Published in final edited form as:

Curr Cancer Drug Targets. 2011 March ; 11(3): 296–306.

Green Tea Polyphenols as Proteasome Inhibitors: Implication in Chemoprevention

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Abstract

Next to water, tea is the most popular beverage in the world. The most abundant and active compound in green tea is (–)-epigallocatechin-3-gallate (EGCG), which is extensively studied for its cancer-preventive and anti-cancer activities as well as its cellular targets. One potential molecular target of EGCG is the proteasome. While molecular docking and structure-activity relationship (SAR) analysis suggests that the ester carbon of EGCG is important for mediating its proteasome-inhibitory activity, EGCG is very unstable under physiological conditions. Therefore, a series of analogs were synthesized aiming to improve stability and bioavailability of EGCG. Among them, peracetate-protected or the prodrug of EGCG was found to have increased bioavailability, stability, and proteasome-inhibitory activities against various human cancer cells and tumors compared to EGCG, suggesting its potential use for cancer prevention and treatment. Epidemiological studies have indicated that green tea consumption is associated with the reduced risk of cancers, especially associated with the reduced risk of late stage of cancers. This risk reduction may be attributed not only to proteasome inhibition, but also to numerous other intracellular molecules targeted by EGCG that are involved in cell cycle regulation, apoptosis, angiogenesis, and metastasis.

Keywords

Proteasome inhibitors; drug discovery; chemoprevention; targeted therapy

INTRODUCTION

Targeting the proteasome has become an attractive approach in cancer prevention and cancer therapy because most of the intracellular proteins involved in carcinogenesis and tumor progression are degraded through the ubiquitin-proteasome pathway [1]. Ubiquitin-

proteasome degradation system involves two successive steps, enzymatic ubiquitination followed by proteolysis through the 26S proteasome. Ubiquitination is a process of conjugating multiple ubiquitin molecules to the protein substrate. Ubiquitin is a highly conserved 76-amino acid protein that becomes covalently ligated to a target protein by a multi-enzymatic system consisting of Ub-activating (E1), Ub-conjugating (E2), and the Ub-ligating (E3) enzymes, which act in a sequential manner. Ubiquitinated proteins are recognized and selectively degraded by the 26S proteasome, the largest protease complex present in the nucleus and cytosol of a eukaryotic cell. The ubiquitin is then released and recycled. The proteolytic core of this catalytic machine, the 20S proteasome, is composed of 28 subunits arranged in four heptameric, tightly stacked rings (α 7, β 7, β 7, α 7) to form a barrel-shaped structure [2]. 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 β 5 subunit), peptidylglutamyl peptide hydrolyzing (PGPH)-like (cleavage after acidic side chains, mediated by the β 1 subunit), and trypsin-like (cleavage after basic side chains, mediated by the β 2 subunit) activities [3]. 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 ubiquitin-proteasome pathway plays a critical role in regulation of essential cellular processes (such as cell cycle progression, proliferation, and apoptosis) and abolishment of unwanted or abnormal proteins that result from oxidative damage and mutations. Furthermore, proteins identified as proteasome substrates include cyclins A, B, D and E, tumor suppressor protein p53, pro-apoptotic protein Bax [4], cyclin-dependent kinase inhibitor p27 [5], and the NF- κ B inhibitor, I κ B- α [6]. Because tumor cells appear to rely more heavily on high proteasome activity than normal cells, proteasome inhibition could contribute to a novel, selective strategy for cancer prevention and therapy [7].

THE PROTEASOME AS A POTENTIAL MOLECULAR TARGET OF GREEN TEA POLYPHENOLS

Tea (*Camellia sinensis*) is the most popular beverage, next to water, consumed in the world. Assumption of green tea, a focus of this review article, is suggested to bring about cancer-preventive and anti-oxidant effects from various green tea polyphenols including (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), and (–)-epigallocatechin-3-gallate (EGCG) (Fig. 1) [8]. Among them, EGCG is the most abundant and potent constituent.

