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Inhibition of Oral Epithelial Cell Growth *in vitro* by Epigallocatechin-3-gallate; Its Modulation by Serum and Antioxidant Enzymes

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Abstract The most abundant tea catechin, epigallocatechin-3-gallate (EGCG), has been reported to inhibit cell proliferation and induce apoptosis in many types of cancer cells. In the present study, effects of EGCG on the growth of oral epithelial cells including CAL-27 oral squamous carcinoma cells and dysplastic oral keratinocytes (DOK) were investigated. EGCG inhibited growth of CAL-27 cells and DOK with IC_{50} of 14.4-21.0 and 5.8-14.2 μM after 24 and 48 hr incubation, respectively. EGCG was significantly less effective in inhibiting DOK growth. The effects of EGCG, however, were dramatically less pronounced in the presence of superoxide dismutase (SOD) and catalase. Inhibitory effects of EGCG on CAL-27 cell growth were also much less pronounced in the presence of fetal bovine serum (FBS). EGCG induced caspase-3 activation in both CAL-27 and DOK cells in a serum free condition without SOD/catalase; in the presence of 10% FBS and SOD/catalase, EGCG, even at 100 μM, did not affect cell growth. The present results indicate that EGCG inhibited oral cell growth with higher potency to more malignant CAL-27 cells than DOK, and the effects were markedly altered by SOD/catalase and serum content in media.

Keywords: epigallocatechin-3-gallate (EGCG), oral cell, fetal bovine serum, superoxide dismutase, reactive oxygen species

Introduction

Tea (*Camellia sinensis*) is one of the most widely consumed beverages worldwide. Several epidemiological studies have reported that consumption of tea is inversely related to incidence of various types of cancer (1,2). Studies using animal models have also demonstrated that the administration of tea have shown tumor suppressive effects at many organ sites (3-6). Epigallocatechin-3-gallate (EGCG) is the most abundant constituent, and is believed to have the most potent anti-carcinogenic activity in green tea. Previous studies have reported the anti-carcinogenic mechanisms of action for EGCG including induction of cancer cell apoptosis, inhibition of transcription factor activation including AP-1, NFκB, and β-catenin, inhibition of growth factor signaling, inhibition of topoisomerase, telomerase, and the proteasome activity, and modulation of arachidonic acid metabolism (6-12).

Many biological effects of EGCG have been observed in studies using cell culture system with 10-100 μM of EGCG (13). Pharmacokinetic studies, however, have shown that the peak plasma concentration of EGCG *in vivo* is usually submicromolar level (14,15). Several cellular events including induction of apoptosis and modulation of certain gene expressions by EGCG have been suggested to be mediated by reactive oxygen species (ROS) generated during EGCG autooxidation (16,17). Such high concentrations of available EGCG, occurrence of EGCG autooxidation, as well as ROS generation are, however, still questionable in most tissues *in vivo*. Accordingly, it is debatable whether these effects observed in a cell culture system allowing EGCG autooxidation and ROS generation from considerably high concentration of EGCG are physiologically reliable.

Oral cancer is the most common head and neck malignancy and one of the most common types of cancer especially in the developing countries (18). In recent decades, the incidence and mortality rates have also been increasing in the Western countries (19,20). Exposure to cigarette smoke and alcohol is considered as major risk factors of this cancer worldwide (18,21). There is increasing evidence that high consumption of fruits and vegetables could be related to reduced risk of oral cancer (21,22). Recently a prospective study from Japan reported that green tea consumption showed a tendency for a reduced oral cancer risk in women (23).

Oral epithelium has different features from most other internal tissues, in that it can be directly exposed to high concentration of EGCG without systemic delivery and to ROS generated by EGCG. In order to understand physiologically-reliable EGCG actions in oral epithelium, the effects of serum and ROS in modulating EGCG activities was investigated. In the present study, effects of EGCG on the growth of CAL-27 oral squamous carcinoma cells and pre-malignant dysplastic oral keratinocytes (DOK) were analyzed. Modulation of EGCG effects in the presence of different serum conditions as well as in the presence of antioxidant enzymes including superoxide dismutase (SOD) and catalase was also investigated.

Materials and Methods

Chemicals and cell lines Pure epigallocatechin-3-gallate (EGCG) (>99% purity) was obtained from Mitsui Norin Co., Ltd. (Shizuoka, Japan). The structure of EGCG is shown in Fig. 1. CAL-27 human oral squamous carcinoma

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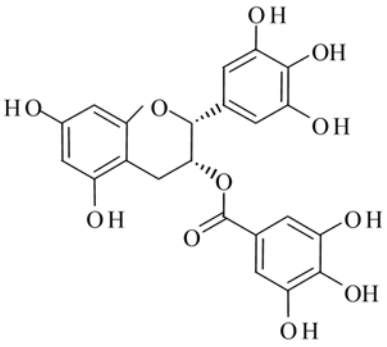

Fig. 1. A structure of epigallocatechin-3-gallate.

cells, human dysplastic oral keratinocytes (DOK), HT-29 human colon adenomcarcinoma, and HeLa human cervical carcinoma cells were obtained from American Type Culture Collection (Manassas, VA, USA). CAL-27 and HeLa cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 unit/mL penicillin, and 0.1 mg/mL streptomycin, and DOK were grown in the same medium containing 100 ng/mL dexamethasone. The cells were kept at 37°C in 95% humidity and 5% CO_2. SOD (from bovine erythrocyte), catalase (from bovine liver), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and all other chemicals were from Sigma-Aldrich (St. Louis, MO, USA).

