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Forum Review

Redox Properties of Tea Polyphenols and Related Biological Activities

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ABSTRACT

Plant polyphenolic compounds are known to be strong antioxidants. Because oxidative stress is believed to contribute to many acute and chronic diseases, these polyphenols have been postulated to have many beneficial health effects, such as the prevention of cancer and cardiovascular diseases. Indeed, some of these beneficial effects have been demonstrated in animal models and in some, but not all, epidemiological studies. Nevertheless, only some of these activities have been demonstrated to be associated with the antioxidative activities of polyphenols. In studies with cell lines in culture, some of the observed activities may be due to superoxide and hydrogen peroxide produced during the autooxidation of polyphenols. Such pro-oxidation-dependent reactions may not happen in tissues where the oxygen partial pressure is much lower than that in cell culture medium. This review will use the well-studied tea polyphenol, (−)-epigallocatechin-3-gallate, as an example to illustrate the redox properties of polyphenols and their influence on signaling pathways related to anti-cancer activities. Existing data suggest, however, that most of the relevant mechanisms of cancer prevention by tea polyphenols are not related to their redox properties, but are due to the direct binding of the polyphenol to target molecules, including the inhibition of selected protein kinases, matrix metalloproteinases, and DNA methyltransferases. *Antioxid. Redox Signal.* 7, 1704–1714.

INTRODUCTION

TEA, made from the leaves of the plant *Camellia sinensis,* is a popular beverage worldwide. In recent years, extensive studies have been conducted on tea and tea constituents to investigate their potential beneficial health effects. A typical green tea beverage, with 2.5 g of tea leaves in 250 mL of hot water for a 3-min brew, usually contains 620–880 mg of water-extractable solids (23). Tea polyphenols, known as catechins, usually account for 30%–42% of the dry weight of the solids in brewed green tea (23). The structures of the four major catechins, (−)-epigallocatechin-3-gallate (EGCG), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epicatechin (EC), are shown in Fig. 1. Of these catechins, EGCG is the most abundant (accounting for 50%–75% of the catechins) and the most biologically active compound.

Although the cancer preventive effect of tea consumption still remains to be convincingly demonstrated in humans, tea and tea preparations have been shown to inhibit tumorigenesis in many animal models, including those for cancer of the skin, lung, oral cavity, esophagus, stomach, small intestine, colon, liver, pancreas, bladder, and prostate (29, 33, 80, 81). Although the cancer preventive activity of tea polyphenols has been demonstrated in many experimental systems (81), the mechanisms of the chemopreventive activity are not clearly understood. In some animal models, for example, the inhibition of ultraviolet (UV) light-induced skin tumorigenesis model in mice, caffeine, not EGCG, has been shown to be the active constituent in tea (27). The antioxidative properties of tea polyphenols are believed to be responsible for the cancer preventive activities of tea by many scientists and especially by the public press. This review will examine the evidence for this thesis. It will also discuss the effects of tea

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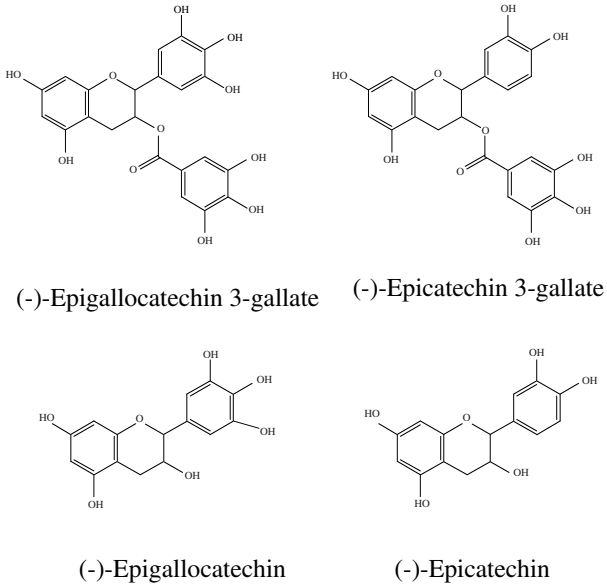

FIG. 1. Structures of major tea catechins.

polyphenols on signal transduction pathways that are related to cancer chemoprevention, but which may not depend on the redox properties of the polyphenols.

