Targeting Multiple Signaling Pathways by Green Tea Polyphenol (−)-Epigallocatechin-3-Gallate

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Abstract

Cell signaling pathways, responsible for maintaining a balance between cell proliferation and death, have emerged as rational targets for the management of cancer. Emerging data amassed from various laboratories around the world suggests that green tea, particularly its major polyphenolic constituent (−)-epigallocatechin-3-gallate (EGCG), possesses remarkable cancer chemopreventive and therapeutic potential against various cancer sites in animal tumor bioassay systems and in some human epidemiologic studies. EGCG has been shown to modulate multiple signal transduction pathways in a fashion that controls the unwanted proliferation of cells, thereby imparting strong cancer chemopreventive as well as therapeutic effects. This review discusses the modulations of important signaling events by EGCG and their implications in cancer management. (Cancer Res 2006; 66(5): 2500-5)

Introduction

Cancer chemoprevention. The multistage process of cancer development (i.e., carcinogenesis) leading to clinically visible and metastasized cancers in humans is a long process, generally taking many years through well-defined stages known as initiation, promotion, and progression. Because advanced metastasized cancers are mostly incurable, an effort to prolong or block the process of carcinogenesis through chemoprevention has become an important and feasible strategy for cancer control and management. The concept of chemoprevention is to control the occurrence of cancer by slowing, blocking, or reversing the development of the disease by the administration of naturally occurring or synthetic compounds. For a variety of reasons, the most important of which is potential human acceptance, naturally occurring dietary substances for chemoprevention are preferred. Many studies with a number of different diet-derived compounds indicate that many such agents are capable of prolonging one or more stages of the carcinogenic process (1).

Cancer chemoprevention through modulation of intracellular signaling network. Center to the cancer biology is disrupted intracellular signaling network, which transmits aberrant signals resulting in abnormal cellular function. Consistent with this notion, targeting deregulated intracellular signaling cascades is considered to be a rational approach in achieving chemoprevention. Recent research is clarifying that many dietary cancer chemopreventive agents exert their effects by modulating one or more cell signaling pathways in a manner that interrupts the carcinogenic process (2).

Tea in chemoprevention of cancer. Tea produced from the leaves of the plant *Camellia sinensis* is, next to water, the most widely consumed beverage in the world. Among all teas consumed in the world, green tea is the best studied for health benefits, including chemopreventive efficacy, because its chemistry compared with other teas is better known (3). A search of literature shows that there are >765 published studies showing the effects of green tea on cancer, mostly dealing with its chemopreventive effects. It is generally agreed that much of cancer chemopreventive effects of green tea are mediated by its polyphenols. The major catechins in green tea are (−)-epigallocatechin-3-gallate (EGCG), (−)-epicatechin-3-gallate, (−)-epigallocatechin, and (−)-epicatechin. EGCG is the major catechin in green tea and accounts for 50% to 80% representing 200 to 300 mg in a brewed cup of green tea.

In recent years, many studies from our and other laboratories have shown strong chemopreventive and possibly cancer chemotherapeutic effects of green tea polyphenols and EGCG against cancers of the skin (UV radiation and chemically induced), lung, breast, colon, liver, stomach, prostate, and other sites (3, 4). The purpose of this brief review is to present recent research data focusing on the modulation of cellular signaling events by EGCG.

Induction of Apoptosis and Cell Cycle Arrest by EGCG

Apoptosis is a highly ordered protective mechanism through which unwanted or damaged cells are eliminated from the system. It is essential for normal development, turnover, and replacement of cells in the living system. In addition, apoptosis serves as a protective mechanism against neoplastic development in an organism by eliminating genetically damaged cells or excess cells that have improperly been induced to divide. The very first study documenting selective effects of EGCG for induction of apoptosis and cell cycle arrest for cancer cells was from our laboratory in which it was shown that EGCG induces apoptosis and cell cycle arrest in many cancer cells without affecting normal cells (5). Many subsequent studies have verified this observation in several cell types, including lung, colon, pancreas, skin, and prostate (4). Various studies show that there is link between cell cycle regulation and cancer and the inhibition of the cell cycle is considered as a target for the management of cancer. Although EGCG has been shown to affect a number of factors associated with cell cycle progression,