Analysis of cell viability Effects of EGCG on oral cell growth were analyzed by measurement of cell viability using MTT. Approximately 3,000 cells were plated at a 96-well plate in a growth medium (DMEM containing 10% FBS and 1% penicillin and streptomycin). Each cell was treated the next day with EGCG at the indicated conditions and concentrations as described in figures and legends. After incubation for 24 and 48 hr, the compound-containing medium was removed, and a medium containing 0.5 mg/mL MTT was added to each well. The cells were further incubated at 37°C for 1 hr. The medium was then removed and replaced by 100 μL of dimethyl sulfoxide (DMSO), and the absorbance was measured at 550 nm using a microplate reader (Spectra Max 250; Molecular Device, Sunnywale, CA, USA).

Analysis of caspase-1 and -3 activities CAL-27 and DOK cells were plated in a 6-well plate with a growth medium for 24 hr. The cells were then incubated with 20 or 50 μM of EGCG or vehicle (0.1% DMSO) under different conditions for 24 hr. The cells were then lysed and centrifuged at 10,000×g for 15 min. Caspase-1 and -3 activities were analyzed using caspase fluorescent assay kit according to the manufacturer's instruction (Promega, Madison, WI, USA). In brief, the supernatant containing approximately 50 μg protein was added to Ac-YVAD-7-amino-4-methylcoumarine or Ac-DEVD-7-amino-4-methylcoumarine and caspase-1 or -3 substrate, respectively. Released fluorescent product, 7-amino-4-methylcourmarine, in 100 μL total reaction volume was detected using a fluorescence microplate reader (Tecan, Männedorf, Switzerland).

Data analysis Statistical significance was evaluated using the Student's *t*-test. IC_{50} (concentration that caused 50% inhibition) values were calculated using the corresponding linear regression equations determined based on the points within a linear range.

Results and Discussion

Effects of EGCG on oral cell growth and its modulation by SOD/catalase Effects of EGCG on the growth of CAL-27 oral squamous carcinoma cells and DOK were investigated using MTT assay. EGCG inhibited CAL-27 cell growth with IC_{50} of 14.4 and 5.8 μM after 24 and 48 hr incubation, respectively (Fig. 2A and 2D). The effect of EGCG on CAL-27 cells was significantly less pronounced in the presence of SOD and catalase (5 and 30 unit/mL, respectively) after 24 hr incubation. At 48 hr incubation with higher than 10 μM EGCG, most CAL-27 cells died regardless of SOD/catalase. DOK were much less sensitive to EGCG. IC_{50} values for DOK after 24 and 48 hr incubation were 1.5 and 2.4 fold higher than those for CAL-27 cells, respectively (Fig. 2B and 2D). In the presence of SOD/catalase, EGCG was also much less effective in inhibiting DOK cell growth. EGCG (10-20 μM) rather slightly increased DOK viability measured at 24 hr incubation. Similar results were also observed in other types of cells. Growth inhibitory effects of EGCG on HT-29 colon adenocarcinoma and HeLa cervical carcinoma cells were also significantly reduced in the presence of SOD/catalase (Fig. 2C). Several previous reports indicated that EGCG was more effective in inhibiting growth of cancer cells than normal cells including oral epithelial cells (24,25). In the present study, EGCG also showed significantly more potent effect in inhibiting CAL-27 cancer cell growth than DOK that was established from the human dorsal tongue epithelium showing a partially transformed and non-malignant phenotype. The results suggest that EGCG is more effective in inhibiting growth of more malignant cells and this selective effect could be a desirable aspect as a chemopreventive agent. To elucidate the mechanisms for decreasing cell viability by EGCG, it was investigated whether EGCG induced apoptotic events in CAL-27 and DOK cells by analyzing caspase activation. In the absence of SOD/catalase, 50 μM of EGCG increased caspase-3 activity of CAL-27 and DOK cells significantly by 106 and 40%, respectively, whereas caspase-1 activity was not increased (Fig. 3). In the presence of SOD/catalase, EGCG did not affect caspase-1 and -3 activities of DOK, but gradually increased caspase-1 and -3 activities in CAL-27 cells (Fig. 3).

