Mechanism of Cancer Chemopreventive Activity of Green Tea (44372)

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Chemoprevention, in recent years, has emerged as a potential strategy for prevention and control of cancer. Chemoprevention is a means by which the use of naturally occurring and/or synthetic compounds completely prevents, blocks or reverses the occurrence of the disease. Prevention of cancer through dietary intervention has received considerable attention in the recent past and is viewed as a potential strategy for the management of cancer (1, 2). Ever since the finding from this laboratory in 1988 and 1989 about anticarcinogenic and antimutagenic activity of polyphenolic agents present in green tea (3–5), prevention of cancer through the consumption of this widely used beverage is receiving increasing attention. Green tea, derived from the plant *Camellia sinensis* (an evergreen shrub of the theaceae family), contains many polyphenolic antioxidants and is one of the most popularly consumed beverages in the world. It is important to emphasize that for many generations in some parts of the world, tea consumption has been considered to possess health-promoting potential (6). Extensive laboratory research and the epidemiological observations published in recent years have revealed that the polyphenolic compound present in green tea may prevent a variety of cancer types. The effectiveness of green tea against cancer has been attributed to the presence of the polyphenolic antioxidants as its major constituents. The major polyphenolic antioxidants present in green tea are (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin-3-gallate, and (−)-epigallocatechin-3-gallate.

History and Consumption of Tea

Thousands of years ago, the plant *Camellia sinensis* was originally discovered and grown in Southeast Asia. According to Chinese mythology, in 2737 BC, emperor Shen Nung discovered tea for the first time. It rapidly spread worldwide and is presently cultivated in at least 30 countries around the world. Tea, consumed worldwide at greatly varying levels, is next to water with a per capita human consumption of approximately 120 ml/day worldwide (7). Many types of tea have a common origin (*Camellia sinensis*) but different processing. The main types of tea include black tea (78%, mainly consumed in western countries and some Asian countries), green tea (20%, mainly consumed in China, Japan, India, and a few countries in North Africa and the Middle East), and oolong tea (2%, consumed in southeastern China and Taiwan) (7).

Tea and Cancer Chemoprevention

The experimental and epidemiological data amassed from several laboratories worldwide have provided convincing evidence that the polyphenolic antioxidants present in green tea afford protection against cancer initiation and its subsequent progression and development (reviewed in Refs. 7–9). A majority of the studies demonstrating cancer chemopreventive effects have been conducted with green tea whereas only a limited number of studies have assessed the chemopreventive potential of black tea (7–9). Studies have shown that oral consumption or topical applications of green tea and/or its polyphenolic constituents afford protection against chemical carcinogen- or ultraviolet radiation-induced skin carcinogenesis in the mouse model (10). In many other animal model systems, the polyphenols isolated from green tea or water extract of green tea have also been shown to afford protection against chemically induced carcinogenesis in lung, fore-stomach, esophagus, duodenum, pancreas, liver, breast, and colon (7–9). Recent studies have suggested that much of the cancer chemopreventive effects of green tea are mediated by its major polyphenolic constituent epigallocatechin-3-gallate (EGCG) (7, 9). It is important to note that many other polyphenols and agents present in green tea also contribute to its cancer chemopreventive effects. The use of green tea for chemoprevention of cancer is supported by several factors: i) a single cup of brewed green tea contains up to 200 mg of EGCG; ii) green tea meets all the qualities of an ideal chemopreventive agent(s) (Fig. 1); and iii) many consumer products such as drinks, ice creams, health-care products, and cosmetics

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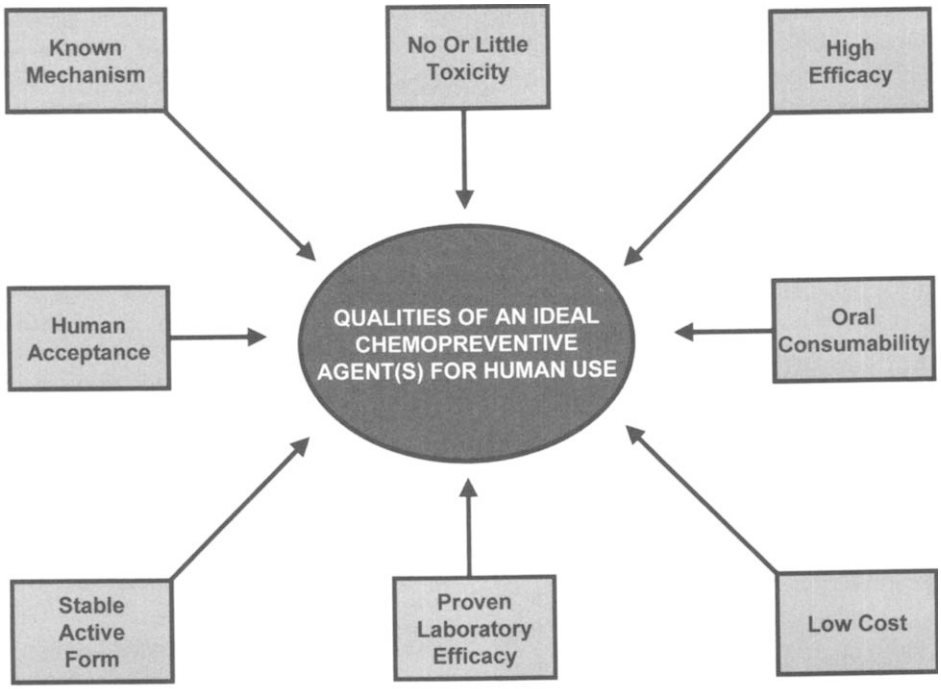

