REVIEW

Mechanistic issues concerning cancer prevention by tea catechins

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The cancer preventive activities of tea (*Camellia sinensis*, Theaceae) have been demonstrated in animal models for cancers at different organ sites and suggested by some epidemiological studies. Many mechanisms for cancer prevention have been proposed based on studies in cell lines, which demonstrated the modulation of signal transduction and metabolic pathways by (−)-epigallocatechin-3-gallate (EGCG), the most abundant and active polyphenol in green tea. These molecular events may result in cellular changes, such as enhancement of apoptosis, suppression of cell proliferation, and inhibition of angiogenesis. Nevertheless, it is not known whether these are the molecular mechanisms of inhibition of carcinogenesis in animals and humans. This article discusses the key issues involved in extrapolating results from cell line studies to mechanistic information in vivo and in translating animal studies to human cancer prevention.

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1 Introduction

Tea, made from the dried leaves of the plant *Camellia sinensis*, is a widely consumed beverage worldwide. The relationship between tea consumption and cancer has been studied in many earlier epidemiological studies and this topic has been reviewed [1]. Research activities on tea and cancer have intensified since 1980s; the cancer preventive activities have been demonstrated in many different animal models and the results have encouraged additional epidemiological studies. Subsequently, numerous cell line studies have been carried out in an attempt to understand the mechanisms of the anti-cancer actions of tea constituents. Most of the studies have been carried out with green tea and green tea polyphenols. Green tea, mainly consumed in China and Japan, is produced by steaming or pan-frying tea leaves, which inactivates the enzymes and stabilizes tea constituents. When green tea is brewed with hot water, about a third of the solid materials are water-extractable, of which about a third (by dry weight) are catechins. (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epicatechin (EC) are the major polyphenols in green tea, collectively known as catechins. Black tea, the major form of tea consumed in Western countries, India and other parts of the world, is produced by crushing the tea leaves to promote enzymatic oxidation and subsequent polymerization of most of the tea polyphenols to form oligomeric polyphenols (theaflavins) and polymeric polyphenols (thearubigins). These higher molecular weight black tea polyphenols have very low or no bioavailability. Green and black tea also contain 2–5% caffeine in the water-extractable materials,

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Abbreviations: ACF, aberrant crypt foci; **AOM,** azoxymethane; **EC,** (−)-epicatechin; **ECG,** (−)-epicatechin-3-gallate; **EGFR,** epidermal growth factor receptor; **EGC,** (−)-epigallocatechin; **EGCG,** (−)-epigallocatechin-3-gallate; **HGF,** hepatocyte growth factor **HGFR,** hepatocyte growth factor receptor; **IGF,** insulin-like growth factor; **IGFR,** insulin-like growth factor receptor; **LR,** laminin receptor; **MMP,** metalloproteinase; **NNK,** 4-methylnitrosamino-1-(3-pyridyl)-1-butanone; **PPE,** polyphenon E; **ROS,** reactive oxygen species; **RXRα,** retinoid X receptor α; **VEGFA,** vascular endothelial growth factor A

and caffeine has been shown to be an active tea constituent in the prevention of skin and lung cancers in animal models.

The inhibitory activities of tea (mainly green tea) and tea catechins against carcinogenesis at different organ sites have been demonstrated in many animal models [1–6]. Mechanisms of action of tea catechins, especially EGCG, the most abundant and active form of catechin, have been extensively investigated, especially in cell culture systems. It remains to be demonstrated whether these mechanisms are involved in cancer prevention in animal models and humans. This chapter reviews the inhibitory effects of tea and tea catechins against tumorigenesis in animal studies as well as possible mechanisms involved. EGCG is used frequently as an example to illustrate some of the issues concerning mechanistic investigations on catechins. Results from our own laboratory are discussed in more detail to serve as examples. Results from other studies are also discussed to illustrate the challenging issues in integrating in vitro studies to gain an understanding of the mechanisms of cancer prevention by tea in animal models and humans.

2 Inhibition of tumorigenesis in animal models by tea and possible mechanisms involved

Tea extracts and green tea polyphenols have been demonstrated to inhibit tumorigenesis in many animal models, including those for cancers of the oral cavity, esophagus, stomach, small intestine, colon, liver, pancreas, lung, bladder, skin, prostate, and mammary glands. Most of the studies were carriedout with green tea or green tea polyphenol preparations, and some were carried out with pure EGCG, administered through the drinking water or diet. The following is a review on studies of some of the organ sites.

2.1 Inhibition of tumorigenesis in the oral-digestive tract

The systemic bioavailability of gallated catechins, such as EGCG, is a limiting factor for their effectiveness against tumorigenesis in internal organs. The epithelial cells in the digestive tract have the advantage of having direct contact with the catechins that are ingested orally. Inhibitory effects of tea against tumorigenesis in the oral cavity, esophagus, stomach, small intestine, and colon have been shown in more than 30 studies.

Tea preparations have been shown to inhibit carcinogenesis in the oral cavity and esophagus. For example, in our studies, tea preparations were shown to inhibit *N*-nitrosomethylurea (NMU)-induced oral carcinogenesis in a hamster model and *N*-nitrosomethylbenzylamine-induced esophageal carcinogenesis in a rat model [7, 8]. EGCG also inhibited tumorigenesis in rat stomach and forestomach induced by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine [9]. Black tea polyphenols (polyphenon B), given at 0.05% in the diet, also effectively inhibited forestomach tumor formation [10]; this inhibition was associated with increased apoptosis as well as reduced cell proliferation, infiltration, and angiogenesis.

