiologically achievable concentration of EGCG inhibited cell cycle progression of human colon adenocarcinoma Caco-2 cells through 67LR without affecting components of Wnt-signaling. Further, we found that EGCG at a physiological concentration decreased the phosphorylation of MRLC at Thr-18/Ser-19 and MYPT1 at Thr-696 in Caco-2 cells through 67LR, suggesting that an activation of myosin phosphatase is involved in antiproliferative effect of EGCG at a physiological concentration.

Materials and methods

Materials and chemicals. EGCG, catalase, superoxide dismutase (SOD), and anti-β-actin antibody were purchased from Sigma Chemical Co. (St. Louis, MO). Anti-67LR (F-18) antibody, anti-MRLC (FL-172) antibody, horseradish peroxidase (HRP)-conjugated anti-mouse IgG and HRP-conjugated anti-goat IgG were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-phospho-MRLC (Thr-18/Ser-19) antibody was obtained from Cell Signaling Technology, Inc. (Beverly, MA). Anti-MYPT1 antibody was purchased from BD Bioscience (San Jose, CA). Anti-phospho-MYPT1 (Thr-696) antibody was purchased from Upstate Biotechnology (Lake Placid, NY). HRP-conjugated anti-rabbit IgG antibody was obtained from ICN pharmaceuticals, Inc. (Costa Mesa, CA). Propidium iodide was obtained from Wako Pure Chemical Industries (Osaka, Japan).

Cell culture and cell proliferation assays. HeLa cells (a human cervical adenocarcinoma) were maintained in Dulbecco's modified Eagle's medium (DMEM) (COSMO BIO Co. Ltd., Tokyo, Japan) supplemented with 10% FBS (Biological Industries, Kibbutz Beit Kaemek, Israel) in a humidified atmosphere with 5% CO_2 at 37 °C. Caco-2 cells (a human colorectal adenocarcinoma) were maintained in DMEM supplemented with 10% FBS and 1% non-essential amino acid (NEAA) (Hyclone Inc., Logan, UT). To assess cell proliferation, cells were plated in 24-well plates at a density of 1×10^4 cells/well, and 24 h later they were treated with the indicated concentrations of EGCG for the indicated time periods in DMEM supplemented with 2% FBS, 1% NEAA (only for Caco-2 cells), 5 mg/mL BSA, 5 U/mL SOD and 200 U/mL catalase. Cell density was adhesive cell number per well.

Flow cytometric analysis. Cells were suspended in 70% ethanol and incubated at −20 °C for more than 4 h. The cells were washed with PBS and resuspended in PBS together with 10 μg/mL RNaseA. Incubation was continued at 37 °C for 20 min. The cellular DNA was then stained by applying propidium iodide (20 μg/mL) for 30 min at room temperature. The stained cells were analyzed by flow cytometry (FACS Calibur; Becton Dickinson, Sunnyvale, CA) for relative DNA content. ModFit LT software (Verity Software House, Inc., Topsham, ME) was used to assess cell cycle distribution.

Western blot analysis. Cells were lysed in cell lysis buffer containing 50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 50 mM NaF, 30 mM $Na_4P_2O_7$, 1 mM phenylmethanesulfonyl fluoride, 10 μg/ml aprotinin, and 1 mM pervanadate. Proteins were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and then transferred onto a nitrocellulose membrane. The membranes were blocked by BSA and incubated with primary antibodies, followed by incubation with HRP-conjugated secondary antibodies. Immunoreactive bands were visualized using the enhanced chemiluminescence Advance Western Blotting Detection kit (GE Healthcare, Little Chalfont, UK). Band intensities were quantified using NIH Image-J software.

RNA interference by shRNA. Target sequences for short hairpin RNAs for 67LR and nonspecific control are as follows: shRNA for 67LR, 5′-GGAGGAATTTCAGGGTGAA-3′; shRNA for nonspecific control, 5′-GCATATGTGCGTACCTAGCAT-3′. The annealed shRNA inserts were cloned into the the psiRNA-hH1neo shRNA expression vector (InvivoGen, San Diego, CA) according to the manufacturer's protocol. For stable transfection, FuGENE6 reagent (Roche, Basel, Switzerland) was mixed with DNA for 20 min in serum-free medium, the mixture was then added to Caco-2 cells in DMEM supplemented with 10% FBS and 1% NEAA. For stable transfection, the transfected cells were grown in medium containing G418.

