Molecules **2007**, *12*, 946-957

molecules

ISSN 1420-3049

www.mdpi.org/molecules

Review

Cancer Preventive Mechanisms of the Green Tea Polyphenol (-)-Epigallocatechin-3-gallate

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Received: 15 March 2007; in revised form: 30 April 2007 / Accepted: 1 May 2007 / Published: 3 May 2007

Abstract: Accumulating evidence indicates that consumption of tea, especially green tea, is good for preventing cancer. To elucidate the cancer preventive mechanisms of green tea, much effort has been devoted to investigating the anticancer effects of (-)-epigallocatechin-3-gallate (EGCG), the major component of green tea. It has been revealed that EGCG restrained carcinogenesis in a variety of tissues through inhibition of mitogen-activated protein kinases (MAPK), growth factor-related cell signaling, activation of activator protein 1 (AP-1) and nuclear factor-B (NF-κB), topoisomerase I, matrix metalloproteinases and other potential targets. Therefore, EGCG is a multipotent anticancer agent, which not only provides solid evidence to support the anticancer potential of green tea, but also offers new clues for discovering multiple-targeted anticancer drugs.

Keywords: Green tea, (-)-Epigallocatechin gallate, Cancer prevention, Mechanisms.

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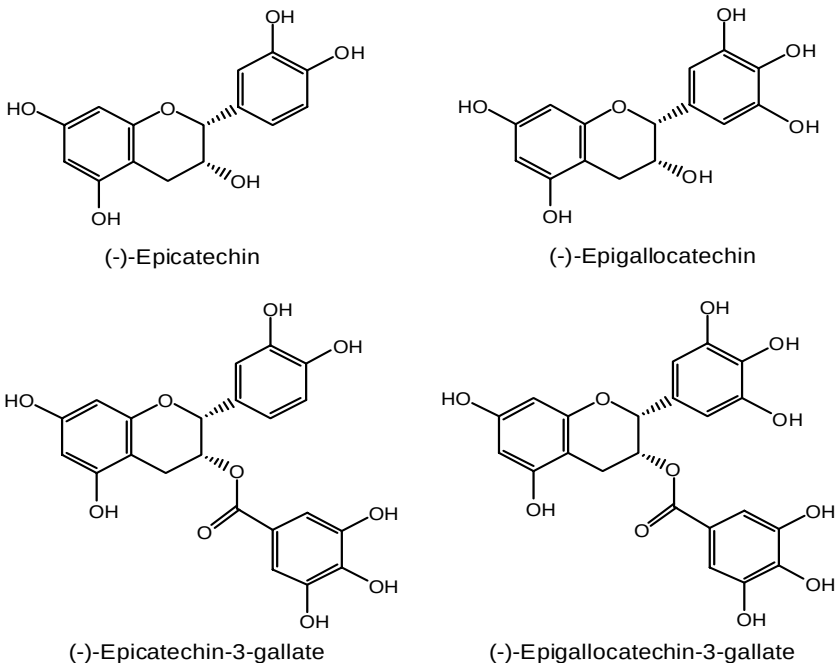
1. Introduction

Tea is one of the most popular beverages consumed in the world. Generally, it is divided into three types: green tea (non-fermented), oolong tea (semi-fermented), and black tea (fermented). Consumption of tea, especially green tea, has been associated with many healthy benefits including cancer prevention [1]. Some epidemiological observations have revealed that there was an inverse

correlation between increased green tea intake and relative risk for cancers. A prospective cohort study with 8,552 subjects from Saitama Prefecture in Japan indicated that green tea had a potentially preventive effect against cancers in many organs including stomach, lung, colorectum and liver [2]. Green tea drinking was also found to protect against stomach cancers in residents of Nagoya in Japan [3], pancreatic and colorectal cancers in Shanghai residents [4] and breast cancers in Saitama, Japan [5].

