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COMMENTARY

Cancer Chemoprevention by Tea Polyphenols through Mitotic Signal Transduction Blockade

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ABSTRACT. Tea is a popular beverage. The consumption of green tea is associated with a lower risk of several types of cancer, including stomach, esophagus, and lung. The cancer chemopreventive effect of tea has been attributed to its major phytopolyphenols. The tea polyphenols comprise about one-third of the weight of the dried leaf, and they show profound biochemical and pharmacological activities including antioxidant activities, modulation of carcinogen metabolism, inhibition of cell proliferation, induction of cell apoptosis, and cell cycle arrest. They intervene in the biochemical and molecular processes of multistep carcinogenesis, comprising tumor initiation, promotion, and progression. Several studies demonstrate that most tea polyphenols exert their scavenging effects against reactive oxygen species (ROS); excessive production of ROS has been implicated for the development of cardiovascular diseases, neurodegenerative disorders, and cancer. Recently, we have found that the major tea polyphenol (-)-epigallocatechin-3-gallate (EGCG) suppresses extracellular signals and cell proliferation through epidermal growth factor receptor binding in human A431 epidermoid carcinoma cells; EGCG also blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of the transcription factor NFκB in macrophages. Furthermore, EGCG blocks the cell cycle at the G1 phase in MCF-7 cells. We have demonstrated that EGCG inhibits the activities of cyclin-dependent kinases 2 and 4; meanwhile, EGCG induces the expression of the Cdk inhibitors p21 and p27. These results suggest that tumor promotion can be enhanced by ROS and oxidative mitotic signal transduction, and this enhancement can be suppressed by EGCG or other tea polyphenols. BIOCHEM PHARMACOL **58**;6:911–915, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. tea; EGCG; EGF; nitric oxide synthase; NFκB; mitotic signal transduction

TEA POLYPHENOLS AS CANCER CHEMOPREVENTIVE AGENTS

Tea (*Camellia sinensis*) is one of the most popular beverages consumed worldwide. The significance of daily tea consumption and its cancer chemoprevention in humans is an important issue. It has been demonstrated that oral administration of tea infusion can inhibit the development of experimental rodent skin tumors [1], growth of implanted tumor cells [2], and invasion and metastasis of malignant tumors [3]. The aforementioned chemopreventive effects of tea against tumorigenesis and tumor growth have been attributed to the biochemical and pharmacological activities of the polyphenols in tea. The most significant properties of tea polyphenols that may affect carcinogenesis are their antioxidant activities [4], modulation of carcinogen-metabolizing enzymes [5], trapping of ultimate carcinogens [6], inhibitory action against nitrosation reactions [7], inhibition of cell proliferation-related activities [8], induction of cell apoptosis and cell cycle arrest [9], blockade of mitotic signal transduction through modulation of growth factor receptor binding [10], and nuclear oncogene expression [11]. From these studies, tea or its major tea polyphenol, EGCG§ has been shown to offer protection against all stages of multistage carcinogenesis, namely tumor initiation, promotion, and progression (Fig. 1). Elucidating the molecular mechanism by which EGCG imparts its protective effects is crucial. We believe that it certainly can provide useful information in clinical application of the purified tea polyphenolic derivatives.

SIGNAL TRANSDUCTION IN CARCINOGENESIS

The biochemical and molecular mechanisms of multistage carcinogenesis, which are very complicated, have been illustrated tentatively in Fig. 1. It has been established that most environmental carcinogens such as aflatoxins, polycyclic aromatic hydrocarbons, and nitrosamines are procar-

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§ *Abbreviations:* EGCG, (-)-epigallocatechin-3-gallate; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; iNOS, inducible nitric oxide synthase; PKC, protein kinase C; TPA, 12-*O*-tetradecanoyl-13-acetate; NFκB, nuclear factor κB; and IκB, κB inhibitor.