In mouse models, the inhibitory effects of tea and tea polyphenols against intestinal tumorigenesis have been consistently observed in different laboratories [11–17]. For example, we showed that administration of EGCG at 0.02%–0.32% in drinking water dose-dependently inhibited small intestinal tumorigenesis in $Apc^{Min/+}$ mice, while caffeine did not have an inhibitory effect [14]. The inhibition was associated with increased levels of E-cadherin on the plasma membrane, as well as decreased levels of nuclear β-catechin, c-Myc, phospho-Akt, and phospho-Erk in the tumors [14]. Administration of green tea extracts (0.6% in drinking fluid) also inhibited the formation of azoxymethane (AOM)-induced aberrant crypt foci (ACF) in CF-1 mice on a high-fat diet [16]. Similarly, EGCG (0.1% in drinking fluid) administration decreased tumor incidence and the number of tumors per tumor-bearing mouse in AOM-treated CF-1 mice (Yang, C. S. et al., unpublished results). Recently, Shimizu et al. [17] demonstrated the inhibition of AOM-induced ACF formation in male C57BL/KsJ-*db/db* mice by EGCG (0.01 and 0.1% in drinking water) by suppressing the activities of the insulin-like growth factor (IGF)/IGF-1R axis. The elevated levels of IGF-1R, p-IGF-1R, P-GSK-3β, and β-catenin in the colonic mucosa were decreased by treatment with EGCG; also decreased were the plasma levels of IGF-1, insulin, triglyceride, cholesterol, and leptin [17].

On the other hand in mice with colonic inflammation, treatment with green tea polyphenols may not produce beneficial effects. For example, colonic damage (measured as colon-shortening), induced by 2% dextran sulfate sodium (DSS), was enhanced by 0.5 and 1% green tea polyphenols in the diet [18]. We recently examined the effect of dietary (0.1 and 0.3%) EGCG on AOM/DDS-induced colon carcinogenesis in female BALB/cJ and CD-1 mice, and inhibition of carcinogenesis was not observed. With 0.3% EGCG, rectal bleeding and enhanced carcinogenesis were observed. In female CD-1 and C57LB/6 mice, which received DSS (1.5% for 1 wk), but with no AOM, 0.1% dietary EGCG appeared to slightly decrease inflammation, but 0.5% EGCG caused rectal bleeding, enhanced inflammation, and loss of body weight (Guan, F., Ju, J. and Yang, C. S., unpublished results).

In rat models, the effects of tea preparations on colon tumorigenesis have not been consistent [19–25]. This inconsistency in colon carcinogenesis is rather surprising, because the intestine is considered to be a promising site for chemoprevention with polyphenols that have low systemic bioavailability [26, 27]. Orally ingested EGCG has only limited systemic bioavailability, with most of it passing

through the colon; and the absorbed EGCG is excreted mostly through the bile into the intestine. Values of 0.5–23.4 μg EGCG per gram of colonic tissues have been reported [28]. Our recent animal study showed that, after injection with AOM, treatment of rats with Polyphenon E (PPE, a standardized green tea polyphenol preparation containing 65% EGCG, 25% other catechins, and 0.6% caffeine), 0.12 or 0.24% in the diet for 8 wk, dose dependently decreased the total number of ACF per rat by 16.3 or 36.9%, respectively. The inhibitory activity was associated with decreased levels of nuclear β-catenin and cyclin D_1, and increased levels of retinoid X receptor α (RXRα), in the ACF with high-grade dysplasia [29]. After treatment with 0.24% PPE for 34 wk, the incidence of adenocarcinoma decreased from 57 to 23%, and the multiplicity of adenocarcinoma and adenoma decreased by 80 and 45%, respectively (Yang, C. S. et al., unpublished). Loss of expression of RXRα was observed in colonic dysplastic ACF, adenomas, and adenocarcinoma, but the RXRα expression was (partially) retained in PPE-treated rats in these lesions. Volate et al. recently reported similar restoration or de-silencing of RXRα by extracts of green tea through decreased CpG methylation in the promoter region of RXRα [30].

There have been suggestions that mixtures of catechins are more effective cancer preventive agents than pure EGCG, because of synergistic actions between catechins; and that ECG is more effective than EGCG. These hypotheses were tested in *Apc*$^{Min/+}$ mice by comparing the activities of PPE, EGCG, and ECG administered in drinking fluid [15]. The tumor multiplicity was decreased by both PPE (0.12%) and the corresponding amount of dietary EGCG (0.08%) by approximately 50%, and no difference was observed between PPE and EGCG. Because of the large standard errors (individual difference of the mice), the statistical power is a limitation of this model. Additional studies are required to further elucidate whether EGCG can interact with other catechins in PPE to generate synergistic actions to inhibit tumorigenesis. Immunohistochemical (IHC) analysis showed that PPE or EGCG treatment increased apoptosis, suppressed cell proliferation, and decreased the levels of phospho-Akt and nuclear β-catenin [15]. ECG (0.08% in drinking fluid), reduced the tumor multiplicity by 33%, but the results were not statistically significant [15]. We also found that the inhibitory activity of PPE was higher when administered in the diet than in the drinking water, both at 0.12%.