REDOX PROPERTIES OF TEA POLYPHENOLS

Because of the di- or trihydroxyl groups on the B-ring and the *meta*-5,7-dihydroxyl groups on the A ring, tea catechins are strong antioxidants. The antioxidative activity is further increased by the presence of the trihydroxyl structure on the D-ring (gallate) in EGCG and ECG (55, 76). Tea preparations have been shown to react with reactive oxygen species, such as superoxide radical, singlet oxygen, hydroxyl radical, peroxyl radical, nitric oxide, nitrogen dioxide, and peroxynitrite. Among tea catechins, EGCG is most effective in reacting with most reactive oxygen species. The B-ring appears to be the principal site of antioxidant reactions (59, 69). The antioxidative reactions are depicted in Fig. 2. The polyphenolic structure allows electron delocalization, conferring high reactivity to quench free radicals. During the reactions of tea polyphenols with free radicals, several oxidation products are formed. Reactions of EGCG and other catechins with peroxyl radicals lead to the formation of anthocyanin-like compounds (30), as well as seven-member B-ring anhydride dimers and

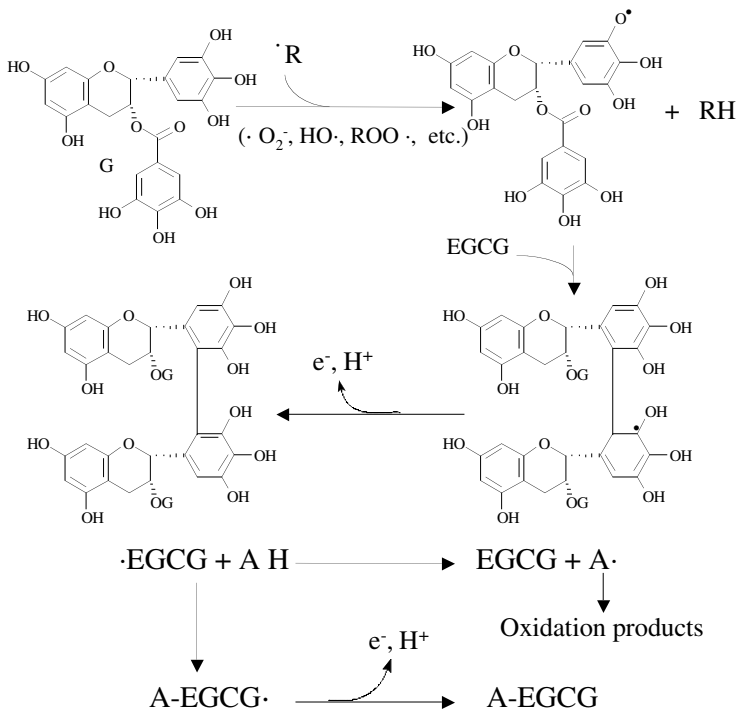

FIG. 2. Proposed antioxidative mechanism of EGCG. As a strong antioxidant, EGCG readily scavenges free radicals, such as superoxide, hydroxyl, and organic peroxide radicals, resulting in the formation of EGCG radical (·EGCG). ·EGCG can react with another EGCG molecule to generate different dimers (the simplest EGCG dimer, theasinensin A (AH), is shown as an example). In biological systems, ·EGCG is likely to be reduced by agents, such as ascorbate, gluthathione, and proteins, to regenerate EGCG. EGCG radical may also react with these agents to form EGCG adducts.

ring-fission compounds (68, 69). A second mechanism for the effective antioxidant activity is due to the metal ion chelating capacity of the vicinal dihydroxyl and trihydroxyl structures, which can prevent the generation of free radicals.

Green tea can inhibit the oxidation of lipoproteins induced by Cu^{2+} *in vitro* (22, 24). Pretreatment of macrophages or endothelial cells with green tea polyphenols reduced cell-mediated low-density lipoprotein oxidation (84). The effects of tea and tea polyphenols on biomarkers of oxidative stress, such as DNA oxidative damage, have been demonstrated in animals after receiving carcinogenic or other types of oxidative stress, but such data on humans are limited (21, 23). In humans, only transient and modest increases in plasma total antioxidant activity after tea ingestion have been observed in some, but not other, experiments (23). Apparently, the bioavailability of tea polyphenols limits the biological activity *in vivo* (73).

Similar to many other antioxidants, EGCG and other tea polyphenols may also act as pro-oxidants. Under cell culture conditions, EGCG is not stable, with a half-life of 0.5–2 h depending on the cultural medium (25, 86). The half-life can be extended several fold by the addition of superoxide dismutase (SOD), suggesting a role for superoxide radical in the oxidation and polymerization of EGCG. A proposed mechanism of EGCG autooxidation is shown in Fig. 3. EGCG and other catechins can be oxidized to form phenolic radicals, superoxide radical, and hydrogen peroxide. These species may trigger a variety of biochemical reactions and biological responses. As will be discussed in subsequent sections, the radical species may contribute to the inactivation of epidermal growth factor (EGF) receptor (EGFR) and telomerase, while hydrogen peroxide may contribute to cell apoptosis. The oxygen partial pressure in a cell culture system (160 mm Hg) is much higher than that in the blood or tissues (<40 mm Hg) (61). It is not clear whether these pro-oxidation EGCG-generated reactions occur in low oxygen partial pressure conditions *in vivo*.