Figure 1.

supplemented with green tea extracts are available on the market.

Modulatory Effect of Tea on Cancer Chemotherapy. In recent years, the biochemical modulations in cancer chemotherapy have been studied extensively. The side effects due to modulatory drugs pose a threat to the patient's life. Also, the use of modulators increases the medication burden on the patients. Therefore, substances consumed through diet and beverages can serve as excellent biochemical modulators to increase the efficacy of therapy. In this regard, Sadzuka *et al.* (11) recently showed that the oral administration of green tea enhanced the tumor-inhibitory effects of doxorubicin on Ehrlich ascites carcinomas implanted in CDF_1 and BDF_1 mice. The study showed that green tea treatment increases the concentration of doxorubicin in the tumor, but not in normal tissue. If these data could be verified in the human population, they may have relevance to cancer chemotherapy. Currently in our laboratory, experiments are underway to investigate the modulatory effect of green tea constituents on the cytotoxicity of many anticancer drugs.

Mechanism(s) of Biological Effects of Tea

Since green tea and its polyphenols have shown preventive effects against a variety of cancer types, a complete understanding of the mechanisms of its biological effects is very important and may be helpful for designing better strategies against cancer. The initial attempts to elucidate the mechanisms of cancer chemoprevention by green tea and its polyphenols largely focused on the following (7, 9): i) prevention against mutagenicity and genotoxicity; ii) inhibition of biochemical markers of tumor initiation; iii) inhibition of biochemical markers of tumor promotion; iv) effects on detoxification enzymes; v) trapping of activated metabolites of carcinogens; and vi) antioxidant and free radical scavenging activity.

Studies to elucidate the biological responses imparted by green tea polyphenols have been aimed toward a variety of biochemical, genetic, and molecular targets. Table I gives a brief summary of the specific mechanistic targets where green tea or the polyphenolic fraction obtained from it or the individual constituents present therein have been shown to

Table I. Cancer Chemopreventive Mechanisms of Green Tea

Effect on metabolism	Antiproliferative effect	Antioxidant potential
Cytochrome P450	ODC	Antioxidant enzyme
Mutagenicity	Apoptosis	Radical scavenging
Genotoxicity	Cell cycle	Immune response
Cyclooxygenase	MAPK	Cytokines
Lipoxygenase	EGFR	NO
Detoxication enzymes	NFκB, AP-1	ROS
Urokinase		

produce effects in a manner that ultimately afforded cancer chemopreventive effects. These targets can be classified broadly under three main categories: i) effect of green tea on metabolism; ii) antiproliferative effects of green tea; and iii) antioxidant potential of green tea. Recent efforts have been aimed toward finding the molecular mechanisms involved in the biological response of green tea polyphenols. The ability of green tea polyphenols to affect multiple targets/pathways may be the reason for its exceptional cancer chemopreventive efficacy. The recent advances in this area are described below.

Green Tea Activates the Mitogen-Activated Protein Kinases (MAPK)-Pathway. In a recent study, the involvement of the MAPK-pathway was documented as a mechanism of biological response of green tea polyphenols. It was shown that the activation of the MAPK-pathway by green tea polyphenols may be a potential signaling pathway in the regulation of antioxidant-responsive element (ARE)-mediated phase II enzyme gene expression (12). Further, it was demonstrated that green tea polyphenol treatment to the human hepatoma HepG2 cells transfected with a plasmid construct containing ARE and a minimal glutathione S-transferase Ya promoter linked to the CAT reporter gene, resulted in an induction of chloramphenicol acetyltransferase (CAT) activity. This indicated that green tea polyphenols stimulate the transcription of Phase II detoxifying enzymes through ARE. The treatment of HepG2 cells with green tea polyphenols was shown to result in a significant activation of MAPK, extracellular signal-regulated kinase 2 (ERK2) as well as c-*jun* N-terminal kinase 1 (JNK1). Green tea polyphenol treatment also increased mRNA levels of the immediate early genes c-*jun* and c-*fos*.

