Effects of tea polyphenols on signal transduction pathways related to cancer chemoprevention

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Abstract

The inhibition of carcinogenesis by tea and tea polyphenols has been demonstrated in different animal models by many investigators. The mechanisms of this inhibitory activity have also been investigated extensively, mostly in cell culture systems, but no clear conclusion can be reached concerning the cancer preventive mechanisms in vivo. In this article, we reviewed the possible mechanisms, which include the inhibition of specific protein kinase activities, blocking receptor-mediated functions, and inhibition of proteases. These events may lead to cell cycle regulation, growth inhibition, enhanced apoptosis, inhibition of angiogenesis, and inhibition of invasion and metastases. The possible complications of translating results obtained in cell culture studies to animals and humans are discussed. It is likely that multiple signal transduction pathways are involved in the inhibition of carcinogenesis by tea constituents. The relative importance of these pathways needs to be determined in vivo.

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Keywords: Tea polyphenol; EGCG; Theaflavins; Signal transduction; Cancer prevention

Abbreviations: AA, arachidonic acid; AP-1, activator protein-1; BMP, bone morphogenic protein; CDK, cyclin-dependent kinase; COX, cyclooxygenase; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-gallate; EGCG, (-)-epigallocatechin-3-gallate; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular regulated kinase; JNK, c-JUN NH₂-terminal kinase; LOX, lipoxygenase; LT, leukotriene; MMP, matrix metalloproteinase; NF-H9260B, nuclear factor-H9260B; PDGF-R, platelet-derived growth factor receptor; PGE₂, prostaglandin E₂; SOD, superoxide dismutase; STAT3, signal transducer and activator of transcription 3; TFdiG, theaflavin-3-3′-gallate; TGF-β, transforming growth factor β

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1. Introduction

Tea (Camellia senensis) is a popular beverage worldwide. Historically, tea has also been used for medicinal purposes. In recent years, extensive studies have been conducted on tea and tea constituents because of their potential beneficial health effects. In addition to caffeine, green tea contains characteristic polyphenol constituents, generally known as catechins, which include (−)-epigallocatechin-3-gallate (EGCG); (−)-epicatechin-3-gallate (ECG); (−)-epigallocatechin (EGC) and (−)-epicatechin (EC). Of these catechins, EGCG is the most abundant and the most biologically active compound. In making black tea, the “fermentation” process causes the oxidation and polymerization of most of the catechins to form theaflavins, including theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate, and theaflavin-3,3'-digallate (TFdG), as well as the poorly characterized larger polymers known as thearubigins as the major products. The structures of some of these compounds are shown in Fig. 1.

Tea has 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 (reviewed in [1–3]). The mechanisms of the chemopreventive activity, however, are not clearly understood. Although the cancer preventive activity of tea polyphenols has been demonstrated in many experimental systems [2], caffeine has been shown to be the active ingredient in some other systems; for example, in the inhibition of UV-light induced skin tumorigenesis in mice [4] and chemically induced lung tumorigenesis in F344 rats [5].

This chapter will discuss the effects of tea polyphenols on signal transduction pathways that are related to cancer chemoprevention. Mechanistic investigations of the cancer chemopreventive activity depend on the methodologies that are available at different time periods. For example, EGCG and other tea polyphenols are well known for their antioxidant activities. Indeed, they have been demonstrated to inhibit carcinogen-induced DNA damage and tumor promoter-induced oxidative stress (reviewed in [1,2,6]). These results are consistent with the commonly mentioned idea that tea prevents cancer because tea polyphenols are antioxidants. It is
unclear, however, whether this is a general mechanism for cancer prevention, especially in human carcinogenesis when strong carcinogens and tumor promoters are not known to be involved. In the 1980s when carcinogen activation and tumor promotion were active areas of research, these events had been proposed as the targets of tea polyphenol action. With the advancement of research on signal transduction, many current efforts have been focused on the effect of tea polyphenols on signal transduction pathways. Most of these studies have been carried out in cell lines. It is not clear whether some of the phenomena observed in cell lines occur in vivo.