Statistical analysis. All values are expressed as means ± SD compared with controls. Statistical analysis was performed by use of Student's t test. A level of $p < 0.05$ was considered significant.

Results

Caco-2 cells exhibited higher 67LR protein expression and sensitivity to EGCG than HeLa cells

We investigated the expression levels of 67LR in cervical adenocarcinoma HeLa cells and colorectal adenocarcinoma Caco-2 cells. Caco-2 cells exhibited higher 67LR protein expression than HeLa cells (Fig. 1A). We then examined antiproliferative effects of EGCG on HeLa cells and Caco-2 cells. EGCG inhibited the growth of Caco-2 cells in a dose-dependent manner (Fig. 1B) and the inhibitory effect was significantly observed even at 1 μM EGCG treatment (Fig. 1C). Compared with Caco-2 cells, HeLa cells were less sensitive to EGCG because its growth was inhibited at only 10 μM EGCG (Fig. 1B). These results suggest that the expression level of 67LR may be elevated in the cells that are sensitive to the effect of EGCG at a physiological concentration. To further determine the possible involvement of EGCG in the regulation of cell cycle, we subjected EGCG-treated cells to FACS analysis using propidium iodide staining to measure DNA content. EGCG dose-dependently decreased the G_0/G_1 and S fractions while increasing the G_2/M fraction in Caco-2 cells (Fig. 1D). The inhibition of cell cycle progression was significantly observed even at 1 μM EGCG. These results indicate that a physiologically achievable concentration of EGCG inhibited cell growth of Caco-2 cells by accumulating the cells in G_2/M phase.

Effect of EGCG on E-cadherin, β-catenin, and c-Myc protein expression in Caco-2 cells

We investigated the effect of EGCG on the protein expression of E-cadherin, β-catenin, and c-Myc, which are important Wnt-signaling components, in Caco-2 cells by Western blot analysis (Fig. 2). E-cadherin protein expression was not affected by EGCG at 1 or 5 μM, though it was slightly reduced by 10 μM EGCG treatment for 72 h. β-Catenin protein expression were slightly affected by EGCG at 1 or 5 μM but dose- and time-dependency were not observed. c-Myc protein expression was slightly reduced by 10 μM EGCG treatment for 72 h. Since these results did not correlate with EGCG-induced cell growth inhibition shown in Fig. 1, it is suggested that E-cadherin, β-catenin, and c-Myc are not involved in antiproliferative effect of EGCG at a physiological concentration on Caco-2 cells.

Effect of EGCG on the phosphorylation of MRLC and MYPT1 in Caco-2 cells and HeLa cells

To elucidate the intracellular mechanism of how EGCG accumulates Caco-2 cells in G_2/M phase, we examined the effect of EGCG on MRLC phosphorylation at Thr-18/Ser-19 by Western blot analysis. EGCG treatment for 24 h dose-dependently reduced the level of MRLC phosphorylation (Fig. 3A). To investigate how EGCG reduced MRLC phosphorylation, we tested the effect of EGCG on the phosphorylation of MYPT1 at Thr-696 and found that EGCG dose-dependently induced the dephosphorylation of MYPT1 at Thr-696 in Caco-2 cells (Fig. 3B). These results suggest that EGCG activated myosin phosphatase by reducing MYPT1 phosphorylation at Thr-696. Moreover, decrease in the phosphorylation of both MRLC and MYPT1 was observed even at 1 μM EGCG and correlated

