Published in final edited form as:
Nutr Cancer. 2009 ; 61(6): 827–835. doi:10.1080/01635580903285049.

Molecular Mechanisms of Green Tea Polyphenols

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Abstract

Tea, next to water, is the most popular beverage in the world. It has been suggested that tea consumption has the cancer-preventive effects. Epidemiological studies have indicated decreased cancer occurrence in people who regularly drink green tea. Research has also discovered numerous mechanisms of action to explain the biological effects of tea. The most abundant and popular compound studied in tea research is (−)-epigallocatechin-3-gallate or (−)-EGCG, which is a powerful antioxidant and can inhibit a number of tumor cell proliferation and survival pathways. Tea polyphenols are known to inhibit metaloproteonases, various protein kinases, and proteins that regulate DNA replication and transformation. We also reported that ester bond-containing tea polyphenols, for example, (−)-EGCG, potently and specifically inhibited the tumor proteasomal activity. We further demonstrated that methylation on green tea polyphenols under physiological conditions decreased their proteasome-inhibitory activity, contributing to decreased cancer-preventive effects of tea consumption. Since (−)-EGCG is unstable under physiological conditions, we also developed the peracetate-protected or prodrug form of (−)-EGCG, Pro-EGCG (1), and showed that Pro-EGCG (1) increases the bioavailability, stability, and proteasome-inhibitory and anti-cancer activities of (−)-EGCG in human breast cancer cells and tumors, demonstrating its potential use for cancer prevention and treatment.

INTRODUCTION

Over 6 million people die due to cancer each year worldwide, which is the largest single cause of death in both men and women. The biochemical and molecular mechanisms of multistage carcinogenesis, namely, tumor initiation, promotion, and progression, are very complicated. The aim of chemoprevention is to arrest multistage carcinogenesis prior to development of malignancy.

Next to water, tea is one of the most popular beverages consumed in the world and is distinguished by the presence of a group of polyphenols called catechins. A growing body of evidence from laboratory animal studies demonstrates that tea consumption has an inhibitory effect on carcinogenesis at various organ sites. For example, oral administration of tea infusion can inhibit the development of experimental rodent skin tumors (1), growth of implanted tumor cells (2), invasion and metastasis of malignant tumors (3,4), and angiogenesis (5,6). The bioavailability and biotransformation of tea polyphenols, however, are the key factors for the previously mentioned chemopreventive effects of tea against tumor-genesis. At present, epidemiological studies have not yielded conclusive evidence of the protective effect of tea consumption against the development of human cancers (7–9). However, limited epidemiological studies have suggested that people drinking more cups of

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tea regularly have a lower risk of prostate (10,11) and breast cancer (12). Four major green tea components are epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC), all of which are also present in black and other teas.

The mechanisms by which tea polyphenols may act include inhibition of mutagenesis (13–15), genotoxicity (16,17), transformation (21–23), cell proliferation (27–36), and angiogenesis (5,6). Each mechanism involves multiple potential molecular targets, which will be discussed in detail below. Many studies have suggested that polyphenol EGCG possesses the most potent antioxidative activity against all stages of multistage carcinogenesis (40–42). It has been found that EGCG supplement is incorporated into human plasma at a maximum concentration of 4,400 pmol/ml (43–45). Therefore, such concentrations of EGCG would be enough to exert antioxidant activity in the blood stream. Previously, we reported that EGCG potently and specifically inhibits the chymotrypsin-like activity of the protea-some in vitro (IC_{50} = 86–194 nM] and in cultured tumor cells (1–10 μM) at the concentrations found in the serum of green tea drinkers (46). Understanding daily tea consumption and its cancer chemoprevention mechanisms in humans is animportant issue to elucidate the beneficial potential of tea beverages on cancer prevention.