There are two major problems in extrapolating results observed in cell lines to animal models. (1) The concentration of the test compound used in cell line systems, for example, EGCG at 20, 50, 100 μM, or higher concentrations, are much higher than those observed in the plasma or tissues in experimental animals or humans after ingestion of tea or related tea preparations [2]. (2) The oxygen partial pressure in a cell culture system (160 mmHg) is much higher than that in the blood or tissues (<40 mmHg) [7]. Under cell culture conditions, EGCG is not stable, with a half-life less than 2 h [8]. 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 auto-oxidation is shown in Fig. 2. Similar to many other antioxidants, EGCG and other tea polyphenols may also act as pro-oxidants. They 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, hydrogen peroxide may contribute to cell apoptosis and the radical species may contribute to the inactivation of epidermal growth factor receptor (EGFR) and telomerase as reported in the literature. It is not clear whether these pro-oxidation of EGCG generated reactions occur in low oxygen partial pressure conditions in vivo in cells, which generally have strong anti-oxidative capacity and low oxygen partial pressure.
Fig. 2. Auto-oxidation of EGCG and generation of reactive species.

Under neutral or slightly alkaline pH in the cell culture medium, EGCG is oxidized by molecular oxygen to form superoxide radical (O$_2^•−$) and EGCG radical (EGCG•). The O$_2^•−$ can then react with another EGCG molecule 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 O$_2^•−$. The O$_2^•−$ can also be converted to H$_2$O$_2$, especially in the presence of superoxide dismutase.

2. Inhibition of mitogen activated protein (MAP) kinases and activator protein-1 (AP-1)

Previously, we found that 5–20 μM of EGCG or theaflavin inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)- or epidermal growth factor (EGF)-induced transformation of mouse epidermal cell line JB6, and the activity was closely related to the inhibition of the activation of the transcription factor AP-1 [9]. This activity was associated with the inhibition of c-Jun NH$_2$-terminal kinase (JNK) phosphorylation, but not ERK1/2 phosphorylation. Since MAP kinases play key roles in growth and neoplasia, the effects of tea polyphenols on this cascade were investigated subsequently. In 30.7b Ras12 cells (H-ras transformed JB6 cells), AP-1 was highly activated, and most tea polyphenols inhibited the transcription activity of AP-1 [10]. 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 TFdiG inhibited the phosphorylation of MEK1/2, ERK1/2, and ELK-1, as well as c-Jun (Fig. 3), but not that of JNK [10,11]. Further studies suggested that EGCG decreased the association between RAF-1 and MEK1, and EGCG 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 [11]. This hypothesis is supported by 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 (5 mg in 200 μl acetone/mouse), decreased phosphorylation of ERK1/2 and JNK and reduced protein level of p38 were observed [12]. In the same mouse strain, green tea polyphenols, applied in topical hydrophilic ointment or through drinking water, prevented UVB-induced depletion of antioxidant enzymes in skin. UVB-induced phosphorylation of ERK1/2, JNK and p38 was also blocked by the treatment [13]. EGCG and theaflavin were also shown to inhibit PI3K pathway by decreasing the levels of PI3K and phosphorylation of Akt in two human prostate cancer cell lines, DU145 and LNCaP cells [14].

There have been several reports of EGCG activating ERK1/2 in different cancer cell lines [14,15]. 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.

A very interesting result, observed in normal human epidermal keratinocytes, is that low concentrations of EGCG (<1 μM) increased cell proliferation and inhibited UV-induced apoptosis, possibly through activating ERK and Akt pathways and changing the Bcl-2/Bax balance [16]. When EGCG was applied to aged human skin, it resulted in increased cell proliferation and thickening of the skin. This leads to the proposal that EGCG may prevent the aging of the skin. Another study showed that adding 100 μM EGCG to normal epidermal keratinocytes did not induce caspase 3; rather, the cells showed clear differentiated features [17]. These data, contrasting the observation in cancer cell lines, deserve further mechanistic investigation.
Fig. 3. EGFR and MAP kinase cascades as targets for tea polyphenols. Activation of EGFR or HER-2/neu elicits the autophosphorylation of the receptors, and the phosphotyrosine residues are recognized and bound by other protein kinases in the cytosol. These kinases transduce the signal by phosphorylating downstream protein kinases. Some of the phosphorylated signal transducers translocate into the nucleus to activate transcription factors (such as AP-1), which regulate the expression of selected sets of genes. EGCG and TFdiG 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, JNK pathways, and the PI3K pathway.