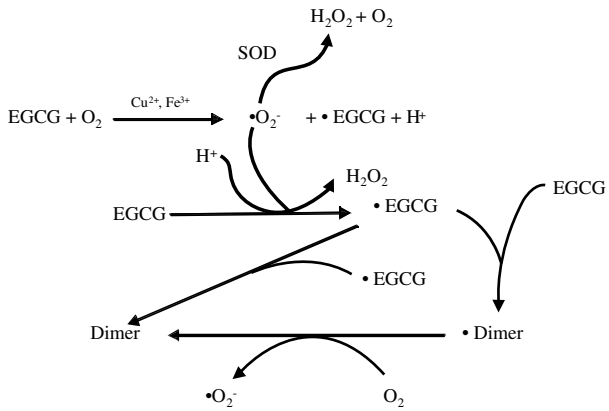

FIG. 3. Proposed mechanism for the generation of reactive species by autooxidation of EGCG. Under neutral or slightly alkaline pH in the cell culture medium, EGCG is oxidized by molecular oxygen to form superoxide radical ($\cdot O_2^-$) and EGCG radical (·EGCG) in a reaction probably catalyzed by trace metal ions such as Cu^{2+} and Fe^{3+}. The $\cdot O_2^-$ can then react with another EGCG molecule to form ·EGCG. ·EGCG may collide with another ·EGCG to form an EGCG dimer. More likely, ·EGCG may react with EGCG to form EGCG dimer radical, which has the potential to react with molecular oxygen to generate EGCG dimer and $\cdot O_2^-$. The $\cdot O_2^-$ can also be converted to H_2O_2, especially in the presence of SOD.

EFFECTS OF TEA POLYPHENOLS ON GROWTH FACTOR RECEPTOR-MEDIATED PATHWAYS

EGFR is often overexpressed in neoplastic cells, activating signal transduction pathways that promote cell proliferation and tumor progression (47, 58). Some of the EGFR signaling pathways are shown in Fig. 4. Inhibition of EGF binding and EGF-induced autophosphorylation of EGFR by EGCG has been observed in A431 epidermoid carcinoma cells (35, 36). EGCG appeared to block the binding of EGF to EGFR. However, a 30-min preincubation of EGCG with the cells was needed to produce these inhibitory effects. *In vitro* kinase assays showed that receptor tyrosine kinase activity was inhibited by EGCG (IC_{50} = 1–2 μ*M*) (35). In recent studies, EGCG has been found to inhibit EGFR autophosphorylation in YCU-N861 and YCU-H891 head and neck carcinoma cells and MDA-MB-231 breast carcinoma cells (45, 46). Downstream events, such as the phosphorylation of extracellular signal-regulated kinase (ERK), signal transducer and activator of transcription 3, and Akt, were also blocked by the treatment with EGCG. The results suggested that blocking the EGFR signaling by EGCG would potentially inhibit cancer cell proliferation. In these experiments, however, the cells were preincubated with EGCG for 6 or 18 h before the addition of transforming growth factor (TGF)-α.

Similarly, green tea and black tea polyphenols inhibited the activation of platelet-derived growth factor (PDGF) receptor (PDGF-R) in A431 cells, mouse NIH3T3 fibroblast cells, and A172 human glioblastoma (36, 56). In rat hepatic stellate cells, PDGF-Rβ activation was also blocked by EGCG (10). The inhibitory effect was correlated with a reduction in proliferation of hepatic stellate cells and an anti-fibrotic activity. It was suggested that cell membrane-incorporated EGCG preferably binds to PDGF-BB, one of the PDGF isoforms, lowering the ligand binding and leading to the inhibition of the PDGF-R signaling pathways (57, 74).

The fact that a 30-min or longer period of preincubation of EGCG with cells is required for demonstrating the inhibition of EGFR signaling led us to study the effect of this preincubation. Similar to previous results, we observed that, with the human esophageal squamous cell carcinoma KYSE 150 cell line, a preincubation of 30–240 min with EGCG was needed to demonstrate a clear-cut inhibition of EGF-activated signal transduction pathway. This preincubation-induced inhibitory effect, however, was prevented if the preincubation was conducted in the presence of SOD or SOD plus catalase, suggesting that the inhibition of the EGFR signaling pathway is related to the autooxidation of EGCG as depicted in Fig. 3. The possible inactivation of EGFR in this situation—for example, by EGCG radicals—consistent with our observation that the