THE POTENTIAL MOLECULAR MECHANISMS RESPONSIBLE FOR THE CANCER-PREVENTIVE EFFECT OF TEA POLYPHENOLS

Inhibition of Mutagenesis and Genotoxicity

Mutations of genes induced by various chemicals are an important phenomenon during the initial events of carcinogenesis (18,19). Using microbial systems and mammalian in vitro and in vivo systems, researchers have shown the antimutagenic activity of tea polyphenols. In an animal model study, Muto et al. (13) demonstrated that drinking green tea can reduce the tumor initiating potency of the potent mutagen benzo[α]pyrene. Okuda et al. (14) demonstrated that ECG and EGCG have significant inhibitory activity against the mutagenicity of Trp-P-2 and *N*-OH-Trp-2 by using *Salmonella typhimurium* TA98 and TA100 with or without rat liver S9 mix. In mammalian cells system, Kuroda (15) showed that 6-thioguanine (6TG)-resistant mutations, induced by 4-nitroquinoline 1-oxide (4NQO) in Chinese hamster V79 cells, were inhibited by ECG and EGCG. Thus, the catechins may act intracellularly as bioantimutagenic blocking agents or suppressive agents.

It has been shown that the oral administration of 0.2% green tea or 0.1% black tea for 28 days decreased the extent of chromosome damage (micronuclei) in the peripheral blood of mice subsequently treated with benzo[α]pyrene (16). However, against the chromosome damaging action of γ-rays, no similar protective effect of green tea or black tea was found (16). Reductions in levels of carcinogen-DNA adducts were also found in rats given extracts of 2% green tea or 1% black tea for 8 wk before oral administration of 2-amino-3-methylimidazo-[4,5-*f*]quinoline (IQ) (17).

Inhibition of Transformation

Transformation of normal cells, which begins the onset of cancer, seems to sensitize cells to treatment by those drugs that work in the DNA synthesis phase of the cell cycle. This sensitivity should be due to various factors including an increased population of S-phase cells, compromise of cell cycle checkpoints, cell cycle dysregulation, and/or alteration of apoptotic regulators (20). In a study using mouse C3H10T1/2 fibroblast cells, Komatsu et al. (21) showed that EGCG can inhibit *x*-ray induced, oncogenic transformation and that the transformation frequency with 15 μM of EGCG was reduced nearly to spontaneous levels. Ionizing radiation provides no DNA adducts, and only promotion is affected by EGCG. This

is supported by the observation that in UV-induced tumorigenesis in vivo, only skin-tumor-promoting activities are inhibited by EGCG (1).

Different tumor-promoting agents are known to induce free radicals in cells. On the other hand, inhibitors of free radical reactions, such as superoxide dismutase (SOD) mannitol, catalase, and tea extracts, have been suggested to suppress tumor promotion (22). Therefore, it is thought that inhibition of oncogenic transformation by EGCG is possibly associated with its antioxidant activities by scavenging free radicals that are generated during promotion (see below). However, a compound named quercetin, which is chemically similar to EGCG and has antioxidant properties, shows no suppressive effect, suggesting that inhibition of free radical production may not be sufficient for inhibition of transformation (23).

Inhibition of Cell Proliferation and Induction of Apoptosis

Studies have shown that cell cycle machinery regulates cell proliferation, and dysregulated cellular proliferation is a hallmark of cancer (24). In the eukaryotes, the regulation of cell cycle, in part, is controlled by a family of protein kinase complexes (25, 26). It has been shown that the involvement of cki-cyclin-cdk machinery during cell cycle is affected by EGCG (27–30). Liang et al. (28) studied the effects of EGCG and other catechins on cell cycle progression. Their results indicated that EGCG inhibited the activities of several key G_1 regulatory proteins such as Cdk2 and Cdk4, and could induce the protein expression of Cdk inhibitors p21 and p27 in human breast carcinoma cells. These results suggest that EGCG may exert its growth-inhibitory effects through modulation of G_1 regulatory proteins.

Green tea extract and EGCG are capable of inhibiting the growth of a variety of mouse and human cancer cell types (31). It has been shown that green tea and its polyphenolic constituents impart inhibitory effects on the activities of many enzymatic and metabolic pathways that are involved in multisteps carcinogenesis (32,33). Additionally, EGCG exerts growth-inhibitory effects on both androgen-dependent and androgen-independent prostate cancer cells, and these antiproliferative effects are demonstrated by dysregulation of cell cycle and induction of apoptosis (34). It has also been shown that EGCG induces apoptosis in human chondrosarcoma cells through the activation of caspase-3-like protease (35). Also, green tea polyphenol EGCG can mediate the retinoblastoma (pRb)-E2F/DP pathway, an important regulator of cell cycle arrest and apoptosis (36).