3. Blocking growth factor receptor-mediated pathways

The inhibition of EGF-induced transformation of JB6 cells and associated MAP kinase signaling could also be explained if we assume that EGCG blocks the function of EGFR. Some of the EGFR signaling pathways are shown in Fig. 3. Indeed, inhibition of EGF binding and EGF-induced autophosphorylation of EGFR have been observed in A431 epidermoid carcinoma cells [18,19]. EGCG appeared to block the binding of EGF to EGFR. Nevertheless, a 30 min pre-incubation 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 (IC50 = 1–2 μM) [19].

EGFR is often overexpressed in neoplastic cells, activating signal transduction pathways that promote cell proliferation and tumor progression [20,21]. Antagonists to EGFR are currently under intensive investigation for cancer therapy [22]. In recent studies, EGCG was also found to inhibit EGFR autophosphorylation in YCU-N861 and YCU-I891 head and neck carcinoma cells and MDA-MB-231 breast carcinoma cells [23,24]. Downstream events, the phosphorylation of ERK, STAT3, and Akt were subsequently blocked by EGCG.
the treatment with EGCG. In these experiments, however, the cells were pre-incubated with EGCG for 6 or 18 h before the addition of TGF-α. The inhibition of EGFR-dependent STAT3 activation subsequently retarded VEGF synthesis in the cancer cells. The inhibitory effect of NF-κB by EGCG also contributed to the overall VEGF down-regulation [24]. The results suggested that blocking the EGFR signaling by EGCG would potentially inhibit both cancer cell proliferation and angiogenesis. Overexpression of HER-2/neu, an oncogene in the EGFR tyrosine kinase superfamily, is observed in many human cancers, and it is recognized as a target for cancer therapy [25]. In cell lines YCU-H891 and BT-474 (a breast carcinoma cell line) EGCG (20 and 60 μM) blocked the HER-2/neu oncogenic pathway, including the activation of STAT3 and c-fos, and cyclin D1 promoter activity [26].

Similarly green tea and black tea polyphenols inhibited the activation of platelet-derived growth factor β (PDGF-β) receptor in A431 cells, mouse NIH3T3 fibroblast cells, and A172 human glioblastoma [18,27]. In rat hepatic stellate cells, PDGF-β receptor (PDGF-Rβ) activation was also blocked by EGCG [28]. The inhibitory effect was correlated with a reduction in proliferation of hepatic-stellate cells and an anti-fibrotic activity. Mechanistic evidence suggested that this inhibition was through the blockade to tyrosine phosphorylation. 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 [29,30].

The fact that a 30 min or longer period of pre-incubation of EGCG with cells is required for demonstrating the inhibition of EGFR signaling led us to study the effect of this pre-incubation. Similar to previous results, we observed that, with human esophageal squamous cell carcinoma KYSE 150 cell line, a pre-incubation of 30–240 min with EGCG was needed to demonstrate a clear-cut inhibition of EGFR-activated signal transduction pathway. This pre-incubation-induced inhibiting effect, however, was prevented if the pre-incubation was conducted in the presence of superoxide dismutase (SOD), suggesting that the inhibition of the EGFR signaling pathway is related to the auto-oxidation of EGCG as depicted in Fig. 2. The possible inactivation of EGFR in this situation, for example by superoxide or EGCG radicals, is consistent with our observation that the EGFR protein level was decreased by 60% after pre-incubation of 240 min. A key question is whether the inhibition or inactivation of growth factor receptor by EGCG occurs in animal tissues, or it is just a phenomenon in cell culture studies due to auto-oxidation of EGCG in the presence of high oxygen partial pressure and absence of an effective anti-oxidative system.