We first reported that ester bond-containing tea polyphenols potently and selectively inhibit the proteasomal chymotrypsin-like but not the trypsin-like activity [9]. Among the green tea polyphenols examined, EGCG showed the strongest inhibitory activity against purified 20S proteasome, 26S proteasome of tumor cell extracts, and 26S proteasome in intact tumor cells. EGCG inhibits the chymotrypsin-like activity of the proteasome *in vitro* with an IC₅₀ value around 86 to 194 nM and *in vivo* at 1–10 μ M [9]. These *in vivo* EGCG concentrations are comparable with the concentrations found in the serum of green tea drinkers. Furthermore, the inhibition of the proteasome *in vivo* was able to accumulate the natural proteasome substrates p27^{Kip1} and I κ B- α as well as induce the arrest of tumor cells in the G₁ phase. A green or black tea extract, which contains significant portions of EGCG (51.5 and 19.7%, respectively) and ECG (14.7 and 14.9%, respectively), also strongly inhibited the chymotrypsin-like activity of the 20S proteasome (IC₅₀ values were 0.1 and 0.3 μ g/ml, respectively) [9]. EGCG not only inhibits proteasomal chymotrypsin-like activity, but also inhibits immunoproteasome BrAAP activity mediated by the β 5i subunit, as shown by other researchers [10, 11].

Structural-Activity Relationship of EGCG Analogs

To determine the potential binding site of EGCG for its mediated proteasome inhibition, cell-free proteasome activity in the presence of naturally occurring green tea polyphenols has been examined. The ester bond-containing tea poly-phenols, such as ECG, gallocatechin-3-gallate (GCG), and catechin-3-gallate (CG) (Fig. 1), as well as EGCG were all strong inhibitors for the chymotrypsin-like activity of the purified 20S proteasome with IC_{50} values at nM ranges [9]. In contrast, the polyphenols without the gallate ester function, such as EGC, EC, gallocatechin (GC), and catechin (C) (Fig. 1) could not inhibit the proteasomal chymotrypsin-like activity [9]. These results indicate that the ester bonds contained in green tea polyphenols are essential for potent inhibition of the proteasomal chymotrypsin-like activity [9].

Consistently, the ester bond carbon of EGCG was found to have the highest susceptibility toward a nucleophilic attack by the Thr1 of the $\beta 5$ subunit of the proteasome among all the other atoms with a value of 0.7, similar to other ester bond-containing polyphenols (ECG, GCG, and CG) [9], whereas low nucleophilic susceptibility was found in non-ester bond-containing polyphenols (EGC EC, GC, and C with values of 0.2~0.3). Thus, the nucleophilic susceptibility of EGCG toward the $\beta 5$ subunit correlates with the biological ability of EGCG to inhibit proteasomal chymotrypsin-like activity. These data support the essential role of poly-phenol ester bonds in the inhibition of the proteasome activity [9].

Our subsequent structure-activity relationship studies showed that the A ring and gallate ester/amide bond were essential for the proteasome-inhibitory function of EGCG [12]. In a mechanistic model, with *in silico* docking calculations, to account for proteasome inhibition, the polyphenol gallate ester, for example, of EGCG was found to bind the active site of the $\beta 5$ subunit of 20S proteasome. The A ring of EGCG acts as the phenyl ring of a phenylalanine mimic, binding to the hydrophobic S1 pocket of the $\beta 5$ subunit (Fig. 2). The ester bond of EGCG is then in reasonable proximity (about 3.18 Å) from the Thr 1 OH, which is responsible for the proteasomal catalytic activity (Fig. 2). Inhibition is then due to the irreversible transfer of the gallate moiety from EGCG to the hydroxy oxygen of Thr 1 [13]. This model was further supported by the results from natural polyphenols and their respective enantiomers [14]. The unnatural enantiomers were equal potent to, or more potent than the natural compounds in inhibiting the chymotrypsin-like activity of the proteasome [14] because they were also able to dock into the S1 pocket. For example, (+)-EGCG (Fig. 1) was oriented in the proteasome $\beta 5$ subunit, with the A–C rings in the S1 pocket and the B ring in solvent. In order for the gallate group of (+)-EGCG to bind in the same relative position to the hydroxyl of Thr 1 as natural (–)-EGCG, the A–C rings of (+)-EGCG had to flip 180° to attain similar orientation/conformation [14]. Similar molecular docking explanations were applied to the homology modeled active subunits of the IFN-gamma inducible proteasome [15].