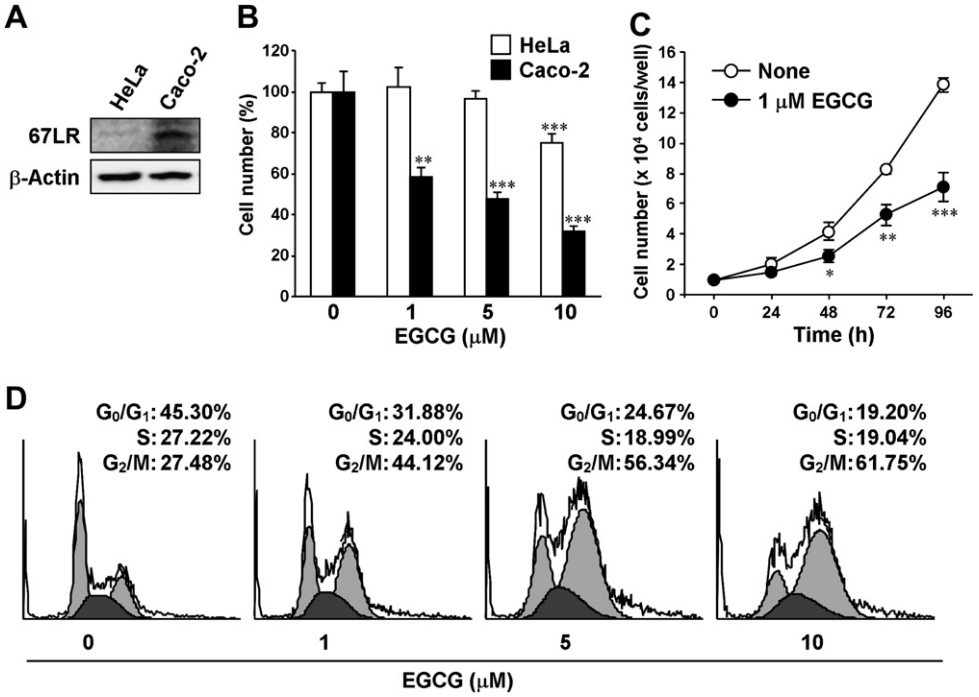

Fig. 1. Caco-2 cells exhibited higher 67LR protein expression and sensitivity to EGCG than HeLa cells. (A) Western blot analysis of 67LR in HeLa cells and Caco-2 cells. (B) The cells were treated with the indicated concentrations of EGCG for 96 h. (C) Caco-2 cells were treated with 1 μM EGCG for the indicated time periods. Data shown are means ± SD for three samples. Data containing asterisk marks are significantly different from the values in untreated control at $^{*}p < 0.05$, $^{**}p < 0.01$, or $^{***}p < 0.001$. (D) FACS analysis of the cell cycle. Cells were treated with the indicated concentrations of EGCG for 96 h. The cells were then evaluated for DNA content after propidium iodide staining.

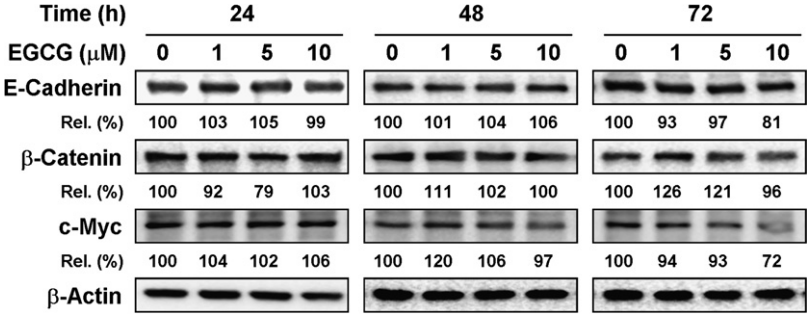

Fig. 2. Effect of EGCG on E-cadherin, β-catenin and c-Myc protein expression in Caco-2 cells. Cells were treated with the indicated concentrations of EGCG for the indicated time periods. Total cellular protein was subjected to Western blot analysis using anti-E-cadherin, anti-β-catenin and anti-c-Myc antibodies. Band intensity of each protein was normalized for β-actin and expressed as a percentage compared with the value of untreated control.

well with EGCG-induced cell growth inhibition shown in Fig. 1. The suppressive effects of EGCG at 1 μM on the phosphorylation of both MRLC and MYPT1 were still observed at 96 h after EGCG treatment in Caco-2 cells (Fig. 3C). In HeLa cells whose growth was not inhibited by 1 μM EGCG as shown in Fig. 1B , 1 μM EGCG did not affect the phosphorylation of either MRLC or MYPT1 (Fig. 3 D). These results suggest that a physiologically achievable concentration of EGCG inhibited cell growth by reducing the phosphorylation of both MRLC and MYPT1 in Caco-2 cells.