4. Cell cycle arrest

Dysregulation of the cell cycle check points and overexpression of growth promoting cell cycle factors such as cyclin D1 and cyclin dependent kinases (CDK) are associated with tumorigenesis [31,32]. Some key proteins involved in cell cycle control are shown in Fig. 4. Studies have shown that EGCG can induce G0/G1 phase cell cycle arrest in several human tumor cell lines, including breast, epidermoid, prostate, and head and neck squamous cell cancers [23,33–37]. Liang et al. reported that treatment of MCF7 breast cancer cells with 30 μM EGCG resulted in G0/G1 phase cell cycle arrest [38]. EGCG also induced expression of p21 and p27, inhibited the activity of CDK2 and CDK4, and caused Rb hypophosphorylation. In prostate cancer cells, EGCG (10–80 μM) increased the expression of p16, p18, p21, and p53, which are associated with negative regulation of cell cycle progression [35,39]. EGCG also reduced the protein expression of cyclin D1, cyclin E, CDK2, CDK4, and CDK6, but not cyclin D2. Head and neck squamous cell carcinoma cells were found to be more sensitive to the effects of EGCG; EGCG induced G0/G1 phase cell cycle arrest at concentrations lower than 20 μM [23]. EGCG induced the expression of p21 and p27 while decreasing the expression of cyclin D1 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 [38]. 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.
Fig. 4. Cell cycle and possible modulation of tea polyphenols. Stimulation of cells with growth factors or stress signals results in the movement from the quiescent G₀ phase of the cell cycle into the G₁ phase, where sequential phosphorylation of Rb first by the cyclin D₁/CDK4 complex and then the cyclin E/CDK2 complex occurs. This allows release of the transcription factor E2F, which is responsible for transcription of early genes necessary for cell division. The cyclin A/CDK2 complex then promotes progression of the cell cycle into the S-phase with replication of cellular DNA. Cyclin A/CDC2 and cyclin B/CDC2 are responsible for promoting movement through G₂ and into mitosis (M) where cellular division occurs. P16 INK4, p27 KIP1, and p21 WAF1 negatively regulate movement of cells through G₁ and into S-phase. Tea catechins, especially EGCG, have been shown to inhibit the activity of CDK2 and 4 and inhibit the phosphorylation of Rb. Further EGCG has been shown to up-regulate the expression of p16, p21, p27, and p53. These activities contribute to the ability of EGCG to inhibit cell cycle progression at G₁.

5. Inhibition of nuclear factor-κB (NF-κB) signaling pathway

The transcription factor NF-κB-induced signaling is well known for its importance in inflammation [40]. Recent results also point to its pivotal roles in suppressing apoptosis in cancer cells [41]. NF-κB is inactive when bound to IκB in the cytosol (Fig. 5). The phosphorylation of IκB by 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 a set 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 [24]. In A431 epidermoid carcinoma cells, treatment of EGCG dose- and time-dependently increased IκB level, and inhibited NF-κB nuclear translocation [42]. UVB irradiation-induced NF-κB activation in normal human epidermal keratinocytes was associated with increased IκB phosphorylation and degradation and EGCG was shown to block NF-κB activation and nuclear translocation [43].

Although reactive oxygen species have been suggested to be involved in the activation of the NF-κB signaling system, and that its inhibition by EGCG is due to the antioxidant activity, direct evidence for this mechanism is lacking. We propose that the inhibition of NF-κB signaling by EGCG can be simply 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 [12].

6. Induction of apoptosis

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
Fig. 5. NF-κB pathway and possible modulation by EGCG. NF-κB is inactive in the cytosol as a result of the binding of p50 and p65 to IκB. When IκB is phosphorylated by IKKs and degraded in a proteasome-dependent pathway, p50 and p65 are set free and are translocated into the nucleus to activate a specific set of genes. This pathway has been shown to be inhibited by EGCG both in vitro and in vivo, possibly by inhibiting IKK-catalyzed phosphorylation of IκB.

cell lines, including leukemia, skin, lung, stomach, and prostate cancer cells [44–46]. 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 are treated with green tea polyphenols, and caspase 3-deficient tumor cells did not undergo apoptosis under the same treatment [47].

Some recent studies have focused on primary events in tea polyphenol-induced apoptosis. The observations that inclusion of catalase in the cell culture system prevented EGCG-induced apoptosis of human lung cancer cell line H661 and H-ras-transformed human bronchial epithelial cell line 21 BES, suggest that H2O2 generated from EGCG plays a role in apoptosis induction [48,49]. Oxidative stress caused by high concentrations (100 μM) of tea polyphenols was found to induce apoptosis, which could be blocked by free radical scavengers (such as N-acetyl-l-cysteine and glutathione) [15,50]. In EGCG-treated LNCaP cells and smooth muscle cells, p53 protein was stabilized, and NF-κB transcription activity was inhibited by EGCG [51,52]. As a consequence, LNCaP cells were arrested in the G0/G1 phase, and the balance between pro- and anti-apoptotic Bcl-2 family proteins favored apoptosis [51]. A recent study with NMR 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 [53]. The BH3 domain was recognized as one of the binding sites of tea polyphenols; however, the functional importance of this binding still requires more investigation.