EGCG Inhibit Urokinase Activity. In a recent study that received appreciable media attention, it was shown that the anticancer activity of the green tea polyphenol EGCG might be due to the inhibition of the enzyme urokinase that is one of the most frequently expressed enzymes in human cancers (13). Employing the molecular modeling techniques, the authors demonstrated that EGCG binds to urokinase thereby blocking His 57 and Ser 195 of the urokinase catalytic triad and extending toward Arg 35 from a positively charged loop of urokinase. This computer-based calculation was verified by quantifying the inhibition of urokinase activity by spectrophotometric amidolytic assay. However, the validity of this study with achievable dose levels was challenged by CS Yang (14).

Green Tea Induces Apoptosis and Cell Cycle-Arrest. Apoptosis, a programmed cell death, is different from necrotic cell death and is regarded as an ideal way of cell elimination and is involved in maintaining homeostasis in the living system. In recent years, apoptosis has become a challenging area of biomedical research because the life span of both normal and cancer cells within a living system is significantly affected by the rate of apoptosis (15). Therefore, the chemopreventive agents that can modulate apoptosis thereby affecting the steady-state cell population may be useful in the management and therapy of cancer. On one hand, a number of cancer chemopreventive agents are shown to induce apoptosis and on the other hand, several tumor promoting agents are demonstrated to inhibit apoptosis (16–18). Therefore, it is reasonable to assume that the chemopreventive agents that have proven effects in animal tumor bioassay systems and/or human epidemiology on the one hand and that can cause induction of apoptosis of cancer cells on the other hand should have wider implication for the management and control of cancer. Only a limited number of chemopreventive agents are known to cause apoptosis (19, 20). In a recent study from our laboratory (21), we demonstrated that EGCG induces apoptosis and cell cycle arrest in human epidermoid carcinoma cells A431. An important observation of this study was that the apoptotic response of EGCG was specific to cancer cells as the induction of apoptosis was also observed in human carcinoma keratinocytes HaCaT, human prostate carcinoma cells DU145, and mouse lymphoma cells LY-R, but not in normal human epidermal keratinocytes.

In another study Chen *et al.* (22) compared the effect of EGCG on the growth of SV40 virally transformed WI38 human fibroblasts (WI38VA) with that of normal WI38 cells. EGCG treatment was found to inhibit the growth of WI38VA cells, but had little or no inhibitory effect on the growth of WI38 cells. In this study, a similar differential growth inhibition was also observed between a human colorectal cancer cell lien (Caco-2), a breast cancer cell line (Hs578T), and their respective normal counterparts. EGCG was also found to induce apoptosis in WI38VA cultures, but not in WI38 cultures. Further, EGCG significantly enhanced serum-induced expression of c-*fos* and c-*myc* genes in W138VA cells, but not in normal WI38 cells. This study suggested that differential modulation of certain genes, such as c-*fos* and c-*myc*, might be responsible for the differential effects of EGCG on the growth and death of cancer cells.

Fujiki *et al.* (23) demonstrated that EGCG and other tea polyphenols inhibit growth of human lung cancer cells (PC-9 cells) with a G2/M phase arrest of the cell cycle. In this study, ^{3}H EGCG administered by p.o. intubation into mouse stomach resulted in small amounts of ^{3}H-activity in various organs (such as skin, stomach, duodenum, colon, liver, lung, and pancreas) where EGCG and green tea extract has been shown to have anticarcinogenic effects. The involvement of the tumor necrosis factor (TNF)-α pathway was suggested as a mechanism of EGCG action.

EGCG Inhibits Cell Proliferation and Tumor Progression Through Epidermal Growth Factor Receptor (EGFR) Binding. In a recent study (24) it was demonstrated that EGCG could significantly inhibit DNA synthesis and protein tyrosine kinase activities of EGF-R, PDGF-R, and FGF-R, but not of $pp60^{v\text{-}src}$ PKC, and PKA in A431 cells. EGCG also inhibited auto-phosphorylation of EGF-R by EGF and blocked the binding of EGF to its receptor. This study suggested that EGCG might inhibit

tumor development *via* blocking cellular signal transduction pathways.