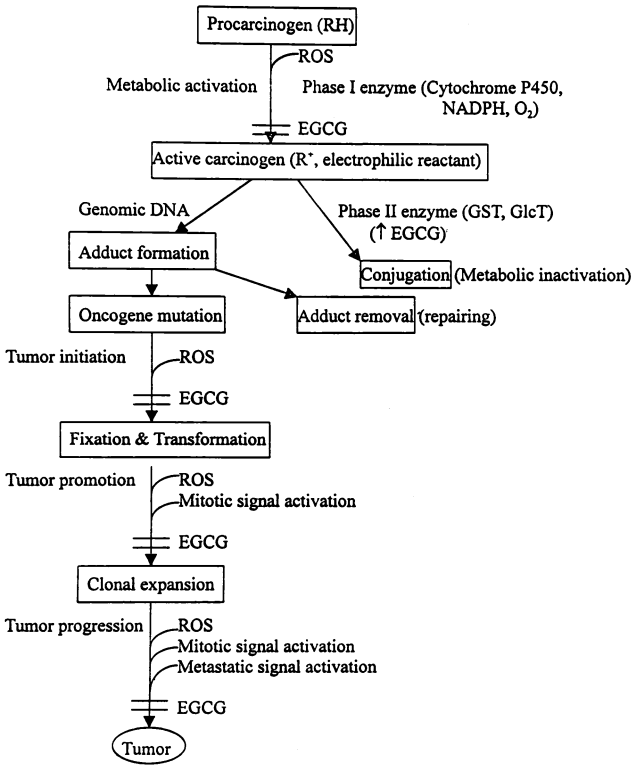

FIG. 1. Blockade of tumor induction by EGCG. Tumor induction is accomplished by a sophisticated multistage carcinogenesis including metabolic activation, initiation (oncogene mutation), promotion (fixation and transformation), and progression (clonal expansion, invasion, and metastasis). Abbreviations: EGCG, (-)-epigallocatechin-3-gallate; GST, glutathione *S*-transferase; GlcT, UDP-glucuronyl transferase; and ROS, reactive oxygen species including superoxide anion, hydrogen peroxide, hydroxyl radical, nitric oxide, peroxynitrite, and nitric dioxide anion. ‡: site of blockade by EGCG.

cinogens (RH), which have to be metabolically activated to electrophilic active carcinogens (R^+). The interaction of R^+ with genomic DNA forms DNA adducts, which may lead to oncogene activation and tumor suppressor gene inactivation. Tumor initiation and promotion may result from oncogene activation, tumor suppressor gene inactivation, or both.

As illustrated in Fig. 1, ROS and mitotic signal activation are considered as major biodeterminants in the processes of tumor development. ROS, of course, arise whenever the cell is involved in oxygen utilization (converting oxygen to water), and this production may be exacerbated by drugs, xenobiotics, and disease [12]. ROS actively participate in the metabolic activation of procarcinogens and the processes of tumor initiation, promotion, and progression, whereas mitotic signal activation may be evoked in tumor promotion and progression. However, metastatic signal activation is involved solely in the process of tumor progression. NADPH is involved in the reaction mechanism of cytochrome P450. The enzyme that uses NADPH to yield the reduced cytochrome P450 is called NADPH-cytochrome P450 reductase. Electrons are transferred from NADPH to NADPH-cytochrome P450 reductase and then to cytochrome P450. This leads to the reactive activation of molecular oxygen (forming ROS), and one atom of oxygen subsequently is inserted into the substrate. This insertion may be intercepted by the presence of EGCG [13]. Other studies have demonstrated that tea polyphenols inhibit, 1,2,4-benzenetriol-generated ROS and induce phase II enzymes [5]. ROS may induce oncogene mutation by modifying the structure of DNA bases [14].

Oncogene mutation that leads to proto-oncogene activation and tumor suppressor gene inactivation may play a crucial role in tumor initiation, promotion, and progression. ROS generally are regarded as having carcinogenic potential and have been associated with tumor promotion. Some tumor cells produce ROS, although the source of these products and their contribution to the transformed phenotype are not known. Certain ROS have been shown to act as essential intracellular second messengers for several cytokines and growth factors [15]. Thus, an antioxidant such as EGCG may be protective against cancer and may inhibit cell proliferation, and intracellular ROS scavengers actually may contribute to suppression of the transformed phenotypes induced by the processes of fixation and transformation (Fig. 1).