Effect of 67LR knockdown on EGCG-induced cell growth inhibition and reduction of the phosphorylation of MRLC and MYPT1 in Caco-2 cells

To investigate whether the inhibitory effect of EGCG on cell cycle progression is mediated by 67LR, we knockdown the expression of 67LR in Caco-2 cells using RNAi-mediated gene silencing. We stably transfected Caco-2 cells with the short hairpin RNA (shRNA) expression vector for 67LR and confirmed the knockdown of 67LR protein expression in the cells (Fig. 4A). 67LR knockdown significantly attenuated 1 μM EGCG-induced inhibition of cell growth (Fig. 4B) and accumulation of the cells in G_2/M phase (Fig. 4C). Furthermore, 1 μM EGCG-induced reduction of the phosphorylation of both MRLC (The-18/Ser-19) and MYPT1 (Thr-696) was also attenuated in 67LR-shRNA expressing cells, suggesting that EGCG activated myosin phosphatase through 67LR (Fig. 4D). These results suggest that 67LR mediates the suppressive effect of EGCG at a physiological concentration on cell cycle progression and the phosphorylation of MRLC and MYPT1.

Discussion

Many mechanisms for anticarcinogenic activities of green tea polyphenol EGCG have been proposed based mainly on studies in cell lines [2,4]. The activities observed with EGCG in cell line studies, however, may not be relevant to the situation *in vivo* because EGCG concentrations used *in vitro* are usually much higher than the achievable levels *in vivo* [1,2]. Therefore, it is important to investigate the activities observed at physiologically achievable

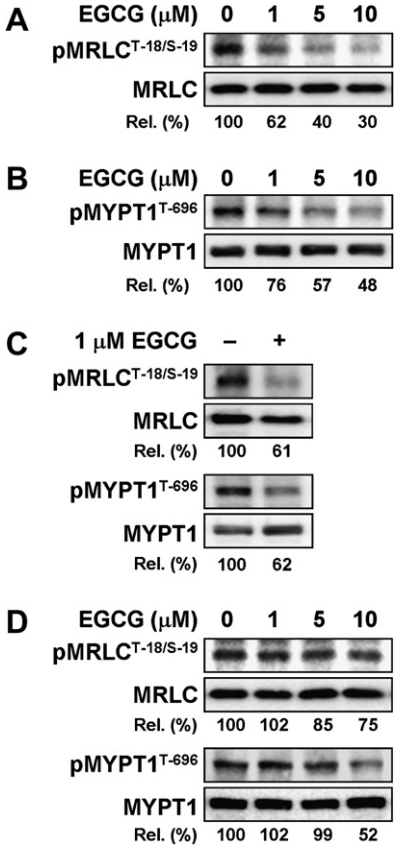

Fig. 3. Effect of EGCG on the phosphorylation of MRLC and MYPT1 in Caco-2 cells and HeLa cells. (A–C) Caco-2 cells were treated with the indicated concentrations of EGCG for 24 h (A and B) or treated with 1 μM EGCG for 96 h (C). (D) HeLa cells were treated with the indicated concentrations of EGCG for 24 h. Total cellular protein was subjected to Western blot analysis using anti-phospho-MRLC (Thr-18/Ser-19) and anti-phospho-MYPT1 (Thr-696) antibodies. Relative band intensity (pMRLC/MRLC or pMYPT1/MYPT1) was expressed as a percentage compared with the value of untreated control.