the direct inhibition of cyclin-dependent kinases is considered as the primary event. The induction of various negative regulators of the cell cycle may be the consequence of this inhibition. EGCG also induces the expression of p21 and p27 while decreasing the expression of cyclin D_1 and the phosphorylation of retinoblastoma. The primary events in green tea polyphenol–induced apoptosis include nuclear condensation, caspase-3 activation, and poly(ADP)ribose polymerase cleavage. In addition, EGCG also invokes Bax oligomerization and depolarization of mitochondrial membranes to facilitate cytochrome *c* release into cytosol. The observation that addition of catalase in the cell culture system prevented EGCG-induced apoptosis suggest that H_2O_2 generated from EGCG plays a role in apoptosis induction. Using nuclear magnetic resonance spectroscopy, the direct binding of tea polyphenols to the BH3 pocket of antiapoptotic Bcl-2 family proteins is shown, suggesting a mechanism for EGCG to inhibit the antiapoptotic function of Bcl-2 proteins. The BH3 domain was recognized as one of the binding sites of tea polyphenols (6).

Modulation of Cell Signaling by EGCG

Inhibition of nuclear factor-κB signaling pathway. Nuclear factor-κB (NF-κB) is an oxidative stress–sensitive transcription factor that plays a critical role in the regulation of a variety of genes important in cellular responses, including inflammation, innate immunity, growth, and cell death. NF-κB is sequestered in the cytoplasm in an inactive form through interaction with IκB (Fig. 1). Phosphorylation of IκB by IκB kinase causes ubiquitination and degradation of IκB, thus releasing NF-κB that then translocates to the nucleus. Phosphorylation and activation of IκB kinase is controlled by an NF-κB-inducing kinase and there is crosstalk between activation of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway, and the NF-κB-inducing kinase/IκB kinase/NF-κB pathway. It has been shown that the galloyl and hydroxyl groups at the 3′ position on EGCG are responsible for its strong anti-inflammatory properties. EGCG has been shown to inhibit NF-κB activity in human colon cancer cells (7). 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 (8). We have identified NF-κB/p65 component of the NF-κB complex as a target for specific cleavage by caspases during EGCG-mediated apoptosis (9). Because based on recent studies, NF-κB is considered as a target for the management of cancer, modulation of this pathway by EGCG could contribute to its chemopreventive potential.

Inhibition of MAPKs and activator protein-1. MAPKs have been implicated in many physiologic processes, including cell proliferation, differentiation, and death. There are three major types of MAPKs in mammalian cells, the extracellular signal-regulated protein kinases (ERK), the p38 MAPKs, and the c-Jun NH_2-terminal kinases (JNK). The major pathways that lie downstream of the membrane-associated receptor tyrosine kinases (RTK) are shown in Fig. 1. In this cascade, Ras interacts with and activates Raf-1, which in turn phosphorylates and activates MAP/ERK kinase 1/2 (MEK1/2). Activated MEK1/2 then phosphorylates ERK1/2. The JNK1/2/3 and p38α/β/γ pathways are parallel MAPK cascades in mammalian cells.