Inhibition of Angiogenesis

Induction of new blood-vessel growth is required for tumor growth and metastasis known as angiogenesis (37). A growing body of evidence supports the central role of angiogenesis in tumor growth and metastasis (38). Studies suggest that the expression of dominantly acting oncoproteins, such as activated Ras, can regulate the angiogenic switch (39). Animal studies showed that EGCG plays an important role in inhibition of the angiogenesis (6), suggesting a possible link between tea consumption and the prevention and treatment of angiogenesis-dependent diseases including cancer. Details molecular mechanisms of tea polyphenols-mediated angiogenesis are discussed below.

MOLECULAR TARGETS OF TEA POLYPHENOLS IN CANCER PREVENTION

Free Radicals

Flavonoids are polyphenolic antioxidants naturally present in vegetables, fruits, and beverages such as tea and wine. Among these diets, green tea contains relatively large amounts of polyphenols. A number of polyphenolic compounds extracted from green tea

leaves have been found to be good antioxidants against lipid peroxidation in phospholipid bilayers (47) and in biological systems (4).

Reactive oxygen species (ROS) are low molecular mass compounds, including superoxide anion radical, hydrogen peroxide, singlet oxygen and hydroxyl radicals, that are associated with normal cellular metabolism (48). Accumulating evidence suggest that ROS produced by either endogenous or exogenous sources is critically involved in multiple stages of carcinogenesis (49). ROS is able to cause damage to genomic DNA, leading to production of mutation, activation of protooncogenes, and inactivation of tumor suppressor genes (49). ROS can also interfere with normal cell signaling through modifying transcription factors and protein kinase cascades (50). It was found that the combination of H_2O_2 and cytochrome c induces lipid peroxidation and DNA strand breaks (51). Since theaflavins, polyphenolic ingredients of black tea, inhibited DNA cleavage induced by H_2O_2in the presence of cytochrome c, they are expected to work as antioxidants in the cells.

Ruch et al. (52) demonstrated that an antioxidant (catechin-enriched) fraction of Chinese green tea was active in directly detoxifying hydrogen peroxide and superoxide radicals and so protected cultured mouse hepatocytes and human keratinocytes. In an in vitro study, Wei et al. (53) showed that extract of green tea and black tea enhanced the scavenging of H_2O_2 and quencing of 8-hydroxy deoxyguanosine (8-OHdG), suggesting the important role of EGCG in the antioxidant activities of tea extracts.

Although tea polyphenols are known antioxidants, it was also reported that tea polyphenols could produce H_2O_2 in cell culture medium (54). Theaflavins (TFs) were also reported to produce H_2O_2 and induce apoptosis in several cell lines (55). Therefore, the relationship between the antioxidant activity of tea polyphenols and the cancer-preventive effects of tea consumption needs further investigation.

Growth Factor Receptor-Mediated Kinases

Cancer cells are characterized by their uncontrolled growth pattern. Activator protein 1 (AP-1) is a transcription factor, and its activity has been associated with invasive and metastatic characteristics of cancer cells. The c-jun and c-fos are the AP-1 encoding oncogenes. They are immediate-early genes, whose transcription is induced rapidly in response to external stimuli. These oncogenes are components of signal transduction pathways that function to stimulate cell proliferation. Researchers have shown that EGCG and TFs inhibit the activity of AP-1 through the inhibition of mitogen-activated protein kinase (MAPK), specifically, through inhibition of c-Jun NH2-terminal kinase (JNK) dependent activity in JB6 cells and H-ras transformed JB6 cells (56).

Binding of growth factors such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) to their receptors result in activation of receptor tyrosine kinases, which then activate Ras, Raf, and MAPK phosphorylation that in turn activates transcription factors such as c-fos, c-jun, c-myc, and other intermediate-early genes (57). Therefore, enhanced activity of PDGF-R and EGF-R has been implicated as a contributing factor in the development of malignant and nonma-lignant proliferative diseases such as cancer (58) and atherosclerosis (59), respectively. Liang et al. (58) showed that under in vivo conditions, EGCG could reduce the autophosphorylation level of EGF-R induced by EGF. In addition, EGCG blocked EGF binding to its receptor. Ahn et al. (60) showed that EGCG inhibited tyrosine phosphorylation by inhibiting the binding of PDGF-BB to its receptor and of EGF to its receptor, thus preventing their downstream signaling transductions cascade. Chung et al. (61) showed that EGCG or theaflavin-3,3′-digallate inhibited the Ras-MAPK signaling pathway. Masuda et al. (62) demonstrated that EGCG inhibited phosphorylation and activation of EGF-R, signal transducer and activator of transcription 3 (Stat 3), and

extracellular regulated kinase (ERK) proteins and also inhibited basal and transforming growth factor-α-stimulated c-fos and cyclin D1 promoter activity.