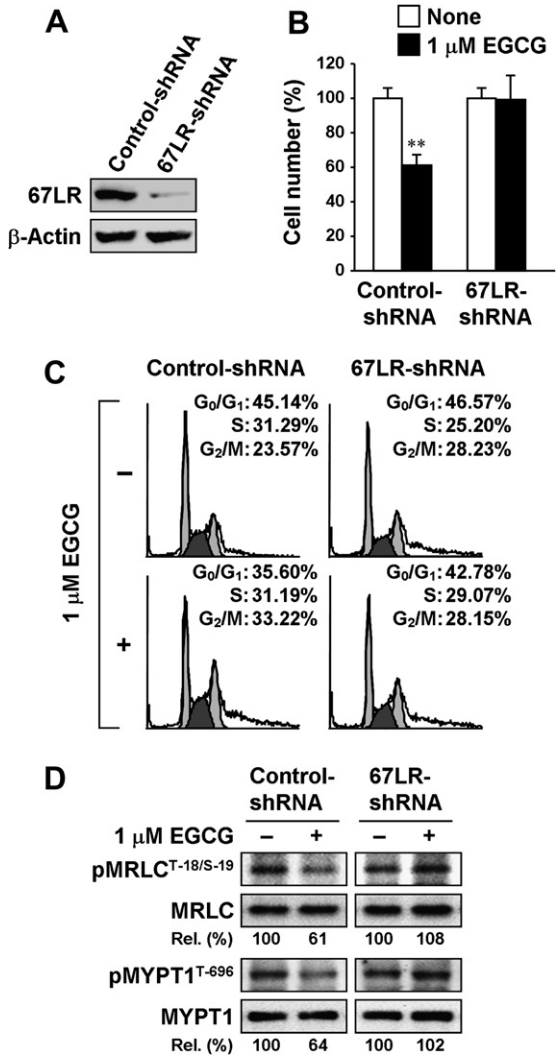

Fig. 4. Effect of 67LR knockdown on EGCG-induced cell growth inhibition and reduction of the phosphorylation of MRLC and MYPT1 in Caco-2 cells. (A) The cells stably transfected with the control-shRNA or the 67LR-shRNA expression vector were lysed and total cellular protein was subjected to Western blot analysis using anti-67LR antibody (F-18). (B) Cells were treated with 1 μM EGCG for 96 h. The results are shown as relative cell number to untreated control and the data presented are means ± SD for three samples. Data containing asterisk marks are significantly different from the values in control at $^{**}p < 0.01$. (C) FACS analysis of the cell cycle. Cells were treated as described in (B) and then evaluated for DNA content after propidium iodide staining. (D) Cells were treated with 1 μM EGCG for 24 h. Total cellular protein was subjected to Western blot analysis using anti-phospho-MRLC (Thr-18/Ser-19) and anti-phospho-MYPT1 (Thr-696) antibodies. Relative band intensity (pMRLC/MRLC or pMYPT1/MYPT1) was expressed as a percentage compared with the value of untreated control.

concentrations of EGCG. In the present study, we found that EGCG at a physiologically achievable concentration (1 μM) significantly inhibited cell growth of Caco-2 cells and accumulated the cells in G_2/M phase through 67LR. We also found that 1 μM EGCG reduced MYPT1 phosphorylation at Thr-696 through 67LR, leading to activation of myosin phosphatase and reduction of MRLC phosphorylation at Thr-18/Ser-19.

Several studies have reported that EGCG suppressed Wnt-signaling by affecting the protein levels of E-cadherin [18] and β-catenin [17], resulting in reduction of c-Myc and cyclin D1 [18]. On the other hand, many studies have reported that EGCG induces cell cycle arrest at G_0/G_1 phase in various cancer cells, including lung, colon, pancreas, skin, and prostate [1,3,5,19]. Since c-Myc and cyclinD1 are important cell cycle regulators particularly in the progression from G_1 to S phase [20], it may be proposed that suppression of Wnt-signaling by EGCG is the mechanism for EGCG-induced cell cycle arrest at G_0/G_1 phase. However, the concentrations of EGCG used in these previous studies are also much higher than those observed in the blood or tissues. In our experiment, a physiologically achievable concentration of EGCG accumulated Caco-2 cells in the G_2/M phase but not G_0/G_1 phase and did not affect the protein levels of E-cadherin, β-catenin and c-Myc. These results suggest that cell growth inhibition induced by a physiological concentration of EGCG in Caco-2 cells is dependent on other mechanisms than suppression of Wnt-signaling.

MRLC phosphorylation in cytokinesis can be regulated by myosin phosphatase and there are several pieces of evidence indicating that myosin phosphatase is involved in cytokinesis. MYPT1 is phosphorylated at the inhibitory sites at the cleavage furrow [21,22] and the MYPT1 phosphorylation is predicted to result in an increase in MRLC phosphorylation at the division site and might provide a signal for cytokinesis. MYPT mutations show effects on cytokinesis. In *Caenorhabditis elegans*, MYPT mutations cause defects in cytokinesis [23]. Mouse embryos lacking MYPT1 have recently been reported to die within 7.5 days post coitus, suggesting that mouse myosin phosphatase may be crucial for cytokinesis *in vivo* [24]. In our experiments, EGCG at a physiological concentration induced reduction of not only MRLC phosphorylation but MYPT1 phosphorylation at Thr-696 through 67LR, suggesting that EGCG activated myosin phosphatase and might prevent cytokinesis. It has also been reported that MYPT1 binds

to many other proteins in addition to phosphorylated MRLC and myosin phosphatase has several additional substrates [25]. Therefore, it is possible that dephosphorylation of not only MRLC but the other substrates by myosin phosphatase is critical for EGCG-induced cell growth inhibition through 67LR.