An outstanding question is why EGCG induced apoptosis more effectively in cancer cells than in normal cells. A possible answer was suggested by Hsu et al. [17,54] who showed that p57/KIP2 was induced by green tea polyphenols only in normal human epithelial cells, which were less responsive to apoptosis.
sis induction by EGCG. In carcinoma cells that were transfected and overexpressing p57/KIP2, resistance to EGCG-induced apoptosis was conferred.

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 [55]. 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 [56]. 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 which were induced by prior irradiation with UVB [57].

7. Novel signaling mechanisms of EGCG identified by expression genomics

Recent results using expression genomics approaches facilitated the identification of biochemical networks and pathways that mediate pharmacological response of cells to various small molecules. Genome-wide scan of the expression profiles of the H-ras transformed human bronchial epithelial 21BES cells in response to EGCG (25 μM) showed distinct temporal changes in gene expression, which can be classified into early, intermediate, and late-response genes (unpublished results). Close to 300 genes were up-regulated and 16 genes were down-regulated. It was shown that H2O2 was produced when EGCG was added to the cell culture system. By treating cells with EGCG in the absence or presence of added catalase, which decomposes H2O2, we further distinguished gene expression changes into those that are mediated by H2O2 from those that are H2O2-independent. We found that many genes and cellular pathways, including genes of the transforming growth factor β (TGF-β)-signaling pathway, were H2O2-dependent, because the effects were abolished by catalase. Gene expression changes of the bone morphogenetic protein (BMP)-signaling pathway were not affected by catalase, which include down-regulation of the type II receptor of BMP, as well as up-regulation of its negative modulators including FK506-binding protein 5 and 8, and SMAD 7 (Fig. 6).

We showed further that H2O2 transactivated TGF-β-response element promoter activity and this transactivation was blocked by catalase; whereas EGCG transactivated 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 [58,59]. BMP, its receptors, and SMADs, are targeted for genetic alteration in various cancers. 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.

8. Inhibition of aberrant arachidonic acid metabolism

Cytosolic phospholipase A2 (cPLA2) is phosphorylated in response to growth stimuli or pro-inflammatory mediators (Fig. 7). The phosphorylated enzyme translocates to the membranes of the endoplasmic reticulum (ER) and nucleus where it catalyzes the release of arachidonic acid (AA) from membrane phospholipids. This free AA then undergoes further metabolism by cyclooxygenase (COX, constitutive COX-1 or inducible COX-2), lipooxygenase (LOX), or cytochrome P450 monooxygenase (Fig. 7). COX-2 mediated metabolism resulting in the formation of prostaglandin E2 (PGE2) and 5-, 12-, or 15-LOX catalyzing the formation of leukotrienes (LT). Overexpression of COX-2, 5-LOX, and other LOX enzymes have been observed in several types of cancer [60].

Green tea and EGCG have been shown to affect some aspects of aberrant AA metabolism in a number of models. EGCG has been shown to inhibit the induction of COX-2 both in vitro and in vivo. Ahmed et al. showed that EGCG (100–200 μM) reduced the protein expression (24–48% decrease) and activity of COX-2 following IL-1β stimulation of human chondrocytes [61]. Oral administration of EGCG to mice (20–50 mg/kg) prior to the application of TPA to the skin significantly inhibited the expression of COX-2 in the skin [62].
Fig. 6. Effects of EGCG on the TGF/BMP signaling mechanisms. The effects on TGF-β pathway are H₂O₂-mediated whereas the effects on the BMP-signaling mechanism are H₂O₂-independent. EGCG appeared to attenuate the BMP signaling pathway by down-regulating BMP receptor type II and up-regulating its negative modulators, including FK506-protein 5, dual specificity phosphatase 5 and 8, and SMAD7.

Lu et al. showed that treatment of SV-40 transformed WI38 human cells with 50 μM theaflavin monogallates results in blockade of serum-induced expression of COX-2 mRNA and protein, but this effect was not observed in non-transformed cells [63]. These effects correlated with the increased sensitivity of the transformed cells to theaflavin monogallatated-induced growth inhibition and apoptosis as compared to the parental non-transformed cell line. Given the poor bioavailability of the theaflavins, the presently used concentrations may only be relevant in the oral and gastrointestinal tract where direct contact between theaflavins in the lumen and the intestinal mucosa is possible [64].