It is reported that EGCG was easily oxidized and generated ROS including H_2O_2 during its autooxidation process in cell culture condition (26). Protection of ROS formation from EGCG could effectively rescue several types of cancer cells from apoptosis (16,27,28). The present result is consistent to the previous reports by that effects of EGCG were much less pronounced in inhibiting growth of both cells in the presence of SOD/catalase. The property of EGCG is quite different from that of curcumin, another well known chemopreventive agent, whose effect on cell growth was significantly enhanced in the presence of SOD and non-thiol antioxidants (29). SOD can stabilize both

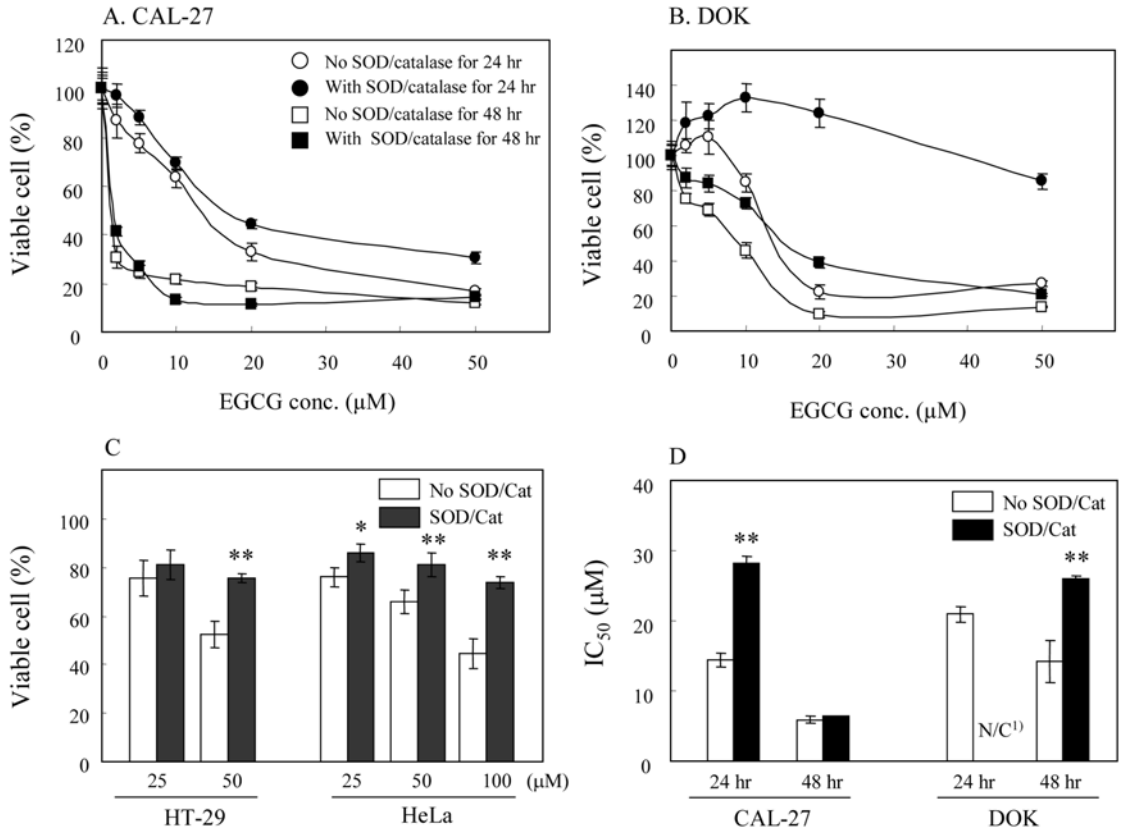

Fig. 2. Effects of EGCG on the growth of CAL-27 (A), DOK (B), HT-29 and HeLa cells (C) in the presence or absence of SOD/catalase in a serum free condition, and changes in the IC_{50} values of EGCG by SOD/catalase (D). Each value represents the mean±SD (n=8). *,**Significantly different from its corresponding control according to Student's t-test (*p<0.05, **p<0.01). [1)]IC_{50} value not calculated (in D).

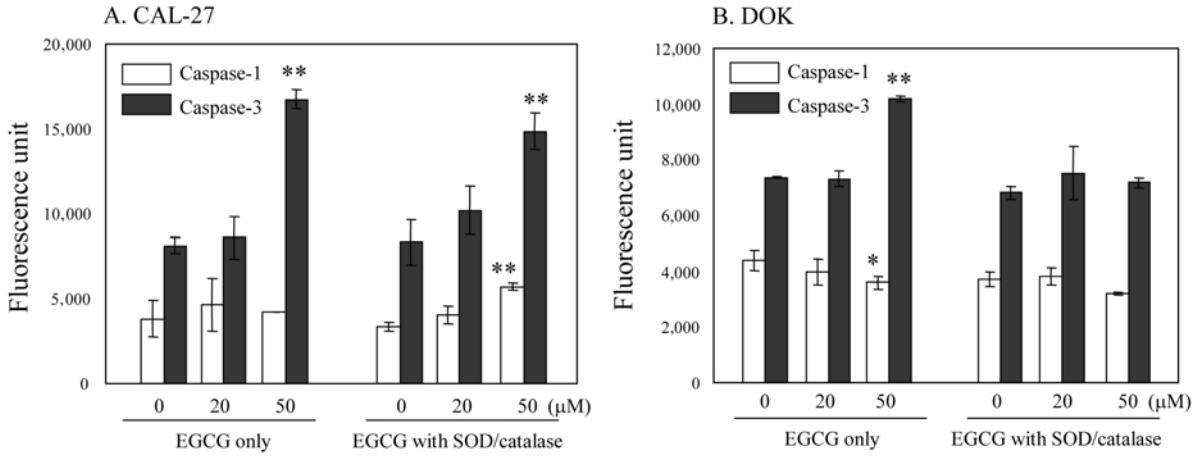

Fig. 3. Effects of EGCG on activation of capase-1 and -3 in the presence or absence of SOD/catalase in a serum free condition. CAL-27 (A) and DOK (B) cells were treated with vehicle (0.1% DMSO), 20 or 50 μM of EGCG for 24 hr. Each value represents the mean±SD (n=3). *,**Significantly different from its corresponding control according to Student's t-test (*p<0.05, **p<0.01).