2.2 Inhibition of lung tumorigenesis

The inhibitory effects of tea preparations against lung tumorigenesis have been demonstrated in at least 20 studies using (4-methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) or benzo[a]pyrene as the carcinogen. Administration of extracts or solutions of green tea, black tea, EGCG, EGC, or theaflavins significantly decreased lung tumorigenesis in rats, mice, or hamsters [31–41]. Treatment with extracts of green or black tea for 60 wk also inhibited the spontaneous formation of lung tumors and rhabdomyosarcomas in A/J mice [42]. In addition, oral administration of green tea infusion reduced the number of lung colonies of mouse Lewis lung carcinoma cells in a metastasis model [43].

Chung and coworkers demonstrated that caffeine was effective, but not as effective as an equal concentration of EGCG, in inhibiting lung tumorigenesis in A/J mice [32]. They also showed that the inhibitory effect of caffeine (680 ppm) was similar to that of 2% black tea (containing 680 ppm caffeine) against NNK-induced lung tumorigenesis in rats, suggesting that caffeine was responsible for the inhibitory effect [34]. This conclusion is different from the experiments with A/J mice, which demonstrated the inhibition of lung tumorigenesis by decaffeinated green and black tea preparations [31]. A possible interpretation of this difference is that the systemic bioavailability of tea polyphenols in mice are much higher than in rats, and green tea polyphenols are more bioavailable than the high-molecular-weight polyphenols in black tea [26]. That is, green tea catechins are bioavailable in the mouse lung; whereas most of the black tea polyphenols are not bioavailable in the rat lung, and caffeine is the major lung cancer preventive agent in black tea.

In our recent study, oral administration of 0.5% PPE or 0.044% caffeine in the drinking water, to tumor-bearing A/J mice (induced by a single dose of NNK 20 wk earlier) for 32 wk, was found to inhibit the progression of lung adenomas to adenocarcinomas [39]. IHC analysis showed that PPE and caffeine treatment inhibited cell proliferation in adenocarcinomas, enhanced apoptosis in adenocarcinomas and adenomas, and decreased levels of c-Jun and phospho-Erk1/2. In the normal lung tissues, neither agent had a significant effect on cell proliferation nor apoptosis, suggesting that the effect is selective against tumor cells. These results demonstrate the broad inhibitory activity of tea preparations in the inhibition of lung neoplasia at different stages of carcinogenesis.

In most studies, the effective doses of green tea or tea polyphenol preparations are much higher than the levels of tea constituents obtained from tea consumption in humans. Therefore, we have been looking for agents that can be used together with tea polyphenols to generate synergistic inhibitory actions. We recently demonstrated the synergistic inhibitory action of a combination of PPE and the cholesterol-lowering agent, atorvastatin (an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, trade name Lipitor), against NNK-induced lung carcinogenesis in A/J mice [44]. The synergistic action of this combination was also demonstrated in human lung cancer H1299 and H460 cells. In both the cell lines and the mouse lung tumors, down-regulation of the anti-apoptotic proteins MCL1 and BCL-X_L and induction of apoptosis were associated with the synergistic inhibitory action [44]. The possible synergistic actions

between atorvastatin and tea in humans warrant future investigation.

2.3 Inhibition of urinary bladder carcinogenesis

Green tea has shown efficacy against rat bladder cancer induced by *N*-(4-hydroxybutyl)-*N*-butyl-nitrosame (OH-BBN) [45, 46]. In our study, PPE was administered (100 or 250 mg/kg b.w./day, intragestrically) to rats at 126 days of age (1 wk after the final dose of OH-BBN of a total of 16 doses in 8 wk). Palpable urinary bladder tumor incidence was reduced from 59% (control) to 40% (by 100 mg/kg) or 18% (by 250 mg/kg) [47]. At 8 h after the 250 mg/kg dose, the serum levels of EGCG, EGC, ECG, and EC were 0.061, 0.440, 0.018, and 2.6 μM, respectively; whereas the urinary levels of EGC and EC were very high, at 70 and 94 μM, respectively; as expected, EGCG and ECG were not detected. Urinary ring-fission metabolites, 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone and 5-(3′,4′,5′-dihydroxyphenyl)-γ-valerolactone, were detected at levels of 2.2 and 20 μM, respectively [47]. Since the non-gallated catechins are mainly excreted in the urine, this may provide additional exposure of bladder epithelial cells to catechins, in addition to the catechins from the systemic circulation. In this observed inhibitory effect [47], however, we do not know the relative contributions of EGCG from systemic circulation versus the urinary EGC, EC and their metabolites that have direct contact with the bladder epithelium.