In contrast to the observed inhibitory effects of green tea components on COX-2 expression, 25 μM EGCG has been reported to significantly increase COX-2 and PGE₂ expression by non-stimulated Raw 264.7 murine macrophages [65]. This effect was preceded by an increase in the levels of phosphorylated ERK and could be attenuated by co-treatment with the MEK inhibitor, PD098059. Further, addition of the protein tyrosine phosphatase inhibitor, sodium orthovanadate, enhanced the effect of EGCG. These data suggest that activation of ERK is critical for up-regulation of COX-2 in Raw 264.7 cells.

Our laboratory reported that catechins or theaflavins (30 μg/mL) inhibited the formation of COX and LOX-dependent metabolites in human normal and tumor microsomes [66]. ECG and EGCG were the most potent inhibitor (62–74% inhibition of LOX and COX). Interestingly, theaflavins inhibited the production of thromboxanes and 12-hydroxyheptadecatrienoic acid, but significantly increased the production of PGE₂. Further investigation showed that although theaflavins inhibited the activity of purified ovine COX-2, it stimulated PGE₂ production in a system consisting of COX-2 and microsomes, suggesting that theaflavins enhance the interaction of COX-2 with PGE₂ synthetase.
Inhibition of aberrant AA metabolism in the colon due to ingestion of green tea has been correlated with reduced aberrant crypt foci in azoxymethane (AOM)-treated mice fed a high-fat diet [67]. In AOM-treated mice, those receiving a high-fat diet had significantly higher colonic levels of 5-LOX, leukotriene A4-hydrolyase, and LTB4 in comparison with those on a low fat diet. Following treatment with 0.6% green tea as the drinking fluid for 10 weeks, there were significantly decreased colonic levels of cPLA2, 5-LOX, and LTB4.

9. Inhibition of protease activities

EGCG has been shown to inhibit at least two important classes of cellular protease activities: the proteasome and matrix metalloproteinases [3]. The proteasome is essential for the degradation of a number of important cellular regulators including IκB, cyclin D1, and other proteins; inhibition of this enzyme has recently been acknowledged as a valid approach to cancer chemotherapy [68]. Nam et al., reported that EGCG and ECG, but not EGC and EC, potently inhibited the chymotryptic activity of the 20s proteasome both in cell-free systems (IC50 = 90–200 nM) and in tumor cell lines (IC50 = 1–10 μM) [69]. Treatment of LNCaP prostate cancer cells with EGCG resulted in G0/G1 cell cycle arrest and accumulation of p27 and IκB, both of which are targets of the proteasome [69]. The decrease in potency from cell-free to whole cell systems may be due to non-specific binding of EGCG to cellular proteins, and thus lowering the effective concentration for in vitro and in vivo studies. Molecular modeling studies showed that EGCG binds to the chymotrypsin
Fig. 8. Activation of MMP2 by MT1-MMP and effects of tea polyphenols. Pro-MMP2 is synthesized in the cytosol and secreted into the extracellular space where it interacts with a complex of TIMP-2 and MT1-MMP. Under low TIMP-2 conditions, the pro-MMP2 is activated by a nearby MT1-MMP not complexed to TIMP-2. Under high TIMP-2 conditions, TIMP-2 prevents this cleavage by occupying the free MT1-MMP2. EGCG has been shown to inhibit secretion of pro-MMP2, up-regulated expression of TIMP-2, and inhibit the proteolytic activity of both MMP-2 and MT1-MMP.

The matrix metalloproteinases (MMPs) (Fig. 8) are involved in tumor progression and metastasis [73]. 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 [74,75]. EGCG and other tea polyphenols have been shown to affect MMP activity both directly and indirectly [3]. Isemura et al. showed that EGCG, ECG, theaflavin, and TFdG inhibit collagenase activity in vitro at concentrations of 5–50 μM [76]. EGCG also inhibited the invasion of Lewis lung cancer cells through a Matrigel basement membrane in vitro. EGCG has been shown to inhibit the activity of secreted MMP2 and MMP9 with IC50 values of 8–13 μM [77]. This inhibition was shown to be independent of Zn2+ concentration. EGCG also increased expression of the tissue inhibitor of metalloproteinases (TIMP) 1 and 2 at even lower concentrations (~1 μM). These proteins are endogenous inhibitors of MMPs (Fig. 8) and the dual activities of EGCG may lead to a more dramatic overall effect on MMP activity. Several researchers showed that EGCG can inhibit the activation of MMPs by MT1-MMP [78–80]. The IC50 for this inhibition was reportedly as low as 19 nM. EGCG has also been shown to inhibit MMP2-induced migration in transfected COS-7 cells [80].