EGCG and curcumin in cell culture system, and stabilized curcumin seems more effective (29,30). In our experimental condition, protection of EGCG autooxidation by SOD interferes with formation of ROS and possible leaking H_2O_2 (possible also due to SOD action) can also be captured by catalase. Significantly decreased EGCG activity in this system suggests that ROS generated by EGCG play an important role in inhibiting oral cell growth or inducing cell death.

There is no clear evidence for formation of ROS by EGCG and occurrence of EGCG autooxidation in internal body. It may be because oxygen level is much lower in most organs and tissues than in cell culture condition, and cells *in vivo* are endowed with various antioxidants and enzymes that protect oxidative stress. Alternatively, it was reported that high concentration of catechins remained in

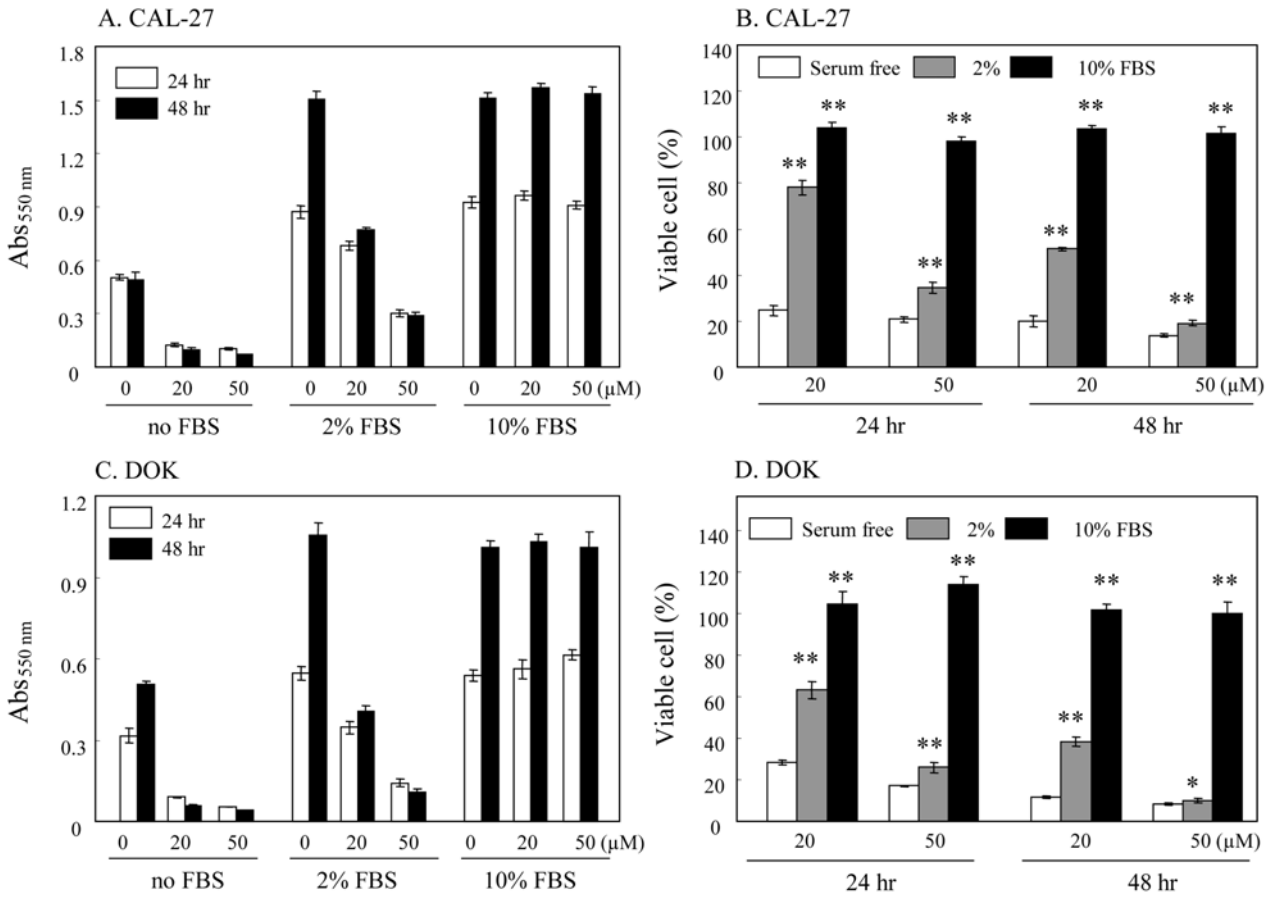

Fig. 4. Effect of EGCG on oral cell growth in different serum conditions. CAL-27 (A and B) and DOK (C and D) cells were incubated with EGCG in a serum free condition or in a medium containing 2 or 10% FBS for 24 and 48 hr. Each value represents the mean±SD (n=8). *,**Significantly different from its corresponding control according to Student's t-test (*p<0.05, **p<0.01).

oral cavity and generated H_2O_2 in saliva after holding or chewing tea leaves (31). Since oral cavity is often directly exposed to atmospheric oxygen, the actions of EGCG found in cell culture system seems to be more reliable in oral epithelium than other internal tissues.