The benefits of green tea with regards to cancer prevention have been attributed, in a large part, to the green tea polyphenols, especially catechins. A cohort study (Iowa Women's Health Study) examined 34,651 postmenopausal women for 12 years and found that food-derived catechin intake was inversely associated with a rectal cancer incidence [6]. The green tea catechins mainly consist of (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin-3-gallate (EGCG) (Figure 1), of which EGCG may be the most effective chemopreventive agent and has been extensively studied with different human cancer cell lines and several cancer animal models. It has been revealed that EGCG inhibited carcinogenesis in a variety of tissues including lung, bladder, skin, small intestine, prostate and breast [7-11]. As to the molecular mechanisms, EGCG has the potential to inhibit the multiple targets implicated in the initiation, promotion and progression stage of cancers. Therefore, EGCG is a multiple-targeted anticancer agent with diverse activities, which stimulated our interest to summarize the current knowledge on this topic in this review.

Figure 1. Main catechin components of green tea polyphenols.



2. Inhibition of the cancer initiation stage by EGCG

It is well known that oncogene mutation and reactive oxygen species (ROS) play important roles in the cancer initiation stage. Oncogene mutation leads to procarcinogen activation by activating some

phase I enzymes such as the cytochrome P450s. ROS actively participate in the metabolic activation of procarcinogens. EGCG can neutralize these procarcinogens by inhibiting the activity of cytochrome P450 enzymes and modulating ROS.

Wang *et al.* [12] investigated the interaction of EGCG with rat hepatic microsomal P450 and found that EGCG significantly inhibited NADPH-cytochrome c reductase activity. An examination of the structure-activity relationships of epicatechin derivatives suggested that the inhibitory effect on the microsomal enzyme system might arise from the galloyl or hydroxyl groups in the molecule. Mukhtar *et al*. [13] also claimed that EGCG could interact with hepatic cytochrome P450 and inhibit the P450-dependent mixed-function oxidase enzymes in skin and liver.

Considerable evidence has demonstrated that EGCG is a powerful antioxidant. The ROS-scavenging effects of EGCG were superior to those of ascorbic acid and α-tocopherol in many cases [14]. Besides, the pyrogallol structure of EGCG also confers the molecule with strong metal-chelating ability. As a result, EGCG can bind with transition metal ions and behave as a preventive antioxidant [15, 16]. Its high affinity towards the lipid bilayers also facilitates the entry of EGCG into the nuclei of cancer cells [17].

3. Inhibition of the cancer promotion stage by EGCG

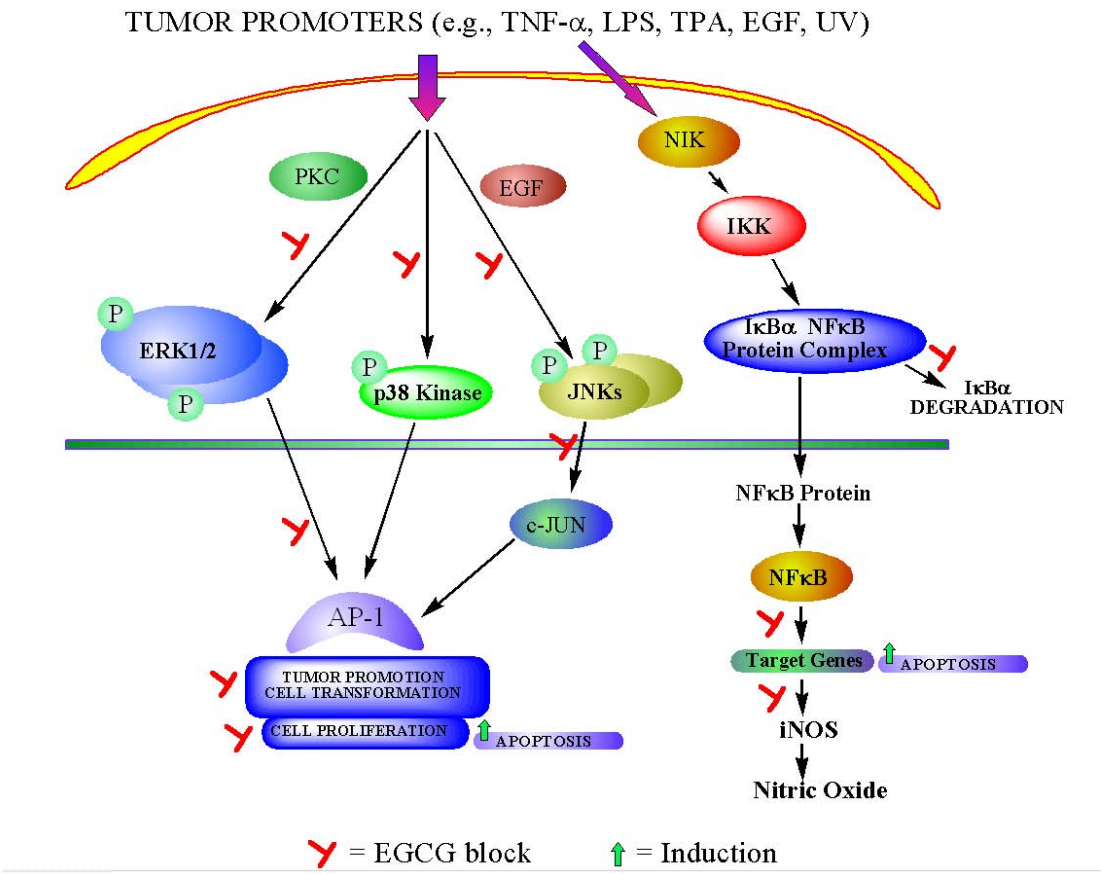
The cancer promotion stage is a reversible and a long-term process, in which some intracellular signaling pathways and proteins associated with cell cycle are involved. EGCG exerts its anticancer effect by interfering with many signaling pathways and modulating cell cycle.