2.4 Inhibition of prostate and mammary tumorigenesis

Administration of a green tea polyphenol infusion (0.1% in drinking water) to transgenic adenocarcinoma of the mouse prostate (TRAMP) mice for 24 wk markedly inhibited prostate cancer development and distant site metastases [48, 49]. The inhibition was associated with decreased cell proliferation and increased apoptosis, decreased IGF-1 level, and restored IGF binding protein 3 (IGF-BP3) in both serum and the dorsolateral prostate [48, 49]. This modulation of IGF-1 and IGF-BP3 levels was associated with reduced levels of phosphotidylinositol 3-kinase (PI3K) as well as phosphorylated forms of Akt, ERK1, and ERK2. The green tea polyphenol treatment also significantly decreased levels of angiogenic and metastatic markers, such as vascular endothelial growth factor A (VEGFA), matrix metalloproteinase (MMP) 2 and MMP 9. These results suggest that the inhibition of the IGF-1 signaling pathway VEGFA and MMPs contribute to the cancer prevention activity of green tea polyphenols. Caporali et al. [50] reported similar inhibitory activity of orally administered green tea catechins on prostate tumor formation in the TRAMP model. Adhami et al. [51] examined the effect of green tea polyphenols (0.1% in drinking water) in the TRAMP mouse model administered at 6, 12, 18, and 28 wk of age and found that the chemoprevention potential decreased with advancing stage of the disease. When green tea polyphenols were initiated at the early stage, IGF-1 and its downstream targets were inhibited [51]. The IGF-1 signaling system appears to be a key target for the inhibition; it is not clear whether tea polyphenols inhibit IGF-1 system by a direct action of tea polyphenols that are present in the prostate or by indirect or systemic actions.

There are at least 10 studies on possible inhibitory effects of tea against mammary tumorigenesis, but the inhibitory activity was not robust. For example, in one study, tea catechin administration in the diet only reduced the volume of mammary tumors [52]; in a second study, green tea was found to increase the latency to first mammary tumor, but did not affect the tumor multiplicity [52]; and in a third study, EGCG failed to suppress mammary tumorigenesis [53]. In our study, even at a high dose of 1000 mg/kg bw/d, i.e. EGCG only slightly decreased mammary tumor incidence and multiplicity (statistically non-significant) in rats treated with NMU [47]. The lack of robust inhibition against mammary tumorigenesis is likely to be due to low bioavailability of tea polyphenols in the mammary tissues (Yang, C. S., unpublished results). The observed inhibitory effect of tea on mammary tumorigenesis may be due to an indirect action of tea. For example, Rogers et al. [54] showed no significant inhibitory effect of black tea administered during the promotion stage of 7,12-dimethylbenz[*a*]anthracene-induced mammary tumorigenesis in rats maintained on AIN76 diet. However, in rats on a high-fat diet, black tea was found to reduce the tumor number and size. The results suggest that black tea may affect fat absorption and metabolism, which subsequently influence estrogen metabolism and mammary tumorigenesis.

Catechins are not the only cancer preventive constituents in tea; caffeine has also been shown to inhibit lung and skin carcinogenesis in mouse models [32, 39, 55]. The mechanism of action of caffeine in the inhibition of skin tumorigenesis has been thoroughly studied and discussed by Conney et al. [55].

3 Activities of green tea polyphenols in vitro

Green tea polyphenols especially EGCG have been studied extensively for their actions against cancer cells and related molecular mechanisms. Most studies have focused on the effects of EGCG on different signaling molecules. However, the chemical mechanisms by which EGCG triggers these molecular changes are often not known. EGCG may exert biological actions through its oxidation–reduction properties or by binding to target molecules, which could be enzymes, receptors, nucleic acids, or plasma membrane. Some of these activities of tea polyphenols are discussed below. Some of the reported activities of EGCG are shown in Fig. 1, and the approximate effective inhibitory concentration of EGCG for each target is also shown.

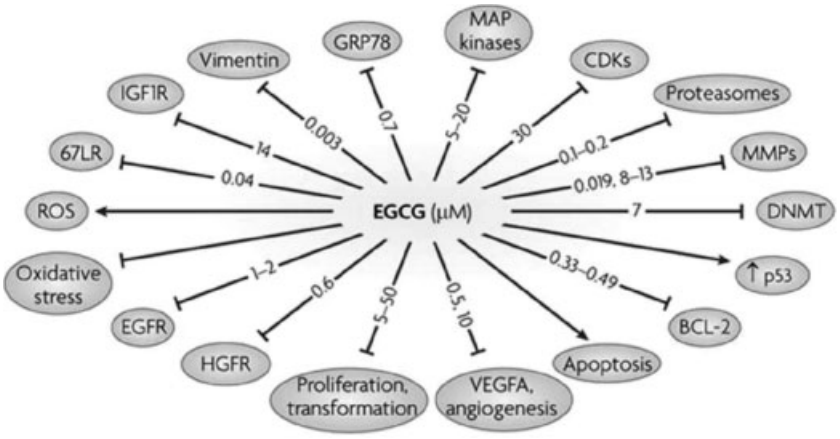

Figure 1. Possible targets for the cancer preventive activity of (−)-epigallocatechin-3-gallate (EGCG). Some of these are direct targets through binding; others are affected indirectly. The reported effective concentrations, in IC_{50}, K_i (inhibition constant) or K_d (dissociation constant) are shown in μM. All these are from studies in vitro. When two values are given, the first value is from cell-free systems and the second value is from studies in cell lines. 67-LR, 67 kDa laminin receptor; CDK, cyclin-dependent kinase; DNMT, DNA methyltransferase; EGFR, epidermal growth factor receptor; GRP78, glucose-related protein 78 kDa; HGFR, hepatocyte growth factor receptor; IGF1R, insulin-like growth factor 1 receptor; MMP, matrix metalloproteinase; ROS, reactive oxygen species; VEGFA, vascular endothelial growth factor A (From [4]).