Modulation of bioactivity of EGCG by serum and SOD/catalase The effects of EGCG on oral cell growth were analyzed in different serum conditions. In serum free condition, the growth of CAL-27 cells was not progressed between at 24 and 48 hr incubation (Fig. 4A). DOK, however, continued growing without serum and showed more population at 48 hr than 24 hr (Fig. 4C). In the presence of FBS, growth of both oral cells continued to more extent. The growth rate, however, was not different under both conditions containing 2 and 10% FBS (Fig. 4A and 4C). The inhibitory effects of EGCG on oral cell growth were much less pronounced if FBS was added to the medium. The IC_{50} of EGCG for CAL-27 cell growth dramatically increased according to increase of serum content (after 24 hr incubation, 39.9 and 69.9 μM in the presence of 2 and 10% FBS, respectively, for CAL-27 cells; 41.3 and 75.2 μM in the presence of 2 and 10% FBS, respectively, for DOK) (Fig. 4B and 4D, Table 1). Although growth rate of cells was not significantly altered, effects of EGCG were significantly more potent in medium containing 2% FBS than in one containing 10% FBS. Addition of 10% FBS to the medium usually increases the protein content by 5-9 mg/mL. Since many polyphenolic compounds can bind to proteins nonspecifically, EGCG may also bind to serum proteins and not be delivered into cell efficiently.

The effects of EGCG on oral cell growth in the presence of FBS and SOD/catalase were also analyzed (Fig. 5). Since both factors significantly altered cytotoxic properties of EGCG in the present cell culture system, the combined condition resulted in markedly less EGCG potency. Especially in the medium containing 10% FBS and SOD/catalase, EGCG was not effective in inhibiting cell growth even up to 100 μM (Fig. 5B and 5D). In the presence of 10% FBS, EGCG slightly increased caspase-3 activity of CAL-27 cells without SOD/catalase; there was no significant difference of caspase-3 activity of both CAL-27 and DOK cells in the medium containing 10% FBS and SOD/catalase (Fig. 6). Interestingly, increased cell growth by EGCG was found in the presence 10% FBS and SOD/catalase (Fig. 5B and 5D). The growth stimulation seems more prominent in CAL-27 cells as compared with DOK. Although the substantial formation of ROS, and oxidative stress has been associated with cell death, many evidence also have suggested that low concentration of ROS are importantly involved in signals to stimulate cell proliferation (32,33). ROS produced by EGCG could be removed by SOD/

Table 1. The calculated IC_{50} values (μM) of EGCG for inhibiting oral cell growth in different culture conditions

Cell	Condition	Without SOD/catalase[1)]		With SOD/catalase	
		24 hr	48 hr	24 hr	48 hr
CAL-27	Serum free	14.4±1.1	5.8±0.5	28.2±1.09	6.3±0.2
	2% FBS	39.9±1.3	21.8±0.7	41.3±3.0	27.9±1.2
	10% FBS	69.9±1.3	68.4±2.1	>>100	>>100
DOK	Serum free	20.9±1.1	14.2±3.0	>>50	25.9±0.4
	2% FBS	41.3±3.0	27.9±1.2	>>100	99.5±5.1
	10% FBS	75.2±1.8	69.3±2.1	>>100	>>100

[1)]Each value represents the mean±SD (n=8).

catalase added in media. The exogenous SOD/catalase, however, might not be accessible into cells; EGCG could generate ROS inside cells and they are likely responsible for its growth stimulatory effect.

The present results indicated that effects of EGCG on cell growth were largely dependent on the presence of serum and SOD/catalase in the media. It is considered that EGCG mainly induces cell death by generating ROS by observing that SOD/catalase effectively rescued cells from EGCG-induced cell death. Our preliminary results indicate that the amount of ROS generated by EGCG in media is not much different regardless of serum content. The inhibitory effect of EGCG on cell growth, however, became markedly less pronounced according to increasing FBS concentration. It is suggested that FBS interferes with EGCG delivery into cells due to its binding properties to serum proteins (34) and cellular level of EGCG is also important for inhibiting oral cell growth. Taken together, EGCG itself in cells as well as ROS generated outside cells might play cooperative roles in modulating cell growth.