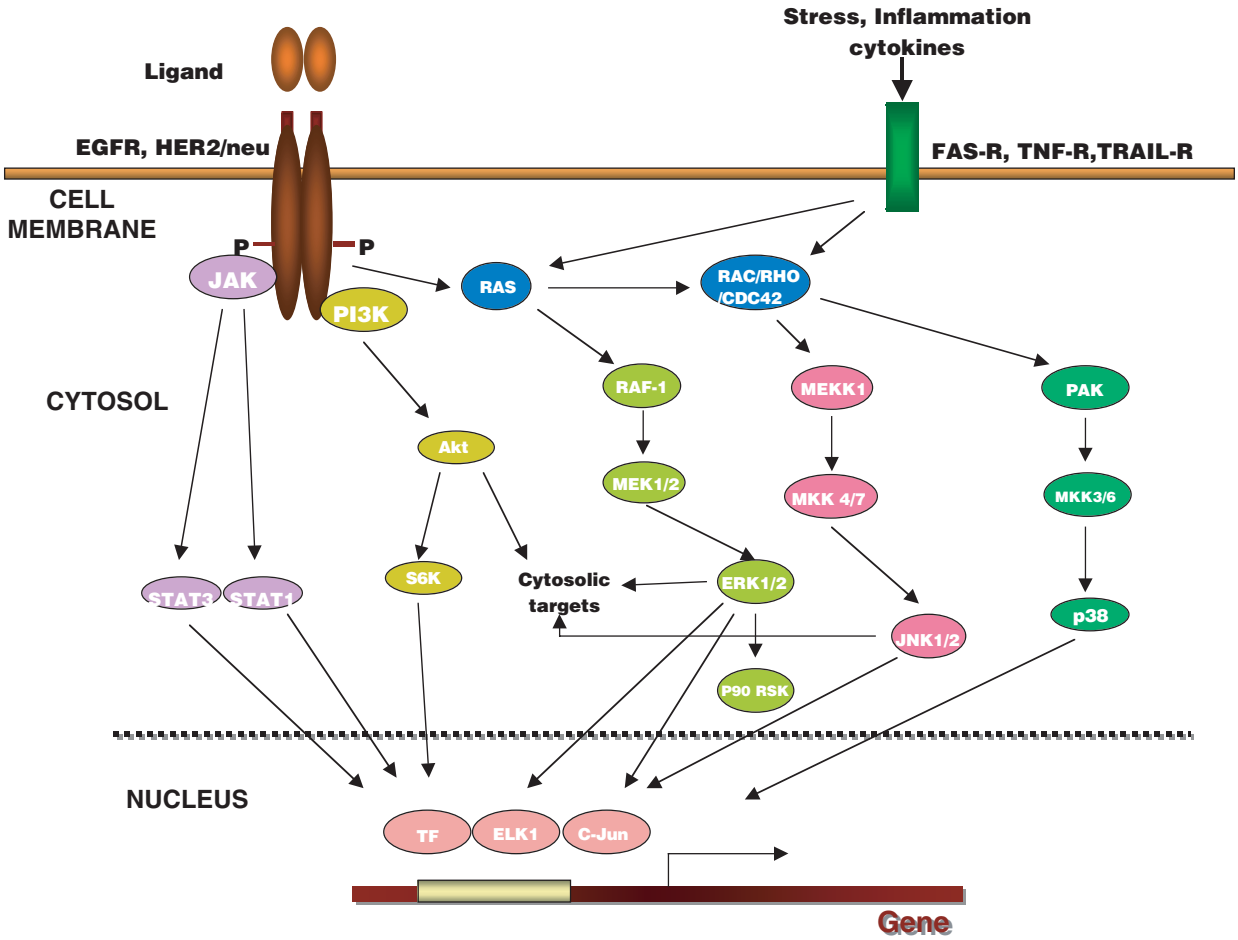

FIG. 4. EGFR signaling transduction pathways as possible targets for tea polyphenols. EGFR or HER-2/neu is phosphorylated when activated, and the phosphotyrosine residues are recognized by cytosolic kinases and serves as docking sites. Intracellular kinases relay the signal by phosphorylation cascades, and eventually transduce the signal into the nucleus to activate transcription factors, by which expression of a certain set of genes is regulated. EGCG and theaflavin-3,3′-digallate have been shown to block the autophosphorylation of EGFR and HER-2/neu as well as the MAP kinase cascade, such as RAS/RAF/MEK/ERK, c-Jun NH_2-terminal kinase (JNK) pathways, and the phosphatidylinositol 3-kinase (PI3K) pathways. FAS-R, Fas receptor; JAK, Janus tyrosine kinase; MEKK, MEK kinase; MKK, MAP kinase kinase; PAK, p21 activated kinase; RSK, ribosomal S6 kinase; S6K, S6 protein kinase; STAT, signal transducer and activator of transcription; TF, tissue factor; TNF-R, TNF receptor; TRAIL-R, tumor necrosis factor-related apoptosis-inducing ligand receptor.

EGFR protein level was decreased after the preincubation. It is conceivable that inactivation preceded and occurred to a greater extent than the degradation of EGFR. A key question is whether the inhibition or inactivation of growth factor receptor by EGCG occurs in animal tissues *in vivo*, or it is just a phenomenon in cell culture studies due to autooxidation of EGCG in the presence of high oxygen partial pressure.