3a. *Interference with intracellular signaling pathway (Figure 2).*

Mitogen-activated protein kinases (MAPKs) pathways are very common in various cells. They are composed of a group of Ser/Thr protein kinases that are activated as a cascade. Three classes of MAPKs are most well known, namely extracellular signal-regulated protein kinases (ERKs), c-Jun N-terminal kinases/stress activated protein kinases (JNKs/SAPKs) and p38 kinases. In general, ERKs are critical transducers of proliferation signals and are often activated by growth-inducing tumor promoters, including 12-O-tetradecanoyl-phorbol-13-acetate (TPA), epidermal growth factor (EGF) and platelet-derived growth factor (PDGF). In comparison, JNKs/SAPKs and p38 kinases are strongly activated by stress-related tumor promoters, such as ultraviolet (UV) irradiation and arsenic. Some studies have shown that EGCG can regulate the important molecules in MAPK pathway, which result in the inhibition of cancer cell survival.

Katiyar *et al*. [18] reported that treatment of H_2O_2 resulted in phosphorylation of ERK1/2, JNK, and p38 in human epidermal keratinocytes. When these cells were pretreated with EGCG, H_2O_2-induced phosphorylation of ERK1/2, JNK, and p38 were found to be significantly inhibited. Maeda-Yamamoto *et al* [19] also reported that EGCG inhibited the phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2), and suppressed p38 MAPK activity in human fibrosarcoma HT1080 cells. These findings demonstrated that EGCG had potential of inhibiting oxidative stress-mediated phosphorylation of MAPK signaling pathways.

Figure 2. Intracellular signaling pathways and its modulation by EGCG.



After activation, MAPKs translocate to the nucleus to activate numerous transcription factors. Two major transcription factors in eukaryotic cells are nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1) [20, 21]. NF-κB is a pleiotropic transcription factor that exists in all cell types and plays an important role in regulating the expression of multiple genes. NF-κB signaling pathway is composed of about a dozen different dimers comprising five structurally related DNA-binding proteins, namely NF-κB1/p50, NF-κB2/p52, RelA/p65, Rel/c-Rel and RelB. It has been well accepted that NF-κB signaling pathway plays a critical role in the control of cell growth and apoptosis [22]. Although there are other important molecules, such as IκB and IKK, within NF-κB signaling pathway, NF-κB is the key factor in this pathway.

Based on the many functions of NF-κB target genes, a close relationship between NF-κB and cancer has been proposed. Some studies have confirmed this hypothesis. Treatment with EGCG (10-40 μmol/L) in a dose- and time-dependent manner was found to inhibit UVB-mediated activation of NF-κB in normal human epidermal keratinocytes [23]. Gupta *et al*. [24] have identified NF-κB /p65 component of the NF-κB complex as a target for specific cleavage by caspases during EGCG-mediated apoptosis.

Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) are important enzymes that mediate inflammatory processes. Improper up-regulation of COX-2 and/or iNOS has been associated with pathophysiology of certain types of human cancers. Surh *et al*. [25] have shown that EGCG could down-regulate the expression of COX-2 and iNOS by suppressing NF-κB activity. These data strongly suggested that NF-κB and its components are potential targets for EGCG cancer prevention. Since NF-

κB is considered as a target for the management of cancer, based on recent studies, modulation of this pathway by EGCG could contribute to its chemopreventive potential.

AP-1 is another important eukaryotic transcription factor. It is a well-characterized transcription factor composed of members of the basic region leucine zipper protein superfamily, i.e., Jun, Fos and activating transcription factor proteins [26]. It regulates the transcription of various genes associated with cellular proliferation and apoptosis [27]. Strong evidence indicates that AP-1 plays a key role in cancer development and it is up-regulated during tumor promotion stage.

Dong *et al*. [28] found that EGCG could inhibit EGF- or TPA-induced cell transformation, as well as AP-1-induced transcriptional activity and DNA binding activity. This study also implicated that the inhibition of AP-1 activation occurred via the inhibition of a JNK-dependent pathway. Huang *et al*. [29] showed that EGCG inhibited arsenite-induced AP-1 transcriptional activation and AP-1 DNA binding activity. Thus, AP-1 serves as another potential target, besides NF-κB, for the cancer prevention effects of EGCG.

In addition, EGCG can also influence epidermal growth factor receptor–mediated signal transduction pathway. Over-expression of growth factor and growth factor receptors such as EGF-Receptor (EGFR), PDGF-Receptor (PDGFR) and others can result in a neoplastic phenotype in tumor cells. EGCG can interfere with cancer promotion by inhibiting growth factor-mediated cell signaling pathway.

Shimizu *et al*. [30] have reported that EGCG (at 10-20 μg/mL) was shown to inhibit the activation of the EGFR, HER2, and multiple downstream signaling pathways in colon cancer cell lines. Tachibana *et al*. [31] found that EGCG bound to a specific metastasis associated 67kDa laminin receptor that was expressed on a variety of tumor cells. It was shown using a subtraction cloning strategy involving cDNA libraries constructed from cells treated or untreated with all *trans*-retinoic acid that the anticancer action of EGCG is mediated by laminin receptor and it allows EGCG to bind to the cell surface. Based on these data, it was suggested that there exist a receptor for EGCG. This suggestion awaits follow-up and confirmation.

In addition, Jung *et al*. [32] reported that 30 μmol/L of EGCG inhibited serum starvation-induced vascular endothelial growth factor (VEGF) expression in HT29 colon cancer cells. This observation may account for the antitumor activity of EGCG against HT29 xenografts in athymic nude mice. The authors observed that EGCG not only decreased tumor growth (58%) and increased tumor cell apoptosis (1.9-fold) but also decreased tumor microvessel density (30%).

3b. Cell cycle modulation

Characterization of the cell cycle has made rapid progress in recent years. The relationship between disrupted cell cycle control and cancer development has been a focus in carcinogenetic mechanism studies. Some research has demonstrated that the anticancer functions of EGCG might be associated with cell cycle modulation.

As an important family of positive cell cycle regulators, cyclins are frequently over-expressed in cancer cells, whereas the negative cell cycle regulators or Cyclin-Dependent Kinase Inhibitors (CKI), such as p16, p21, p27, p53 and P73, are under-expressed. Some investigators found that EGCG could

prevent the cancer progression by up-regulating CKI, and inhibiting the activities of Cyclin-Dependent Kinase (CDK) such as CDK1 and CDK2.