3.1 Anti-oxidant and pro-oxidative activities of tea catechins

The anti-oxidant activities of tea catechins are well established in vitro [2]. However, such activities in vivo are only observed under circumstances when the animals are under oxidative stress. For example, EGCG administration has been found to decrease the levels of lipid peroxidation and protein carbonylation in old rats, but not in young rats [56, 57]. In experimental models for carcinogenesis, reactive oxygen species (ROS) are induced by the treatment with carcinogens, and EGCG has been demonstrated to reduce, for example, the formation of 8-hydroxydeoxyguanosine (8-OHdG), a well-established marker for oxidatives stress [32]. As endogenously formed ROS are also important in promoting carcinogenesis, tea polyphenols may have important roles in quenching these species at different stages of carcinogenesis, but this action remains to be demonstrated. In human studies, administration of green tea to smokers for 4 wk significantly has been shown to reduce the number of 8-OHdG-positive cells [58].

As can be readily observed in cell culture medium, tea catechins can also be oxidized to generate ROS and cause cell death [59, 60]. After entering the cells, EGCG may also induce the production of ROS in the mitochondria, and the role of these ROS in cancer prevention is under active investigation. In our recent studies, oral administration of EGCG to mice bearing human lung cancer H1299 cells, inhibited xenograft tumor growth, enhanced tumor cell apoptosis, and also produced ROS in tumor cells [61]. The detection of ROS in tumor cells is probably due to the lack of sufficient anti-oxidant enzymes in H1299 cells. It remains to be demonstrated whether the production of ROS mediates the induction of apoptosis. At modest doses (e.g. 0.5% EGCG in the diet), production of ROS and toxicity was not observed in the liver, kidney and other organs [61]. At high doses, e.g. 750 mg/kg, i.e. however, hepatotoxicity and ROS were observed [62]. This toxicity is probably similar to the reported liver toxicity in individuals who took excessive amounts of tea extracts in dietary supplements used for the purpose of weight reduction [63, 64].

ROS may also activate the nuclear factor erythroid 2-related factor 2 (Nrf2) anti-oxidant-responsive element pathway to activate anti-oxidant and detoxifying enzymes [65]. Oral gavage of EGCG (200 mg per kg) to C57BL/6J mice upregulated gene expression of γ-glutamyltransferase, glutamate cysteine ligase and haemoxygenase 1 in the liver and colon [66]. Similarly, treatment of human volunteers for 4 wk with 800 mg PPE per day increased glutathione *S*-transferase-P activity in lymphocytes [67]. Interestingly, in a recent intervention study in a high aflatoxin exposure area in China, supplementation with 500 or 1000 mg green tea polyphenols per day for 3 months increased the median urinary aflatoxin B1-mercapturic acid levels by more than tenfold compared with the baseline [68]. It appears that lower levels of ROS produced by moderate doses of tea polyphenols activate Nrf2 to suppress oxidative stress, but high doses of tea polyphenols can produce high levels of ROS, which induce toxicity. Thus, the biological effect of EGCG depends on the context of the biological systems and the dose of EGCG used.

3.2 Binding to proteins and inhibition of enzymes and receptors

3.2.1 High-affinity binding to proteins

The multiple phenolic groups of EGCG can serve as hydrogen bond donors to many biomolecules; for example

in the binding of EGCG to salivary proline [69]. In our previous work, the binding of EGCG to DNA methyltransferase has been proposed to involve five hydrogen bands based on molecular modeling [70]. Using NMR spectroscopy, EGCG was demonstrated to directly bind to the BH3 pocket of anti-apoptotic Bcl-2 proteins – with inhibition constant (K_i) = 0.33–0.49 μM [71]. However, higher EGCG concentrations (by two orders of magnitude) were needed to induce apoptosis. Using an EGCG–Sepharose 4B column, Dong et al. identified vimentin [72], IGF-1R [73], FYN [74], glucose-regulated protein 78 kDa (GRP78) [75], ZAP70 [76] and Ras-GTPase-activating protein SH3 domain-binding protein 1 (G3BP1) [77] as high-affinity EGCG binding proteins (Fig. 1). All of these proteins were demonstrated to be important for the inhibitory activity of EGCG in certain cell lines. However, much higher EGCG concentrations (than the K_d values) were needed to elicit a cellular response. For example, vimentin bound to EGCG with a K_d of 3.3 nM, and functional studies showed that EGCG inhibited the phosphorylation of vimentin with IC_{50} = 17 μM. The reason for the difference in effective concentrations is not fully understood, and the non-specific binding of EGCG to other proteins may be a contributing factor. Although the discovery of the aforementioned high-affinity EGCG-binding proteins is important, the direct involvement of these proteins in cancer prevention remains to be investigated.

3.2.2 Inhibition of enzyme activities

Tea polyphenols have been shown to bind and inhibit the activities of a variety of enzymes. We previously observed that EGCG, at concentrations of 5–20 μM, inhibited the phosphorylation of JNK (JUN N-terminal kinase), JUN, MEK1, MEK2, ERK1, ERK2, and ELK1 (Ets-like protein 1) in JB6 epidermal cell lines [78]. This inhibition was associated with the inhibition of AP1 transcriptional activity or cell transformation. Additional studies with in vitro kinase assays suggested that EGCG inhibited MEK1 phosphorylation by decreasing its association with the kinase RAF1 [79]. Moreover, EGCG seems to inhibit the phosphorylation of ELK1 by competing with the binding site for ERK1/2 [80]. There were also studies showing that EGCG activated ERK1/2 and other MAP kinases through the generation of ROS. These results could be an in vitro artifact, as EGCG and green tea polyphenols have been shown to inhibit the phosphorylation of JUN and ERK1/2 in lung carcinogenesis models [39].