Using in vitro kinase assays, Liang et al. (58) demonstrated that EGCG strongly inhibited the protein tyrosine kinase (PTK) activities of EGF-R, PDGF-R, and FGF-R. But by contrast, EGCG scarcely inhibits serine- and threonine-specific protein kinases such as protein kinases A (PKA) and protein kinase C (PKC).

The Her-2/neu (or c-erbB-2) is the second member of the EGF-R family (EGF-R2). Control of growth and transformed phenotype of Her-2/neu-overexpressing cells is mediated through PI 3-kinase to serine/threonine kinase, Akt/protein kinase B to NF-κB signaling pathway (63). A study by Pianetti et al. (64) shows that EGCG inhibited Her-2/neu tyrosine phosphorylation. As a result, reduced expression of its downstream signaling transduction pathway occurred.

Vascular endothelial growth factor (VEGF) and its high affinity signaling receptor, Flk-1 (VEGF receptor-2), play a major role in tumor angiogenesis in a variety of tumors, including glioma, various carcinoma, and hemangioblastoma (65). In addition, VEGF may act as a survival factor for immature tumor blood vessels (66). Erk-1 and Erk-2 are the most important MAPKs required for growth factor-induced cell proliferation. Cao and Cao (6) demonstrated that green tea and EGCG inhibit angiogenesis in a chick CAM assay. Jung et al. (68) showed that EGCG inhibits angiogenesis through blocking the induction of VEGF in human colon carcinoma cells. Another study shows that ECGC can block VEGF and VEGF-dependent tyrosin phosphorylation of VEGFR-2, thus inhibiting VEGF-dependent angiogenesis (69).

We have also found that growth-arrested prostate cancer cells expressed high levels of a hyperphosphorylated Bcl-X_L in mitochondria. Treatment with tea polyphenols or EGCG blocked expression of the hyperphosphorylated, but not hypophosphorylated, Bcl-X_L in mitochondria, accompanied by cytochrome c release, caspase activation, and apoptosis. Studies using specific inhibitors suggest that tea inhibits p38 mitogen-activated protein kinase and the proteasome activities, leading to inhibition of Bcl-X_L phosphorylation and induction of prostate cancer cell death (70).

Transcription Factors

NF-κB is an oxidative stress sensitive transcription factor, predominantly existing in the cytoplasm in an inactive state bound to a member of the IκB family of inhibitory proteins. Phosphorylation of IκB by PKC or IκB kinase (IKK) results in its degradation and dissociation from the NF-κB complex (73). The released NF-κB then translocates to the nucleus, where it activates transcription from κB sites (74). Pan et al. (75) showed that black tea derivative theaflavin-3,3′-digallate (TF-3) or EGCG block the phosphorylation of IκB; however, they did not determine whether these polyphenols blocked the activation of IKK or inhibited the activity of IKK. Both mechanisms would lead to a reduction in IκB phosphorylation. Yang et al. (76) shows that EGCG can inhibit the activity of IKK, which lead to a reduction in IκB phosphorylation.

Enzymes Involved in DNA Replication

Previously, we have shown that EGC inhibits DNA replication in three leukemia cancer cell lines, Jurkat T, HL-60, and K562. In comparison to other tea polyphenols, EGC was the most potent in accumulation of S-phase cells and inhibition of the S-G2 progression. In addition, EGC-mediated inhibition of S-phase progression results in induction of apoptosis, as determined by sub-G1 cell population, breakage of endonuclear DNA, cleavage of poly (ADP-ribose) polymerase (PARP), and loss of cell viability (30).