INHIBITION OF TELOMERASE BY TEA POLYPHENOLS

Telomerase is important in maintaining the telomere nucleoprotein end caps of the chromosome structure (60). This enzyme has been shown to be overexpressed in many human cancers and may be responsible for an increased replicative lifetime (7). It was recently reported that EGCG inhibited telomerase *in vitro* and in cancer xenografts (48, 50, 51). Naasani *et al.* (50) reported that at neutral pH, EGCG inhibited telomerase activity in a cell-free system with high nanomolar to low micromolar potency. Long-term treatment with slightly higher concentrations (5–10 μ*M*) of EGCG was sufficient to inhibit telomerase and induce cell senescence. Treatment of mice bearing telomerase-positive colon cancer xenografts with 1.2 mg/day EGCG resulted in a 50% inhibition of tumor growth as compared to vehicle-treated control animals. Mice bearing telomerase-negative tumors of the same parental cell line were unresponsive to EGCG treatment (51). Mechanistically, the authors suggested that EGCG undergoes decomposition to form a galloyl radical, which can covalently modify telomerase. This appears to be similar to the EGCG autooxidation-induced reactions as we

discussed previously. It has not been demonstrated, however, if this phenomenon occurs *in vivo* where the concentration of oxygen is considerably reduced compared to cell culture systems.

INDUCTION OF APOPTOSIS BY TEA POLYPHENOLS

The balance between survival and apoptosis often tips towards the former in cancer cells. Tea polyphenol treatment has been shown to induce apoptosis in many cell lines, including leukemic, skin, lung, stomach, and prostate cancer cells (4, 49, 79). The requirement of caspase 3 as an executor in green tea polyphenol-induced apoptosis was demonstrated both directly and indirectly. Caspase 3 activity was augmented when cells were treated with green tea polyphenols, and caspase 3-deficient tumor cells did not undergo apoptosis with the same treatment (26).

Some recent studies have focused on the primary events in tea polyphenol-induced apoptosis. It was observed that inclusion of catalase in the cell culture system prevented EGCG-induced apoptosis of different cell lines, suggesting that H_2O_2 generated from EGCG plays a role in apoptosis induction (77, 82, 83). Oxidative stress caused by high concentrations (100 μ*M*) of tea polyphenols was found to induce apoptosis, which could be blocked by free radical scavengers (such as *N*-acetyl-L-cysteine and glutathione) (11, 31). It is possible that in some cell lines, EGCG can induce apoptosis by two mechanisms: One is H_2O_2-dependent, and the other still occurs in the presence of catalase (77, 82, 83). A recent study with nuclear magnetic resonance spectroscopy showed the direct binding of tea polyphenols to the BH3 pocket of anti-apoptotic Bcl-2 family proteins, suggesting a mechanism for EGCG to inhibit the anti-apoptotic function of Bcl-2 proteins (34). The functional importance of this binding still requires more investigation.

In vivo studies showed that 0.6% green tea in drinking fluid (6 mg of tea solids/ml) increased the apoptosis index in lung adenoma in chemically induced lung tumors in the A/J mouse model (38). An increase in the number of apoptotic epidermal cells was also observed in an experiment in which SKH-1 mice were administered 0.6% green tea in drinking fluid prior to UVB exposure (43). In this experiment, caffeine may be the key active ingredient. Nevertheless, topical application of EGCG (6.5 μmol) to the skin of SKH-1 mice increased the number of caspase-3-positive apoptotic tumor cells, induced by prior irradiation with UVB (44). It remains to be determined whether redox reactions of EGCG are involved in the induction of apoptosis.

SIGNALING MECHANISMS OF EGCG IDENTIFIED BY EXPRESSION GENOMICS

A genome-wide scan of the expression profiles of H-*ras*-transformed human bronchial epithelial 21BES cells in response to EGCG (25 μ*M*) showed distinct temporal changes in gene expression, which can be classified into early-, intermediate-, and late-response genes (71). Close to 300 genes were up-regulated, and 16 genes were down-regulated. It was shown that H_2O_2 was produced when EGCG was added to the cell culture system. By treating cells with EGCG in the absence or presence of added catalase, we further distinguished gene expression changes into those that are mediated by H_2O_2 from those that are H_2O_2-independent. We found that many genes and cellular pathways, including genes of the TGF-β signaling pathway, were H_2O_2-dependent. Gene expression changes of the bone morphogenetic protein (BMP) signaling pathway were not affected by catalase. These included down-regulation of the type II receptor of BMP, as well as up-regulation of its negative modulators including FK506-binding protein 5, dual-specificity phosphatase 5 and 8, and small mother against decapentaplegic 7 (Fig. 5).

We showed further that H_2O_2 transactivated TGF-β-response element promoter activity and this transactivation was blocked by catalase, whereas EGCG transactivated the BMP-response element promoter, and this effect was not influenced by catalase. These results suggest that in addition to the network of pathways described in the previous sections, the BMP signaling pathway may be a novel target for EGCG to influence cell growth and differentiation. Recent advances in the molecular biology of TGF-β/BMP superfamily of cytokines reveal their roles in tumorigenic progression (63, 72). BMP, its receptors, and SMADs are targeted for genetic alteration in various cancers. The expression profile of genes of the BMP pathways in response to EGCG suggests possible mechanisms for the cancer preventive effect of EGCG for further investigation.