Structure activity relationships of Bn-EGCG, an EGCG analog synthesized with all eight hydroxyls protected by benzyl groups have also been studied. The benzyl analog theoretically eliminated any hydrogen bonding created by the hydroxyls in EGCG while maintaining the integrity of the ester bond. The protection of the hydroxyl groups on EGCG renders the compound completely inactive to inhibit either purified 20S proteasome or cellular 26S proteasome, indicating the hydroxyl groups on EGCG are important for mediating proteasome inhibition [16]. *In silico* docking was performed to elucidate the role of the EGCG hydroxyl groups and it was determined that the two hydroxyl groups at the C5 and C7 positions of the A ring on EGCG binding to Lys 32 and Ala 146 in the hydrophobic S1 pocket of the $\beta 5$ subunit of the proteasome. This binding allows for the electrophilic carbonyl carbon of EGCG to be oriented in a suitable position for nucleophilic attack by the hydroxyl group of N-terminal Thr 1 of the $\beta 5$ subunit of the proteasome, inhibiting the

proteasomal chymotrypsin-like activity [17]. In addition, the hydroxyl groups on the D ring forms hydrogen bonds with Gly 47 and Ser 131 of the proteasome that further contributes to binding stability of EGCG to the proteasome [17].

Detailed structure–activity relationship analysis by using a number of synthetic green tea polyphenol analogs involving modifications of the EGCG A ring and B ring revealed that a decrease in the number of B ring OH groups led to decreased potency. Introduction of a hydrophobic benzyl group into the 8 position of the A ring did not significantly affect the proteasome-inhibitory potency [17].

Synthetic EGCG Analogs and Prodrugs

Green tea has been shown to affect a variety of biological systems, including metabolism, angiogenesis, and cell proliferation. Unfortunately, the most abundant green tea polyphenol, EGCG, is very unstable in neutral or alkaline conditions leading to low bioavailability. To find more stable and more potent green tea polyphenols that target the proteasome, several novel EGCG analogs have been synthesized following strategies that include hydroxyl group removal in various positions, acetate protection, para-amino group addition, and fluoro-substitutions.

EGCG Peracetate as Prodrug—EGCG analogs with the OH groups eliminated from the B and/or D rings as well as their putative prodrugs with OH groups protected as the acetates, have been tested *in vitro* and in whole cells [18, 19]. Compared to non-protected EGCG analogs, acetate-protected analogs exhibit greater potency to inhibit proliferation and induce apoptosis in human leukemic, prostate, and breast cancer cells [20]. HPLC analysis elucidated that the acetate protected EGCG analog (Pro-EGCG) (Fig. 1) is six times more stable than natural EGCG when incubated with RPMI 1640 culture medium at 37 °C. Various bioassays indicate that an intercellular conversion, from the acetate-protected to the parent unprotected hydroxyl form occurs within cells. These assays showed that the Pro-EGCG has no inhibitory activity against a purified 20S proteasome *in vitro*, as expected, but exhibits increased proteasome-inhibitory activity associated with cell death in intact leukemic cells over natural EGCG. Furthermore, in human breast MDA-MB-231 xenograft nude mice treated with Pro-EGCG or with EGCG it was found that Pro-EGCG resulted in much more significant inhibition of tumor growth than unprotected EGCG (56% *vs.* 23%), associated with increased levels of proteasome inhibition and tumor cell apoptosis *in vivo* [21]. These results indicate that Pro-EGCG may function as a viable and novel proteasome inhibitor prodrug.