Catechin derivatives ECG, EGCG, EGC, and green tea extract were also found to inhibit the activities of cloned HIV type 1 reverse transcriptase (HIV-1 RT), duck hepatitis B virus replication complexes reverse transcriptase, herpes simplex virus 1 DNA polymerase, and cow thymus DNA polymerase alpha (77,78). The strongest inhibition by EGCG and ECG were observed with HIV-1 RT. DNA polymerase alpha and beta were also strongly inhibited. The mode of inhibition of reverse transcriptase and other DNA polymerases was competitive with respect to the template primer, whereas the mode of inhibition of RNA polymerase was competitive with respect to the nucleotide substrate. Bovine serum albumin significantly reduced the inhibitory effects of catechin analogues and green tea extract on HIV-1 RT (77). In tissue culture, green tea extract inhibited the cytopathic effect of coxsackie B3 virus but did not inhibit the cytopathic effects of HSV-1, HSV-2, influenza A, or influenza B viruses (77).

DNA topoisomerases (topo) I and II play important roles in DNA metabolism and structure for cell survival. The importance of topo-mediated DNA cleavage in tumor cell death has been recognized as an effective molecular target for many antitumor drugs (79). EGCG inhibits topo I but not topo II in human colon cancer cell lines (80). The substitution of gallic acid at the 3 position of EGCG increased the inhibition against topo I from calf thymus gland and topo II from human placenta, and the substitution of a hydroxyl group at the 3′ position also increased the inhibition against topo I. These results suggest that the 3 and 3′ positions of the EGCG molecule play important roles in the process of inhibition of topo I and II (81).

Telomerase, the unique reverse transcriptase responsible for maintaining the telomeres, the end of chromosomes, has become a field of interest in cancer biology. Telomerase activity has been observed in more than 85% of all cancer cells, whereas in most somatic cells, it appears in undetectable level (82). Naasani et al. (83) showed that in cell free system and in living cells, EGCG potently inhibited telomerase activity with concentrations of 1 μM (IC_{50}) and 15 μM.

THE UBIQUITIN-PROTEASOME PATHWAY

We have found that EGCG potently and specifically inhibits the chymotrypsin-like activity of the proteasome in vitro (IC_{50} = 86–194 nM) and in cultured tumor cells (1–10 μM) at the concentrations found in the serum of green tea drinkers (46). Recently, proteasome inhibition has become increasingly important in cancer and drug resistance research. The vast majority of regulated proteolysis in eukaryotic cells occurs through the actions of the ubiquitin-proteasome pathway (84). Although it would seem disastrous to alter the activity of this crucial protein degradation system, proteasome inhibition has been well established as a rational strategy for multiple myeloma (85– 86), non-Hodgkin lymphoma (87), and some other solid tumors (88). Understanding the involved mechanism of action has led to integration into combination regimens using both proteasome inhibitors and standard chemotherapeutics.

The ubiquitin-proteasome pathway involves two successive steps: conjugation of multiple ubiquitin molecules to the protein substrate and degradation of the tagged protein by the 26S proteasome. Ubiquitin is a highly conserved 76-amino acid protein that becomes covalently ligated to a target protein by a multienzymatic system consisting of Ub-activating (E1), Ub-conjugating (E2), and Ub-ligating (E3) enzymes, which act in a sequential manner. This is a three-stage process that starts with activation of ubiquitin by the E1 enzyme in an ATP-requiring reaction that generates a high-energy thiol ester intermediate, E1-S∼ubiquitin. Activated ubiquitin is then transferred from E1 by one of several ubiquitin-conjugating enzymes, E2, via an additional high-energy thiol-ester intermediate, E2-S∼ubiquitin. From

E2 to the E3-bound substrate, the activated ubiquitin can be then transferred directly or via a third high-energy thiol ester intermediate, E3-S~ubiquitin (84).