EGCG has also been reported to inhibit the chymotryptic activity of 20S proteasomes [81]. The difference in effective concentrations in cell-free systems (IC_{50} = 0.09–0.2 μM) and in cell lines (IC_{50} = 1–10 μM) [81] suggests that EGCG may bind non-specifically to proteins or other macromolecules in the cells and therefore elevate the concentrations of EGCG needed to exert its activity in studies with cell lines. EGCG and other catechins have been shown to inhibit the activity of secreted matrix MMP2 and MMP9 was inhibited with IC_{50} values of 8–13 μM [82, 83]. EGCG has also been shown to increase the expression of the tissue inhibitor of MMPs (TIMP1 and TIMP2) at lower concentrations (~1 μM) [83] as well as to inhibit the activation of pro-MMP2 by membrane-type MMP [84]. These activities may contribute to the reported inhibition of metastasis and invasion following treatment of tumor-bearing mice with green tea or EGCG [85]; however, additional in vivo studies are needed to verify this mechanism.

We reported previously that EGCG inhibited DNA methyltransferase (K_i = 7 μM) from KYSE 510 human esophageal cancer cells and this resulted in the demethylation and reactivation of the hypermethylated promoters of the tumor suppressor gene INK4A, retinoic acid receptor-β, as well as the DNA repair genes, MLH1, and methylguanine methyltransferase [70]. Reactivation of some of these genes was also observed in HT29 colon and PC3 prostate cancer cells. Pandey et al. reported that green tea polyphenols reactivated GSTP1 in human prostate cancer cells by causing promoter demethylation and chromatin remodeling [86]. EGCG has also been reported to inhibit dihydrofolate reductase [87], glucose-6-phosphate dehydrogenase [88] glyceraldehyde-3-phosphate dehydrogenase [89], and carbonyl reductase 1 [90]. These enzyme inhibition results are interesting biochemically, but their relevance in cancer prevention remains to be demonstrated.

3.2.3 Inhibition of receptor-dependent signaling pathways

Tea catechins have been shown to affect many receptor related activities and their inhibitory actions against receptor tyrosine kinases have been reviewed recently by Larsen et al. [91]. IGF/IGF-1R axis has been reported to be targets of EGCG in human colon and hepatocellular carcinoma cells [92, 93]. EGCG also inhibits IGF-1R phosphorylation and increases expression of transforming growth factor-β2 (TGFβ2) in human colon cancer SW837 cells [92], which is consistent with our observations in HRAS-transformed human bronchial epithelial 21BES cells [94]. IGF-1R activation can induce cell proliferation and survival, transformation, metastasis, and angiogenesis as well as inhibit apoptosis in different cancer cell lines [95]. As discussed previously, inhibition of the IGF/IGF-1R axis by orally administrated EGCG and green tea polyphenols has been demonstrated in a colon carcinogenesis model in *db/db* obese mice [17] and in the TRAMP mouse model [48]. Inhibition of the IGF/IGF-1R axis could be a very important mechanism for cancer prevention by green tea polyphenols.

Members of the epidermal growth factor receptor (EGFR) family are frequently overexpressed in human cancers and are associated with poor prognosis [96]. Many studies have demonstrated the inhibitory effects of EGCG on the EGFR

signaling pathways [59, 97–100]. Different mechanisms for the inhibition of EGFR have been proposed. These include (i) interfering with the binding of EGF to EGFR and inhibiting EGFR tyrosine kinase activity [97], (ii) altering lipid organization in the plasma membrane (lipid rafts) and inhibiting EGF binding to EGFR [99], and (iii) inducing internalization of EGFR into endosomes [100]. The synergistic action of EGCG and erlotinib, an EGFR tyrosine kinase inhibitor, against head and neck cancer cell growth has been reported [101]. Inhibition of EGFR signaling has also been shown to decrease the production of VEGFA in cancer cells [102]. In addition, EGCG (0.5–10 μM) has been shown to disrupt VEGFA-induced VEGFR2 dimerization in human umbilical vein endothelial cells [103]. EGCG has also been shown to inhibit growth and activation of VEGF/VEGFR axis in human colorectal cancer cells [104]. We have previously observed the downregulation of VEGFA expression and suppression of angiogenesis by treatment with green tea (0.6% green tea solid in drinking fluid) in the NNK-induced lung tumorigenesis model [37]. In a murine gastric tumor model, EGCG (1.5 mg per day per mouse, administered intraperitoneally for 28 days) suppressed VEGFA protein expression and tumor microvessel density [105].

Deregulation of the hepatocyte growth factor (HGF) – HGFR (a receptor tyrosine kinase also known as Met) pathway occurs in several types of human cancers and can lead to increased tumorigenesis and metastasis [106]. HGFR and HGF play key roles in epithelial–mesenchymal transition, which is associated with tumor invasion [107]. It has been shown in MDA-MB-231 cells that the HGF-induced phosphorylation of HGFR and AKT1 was completely blocked by 0.6 μM EGCG, and that cell invasion was significantly decreased by 5 μM EGCG [108]. Larsen et al. have provided evidence for the binding of EGCG to the ATP-binding site of HGFR [109]. In a study with FaDu hypopharyngeal carcinoma cells, 1 μM EGCG prevented HGF-induced motility in an in vitro wound healing assay [110]. Milligan et al. [111] studied the inhibitory effect of EGCG, with or without HGFR or EGFR inhibitors in a series of non-small cell lung cancer cell lines, and found that EGCG was a potent inhibitor of cell proliferation independent of EGFR inhibition. EGCG appeared to be a more effective inhibitor against c-Met than EGFR [111].