Once activated, MAPKs (ERK, JNK, and p38) activate ELK and c-Jun. Phosphoinositide 3-kinase (PI3K) is activated by RTKs and it synthesizes the second messenger, phosphatidyl inositol-3,4,5-triphosphate, which is necessary for phosphorylation of Akt. Akt directly phosphorylates the proapoptotic protein Bad, thus enhancing the antiapoptotic function of Bcl-x_L. In JB6 mouse epidermal cell line, it was shown that EGCG (5-20 μmol/L) inhibited the MAPK pathway (10). The treatment of EGCG (10-40 μmol/L) to NHEK before UVB exposure was shown to inhibit UVB-induced hydrogen peroxide production concomitant with the inhibition of UVB-induced phosphorylation of ERK1/2, JNK, and p38 proteins (8). Recently, EGCG (10-20 μg/mL) has been shown to inhibit MAPK pathway and activator protein-1 (AP-1) activity in human colon cancer cells (7). We have shown that p.o. feeding of EGCG containing green tea polyphenols inhibits PI3K pathway in transgenic adenocarcinoma of the mouse prostate (TRAMP) model system (11). Because the deregulation of the MAPK pathway is frequently seen in a variety of human cancers, modulation of MAPKs by EGCG may provide novel strategies for the prevention or treatment of cancer.

AP-1 transcription factor is a protein dimer composed of members of the basic region leucine zipper protein superfamily, specifically, the Jun, Fos, and activating transcription factor proteins. High AP-1 activity has also been shown to be involved in the tumor promotion and progression of various types of cancers, such as lung, breast, and skin cancer. EGCG was shown to inhibit 12-*O*-tetradecanoylphorbol-13-acetate or epidermal growth factor–induced transformation of mouse epidermal cell line JB6, and the inhibitory activity was closely related to the inhibition of AP-1 (10).

Inhibition of epidermal growth factor receptor–mediated signal transduction pathway. The epidermal growth factor receptor (EGFR) is a plasma membrane glycoprotein with an extracellular ligand-binding domain, a single transmembrane region, and an intracellular domain that exhibits intrinsic tyrosine kinase activity. Overexpression of EGFR produces a neoplastic phenotype in tumor cells. EGCG (10-20 μg/mL) recently was shown to inhibit the activation of the EGFR, HER2, and multiple downstream signaling pathways in colon cancer cell lines (7). EGCG binds to a specific metastasis associated 67 kDa laminin receptor that is 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 (12). The cell-laminin interaction via the 67LR is an important step in several signal transduction pathways and 67LR is involved in kinase-phosphatase cascades. There is an association between 67LR and the integrin α_6 subunit, which is a part of laminin-binding integrins $\alpha_6\beta_4$ and $\alpha_6\beta_1$. Recent evidence has also suggested the involvement of MAPK in the laminin signaling pathway in metastatic human melanoma cells (13). Based on these data, it was suggested that there exist a receptor for EGCG. This suggestion awaits follow-up and confirmation.