REDOX-INDEPENDENT ACTIVITIES OF TEA POLYPHENOLS

Inhibition of specific protein kinases

The transcription factors activator protein-1 (AP-1) and nuclear factor-κB (NF-κB) play important roles in carcinogenesis (12, 15, 32, 40, 78, 85). Many studies have suggested that inhibition of the activation of AP-1 and NF-κB as a mechanism for the anti-cancer activities of EGCG and tea. Although reactive oxygen species have been suggested to be involved in the activation of the NF-κB signaling system, and its inhibition by EGCG is due to antioxidant activity, direct evidence for this mechanism is lacking. We propose that such inhibitions could be interpreted by the effect of EGCG on specific protein kinases.

In 30.7b Ras12 cells (H-*ras*-transformed JB6 cells), mitogen-activated protein (MAP) kinases were highly activated, and activity was inhibited by the tea polyphenols (13). The presence of a galloyl group in the catechin structure was associated with a high inhibitory ability. The presence of a trihydroxy structure on the B-ring, such as in EGCG, conferred a higher inhibitory activity than those with a dihydroxy structure, such as in ECG. EGCG and theaflavin-3,3′-digallate (from black tea) inhibited the phosphorylation of MAP kinase kinase (MEK)1/2, ERK1/2, and ELK-1, as well as c-Jun (13, 14). Further studies suggested that EGCG decreased the association between RAF-1 and MEK1, and EGCG

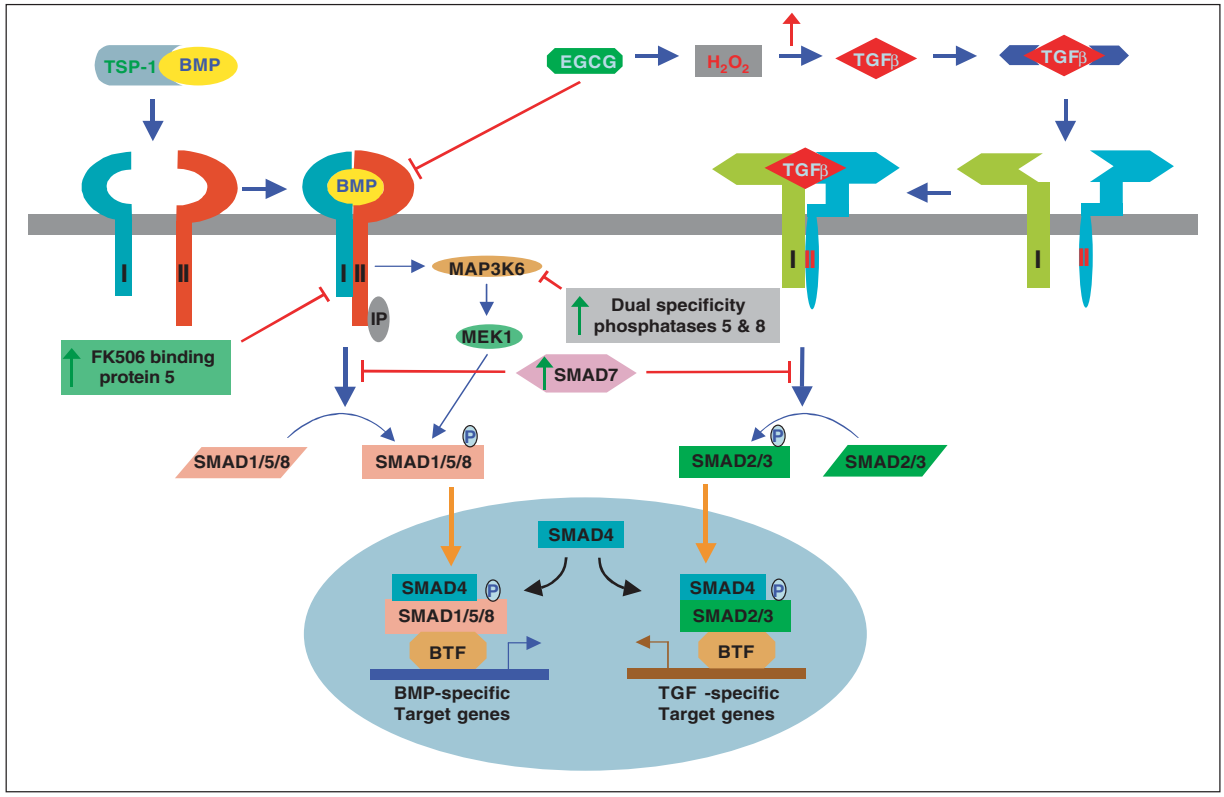

FIG. 5. Effects of EGCG on the TGF/BMP signaling pathway. The effects on the TGF pathway are H_2O_2-dependent, whereas the effects on the BMP signaling mechanism are H_2O_2-independent. EGCG appeared to attenuate the BMP signaling pathway by down-regulating BMP receptor type II and up-regulating its negative modulators, including FK506-binding protein 5, dual-specificity phosphatase 5 and 8, and small mother against decapentaplegia (SMAD)7. IP, inositol phosphate.