The implication of ROS (such as superoxide anion) as mediators of Ras-induced cell cycle progression independent of MAPK and JNK suggests another possible mechanism for the effect of antioxidants including EGCG against Ras-induced cellular transformation in tumor promotion [16]. This is the first evidence that puts superoxide firmly into the Ras pathway, which is one of the most important growth-stimulating pathways of the cell. The Ras pathway can contribute to cancer development (tumor promotion) if it becomes overactive, which may happen as a result of mutation in *ras*, the gene that encodes Ras, or changes in other oncogenic proteins that also send their signals through the Ras pathway. Consequently, the elements (signal transducers such as oxidase, superoxide, Raf, MAPK, and MAPK kinase) in this pathway are all good anticancer drug targets.

MECHANISMS OF CANCER CHEMOPREVENTION

In discussing the molecular mechanisms of cancer chemoprevention of tea polyphenols, we would like to elaborate on the inhibitory effects of these compounds on the process of tumor promotion. Several biomarkers in TPA-induced tumor promotion have been investigated and are illustrated in Table 1. Most of these biomarkers are found to be induced by TPA [17, 18] and inhibited by EGCG [19, 20].

These biomarkers are active mitotic signal transducers. The identification of the jun and fos oncogene products as components of the AP-1 transcription factor provides a clear function for these oncogenes as components of signal transduction pathways that function to stimulate cell proliferation. It is conceivable that one of the mechanisms of cancer chemoprevention exerted by EGCG may be through

TABLE 1. Biomarkers in tumor promotion*

1. Elevation in ROS, xanthine oxidase, NADPH oxidase, and peroxidase.
2. Elevation in PKC, calcium release, and calcium channel activation.
3. Elevation in ornithine decarboxylase and polyamine biosynthesis.
4. Elevation in cyclooxygenase I and II, arachidonic acid metabolism, prostaglandins, and thromboxanes.
5. Elevation in MAPK cascades.
6. Activation of AP-1 (TPA-response element) and NFκB (IκB kinase, PKC, ROS).
7. Overexpression of c-Jun, c-Fos, c-Myc, Bax, and Cdks (oncogene activation).
8. Down-regulation of p53, Rb, Bcl-2, p21, and p27 (tumor suppressor inactivation).

*Most of these biomarkers are active mitotic signal transducers and are stimulated by the phorbol tumor promoter TPA in the promoting cells. They are inhibited effectively by the presence of EGCG; therefore, EGCG is considered as an active inhibitor of tumor promotion.

inhibiting the elevation of these biomarkers in tumor promoting cells.

Several mechanisms of anticancer activity of tea polyphenols have been postulated, but none seems well established [19, 21]. We have demonstrated that EGCG inhibits not only peroxy radical generation [22] but also environmental mutagen-induced mutagenicity; EGCG can block EGF binding to its receptor in A431 cells and then can inhibit the receptor tyrosine kinase activity both *in vivo* and *in vitro* [10]. We have found that the IC_{50} values for EGF, PDGF, and FGF receptor tyrosine kinases are 0.51, 1.04, and 1.03 μg/mL, respectively [10]. Most mitogenic signals initiated by the binding of a growth factor to its cell surface receptor are transmitted to the cell nucleus, leading to a change in gene expression, DNA synthesis, and cell proliferation. Therefore, it is suggested that EGCG can suppress the EGF, PDGF, or FGF receptor-mediated extracellular signals and then inhibit tumor promotion. We have studied the effects of EGCG and other catechins on cell cycle progression [23]. The results indicate that EGCG (30 μM) blocks the cell cycle at the G_1 phase in MCF-7 cells, and the Rb protein changes from the hyper- to the hypophosphorylated form; EGCG inhibits the activities of cyclin-dependent kinases 2 and 4, whereas the levels of the Cdk inhibitors p21 and p27 are induced by EGCG. These results suggest that EGCG may exert its growth-inhibitory effects through modulation of G_1 regulatory proteins such as Cdk2 and Cdk4 [23]. Furthermore, we also found that EGCG can block the induction of nitric oxide synthase (iNOS) by down-regulating the activity of transcription factor NFκB in macrophages [11]. The NFκB transcription factor consists of two subunits (p50 and p65) that are complexed with IκB in the cytoplasm; phosphorylation of IκB by PKC or IκB kinase results in its degradation and dissociation from the complex [24]. The released NFκB then translocates to the nucleus, where it activates transcription from κB sites [25]. We have found that 10 μM EGCG can inhibit about 60% of iNOS protein production. The degradation of IκB was found to be suppressed by the presence of EGCG in the lipopolysaccharide-stimulated macrophage [11]. Inhibition of iNOS activity may suppress the formation of endogenous carcinogenic *N*-nitroso compounds, peroxynitrite, or hydroxy radicals and thus inhibit tumor initiation and promotion.