Inhibition of insulin-like growth factor-I–mediated signal transduction pathway. Insulin-like growth factor (IGF) family of ligands, binding proteins, and receptors is an important growth factor system involved in the maintenance of normal functions of cells. The binding of free IGFs to IGF-I results in
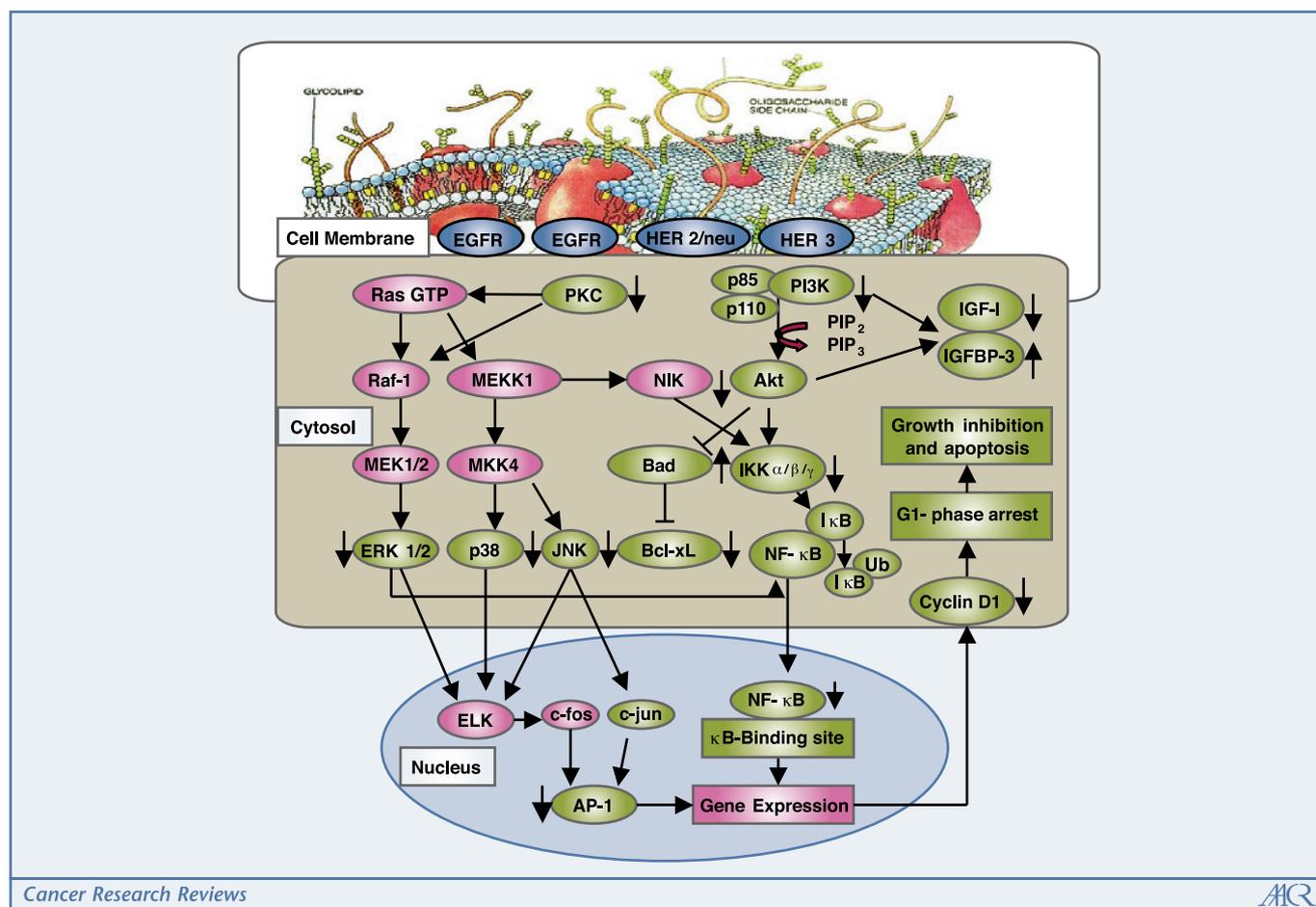
Figure 1. Effect of EGCG on EGFR, MAPK cascades, and activation of the transcription factors AP-1 and NF-κB. Activation of EGFR or HER-2/neu elicits the autophosphorylation of the receptors and the phophotyrosine residues are bound by other protein kinases in the cytosol. The activated RTKs then phosphorylate several downstream molecules, thus activating several signaling pathways. Activation of Ras, Raf-1, and PI3K stimulates several intracellular processes. The activated Raf-1 stimulates MAPK kinase and MEK1/2 cascade, which then phosphorylate the MAPK protein ERK1/2. When activated, MAPKs activates various transcription factors, including ELK and c-Jun. The binding of free IGFs to IGF-I results in intramolecular receptor autophosphorylation and phosphorylation of critical downstream targets. This leads to activation of several signaling pathways, including the PI3K/Akt pathway and the Ras/MAPK pathway, thus inducing activation of specific genes, DNA synthesis, and cell proliferation. Her-3 activates PI3K and this causes synthesis of phosphatidyl inositol-3,4,5-triphosphate (PIP_3), which activates downstream pathways that involve Akt. NF-κB-inducing kinase and Akt phosphorylate and activate IκB kinase. Activated IκB kinase (*IKK*) phosphorylates IκB, which triggers ubiquitinylation and subsequent degradation of the IκB. NF-κB-inducing kinase (*NIK*) enhances the NF-κB activity through activation of MEK1/2 and ERK1/2. EGCG blocks the autophosphorylation of EGFR and Her-2/neu as well as the MAPK cascade, such as RAS/RAF/MEK/ERK/JNK pathways and PI3K pathways. EGCG also caused substantial reduction in the levels of IGF-I and significant increase in the levels of IGFBP-3. *Green,* molecules on which both the *in vivo* and *in vitro* effects of EGCG have been shown; *pink,* molecules on which only *in vitro* effects of EGCG have been shown.