Nihal *et al*. [33] found that EGCG treatment of human melanoma cells resulted in a significant, dose-dependent decrease in cyclin D1 and CDK2 protein levels and induction of p16, p21 and p27. Kavanagh *et al*. [34] found that p27, which could promote G1/S phase growth arrest, was induced in breast cancer cells by treatment with EGCG. Hastak *et al*. [35] have clearly demonstrated that EGCG activated growth arrest, primarily via a p53-dependent pathway that involved the function of both p21 and Bax such that down-regulation of either molecule conferred a growth advantage to prostate carcinoma cells. These results indicated that EGCG might exert its growth-inhibitory effects through modulation of cell cycle regulatory proteins.

Berger *et al.* [36] have demonstrated that EGCG selectively inhibited the activity of topoisomerase I (but not topoisomerase II), which plays a role in DNA replication, transcription, and chromosome condensation in human colon cancer cell lines. The doses of EGCG necessary for this inhibition (10-17 μmol/L) were lower than those necessary for inhibition of cell growth (IC_{50} = 10-90 μmol/L).

Telomerase is an enzyme essential for unlocking the proliferative capacity of cancer cells and it is lacking in normal somatic cells. It plays a salient role in the process of cancer. In most cancers, the maintenance of telomeres is achieved through the expression of telomerase, which stabilizes and elongates telomeres by the *de novo* synthesis of telomeric DNA. The role of telomerase in immortalization was confirmed recently by the findings that the ectopic expression of telomerase in various normal cells resulted in the extension of the life span of these cells [37, 38]. Naasani *et al.* [39] demonstrated that EGCG after structural rearrangements at physiologically permissible conditions increased remarkably telomerase inhibition. In nude mice models bearing both telomerase-dependent- and independent xenograft tumors cloned from a single human cancer progeny, only the telomerase-dependent tumors responded to prolonged oral administration of EGCG. It is also reported that EGCG strongly inhibited telomerase activity, and thus induced senescence, limited the life span of cancer cells, in both leukemias and solid tumors [40]. Therefore, telomerase inhibition could be one of the mechanisms underlying the anticancer effects of EGCG.

4. Inhibition of the cancer progression stage by EGCG

During the complicated processes of cancer progression, apoptosis and some enzymes such as urokinase and matrix metalloproteinases (MMPs) play a key role. Accumulating evidence indicates that EGCG can inhibit the growth of malignant cells by inducing apoptosis and inhibiting the activity of some such enzymes.

Apoptosis is an active form of cell suicide controlled by a network of genes, in which the Bcl-2 family of proteins plays important roles in control of apoptosis via regulating mitochondrial permeability and releasing of cytochrome c, which activates the caspase cascade. Apoptosis is an essential process which plays a critical role in the pathogenesis of diseases including cancer. EGCG has been shown to induce the apoptosis in a number of cancer cells.

Hwang *et al*. [41] demonstrated that a treatment of chemoresistant HT-29 human colon cancer cells with 100 μmol/L EGCG inhibited cell proliferation by inducing apoptosis. Nishikawa *et al*. [42] showed that EGCG inhibited the growth of HLE cells (an undifferentiated human hepatocellular

carcinoma cell line) *in vitro* and *in vivo*. The inhibition was caused by the induction of apoptosis as a result of the activations of caspase-8, -9 and -3. These caspases appeared to be activated by the down-regulation of Bcl-2α and Bcl-xl. Thangapazham *et al*. [10] found that EGCG treatment inhibited proliferation and induced apoptosis of MDA-MB-231 cells *in vitro* and *in vivo*. Lin *et al*. [43] clearly identified the inhibitory effect of EGCG on differentiation and the induction of apoptosis in adipocytes in vitro. Islam *et al*. [44] showed that EGCG displayed strong inhibitory effects on the proliferation and viability of HTB-94 human chondrosarcoma cells by inducing apoptosis.

Qin *et al*. [45] found that EGCG treatment induced apoptosis in the T24 human bladder cancer cell line by inhibiting phosphatidylinositol 3'-kinase/Akt activation that, in turn, resulted in modulation of Bcl-2 family proteins, leading to enhanced apoptosis of T24 cells. Recent studies find that CKI-p53, p73 play an important role for the anticancer function of EGCG.