It is currently still unclear how EGCG exactly reduces MYPT1 phosphorylation at Thr-696 through 67LR. Most recently, we have identified eukaryotic translation elongation factor 1A (eEF1A) as a component responsible for the antiproliferative activity of EGCG [12]. Intriguingly, it has been reported that eEF1A binds to MYPT1 [26] and is localized in the cleavage furrow during cytokinesis [27], suggesting its involvement in cell division process. Although we have reported that eEF1A was involved in the signaling pathway mediated after binding of EGCG to 67LR, further studies are needed to define the role of eEF1A in decrease of MYPT1 phosphorylation at Thr-696. Future investigation on this matter and the *in vivo* role of 67LR, eEF1A and MYPT1 in EGCG-induced cell growth inhibition may provide new therapeutic approaches for the treatment of cancer.

Acknowledgment

This work was supported in part by Grants-in-Aid for Scientific Research (A) (to H.T.).

References

[1] C.S. Yang, P. Maliakal, X. Meng, Inhibition of carcinogenesis by tea, Annu. Rev. Pharmacol. Toxicol. 42 (2002) 25–54.
[2] C.S. Yang, S. Sang, J.D. Lambert, Z. Hou, J. Ju, G. Lu, Possible mechanisms of the cancer-preventive activities of green tea, Mol. Nutr. Food Res. 50 (2006) 170–175.
[3] S. Bettuzzi, M. Brausi, F. Rizzi, G. Castagnetti, G. Peracchia, A. Corti, Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study, Cancer Res. 66 (2006) 1234–1240.
[4] N. Khan, F. Afaq, M. Saleem, N. Ahmad, H. Mukhtar, Targeting multiple signaling pathways by green tea polyphenol (−)-epigallocatechin-3-gallate , Cancer Res. 66 (2006) 2500–2505.
[5] H. Tachibana, K. Koga, Y. Fujimura, K. Yamada, A receptor for green tea polyphenol EGCG, Nat. Struct. Mol. Biol. 11 (2004) 380–381.
[6] M.A. Shammas, P. Neri, H. Koley, R.B. Batchu, R.C. Bertheau, V. Munshi, R. Prabhala, M. Fulciniti, Y.T. Tai, S.P. Treon, R.K. Goyal, K.C. Anderson, N.C. Munshi, Specific killing of multiple myeloma cells by (−)-epigallocatechin-3-gallate extracted from green tea: biologic activity and therapeutic implications, Blood 108 (2006) 2804–2810.
[7] F. Matsumura, Regulation of myosin II during cytokinesis in higher eukaryotes, Trends Cell Biol. 15 (2005) 371–377.
[8] A.P. Somlyo, A.V. Somlyo, Ca^{2+} sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase, Physiol. Rev. 83 (2003) 1325–1358.
[9] D.J. Hartshorne, M. Ito, F. Erdödi, Role of protein phosphatase type 1 in contractile functions: myosin phosphatase, J. Biol. Chem. 279 (2004) 37211–37214.
[10] D. Umeda, H. Tachibana, K. Yamada, Epigallocatechin-3-*O*-gallate disrupts stress fibers and the contractile ring by reducing myosin regulatory light chain phosphorylation mediated through the target molecule 67 kDa laminin receptor, Biochem. Biophys. Res. Commun. 333 (2005) 628–635.
[11] Y. Fujimura, D. Umeda, Y. Kiyohara, Y. Sunada, K. Yamada, H. Tachibana, The involvement of the 67 kDa laminin receptor-mediated modulation of cytoskeleton in the degranulation inhibition induced by epigallocatechin-3-*O*-gallate, Biochem. Biophys. Res. Commun. 348 (2006) 524–531.
[12] D. Umeda, S. Yano, K. Yamada, H. Tachibana, Green tea polyphenol epigallocatechin-3-gallate (EGCG) signaling pathway through 67-kDa laminin receptor, J. Biol. Chem. 283 (2008) 3050–3058.