The IC_{50} values of EGCG effects on the oral cells in different conditions are summarized in Table 1. The

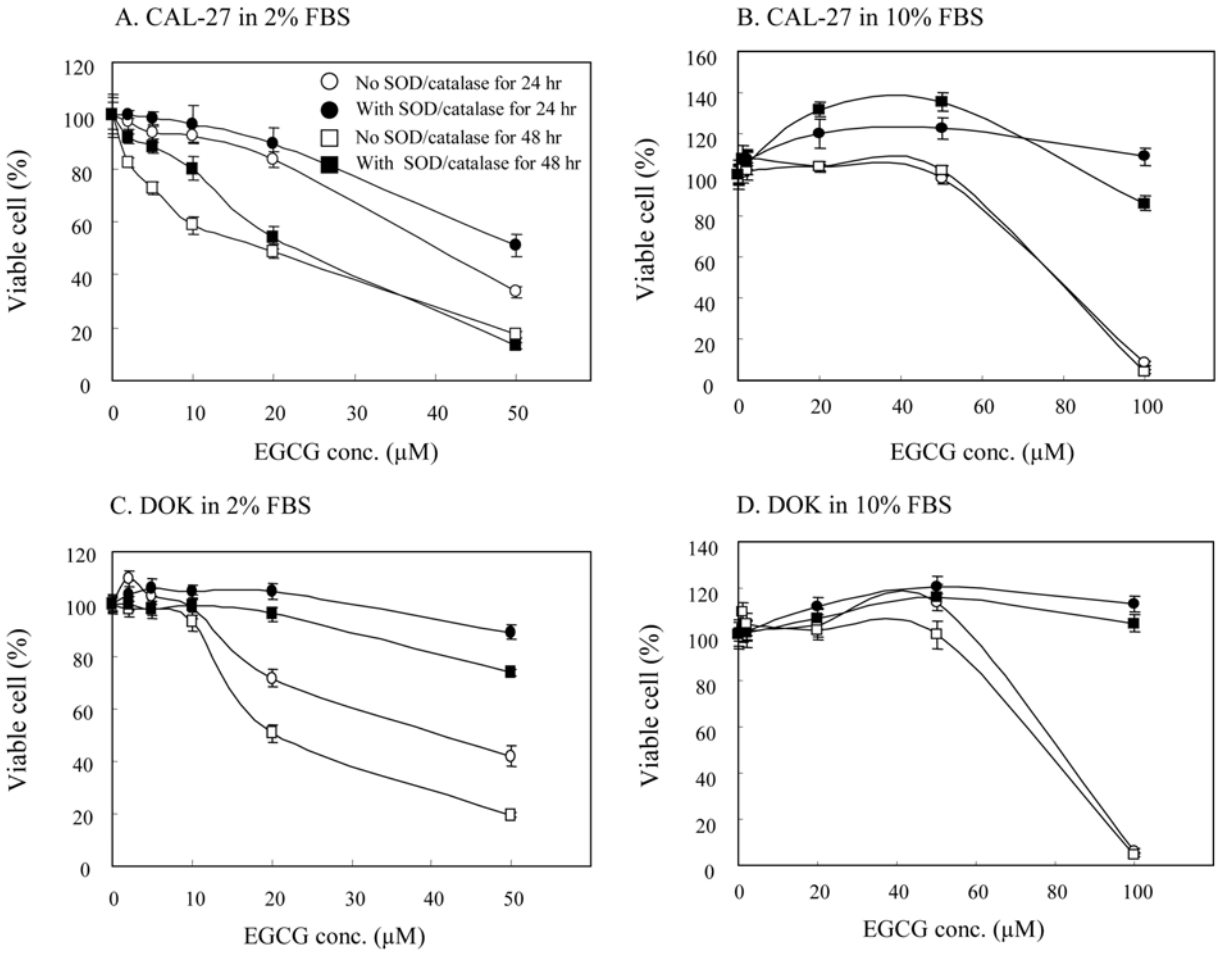

Fig. 5. Changes in EGCG effects on oral cell growth by FBS and SOD/catalase. CAL-27 (A and B) and DOK (C and D) cells were treated with EGCG in the presence (filled) or absence (hollowed) of SOD/catalase in a medium containing 2 (A and C) or 10% (B and D) FBS for 24 (circle) or 48 hr (square). Each value represents the mean±SD (n=8).