The complex process of multistep carcinogenesis is subject to multiple levels of control. These include the *in vivo* mechanisms of carcinogen metabolism, DNA modification (Fig. 1), oncogene activation, tumor suppressor gene inactivation, growth factor binding modulation, and mitotic signal transduction. The previous discussion on cancer chemoprevention has centered mainly on the level of tumor promotion (Table 1), but it should be emphasized that there are also extensive involvements of tumor initiation and progression in tumor development. In considering the biological characteristics, tumor initiation is irreversible and takes place in a very short time, while tumor promotion is reversible and takes a long time for completion. For this reason, the promotion stage provides us better opportunities for blocking cell transformation and tumor development. Tumor progression is certainly too late for chemopreventive intervention. Therefore, in this commentary, we have pointed out that although multistep carcinogenesis is induced by multiple factors, the abnormal production of ROS and the activation of mitotic signal transduction are considered as two important biodeterminants that control the carcinogenic process (Fig. 1). These two biodeterminants are relevant to each other but are still distinctive. ROS are low molecular mass compounds that include superoxide, hydrogen peroxide, hydroxyl radical, nitric oxide, and peroxynitrite. Chemically, ROS can react with bases in DNA, amino acids in protein, and unsaturated fatty acids in lipids and finally lead to structural alterations of the cell membrane and genome [14]. Biologically, ROS can induce several mitotic signal transduction pathways [15, 16].

Cancer cells are characterized by diminished or unrestrained control of growth. Certain genes or enzymes controlling growth and interactions with other normal cells are apparently abnormal in structure or regulation in cancer cells. Little is known about the biochemical mechanisms of this abnormality. Further research on oncogenes, tumor suppressor genes, growth factors and their receptors, DNA repair systems, and regulation of the cell cycle will provide insight into the nature of the disturbed control of growth, proliferation, differentiation, and cell–cell interaction exhibited by cancer cells.

Growth factors must transmit a message across the plasma membrane to the interior of the cell (transmembrane signal transduction). Most growth factors have high-affinity protein receptors on the plasma membrane of target cells. Growth factor binding activates receptor tyrosines kinases, resulting in activation of Ras, Raf, and MAPK phosphorylation [26], which then activates transcription