intramolecular receptor autophosphorylation and phosphorylation of critical downstream targets. This leads to activation of several signaling pathways, including the PI3K/Akt pathway and the Ras/MAPK pathway, thus inducing activation of specific genes, DNA synthesis, and cell proliferation (Fig. 1). We have shown that green tea polyphenols resulted in substantial reduction in the levels of IGF-I and significant increase in the levels of IGF binding protein 3 (IGFBP-3) in TRAMP mice (11). Therefore, targeting the IGF-I signaling pathway by EGCG may be an effective strategy for the prevention and treatment of some cancers.

Inhibition of overexpression of cyclooxygenase-2. Cyclooxygenase-2 (COX-2) is induced by a variety of factors, such as cytokines, growth factors, and tumor promoters. Inappropriate COX-2 activity has been observed in many pathologic conditions, including cancer. We have shown that EGCG (10-100 μmol/L) inhibits mitogen-stimulated COX-2 expression

in androgen-sensitive LNCaP and androgen-insensitive PC-3 human prostate carcinoma cells (14). It has been shown that EGCG reduced the protein expression and activity of COX-2 following interleukin-1β stimulation of human chondrocytes (15). Most research findings strongly suggest that development of chemopreventive compounds, which can block COX-2 expression preferably without affecting COX-1, is a high priority for cancer research.

Inhibition of proteasome activity. The proteasome is a massive multicatalytic protease complex that is responsible for degrading most of the cellular proteins. The ubiquitin/proteasome–dependent degradation pathway plays an essential role in up-regulation of cell proliferation, down-regulation of cell death, and development of drug resistance in human tumor cells, suggesting the use of proteasome inhibitors as potential novel anticancer drugs. Nam et al. (16) revealed that EGCG potently and specifically inhibits the chymotrypsin-like but not

trypsin-like activity of the proteasome *in vitro* and *in vivo*. Inhibition of the chymotrypsin-like activity of the proteasome has been associated with induction of tumor cell apoptosis. EGCG was also found to block the catalytic activities of the 20S/26S proteasome complex, resulting in intracellular accumulation of IκBα and subsequent inhibition of NF-κB activation. This suggests that the proteasome is a cancer-related molecular target of EGCG and that inhibition of the proteasome activity by EGCG may contribute to its cancer-preventive effect.

Modulation of Cell Signaling Associated with Angiogenesis, Metastasis, and Migration by EGCG

Inhibition of vascular endothelial growth factor. Vascular endothelial growth factor (VEGF) is a mitogen for endothelial cells and is associated with tumor-induced angiogenesis. VEGF binds to VEGF receptors 1 and 2, the latter being responsible for most of its mitogenic and chemotactic effects. Recently, EGCG was found to decrease VEGF receptor phosphorylation and induces apoptosis in chronic lymphocytic leukemia B cells (17). Treatment with EGCG (5-50 μmol/L) was shown to result in an inhibition of human umbilical arterial endothelial cell (HUAEC) mitogenesis. The signal transduction pathways of VEGF in HUAEC, including autophosphorylation of VEGF receptors 1 and 2, phosphorylation of ERK1/2, and mRNA expression of the early growth response factor-1 were also inhibited in EGCG-pretreated cells (18). Thus, the inhibition of VEGF binding to its receptor may contribute to the antiangiogenic and cancer chemopreventive effects of EGCG.