Ubiquitinated proteins are recognized by the 26S proteasome, a large multisubunit protease complex that is localized in the nucleus and cytosol and selectively degrades intracellular proteins. In almost all of the cases, only proteins containing polyubiquitin chains on sequential lysine residues are recognized and degraded by the proteasome, and the ubiquitin is released and recycled. The proteolytic core of this complex, the 20S proteasome, contains multiple peptidase activities and functions as the catalytic machine. This core is composed of 28 subunits arranged in four heptameric, tightly stacked rings (α7, β7, β7, α7) to form a cylindrical structure (89). The α-subunits make up the two outer, and the β-subunits the two inner, rings of the stack. The entrance of substrate proteins to the active site of the complex is guarded by the α subunits that allow access only to unfolded and extended polypeptides. The proteolytic activities are confined to the β subunits conferring the unique and distinguishing proteasome feature of multiple peptidase activities that include chymotrypsin-like (cleavage after hydrophobic side chains, mediated by the β5 subunit), peptidylglutamyl peptide hydrolyzing-like or PGPH-like (cleavage after acidic side chains, mediated by the β1 subunit), and trypsin-like (cleavage after basic side chains, mediated by the β2 subunit) activities (89).

The ubiquitin-proteasome pathway is vital in the degradation of proteins involved in cell cycle progression, proliferation, and apoptosis and a vast majority of abnormal proteins that result from oxidative damage and mutations. The proteasome can therefore contribute to the pathological state of several human diseases including cancer, in which some regulatory proteins are either stabilized due to decreased degradation or lost due to accelerated degradation (90). Many important target proteins of the proteasome have been identified, including cyclins A, B, D, and E; tumor suppressor protein p53; proapoptotic protein Bax (91); cyclin-dependent kinase inhibitor p27 (92–93); and the NFκB inhibitor, IκB-α (94). Since inhibition of the ubiquitin-proteasome pathway in tumor cells results in accumulation of tumor suppressor and proapoptotic proteins, the possibility of targeting this pathway in cancer therapy is a viable option.

GREEN TEA AND (−)-EGCG INHIBIT TUMOR CELLULAR PROTEASOME ACTIVITY

It has been suggested that proteasomal activity is essential for tumor cell proliferation and drug resistance development (95). Therefore, the proteasome-mediated degradation pathway has been considered to be an important target for cancer therapy and prevention. We and others have reported that inhibition of the proteasomal chymotrypsin-like activity is associated with induction of apoptosis in tumor cells (96–97). The proteasome inhibitor Bortezomib (Velcade, PS-341) has been used in clinical trials and its antitumor activity has been reported in a variety of tumor models (88, 97, 98).

We have shown that ester bond-containing tea polyphenols, for example, (−)-EGCG, potently and specifically inhibit the proteasomal chymotrypsin-like (β5) and PGPH-like (β1), but not trypsin-like (β2), activities of the proteasome (46). Using an in silico docking method, we have also shown that inhibition of the chymotrypsin activity of the 20S proteasome may be due to acylation of the β5-subunit's catalytic N-terminal threonine (Thr 1) (100). Furthermore, EGCG appears to bind the chymotrypsin site in an orientation and conformation that is suitable for a nucleophilic attack by Thr 1. Our in silico model has been corroborated by comparing the predicted and actual activities of several EGCG analogs. In the biological setting, EGCG exhibits stronginhibitory activity against a purified 20S proteasome and 26S proteasome in intact tumor cells. These inhibitory concentrations are

similar to those found in the serum of greentea drinkers. EGCG induced proteasome inhibition in whole cells has been shown to accumulate the natural proteasome substrates p27 and IκB-α as well as induce arrest of tumor cells in theG1 phase while having little to no effect on normal, nontransformed cells (46, 101–102). Based on our studies, the cancer-preventative properties of green tea could be attributed, at least in part, to its ability to inhibit proteasomal activity and the low toxicityof EGCG points to its potential use as an adjuvant to current anticancerdrugs.

We also found that synthetic (−)-EGCG amides and (−)-EGCG analogs with modifications in the A-ring, C-ring, or ester bond inhibited the chymotrypsin-like activity of purified 20S proteasome with altered potencies, induced growth arrest in the G1 phase of the cell cycle in leukemia Jurkat T cells, and suppressed colony formation of human prostate cancer LNCaP cells (103).