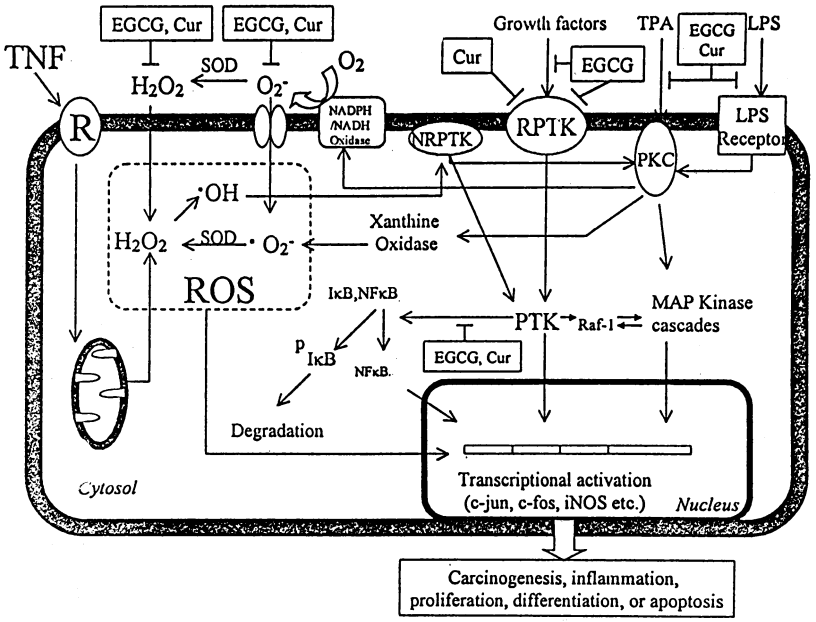

FIG. 2. Oxidative signal transduction pathways in cell proliferation, inflammation, carcinogenesis, differentiation, or apoptosis. Abbreviations: ROS, reactive oxygen species; TNF, tumor necrosis factor; R, TNF receptor; SOD, superoxide dismutase; Cur, curcumin (a phytopolyphenol with cancer chemopreventive effect); NRPTK, non-receptor protein tyrosine kinase; RPTK, receptor protein tyrosine kinase; TPA, 12-O-tetradecanoylphorbol-13-acetate; LPS, lipopolysaccharide; PKC, protein kinase C; PTK, protein tyrosine kinase; Raf-1, ras activating factor-1; MAP kinase; mitogen-activated protein kinase; NFκB, nuclear factor κB (p50 + p65); IκB, κB inhibitor. ⊥: inhibition sign. The oxidative stimulus signals include growth factors, TPA, LPS, TNF, molecular oxygen (O_2), hydrogen peroxide (H_2O_2), and superoxide anion. Both molecular oxygen and hydrogen peroxide are permeable to plasma membrane, while superoxide is impermeable and only can penetrate into the cell through a specific channel. The extracellular signals are transmitted by various intracellular transducers, such as RPTK, NRPTK, PKC, MAPK, Raf-1, PTK, and NFκB, into the nucleus and then initiate transcription activation of various early genes and oncogenes (c-*jun*, c-*fos*, and c-*myc*), leading to the processes of proliferation, carcinogenesis, differentiation, or apoptosis.

factors, leading to transient induction of c-*fos*, c-*jun*, c-*myc*, and other intermediate-early genes.

Transcription factor proto-oncogenes are thus terminal elements of signal transduction pathways that activate expression of critical target genes, thereby converting the transient action of extracellular growth factors into a longer-term alteration in gene expression and ultimately resulting in malignant proliferation. Based on these findings, a general scheme for oxidative mitotic signal transduction pathways leading to cell proliferation, carcinogenesis, inflammation, differentiation, or apoptosis is illustrated in Fig. 2. Many transducers such as RPTK, NRPTK, PKC, MAP kinase, Raf-1, PTK, NFκB, and others involved in the signaling pathway are indicated. The sites of action of EGCG and its targeting molecules along these signaling pathways are also indicated. It is conceivable that the process of cell proliferation and carcinogenesis can be suppressed by EGCG or other phytopolyphenols mainly through blocking oxidative mitotic signal transduction.

CONCLUDING REMARKS

Tea ranks second only to water as a major component of fluid intake worldwide. It is a safe beverage, since it generally is made with boiling water, an important consideration in places where pure uncontaminated water is not available. Yet detailed research on the health effects of tea, particularly regarding its role in cancer chemoprevention, is quite recent [27]. Consumption of green tea in association with a lower risk of cancer of the stomach, esophagus, and lung has been confirmed in some but not all studies; tea drinking also has been found to reduce cardiovascular disease rate. In animal models of cancer of the skin, lung, esophagus, mammary glands, and colon, intake of green or black tea as the sole drinking fluid lowers incidence, multiplicity, and volume of the induced tumors compared with animals on water. Thus, international research on the positive health effects of this important beverage consumed as a warm or cold drink suggests that tea should become a part of our dietary to lower the incidence of major chronic diseases including cancer [27].

For years, health-conscious consumers have been eating lots of vegetables and fruits as much for their antioxidants as for their fiber and vitamin content. A single cup of tea contains 150 mg EGCG [19], which is equivalent to 300 μmole. The blood volume of a 60-kg human is approximately 5 L; then, after drinking a cup of tea, the calculated maximum blood concentration of EGCG may reach 60 μM. Therefore, tea polyphenols such as EGCG may be considered an important source of dietary antioxidants. Antioxidant-bearing foods are supposed to reduce the risk of cancers and other chronic diseases by helping rid the

body of oxygen free radicals, highly reactive molecules thought to contribute to cancer development by damaging DNA. Already, several research teams have demonstrated that oxidizing agents such as ROS affect key transcription factors, some of which help regulate cell growth. They activate NFκB, which turns on the genes for a variety of transducer molecules leading to inflammation and tumor promotion [28]. Based on these findings, it is suggested that suppressing ROS production and blocking the mitotic signaling pathways by EGCG may provide important molecular mechanisms for cancer chemoprevention (Fig. 1).

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