Inhibition of matrix metalloproteinase. The progression of human tumors involves the matrix metalloproteinase (MMP) family. Two particular members of this family, MMP-2 and MMP-9, seem to play an important role in tumor invasion and metastasis. They are involved in the turnover of basement membrane collagen under basal conditions and of other matrix proteins during angiogenesis, tissue remodeling, and repair. EGCG has been shown to affect MMP activity both directly and indirectly. We 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 (11). EGCG (25-100 μmol/L) has been also been reported to inhibit the MMP-2 and MMP-9 in endothelial cells (19). Thus, it seems that EGCG could inhibit or delay cancer invasion, metastasis, and angiogenesis via modulations in MMPs.

Inhibition of urokinase-plasminogen activator. Urokinase-plasminogen activator (uPA) is a trypsin-like protease that converts the zymogen plasminogen into active plasmin. It has the ability to prevent apoptosis, stimulate angiogenesis, mito-genesis, cell migration, and to modulate cell adhesion. Inhibition of urokinase can decrease tumor size or even cause complete remission of cancers in mice. The known urokinase inhibitors are unlikely to be used in anticancer therapy because of their weak inhibitory activity or high toxicity. Jankun et al. (20) showed that EGCG inhibits the activity of uPA. With the use of molecular modeling, the authors showed that EGCG binds to urokinase, blocking His[57] and Ser[195] of the urokinase catalytic triad, and extending toward Arg[35] from a positively charged loop of urokinase. Thus, it was suggested that the

anticancer activity of EGCG is mediated by inhibition of uPA, one of the most frequently overexpressed enzymes in human cancers.

EGCG in Adjuvant Settings

Several studies have suggested that EGCG may reduce the toxicity of certain anticancer drugs. These studies suggest that EGCG could be used in adjuvant settings for cancer management. The synergistic effects of EGCG with sulindac on the inhibition of intestinal tumors in multiple intestinal neoplasia (Min) mice have been reported. The results also indicated that green tea extract inhibited tumor growth in Min mice almost as potently as achieved by sulindac itself (21). The effects of green tea and EGCG were also tested in Kaposi sarcoma tumor model and on endothelial cells. Green tea or purified EGCG when administered to mice in the drinking water inhibited angiogenesis *in vivo* in the Matrigel sponge model and restrained Kaposi sarcoma tumor growth. Histologic analyses of the tumors were consistent with an antiangiogenic activity of EGCG and green tea (19). These data suggest that green tea or EGCG may find use in the prevention and treatment of vascular tumors in a chemoprevention or adjuvant setting.

Clinical Trials

EGCG delivered in the form of capsule (200 mg p.o. for 12 weeks) has been reported to be effective in the patients with human papilloma virus–infected cervical lesions (22). The antineoplastic effects of green tea were seen in patients with androgen-independent prostate carcinoma (23). A prospective cohort study with over 8,000 individuals revealed that the daily consumption of green tea resulted in delayed cancer onset and a follow-up study of breast cancer patients found that stages I and II breast cancer patients experienced a lower recurrence rate and longer disease-free period (24). The positive results observed in phase II and phase III clinical trials along with exciting preclinical results indicate that ways and means to take EGCG "from bench to real-life situations" are on the horizon.