Although (−)-EGCG remains to be the most potent polyphenol in green tea, it is unstable in neutral or alkaline conditions (i.e., physiologic pH). In an effort to discover more stable polyphenol proteasome inhibitors, we synthesized several novel (−)-EGCG analogs with −OH groups eliminated from the B- and/or D-rings. In addition, we also synthesized their putative prodrugs with -OH groups protected by acetate that can be removed by cellular cytosolic esterases. We first examined the structure-activity relationship of these unprotected and protected compounds with respect to their proteasome inhibitory potentials. We found that decreasing the number of -OH groups from either the B- or D-ring leads to diminished proteasome inhibitory activity in vitro. However, in cultured tumor cells, the protected analogs were capable of potently inhibiting the proteasomal chymotrypsin-like activity by as much as 97% (102). Furthermore, we found that, compared to (−)-EGCG, protected analogs exhibited greater potency to inhibit proliferation and induce apoptosis in human leukemic, prostate, breast, and simian virus 40-transformed cells (101). The protected analogs were nontoxic to human normal and nontransformed cells (101).

We have also provide evidence that when cultured human breast cancer MDA-MB-231 cells were treated with the prodrug of (−)-EGCG, Pro-EGCG (**1**), (−)-EGCG not only had been converted but also accumulated, accompanied by enhanced levels of proteasome inhibition, growth suppression, and apoptosis induction, compared to cells treated with natural (−)-EGCG. To investigate the potential use of Pro-EGCG (**1**) as a novel prodrug that converts to a cellular proteasome inhibitor and anticancer agent in vivo, MDA-MB-231 tumors were induced in nude mice, followed by treatment with Pro-EGCG (**1**) or (−)-EGCG for 31 days. Results of this in vivo study demonstrated a significant inhibition of breast tumor growth by Pro-EGCG (**1**), compared to (−)-EGCG, associated with increased proteasome inhibition and apoptosis induction in tumor tissues (104). Therefore, we have shown that Pro-EGCG (**1**) increases the bioavailability, stability, and proteasome-inhibitory and anticancer activities of (−)-EGCG in human breast cancer cells and tumors, suggesting its potential use for cancer prevention and treatment.

Under physiological conditions, biotransformation reactions, such as methylation, can modify green tea polyphenols and therefore limit their in vivo cancer-preventive activity. Although a recent case-control study suggested that methylated polyphenols are less cancer-protective, the molecular basis for this observation is unknown. We hypothesize that methylated green tea polyphenols have decreased proteasome-inhibitory abilities. To test this hypothesis, methylated (−)-EGCG and (−)-ECG analogs that can be found in vivo were synthesized and studied for their structure-activity relationships (SARs) using a purified 20S proteasome. The addition of a single methyl group on (−)-EGCG or (−)-ECG led to decreased proteasome inhibition and, as the number of methyl groups increased, the inhibitory potencies further decreased. These SARs were also supported by our findings

from in silico docking analysis. As mentioned above, we synthesized a peracetate-protected (−)-EGCG molecule, Pro-EGCG (**1**), to enhance its cellular permeability and stability, and our HPLC analysis confirmed conversion of Pro-EGCG (**1**) to (−)-EGCG in cultured human leukemic Jurkat T cells. Furthermore, peracetate-protected forms of methylated green tea polyphenols were added in intact Jurkat T cells to observe the intracellular effects of methylation. Peracetate-protected, monomethy-lated (−)-EGCG induced greater cellular proteasome inhibition and apoptosis than did peracetate-protected, trimethylated (−)-EGCG, consistent with the potencies of the parent methylated analogs against a purified 20S proteasome (105). Therefore, methylation on green tea polyphenols, under physiological conditions, could decrease their proteasome-inhibitory activity, contributing to decreased cancer-preventive effects of tea consumption.

CONCLUSION

There is no observed side effect of tea consumption so far. Beneficial effects of tea consumption have been shown using animal model studies. Consumption of tea is associated with lower risk of cancer. However, due to limited human epidemiological studies, the beneficial effect of tea consumption to human re-mains inconclusive. Tea polyphenols are known to be potent antioxidants and have a wide range of molecular targets that influence cell growth and death as well as angiogenesis. The chemopreventive mechanism of tea is still unclear, although different mechanisms have been suggested. A major challenge of cancer prevention is to integrate new molecularfindings into clinical practice. Identification and validation of molecular targets or biomarkers for tea polyphenols is paramount to cancer prevention and treatment by green tea and will greatly assist in a better understanding of its anticancer mechanisms.

Acknowledgments

This work is partially supported by research grants from the National Cancer Institute-National Institutes of Health (to Q Ping Dou; 1R01CA120009; 5R03CA112625).

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