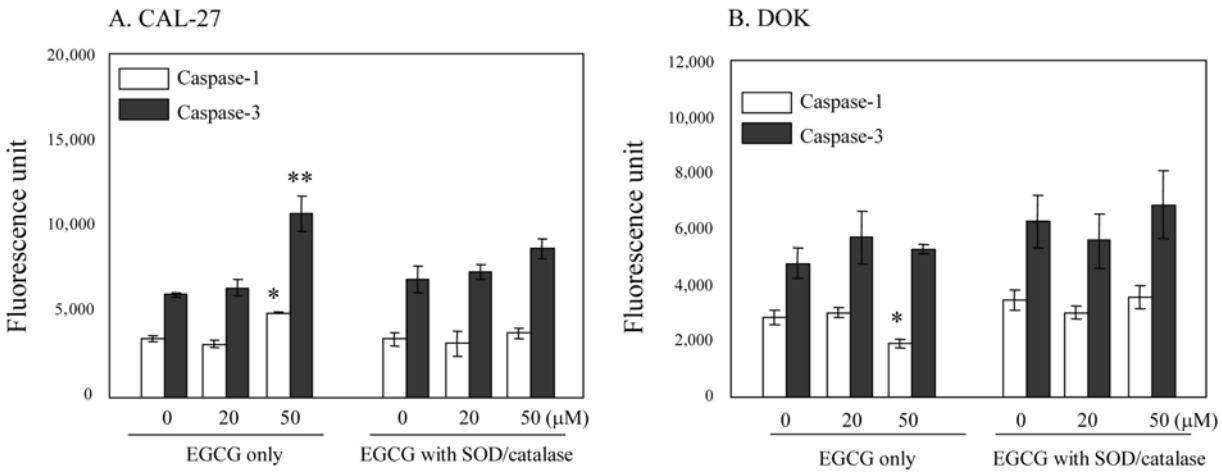

Fig. 6. Effects of EGCG on activation of capase-1 and -3 in the presence or absence of SOD/catalase in a medium containing 10% FBS. CAL-27 (A) and DOK (B) cells were treated with vehicle (0.1% DMSO), 20, or 50 μM of EGCG in a medium containing 10% FBS for 24 hr. Each value represents the mean±SD (n=3). *,**Significantly different from its corresponding control according to Student's t-test (*p<0.05, **p<0.01).

present results indicate that EGCG inhibited oral cell growth effectively without FBS and SOD/catalase. Oral epithelium is often exposed freely to air, which provides a condition for EGCG autooxidation (accordingly ROS generation), and is exposed to EGCG administered directly without delivering through systemic circulations. The present study suggests that oral epithelium is a favorable target tissue where EGCG shows its cancer preventive actions and oral cell system can be a desirable model for evaluating chemopreventive mechanisms of EGCG. The precise anti-carcinogenic mechanism of EGCG in the oral epithelium needs to be explored further.

Acknowledgments

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References

1. Blot WJ, Chow WH, McLaughlin JK. Tea and cancer-a review of the epidemiological evidences. Eur. J. Cancer Prev. 5: 425-438 (1996)
2. Liu J, Xing J, Fei Y. Green tea (*Camellia sinensis*) and cancer prevention: A systematic review of randomized trials and epidemiological studies. Chin. Med. 3: 12 (2008)
3. Katiyar SK, Agarwal R, Mukhtar H. Protective effects of green tea polyphenols administered by oral intubation against chemical carcinogen-induced forestomach and pulmonary neoplasia in A/J mice. Cancer Lett. 73: 167-172 (1993)
4. Yang CS, Liao J, Yang GY, Lu G. Inhibition of lung tumorigenesis by tea. Exp. Lung Res. 31: 135-144 (2005)
5. Gupta S, Hastak K, Ahmad N, Lewin JS, Mukhtar H. Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols. P. Natl. Acad. Sci. USA 98: 10350-10355 (2001)
6. Ju J, Hong J, Zhou JN, Pan Z, Bose M, Liao J, Yang GY, Liu YY, Hou Z, Lin Y, Ma J, Shih WJ, Carothers AM, Yang CS. Inhibition of intestinal tumorigenesis in $Apc^{min/+}$ mice by (-)-epigallocatechin-3-gallate, the major catechin in green tea. Cancer Res. 65: 10623-10631 (2005)
7. Chung JY, Park, JO, Phyu H, Dong Z, Yang CS. Mechanisms of inhibition of the Ras-MAP kinase signaling pathway in 30.7b Ras 12 cells by tea polyphenols (-)-epigallocatechin-3-gallate and theaflavin-3,3'-digallate. FASEB J. 15: 2022-2024 (2001)
8. Shimizu M, Deguchi A, Hara Y, Moriwaki H, Weinstein IB. EGCG inhibits activation of the insulin-like growth factor-1 receptor in human colon cancer cells. Biochem. Bioph. Res. Co. 334: 947-953 (2005)
9. Berger SJ, Gupta S, Belfi CA, Gosky DM, Mukhtar H. Green tea constituent (-)-epigallocatechin-3-gallate inhibits topoisomerase I activity in human colon carcinoma cells. Biochem. Bioph. Res. Co. 288: 101-105 (2001)
10. Nam S, Smith DM, Dou QP. Ester bond-containing tea polyphenols potently inhibit proteasome activity *in vitro* and *in vivo*. J. Biol. Chem. 276: 13322-13330 (2001)
11. Naasani I, Oh-Hashi F, Oh-Hara T, Feng WY, Johnston J, Chan K, Tsuruo T. Blocking telomerase by dietary polyphenols is a major mechanism for limiting the growth of human cancer cells *in vitro* and *in vivo*. Cancer Res. 63: 824-830 (2003)
12. Hong J, Yang CS. Effect of purified green tea catechins on cytosolic phospholipase A_2 and arachidnoic acid release in human gastrointestinal cancer cells. Food Sci. Biotechnol. 15: 799-804 (2006)
13. Lambert, JD, Hong J, Yang GY, Liao J, Yang CS. Inhibition of carcinogenesis by polyphenols: Evidence from laboratory investigations. Am. J. Clin. Nutr. 81: 284S-291S (2005)
14. Chen L, Lee MJ, Li H, Yang CS. Absorption, distribution, elimination of tea polyphenols in rats. Drug Metab. Dispos. 25: 1045-1050 (1997)
15. Lee MJ, Maliakal P, Chen L, Meng X, Bondoc FY, Prabhu S, Lambert G, Mohr S, Yang CS. Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidem. Biomar. 11: 1025-1032 (2002)
16. Yang GY, Liao J, Li C, Chung J, Yurkow EJ, Ho CT, Yang CS. Effect of black and green tea polyphenols on c-jun phosphorylation and H_2O_2 production in transformed and non-transformed human bronchial cell lines: Possible mechanisms of cell growth inhibition and apoptosis induction. Carcinogenesis 21: 2035-2039 (2000)
17. Vittal R, Selvanayagam ZE, Sun Y, Hong J, Liu F, Chin, KV, Yang CS. Gene expression changes induced by green tea polyphenol (-)-epigallocatechin-3-gallate in human bronchial epithelial 21BES cells analyzed by DNA microarray. Mol. Cancer Ther. 3: 1091-1099 (2004)
18. Vokes EE, Weichselbaum RR, Lippman SM, Hong WK. Head and neck cancer. New Engl. J. Med. 328: 184-194 (1993)
19. La Vecchia C, Lucchini F, Negri E, Levi F. Trends in oral cancer