Conclusions and Future Prospects

Currently, there is much interest in the design and development of chemopreventive agents that act on specific molecular and cellular targets. The broader outlook of this goal is to define a chemopreventive/chemotherapeutic agent that can target most, if not all, targets in a manner that control unwanted cellular proliferation. As depicted in Fig. 1, EGCG is an agent, which, both under *in vitro* and *in vivo* situations, can target multiple pathways. However, it should be noted that most of the effects of EGCG in cell culture systems have been obtained with relatively high concentrations than observed in the plasma or tissues of animals or in human plasma after administration of green tea or EGCG. The pharmacokinetic studies in humans indicate that the peak plasma concentration after single p.o. dose of EGCG is <1.0 μmol/L. Therefore, it is not clear whether the activities observed with high EGCG concentrations in cell lines can be observed *in vivo*. However, it is possible that extended use

of green tea by humans could build sufficient EGCG in the plasma. The activities observed at submicromolar concentrations of EGCG may be more relevant to the *in vivo* situations. EGCG is autooxidized under cell culture conditions and the half-life of EGCG is rather short, forming dimers that are also subjected to autooxidation. During this process, superoxide is generated and hydrogen peroxide is produced. Under normal conditions, the oxygen partial pressure in the internal organs (40 mm Hg) is much lower than that under cell culture conditions (160 mm Hg) and cells are endowed with antioxidative enzymes, such as superoxide dismutase and glutathione peroxidase. It remains to be determined whether EGCG autooxidation occurs in target tissues, at sites of inflammation, or in cancer cells.

Although there are several studies supporting the preventive potential of EGCG against cancer, a proper understanding of the mechanisms by which they reduce the risk is necessary to establish the efficacy. Here, we provide evidence that the inhibitory effects of EGCG on carcinogenesis are mediated through the regulation of cell signaling pathways. To better understand the mechanisms responsible for the chemopreventive efficacy of EGCG, it is crucial to identify, in animal models and human clinical trials, molecules in the signaling network that are affected as the deregulation of the intracellular cascades leads to the development of many diseases including cancer. By modulating cell signaling pathways, EGCG activate cell death signals and induce apoptosis in precancerous or cancer cells, resulting in the inhibition of cancer development or progression.

The understanding of the cell signaling pathways and the molecular events leading to carcinogenesis will provide more insight into the identification and development of potent chemopreventive/chemotherapeutic agents that specifically target these pathways. Future studies from cell cultures should be integrated with studies *in vivo*, especially in ongoing clinical trials, to evaluate the applicability of these mechanisms in cancer prevention in humans. To fully elucidate the molecular mechanisms of action of EGCG in future studies, more in-depth *in vitro* and *in vivo* experiments are needed. Furthermore, because EGCG can modulate multiple pathways, it seems to be an attractive agent for a combination chemoprevention/chemotherapeutic approach, which seems ideal for the management of cancer.

Summary

Naturally occurring substances that are derived from diet provides a new insight in cancer therapy. The mechanisms of action of several chemopreventive agents derived from edible plants have gained considerable attention in cancer research. Tea is the most widely consumed beverage worldwide. There is extensive research going on in elucidating the molecular mechanisms of cancer chemoprevention by green tea. Although there are several studies supporting the preventive potential of EGCG against cancer, a proper understanding of the mechanisms by which EGCG reduces the risk is necessary to establish its efficacy for the population where it could be most useful. Several mechanisms to explain the chemopreventive effects of EGCG have been presented, among which its effect to target specific cell signaling pathways have received considerable attention for regulating cellular proliferation and apoptosis. The diversified effects of EGCG may explain its broad pharmacologic effects in modulating cell signaling pathways. EGCG, in addition to other mechanisms, at human achievable dose, is known to activate cell death signals and induce apoptosis in precancerous or cancer cells, resulting in the inhibition of cancer development and/or progression. Importantly, these antiproliferative and proapoptic effects of EGCG have been shown to be selective for cancer cells, as normal cells were not affected by this treatment. In cancer cells, EGCG also causes inhibition of the activity of specific receptor tyrosine kinases and related downstream pathways of signal transduction. This review summarizes recent research data focusing on the green tea polyphenols especially on EGCG-induced cellular signal transduction events that seems to have implications in the inhibition of cell proliferation and transformation, induction of apoptosis of preneoplastic and neoplastic cells as well as inhibition of angiogenesis, tumor invasion, and metastasis.

Acknowledgments

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