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 [81,82]. The concentrations used in many of these studies are quite low. Further studies in vivo analyzing expression of markers such as TIMP or activities of MMPs will provide better insight into the relative importance of this mechanism.

10. Other possible targets

EGCG has been shown to have activity against many potentially important targets for cancer prevention and therapy. For example, in a recent communication, it was reported that, in MCF-7 cells, expre...
sion of the metastasis-associated 67 kDa laminin receptor conferred EGCG responsiveness at low micromolar concentrations [83]. Binding of EGCG to the 67 kDa laminin receptor with a nanomolar Kd value was observed with surface plasmon resonance experiment. We recently reported that EGCG (10–50 μM) inhibited DNA methyltransferases and this inhibition resulted in the reactivation of key tumor suppression gene p16, retinoic acid receptor α, the DNA repair gene hMLH1, and methyguanine methyltransferase, which were inactivated by promoter hypermethylation in human esophageal squamous cell carcinoma cell line KYSE 510 [84]. Some of these genes were also reactivated in colon cancer cells HT29 and prostate cancer cells PC3.

In other studies, Berger et al. reported that EGCG inhibited topoisomerase I activity in various human colon cancer cells with IC50 values of 9–17 μM [85]. These concentrations were similar to those necessary to inhibit cell growth. No inhibition of topoisomerase II was observed even at concentrations greater than 100 μM. Suzuki et al. reported that EGCG inhibited not only calf thymus topoisomerase I (IC50 = 5 μM), but also human placental topoisomerase II (IC50 = 3 μM) [86]. The reason for this discrepancy and the significance of these observations remain to be determined.

Telomerase is important in maintaining the telomere nucleoprotein end caps of the chromosome structure [87]. This enzyme has been shown to be overexpressed in many human cancers and may be responsible for increased replicative life-time [88]. It was recently reported that EGCG inhibited telomerase in vitro and in human cancer cells with IC50 values of 9–17 μM [85]. Long-term treatment with slightly higher concentrations (5–10 μM) 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 [89]. Mechanistically, the authors suggest that EGCG undergoes decomposition to form a galloyl radical which can covalently modify telomerase. This appears to be similar to the EGCG auto-oxidation induced reactions as we discussed previously. It has not been demonstrated, however, if this phenomenon occurs in vivo.

11. Concluding remarks

As was discussed in preceding sections, many signal transduction pathways have been proposed as targets for the cancer chemopreventive activity of tea polyphenols. Nevertheless, it is difficult to determine which are the more relevant or important mechanisms in vivo. Some considerations are as follows:

1. It is possible that multiple mechanisms are involved in the cancer preventive action and 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. We have discussed the inhibition of protein kinases, including MAP kinases, IKKs, and CDKs, by EGCG as a possibility 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.

2. Some of the biological effects of the tea polyphenols observed in vitro might not occur in vivo. In many studies with cell culture systems, rather high concentrations of tea polyphenols, such as EGCG, are utilized. The blood and tissue concentrations of EGCG may be much lower than the effective concentrations observed in vitro. 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 auto-oxidation of tea polyphenols may not occur in tissues, which usually have a strong anti-oxidative capacity.

3. Because of the limited bioavailability of EGCG, reactions that are affected by low concentrations of EGCG are more likely to occur in vivo than those that require high concentrations. Nevertheless, it is difficult to translate information concerning the effective concentration from in vitro studies, especially those with pure or isolated proteins, to in...
vivo situations, because of the nonspecific binding of EGCG to cellular molecules.

4. Based on the above discussions, it is important to demonstrate the proposed mechanisms in vivo, especially in short-term experiments. For example, if we propose that EGCG directly inhibits MAP kinases, then this effect should be seen in vivo a few hours after the administration of EGCG.

References

[22] A. Chen, L. Zhang, The antioxidant (−)-epigallocatechin-3′-gallate inhibits rat hepatic sulfate cell proliferation in vitro by