mortality in Europe. Oral Oncol. 40: 433-439 (2004)
20. Kingsley K, O'Malley S, Ditmyer M, Chino M. Analysis of oral cancer epidemiology in the US reveals state-specific trends: Implications for oral cancer prevention. BMC Public Health 8: 87 (2008)
21. La Vecchia C, Tavani A, Franceschi S, Levi F, Corrao G, Negri E. Epidemiology and prevention of oral cancer. Oral Oncol. 33: 302-312 (1997)
22. Hsu SD, Singh BB, Lewis JB, Borke JL, Dickinson DP, Drake L, Caughman GB, Schuster GS. Chemoprevention of oral cancer by green tea. Gen. Dent. 50: 140-146 (2002)
23. Ide R, Fujino Y, Hoshiyama Y, Mizoue T, Kubo T, Pham TM, Shirane K, Tokui N, Sakata K, Tamakoshi A, Yoshimura T. A prospective study of green tea consumption and oral cancer incidence in Japan. Ann. Epidemiol. 17: 821-826 (2007)
24. Babich H, Krupka ME, Nissim HA, Zuckerbraun HL. Differential *in vitro* cytotoxicity of (-)-epicatechin gallate (ECG) to cancer and normal cells from the human oral cavity. Toxicol. In Vitro 19: 231-242 (2005)
25. Mohan KV, Gunasekaran P, Varalakshmi E, Hara Y, Nagini S. *In vitro* evaluation of the anticancer effect of lactoferrin and tea polyphenol combination on oral carcinoma cells. Cell Biol. Int. 31: 599-608 (2007)
26. Hong J, Lu H, Meng X, Ryu JH, Hara Y, Yang CS. Stability, cellular uptake, biotransformation, and efflux of tea polyphenol (-)-epigallocatechin-3-gallate in HT-29 human colon adenocarcinoma cells. Cancer Res. 62: 7241-7246 (2002)
27. Chen C, Shen G, Hebbar V, Hu R, Owuor ED, Kong AN. Epigallocatechin-3-gallate-induced stress signals in HT-29 human colon adenocarcinoma cells. Carcinogenesis 24: 1369-1378 (2003)
28. Nakagawa H, Hasumi K, Woo JT, Nagai K, Wachi M. Generation of hydrogen peroxide primarily contributes to the induction of Fe(II)-dependent apoptosis in Jurkat cells by (-)-epigallocatechin gallate. Carcinogenesis 25: 1567-1574 (2004)
29. Hong J. Curcumin-induced growth inhibitory effects on HeLa cells altered by antioxidant modulators. Food Sci. Biotechnol. 16: 1029-1034 (2007)
30. Hou Z, Sang S, You H, Lee MJ, Hong J, Chin KV, Yang CS. Mechanism of action of (-)-epigallocatechin-3-gallate: Auto-oxidation-dependent inactivation of epidermal growth factor receptor and direct effects on growth inhibition in human esophageal cancer KYSE 150 cells. Cancer Res. 65: 8049-8056 (2005)
31. Lambert JD, Kwon SJ, Hong J, Yang CS. Salivary hydrogen peroxide produced by holding or chewing green tea in the oral cavity. Free Radical Res. 41: 850-3 (2007)
32. Baas AS, Berk BC. Differential activation of mitogen-activated protein kinases by H_2O_2 and O_2- in vascular smooth muscle cells. Circ. Res. 77: 29-36 (1995)
33. Burdick AD, Davis JW, Liu KJ, Hudson LG, Shi H, Monske ML, Burchiel SW. Benzo(a)pyrene quinones increase cell proliferation, generate reactive oxygen species, and transactivate the epidermal growth factor receptor in breast epithelial cells. Cancer Res. 63: 7825-7833 (2003)
34. Maiti TK, Ghosh KS, Dasgupta S. Interaction of (-)-epigallocatechin-3-gallate with human serum albumin: Fluorescence, Fourier transform infrared, circular dichroism, and docking studies. Proteins 64: 355-362 (2006)