competitively inhibited the phosphorylation of ELK-1 by ERK1/2, possibly by competing for the binding site on ERK1/2. These results led us to hypothesize that EGCG inhibits selected protein kinase activities by competitively binding to the protein substrate binding site, possibly involving proline-rich sequences (14). This hypothesis is consistent with the recent finding that, in the prevention of UV-induced damage in the skin of SKH-1 hairless mice by topically applied green tea polyphenols, decreased phosphorylation of ERK1/2 and c-Jun NH_2-terminal kinase was observed (70). EGCG and the black tea polyphenols, theaflavins, were also shown to inhibit the phosphatidylinositol 3-kinase pathway by decreasing the levels of phosphatidylinositol 3-kinase and phosphorylation of Akt in two human prostate cancer cell lines, DU145 and LNCaP cells (62). The effect of EGCG on MAP kinases and AP-1, however, can be complicated. There have been several reports of EGCG activating ERK1/2 in different cancer cell lines (11, 62). Antioxidants, such as glutathione and *N*-acetyl-L-cysteine, were able to block this activation, suggesting that ERK activation was caused by oxidative stress induced by EGCG. It is unclear whether such an effect occurs *in vivo*.

NF-κB is inactive when bound to inhibitory-κB (IκB) in the cytosol. The phosphorylation of IκB by IκB kinases (IKKs) leads to proteasome-dependent degradation of IκB, setting NF-κB free. NF-κB can then translocate into the nucleus to activate the expression of NF-κB responsive genes. EGCG has been shown to inhibit the constitutive activation of NF-κB in H891 head and neck carcinoma cells and MDA-MB-231 breast carcinoma cells (46). In A431 epidermoid carcinoma cells, treatment of EGCG dose- and time-dependently increased the IκB level and inhibited NF-κB nuclear translocation (19). UVB irradiation-induced NF-κB activation in normal human epidermal keratinocytes was associated with increased IκB phosphorylation and degradation; EGCG was shown to block NF-κB activation and nuclear translocation (2). We propose that the inhibition of NF-κB signaling by EGCG can be interpreted by the inhibition of IKK-catalyzed phosphorylation of IκB. Consistent with this proposal is the observation that topical application of green tea polyphenols to UVB-irradiated SKH-1 hairless skin decreased phosphorylation and degradation of IκB and the subsequent activation of NF-κB (3).

EGCG can induce G_0/G_1-phase cell cycle arrest in several human tumor cell lines, including breast, epidermoid, prostate, and head and neck squamous cell cancers (5, 19, 39, 41, 45). Liang *et al*. (37) reported that treatment of MCF7 breast cancer cells with 30 μ*M* EGCG resulted in G_0/G_1-phase cell cycle arrest, overexpression of p21 and p27, lowered activity of cyclin-dependent kinase (CDK)2 and CDK4, and Rb hypophosphorylation. In prostate cancer cells, EGCG (10–80 μ*M*) increased the expression of p16, p18, p21, and p53, which are associated with negative regulation of cell cycle

progression (18, 19). Head and neck squamous cell carcinoma cells were found to be more sensitive to the effects of EGCG; EGCG induced G_0/G_1-phase cell cycle arrest at concentrations lower than 20 μ*M* (45). EGCG induced the expression of p21 and p27 while decreasing the expression of cyclin D_1 and the phosphorylation of Rb. Although EGCG has been shown to affect a number of factors associated with cell cycle progression, it is unclear which of these are direct effects and which are indirect effects. EGCG has been shown to directly inhibit CDKs (37). It may be proposed that the inhibition of the CDKs is the primary event. The induction of various negative regulators of the cell cycle may be the consequence of this inhibition. The concentrations used in these studies were higher than those observed in blood and tissues following consumption of tea. It remains to be demonstrated whether this cell cycle arrest mechanism by EGCG occurs *in vivo*.

Inhibition of protease activities

EGCG has been shown to inhibit at least two important classes of cellular protease activities: the proteasome and matrix metalloproteinases (MMPs) (33). The proteasome is essential for the degradation of a number of important cellular regulators including IκB, cyclin D_1, and other proteins (1). Nam *et al.* (52) reported that EGCG and ECG, but not EGC and EC, potently inhibited the chymotryptic activity of the 20S proteasome both in cell-free systems (IC_{50} = 90–200 n*M*) and in tumor cell lines (IC_{50} = 1–10 μ*M*). Molecular modeling studies showed that EGCG binds to the chymotrypsin site of the proteasome (64). An interaction was proposed between the carbonyl carbon of EGCG and the active site amino acid Thr^1. The decrease in potency from cell-free to whole-cell systems may be due to nonspecific binding of EGCG to cellular proteins, and thus lowering the effective concentration of EGCG to the target enzyme. This issue adds complications for correlating effective concentrations for *in vitro* and *in vivo* studies.