Binding of EGCG to the 67-LR (with a K_d value of 0.04 μM) was first observed by Tachibana et al. using a surface plasma on resonance assay [112]. Expression of the metastases-associated 67-LR increased the responsiveness of MCF-7 cells to low micromolar concentrations of EGCG [112]. Furthermore, RNA interference (RNAi)-mediated silencing of 67-LR abrogated EGCG-induced apoptosis in multiple myeloma cells [113]. Recent study by this research group also demonstrated the critical role of 67-LR in mediating anti-inflammation action of EGCG (1 μM) in macrophages [114]. Anti-67-LR antibody treatment or RNAi-mediated silencing of 67-LR resulted in abrogation of the inhibitor action of EGCG on lipopolysaccharide-induced activation of TLR4 and downstream signaling of inflammation.

3.3 Other mechanism

3.3.1 Binding to lipids and nucleic acids

The possibility that EGCG may alter lipid organization in the plasma membrane (lipid rafts) and affect protein distribution and receptor functions has been proposed for the inhibition of the functions of EGFR [99], c-Met [115], and 67-LR [116]. Although interesting, it remains to be determined whether the effects occur in normal cell, whether EGCG also alters the lipid rafts of cancer cells in vivo, and what concentration of EGCG is required to exert a desirable effect in vivo.

Based on the physical binding of EGCG to nucleic acids, it has been suggested that DNA and RNA can also be targets of action of green tea catechins [117]. However, the relevance of this proposed binding depends on whether the catechins can bind selectively to specific nucleic acid species in cancer or premalignant cells without affecting normal cells.

3.3.2 Effect on microRNA through binding to HIF1α

MicroRNAs are small (about 22 bases), single-stranded, endogenous, non-coding RNAs that negatively regulate the translation and/or stability of mRNA [118]. MicroRNA levels could be altered by EGCG to cause subtle changes in multiple molecular targets and pathways. It has been reported recently that EGCG upregulated miR-16 in human hepatocellular carcinoma HepG2 cells, and this led to the downregulation of Bcl-2 and induction of apoptosis [119]. In our recent work, we found that, in both human and mouse lung cancer cells in culture, EGCG specifically upregulated the expression of mir-210, a major microRNA regulated by HIF1α (Wang, H. and Yang, C. S., unpublished results). Furthermore, we found that overexpression of mir-210 led to reduced cell proliferation rate and anchorage-independent growth as well as reduced sensitivity to EGCG. The upregulation of mir-210 was found to be correlated with the transiently stabilized HIF1α in lung cancer cell lines after EGCG treatment. We also demonstrated that EGCG could bind to the oxygen-dependent degradation (ODD) domain of the hypoxia-response element of HIF1α promoter and prevented the hydroxylation-dependent, ubiquitination, and proteasome-mediated degradation of HIF1α. The in vivo relevance of this observation remains to be demonstrated.

4 Challenges in the elucidation of the cancer prevention mechanisms

Many mechanisms have been proposed for cancer prevention by tea and tea constituents. The mechanisms summarized in Fig. 1 are only a partial list for the action of EGCG. Figure 2 shows same examples on possible

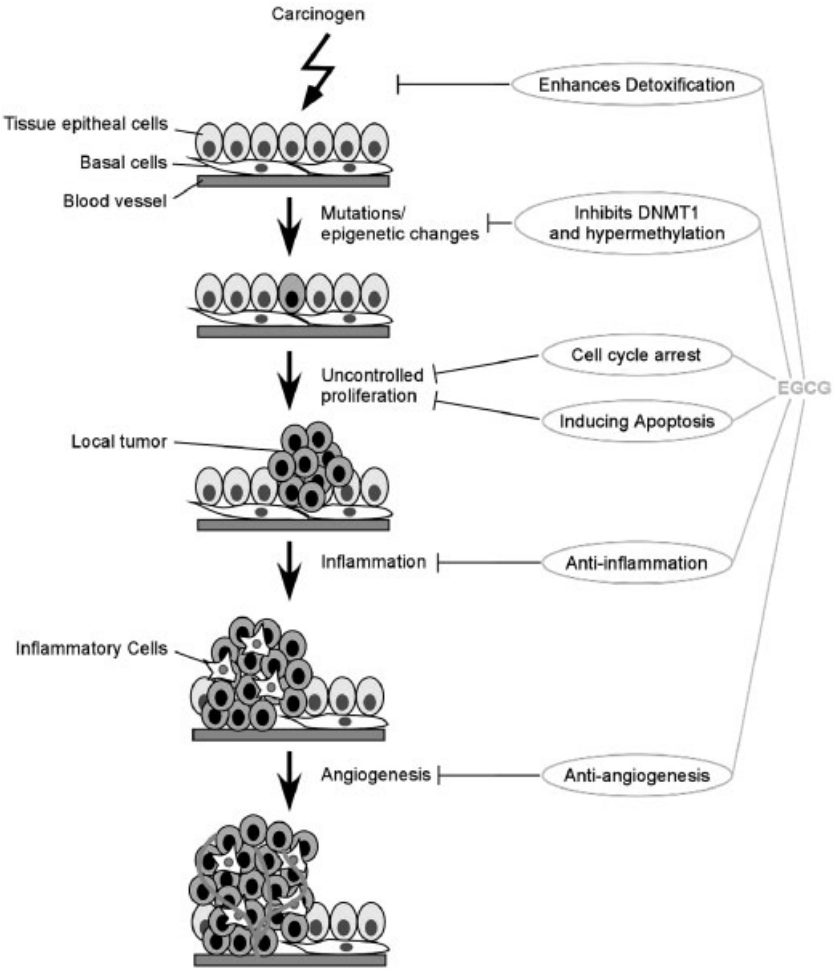

Figure 2. Examples showing how EGCG may affect cellular processes that are important for carcinogenesis.