EGCG Blocks the Induction of Nitric Oxide (NO)-Synthase by Downregulating Lipopolysaccharide-Mediated Activity of Transcription Factor Nuclear Factor-κB (NFκB). Since NO plays an important role in inflammation and carcinogenesis, Lin and Lin (25) assessed the effects of green tea polyphenols on the induction of NO-synthase in thioglycollate-elicited and lipopolysaccharide (LPS)-activated peritoneal macrophages. In this study, Gallic acid (GA), EGC, and EGCG were found to inhibit inducible NO-synthase protein and NO generation. In this study, EGCG was also found to block the activation of NFκB, which is associated with the induction of inducible NO-synthase (iNOS). Taken together, the data from this study suggested that EGCG blocks the early event of NO-synthase induction *via* inhibiting the binding of transcription factor NFκB to the iNOS promoter, which in turn, inhibits the induction of iNOS transcription.

In another study (26), Chan *et al.* demonstrated that in a cell culture system, EGCG inhibits lipopolysaccharide (LPS)- and interferon (IFN) γ-activated iNOS and mRNA expression. EGCG also inhibited the enzyme activities of iNOS and neuronal NO-synthase (nNOS).

EGCG Inhibits Tumor Promoter-Induced Activator Protein-1 (AP-1) Activation and Cell Transformation. Employing the JB6 mouse epidermal cell line, a system that has been used extensively as an *in vitro* model for tumor promotion studies, Dong *et al.* (27) investigated the anti-tumor promotion effects of EGCG and theaflavins. EGCG and theaflavins were found to inhibit EGF- or TPA-induced cell transformation in a dose-dependent manner. EGCG and theaflavins also inhibited AP-1-dependent transcriptional activity and DNA binding activity. This study further demonstrated that the inhibition of AP-1 activation occurs through the inhibition of a c-*jun* NH_2-terminal kinase-dependent but not ERK1- or ERK2-dependent pathway. The downregulation of AP-1 activity as a general therapeutic strategy against cancer is now being increasingly appreciated (28).

Antiproliferative Effects of EGC are Mediated by Inhibition of Protein Tyrosine Kinase Activity, c-*jun* mRNA Expression, and JNK1 Activation. In a recent study some possible mechanisms of the antiproliferative effect of EGC were studied by Lu *et al.* (29). Employing rat aortic smooth muscle cells (A7r5 cells), Lu *et al.* demonstrated that the membranous protein tyrosine kinase activity stimulated by serum is significantly inhibited by EGC. In this study it was also shown that EGC reduced the levels of many tyrosine-phosphorylated proteins with different molecular weights, indicating that EGC may inhibit the protein tyrosine kinase activity or stimulate the protein phosphatase activity. Reverse transcription-polymerase chain reaction analysis demonstrated that the c-*jun* mRNA level was significantly reduced by EGC. The study further demonstrated that the level of phosphorylated JNK1 as well as JNK1-kinase activity was also reduced by EGC. These results demonstrated that the antiproliferative effect of epigallocatechin might be mediated partly through inhibition of protein tyrosine kinase activity, reducing c-*jun* mRNA expression and inhibiting JNK1 activation.

In a study by Kennedy *et al.*, the effect of various tea polyphenols was examined on the viability of Ehrlich ascites tumor cells, and a possible association with tyrosine phosphorylation was determined (30). EGC and EGCG treatments resulted in a significant decrease in cell viability. EGC but not EGCG was found to cause a stimulation of protein tyrosine kinase (PTK) activity. EGC also resulted in the tyrosine phosphorylations of 42- and 45-kDa proteins and activity of ornithine decarboxylase (ODC)—a key enzyme in polyamine biosynthesis in cells.

Conclusion and Future Directions

Almost one-third of cancers are caused by dietary substances, and the strategy of manipulating diet is increasingly being recognized as a practical approach for cancer prevention (7–9, 31). The possibility of using tea, especially green tea, as a cancer chemopreventive agent has only been appreciated in the last 10 years. Tea is one of the most popular beverages in the world, and it has been shown to afford protection against a variety of cancers. Epidemiological as well as laboratory studies have indicated that tea consumption offers beneficial effects against development of certain cancer types. Although compelling evidence is now available to support the cancer preventive potential of tea, a clear understanding of the mechanisms by which tea polyphenols prevent cancer is far from complete. Defining the molecular mechanism(s) involved in the anticarcinogenic property of green tea polyphenols may be useful in devising other, even better strategies against cancer.

In view of the available data from laboratory and epidemiological studies, it will be appropriate to evaluate the usefulness of green tea and the well-defined naturally occurring polyphenols found therein the clinical trials with humans. Phase I Clinical Trials, to evaluate the possible efficacy of formulated green tea in patients with advanced solid tumors, are currently underway at M.D. Anderson Cancer Center and Memorial Sloan-Kettering Cancer Center. Indeed, this important step may be a turning point in the area of cancer chemoprevention. However, more clinical trials are still warranted to take green tea from laboratory to clinics for cancer therapy and from laboratory to common household for cancer prevention. Further research is also needed to define the molecular mechanism(s) of green tea action that may result in devising better strategies against a variety of cancer types.

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