[13] E.E. Calle, C. Rodriguez, K. Walker-Thurmond, M.J. Thun, Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults, N. Engl. J. Med. 348 (2003) 1625–1638.
[14] J.M. Yuan, Y.T. Gao, C.S. Yang, M.C. Yu, Urinary biomarkers of tea polyphenols and risk of colorectal cancer in the Shanghai Cohort Study, Int. J. Cancer 120 (2007) 1344–1350.
[15] F. Radtke, H. Clevers, Self-renewal and cancer of the gut: two sides of a coin, Science 307 (2005) 1904–1909.
[16] S. Orsulic, O. Huber, H. Aberle, S. Arnold, R. Kemler, E-cadherin binding prevents beta-catenin nuclear localization and beta-catenin/LEF-1-mediated transactivation, J. Cell Sci. 112 (1999) 1237–1245.
[17] W.M. Dashwood, O. Carter, M. Al-Fageeh, Q. Li, R.H. Dashwood, Lysosomal trafficking of beta-catenin induced by the tea polyphenol epigallocatechin-3-gallate, Mutat. Res. 591 (2005) 161–172.
[18] J. Ju, J. Hong, J.N. Zhou, Z. Pan, M. Bose, J. Liao, G.Y. Yang, Y.Y. Liu, Z. Hou, Y. Lin, J. Ma, W.J. Shih, A.M. Carothers, C.S. Yang, Inhibition of intestinal tumorigenesis in $Apc^{min/+}$ mice by (−)-epigallocatechin-3-gallate, the major catechin in green tea, Cancer Res. 65 (2005) 10623–10631.
[19] M. Shimizu, A. Deguchi, J.T. Lim, H. Moriwaki, L. Kopelovich, I.B. Weinstein, (−)-Epigallocatechin gallate and polyphenon E inhibit growth and activation of the epidermal growth factor receptor and human epidermal growth factor receptor-2 signaling pathways in human colon cancer cells, Clin. Cancer Res. 11 (2005) 2735–2746.
[20] S. Pelengaris, M. Khan, G. Evan, C-Myc: more than just a matter of life and death, Nat. Rev. Cancer 2 (2002) 764–776.
[21] Y. Kawano, Y. Fukata, N. Oshiro, M. Amano, T. Nakamura, M. Ito, F. Matsumura, M. Inagaki, K. Kaibuchi, Phosphorylation of myosin binding subunit (MBS) of myosin phosphatase by Rho-kinase *in vivo*, J. Cell Biol. 147 (1999) 1023–1037.
[22] T. Yokoyama, H. Goto, I. Izawa, H. Mizutani, M. Inagaki, Aurora-B and Rho-kinase/ROCK, the two cleavage furrow kinases, independently regulate the progression of cytokinesis: possible existence of a novel cleavage furrow kinase phosphorylates ezrin/radixin/moesin (ERM), Genes Cells 10 (2005) 127–137.
[23] A.J. Piekny, P.E. Mains, Rho-binding kinase (LET-502) and myosin phosphatase (MEL-11) regulate cytokinesis in the early *Caenorhabditis elegans* embryo, J. Cell Sci. 115 (2002) 2271–2282.
[24] R. Okamoto, M. Ito, N. Suzuki, M. Kongo, N. Moriki, H. Saito, H. Tsumura, K. Imanaka-Yoshida, K. Kimura, A. Mizoguchi, D.J. Hartshorne, T. Nakano, The targeted disruption of the MYPT1 gene results in embryonic lethality, Transgenic Res. 14 (2005) 337–340.
[25] F. Matsumura, D.J. Hartshorne, Myosin phosphatase target subunit: many roles in cell function, Biochem. Biophys. Res. Commun. 369 (2008) 149–156.
[26] T. Izawa, Y. Fukata, T. Kimura, A. Iwamatsu, K. Dohi, K. Kaibuchi, Elongation factor-1 alpha is a novel substrate of rho-associated kinase, Biochem. Biophys. Res. Commun. 278 (2000) 72–78.
[27] O. Numata, Y. Kurasawa, K. Gonda, Y. Watanabe, Tetrahymena elongation factor-1 alpha is localized with calmodulin in the division furrow, J. Biochem. 127 (2000) 51–56.