Amin *et al*. [46] have identified SHP-2 (a kind of tyrosine phosphatase) as a protective factor of cells lacking functional p53 from EGCG-induced apoptosis. Moreover, they revealed a number of targets for EGCG-induced apoptosis which were expressed in the absence of functional p53. This had not previously been reported to be so induced. At the same time, they also identified a crucial role for p73 in EGCG-induced apoptosis and a number of previously unidentified p73 target genes. Manna *et al*. [47] suggested that *in vivo* EGCG could induce apoptosis in Sarcoma180 (S180) cells through alteration in G2/M phase of the cell cycle by up-regulation of p53, bax and down-regulation of c-myc, bcl-2 and U1B, U4-U6 UsnRNAs (uridylic acid rich small nuclear RNAs).

In addition, some researchers found that the pro-apoptotic activity of EGCG appeared to depend on the cell type and the dosage treated. Up-regulation of p57 was shown in both normal and oral cancer cells, but apoptosis was induced only in malignant cells, suggesting that EGCG affected a p57-mediated survival pathway in normal epithelial cells, while induced a pro-apoptotic pathway in oral cancer cells [48, 49].

Cancer invasion and metastasis are multifactorial processes and require the coordinated action of cell-secreted proteolytic enzymes and their inhibitors [50]. EGCG can also prevent the cancer progression stage by influencing urokinase and matrix metalloproteinases.

Urokinase is a kind of hydrolase. The urokinase plasminogen activator (uPA) is commonly overexpressed in many different human cancers [51]. Inhibition of urokinase-type plasminogen activator (uPA) activity can reduce tumor size or even cause complete remission of tumors in mice [52]. Jankun *et al*. [53] used computer-aided molecular modeling to demonstrate that EGGC bound to urokinase, blocked the histidine 57 and serine 195 residues of the urokinase catalytic triad and extended towards arginine 35 from a positively charged loop of urokinase. Binding of EGCG at such a location would interfere with the ability of uPA to recognize its substrates, thereby inhibiting its enzymatic activity. Kim *et al*. [54] indicated that the inhibition of uPA expression by EGCG was important for the anti-invasive function of EGCG.

MMPs are a family of tightly regulated zinc-dependent proteases that can degrade nearly all components of the extracellular matrix. A large body of experimental evidence suggested that MMPs essentially contributed to the maintenance of tumor growth in primary and metastatic sites [55-57]. Adhami *et al*. [58] have shown that p.o. administered green tea polyphenols (0.1% in drinking water) caused marked inhibition of MMP-2 and MMP-9 in the prostate in TRAMP mice. Fassina *et al*. [59] found that EGCG (25-100 μmol/L) could inhibit the MMP-2 and MMP-9 in endothelial cells. Annabi

et al. [60] have shown that EGCG likewise inhibited the activity and expression of membrane-type matrix metalloproteinase 1-MMP (MT1-MMP), a protein responsible for the activation of MMP. Thus, it seems that EGCG could inhibit or delay cancer invasion, metastasis, and angiogenesis via modulations in MMPs.

Recently, by employing surface plasmon resonance assay (Biacore) and cold spray ionization-mass spectrometry, Kuzuhara *et al*. [61] claimed that DNA and RNA served as new binding targets of EGCG. Their results suggested that multiple binding sites of EGCG were present in DNA and RNA oligomers and revealed for the first time the link between catechins and polynucleotides. These findings will promote our understanding of the effects of catechins on DNA in terms of cancer prevention.

5. Conclusions

The above description illuminates the cancer preventive mechanisms of EGCG. Since the development of cancer is a dynamic and multistage process, in which a large number of genes and proteins are involved, to combat the disease, we will have to shift the drug-discovery paradigm from "one-drug, one-target" to "one-drug, multiple-targets". Hence, the potential of EGCG in hitting multiple targets implicated in various stages of cancer development not just provides solid evidence to support the anticancer effects of green tea, but also offers new evidence to justify the multiple-targeted antitumor strategy. In addition, as the structure of EGCG holds the structural secrets of multipotent anticancer agents, EGCG is expected to serve as a promising starting point to derive novel anticancer drugs.

Acknowledgements

This study was supported by the National Basic Research Program of China (grant 2003CB114400) and the National Natural Science Foundation of China (grant 30100035 and 30570383).

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Sample Availability: Not applicable.