The MMPs are involved in tumor progression and metastasis (9). Overexpression of these zinc-dependent proteases has been shown to increase the invasive and metastatic potential of tumor cells. Conversely, pharmacological or genetic ablation of these enzymes has been shown to inhibit tumor growth and invasion (53, 75). EGCG and other tea polyphenols have been shown to affect MMP activity both directly and indirectly (33). Isemura *et al.* (28) showed that EGCG, ECG, theaflavin, and theaflavin-3,3′-digallate inhibit collagenase activity *in vitro* at concentrations of 5–50 μ*M*. EGCG has been shown to inhibit the activity of secreted MMP2 and MMP9 with IC_{50} values of 8–13 μ*M* (17). Several researchers showed that EGCG can inhibit the activation of MMPs by membrane-type 1-MMP (6, 54). The IC_{50} for this inhibition was reportedly as low as 19 n*M*. EGCG has also been shown to inhibit MMP2-induced migration in transfected COS-7 cells (6). These data may provide a mechanistic basis for the observed inhibition of metastasis and invasion observed following treatment of tumor-bearing mice with green tea or EGCG (42, 67). The concentrations used in many of these studies are quite low. Further studies *in vivo* analyzing expression of markers, such as tissue-type inhibitor of metalloproteinases or activities of MMPs, will provide better insight into the relative importance of this mechanism.

Other possible targets

We recently reported that EGCG (10–50 μ*M*) inhibited DNA methyltransferase, and that this inhibition resulted in the reactivation of key tumor suppression gene p16, retinoic acid receptor β, the DNA repair gene hMLH1, and methylguanine methyltransferase, which were inactivated by promoter hypermethylation in the human esophageal squamous cell carcinoma cell line KYSE 510 (16). Some of these genes were also reactivated in HT29 colon cancer cells and PC3 prostate cancer cells.

In a recent communication, it was reported that in MCF-7 cells, expression of the metastasis-associated 67-kDa laminin receptor conferred EGCG responsiveness at low micromolar concentrations (66). Binding of EGCG to the 67-kDa laminin receptor with a nanomolar K_D value was observed with surface plasmon resonance experiment.

In other studies, Berger *et al.* (8) reported that EGCG inhibited topoisomerase I activity in various human colon cancer cells with IC_{50} values of 9–17 μ*M*. These concentrations were similar to those necessary to inhibit cell growth. No inhibition of topoisomerase II was observed even at concentrations greater than 100 μ*M*. Suzuki *et al.* (65) reported that EGCG inhibited not only calf thymus topoisomerase I (IC_{50} = 5 μ*M*), but also human placental topoisomerase II (IC_{50} = 3 μ*M*). The reason for this discrepancy and the significance of these observations remain to be determined.

CONCLUSIONS

Many signal transduction pathways have been proposed as targets for the cancer chemopreventive activity of tea polyphenols based on studies in cell culture systems. Nevertheless, some of the biological effects of the tea polyphenols observed *in vitro* might not occur *in vivo*, because the effective concentrations observed in the cell culture systems were much higher than the tissue concentrations of these polyphenols after tea ingestion. In addition, the oxygen partial pressure in the tissue culture system is higher than those in the internal organs. Some of the effects caused by the autooxidation of tea polyphenols may not occur in animals or humans.

It is possible that multiple mechanisms are involved in the cancer preventive action and that the relative importance of the different mechanisms may depend on the experimental systems studied. It is unlikely for EGCG to have a specific receptor that mediates all the cancer preventive activity in all experimental systems. Among the numerous mechanisms proposed in the literature, it is important to determine which are the primary events and which are the subsequent events. Although tea polyphenols are strong antioxidants, evidence for an antioxidative mechanism for cancer prevention by tea is lacking. Direct binding to key proteins is a more likely mechanism. We have discussed the inhibition of specific protein kinases (MAP kinases, IKKs, and CDKs) and proteases (MMPs and proteasomes) by EGCG as possibilities to illustrate the point that a biochemical mechanism is needed in

order for tea polyphenols to alter the cellular signal transduction pathways. Nevertheless, this hypothesis needs to be carefully examined, especially in animal or human tissues *in vivo*.

ACKNOWLEDGMENTS

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ABBREVIATIONS

AP-1, activator protein-1; BMP, bone morphogenetic protein; CDK, cyclin-dependent kinase; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin-3-gallate; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; IκB, inhibitory-κB; IκK, inhibitory-κB kinase; MAP, mitogen-activated protein; MEK, mitogen-activated protein kinase kinase; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; PDGF, platelet-derived growth factor; PDGF-R, platelet-derived growth factor receptor; SOD, superoxide dismutase; TGF, transforming growth factor; UV, ultraviolet.

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