mechanisms by which EGCG affects cellular processes that are important for carcinogenesis. An often asked question is that whether all these mechanisms are relevant for cancer prevention in vivo. Apparently, mechanisms suggested by cancer prevention studies in animal models should be considered to be more relevant. These include the induction of apoptosis in different animal models, inhibition of the phosphorylation of c-Jun and Erk1/2 in lung tumorigenesis model, suppression of phospho-Akt, and nuclear β-catenin levels in colon cancer models, inhibition of the IGF/IGF-1R axis in colon and prostate cancer models, and suppression of VEGF-dependent angiogenesis in lung and prostate cancer models [14, 17, 37, 39, 49]. It is still unclear whether these molecules are direct targets for EGCG or downstream events of the primary action. In theory, in vitro experiments could provide more information about the detailed mechanisms. It is reasonable to assume that the high-affinity binding proteins as discussed in Section 3 could serve as initial targets, but this point remains to be substantiated in animal models.

To extrapolate data obtained from cell line systems to mechanistic information on cancer prevention in vivo, however, is a challenging task. A commonly raised concern is that experimental systems with cancer cell lines are very different from carcinogenesis models. This concern can be addressed if we examine whether the key molecular events (that are modulated by EGCG) also occur in carcinogenesis models and whether the proposed mechanisms can be demonstrated in vivo. In relating in vitro observations to events in vivo, an important issue is the difference in effective concentrations. For example, in most experiments, in which an inhibitory effect of EGCG or tea can be demonstrated in animal models, the measured EGCG in the blood and tissues were usually in the sub-micromolar levels. How do we evaluate the relevance of an observation made in cell lines, in which EGCG inhibits a certain enzyme activity with $IC_{50} = 20$ or 50 μM? To address the relationship between the effective concentrations in vivo versus in vitro, we have measured the EGCG levels in blood and xenograft tumors from H1299 cells, whose growth was inhibited approximately 50% by dietary EGCG (0.5%), and the tumor EGCG concentration was 0.18 μM. This value is two orders of magnitude lower than the IC_{50} values of EGCG for the inhibition of growth of H1299 cells in culture [61]. One

possible reason for the observed discrepancy between the cell culture system and the xenograft model is the short-term exposure to EGCG in the cell culture study (for example, 24 or 48 h) versus the long-term treatment in studies with animal models. It has been reported that prolonging the treatment period can reduce the effective concentration of EGCG in a cell culture system [98]. The generation of active metabolites of EGCG in vivo is also a possibility, although our previous results indicated that some of the metabolites of tea catechins that we investigated have lower biological activities than their parent compounds [4]. The environment for cells in culture is also very different from that in xenograft tumors. Therefore, we cannot rule out a mechanism just because the in vitro effective concentrations of EGCG are higher than that we observed in vivo. However, it is reasonable to assume that activities affected by low concentrations of EGCG are likely to be more relevant than activities that only respond to higher concentrations. The activities that can only be observed with very high concentrations of EGCG in cell lines may not be relevant to cancer prevention.

Then, what are the molecular mechanisms for cancer prevention by tea constituents? Because of the broad cancer prevention activities of tea constituents, or even pure EGCG, in different animal models, multiple mechanisms are likely to be involved, dependent on the experimental conditions, including the carcinogens or transgenes used and the organ site involved. Even in the same experimental system, one tea constituent, such as EGCG, may inhibit carcinogenesis via a few or several mechanisms. The possibility that these mechanisms may work synergistically to exert the cancer preventive activity is interesting and needs to be substantiated.

In contrast to the strong evidence for the cancer preventive activity of tea constituents in animal models, results from epidemiological studies have not been consistent concerning the cancer preventive effect of tea consumption in humans. The difference between the results from animal and human studies is likely to be due to: (i) the relatively weak cancer preventive effect of tea in humans, because the lower quantities of human tea consumption as compared to the doses used in animal studies and (ii) confounding factors in epidemiological studies, which reduce the power for detecting a cancer preventive effect in a population; whereas in animals studies, the conditions are controlled to maximize the opportunity to detect a cancer prevention effect. Because of these reasons, precise information about the mechanisms of cancer prevention by tea in humans is even more difficult to obtain. From the limited human studies that are available, action of tea constituents in reducing oxidative stress and enhancing the elimination of carcinogens [67, 68] may be important.

With the strong evidence provided in laboratory studies for the cancer preventive activities of tea constituents, although the results from epidemiological studies are not conclusive, tea preparations can still be used for the prevention of certain types of human cancer. The results from laboratory studies will help us to design the optimal conditions for cancer prevention trials as well as for interpreted results from epidemiological studies.

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