D-Ring Analogs—Additional EGCG analogs containing only a para-hydroxybenzoate ester group (Para-OH EGCG) [22] or para-aminobenzoate group to replace the gallate ester have also been examined. We have compared the IC₅₀ of EGCG with para-OH EGCG, which is a naturally-occurring catechin isolated from *Cistus salvifolius*, for their inhibitory activity against the chymotrypsin-like activity of a purified proteasome *in vitro*. It was found that the loss of the two OH groups in the D-ring of para-OH EGCG led to much reduced inhibitory activity of about 200-fold. On the other hand, EGCG analogs possessing a *p*-NH₂ (Para-amino EGCG) (Fig. 1) or *p*-NHBoc (Boc; *tert*-butoxycarbonyl) D ring act as novel proteasome inhibitors and apoptosis inducers with potency similar to pro-EGCG in Raji B cells [23]. The para-aminobenzoate moiety is a normal constituent of folic acid and may offer the possibility of novel and powerful anticancer agents.

Several novel fluoro-substituted benzoates of EGC, or EGCG analogs with OH groups eliminated from the D ring and replaced with one or two fluorine(s) called F-EGCGs (Fig. 1), and their acetate protected counterparts have also been analyzed [24]. Compared to

acetate protected EGCG, acetate protected 3,4-difluorobenzoate EGCG (Pro-F-EGCG4) exhibited greater potency against proteasomal chymotrypsin-like activity, suppressed cell proliferation, and induced apoptosis in human leukemia Jurkat T cells [24]. Administration of Pro-F-EGCG4 or acetate protected fluoro-substituted benzoates of EGCG at the meta position (Pro-F-EGCG2) in human breast cancer MDA-MB-231 xenografts showed that the two fluoro-substituted EGCG analogs inhibited tumor growth with similar potency to that of the acetate protected EGCG. MDA-MB-231 tumors treated with each fluoro-substituted EGCG analog showed proteasome inhibition and apoptotic cell death, suggesting that the proteasome might be one of the cellular targets of fluoro-EGCGs and that proteasome inhibition is partially responsible for the observed antitumor activity [25]. Similar to the previously mentioned EGCG analogs, the EGCG acetate-protected, fluoro-substituted benzoates also have potential as novel anti-cancer and cancer-preventive agents.

C-Ring Carbocyclic Analogs—We have found that the unnatural enantiomer (+)-EGCG (Fig. 1) as well as other catechin gallates were equal potent to, or more potent than the natural compounds in inhibiting the chymotrypsin-like activity of the proteasome [14, 16]. *In silico* studies suggested that they were also docked to the S1 pocket in the proteasome $\beta 5$ subunit, with the A–C rings of (+)-EGCG flipped by 180° to attain similar orientation/conformation [13]. This suggested that the binding site may be pseudo-symmetrical and may be amenable to binding to analogs which are symmetrical. We have thus synthesized the EGCG analog which has a carbocyclic B ring as well as simpler analogs [12]. The IC₅₀ of this EGCG analog was found to be almost as potent as EGCG for their inhibitory activity against the chymotrypsin-like activity of a purified proteasome *in vitro*. Recently, naphthalene-2,3-diyl digallates and analogs had been synthesized and reported to have cancer cell cytotoxicity and postulated to function as tea polyphenol analogs [26]. Their inhibitory action against proteasome had not been determined however.

Biotransforming Modifications of EGCG

Following absorption EGCG is subjected to modification through major biotransformation reactions including methylation, glucuronidation, and sulfonation, resulting in reduced biological activities of EGCG *in vivo* [27, 28]. Methylation of EGCG occurs *via* catechol-O-methyltransferase (COMT) activity, an enzyme ubiquitously distributed in the body. In humans, a single gene for COMT encodes both a soluble COMT (S-COMT) and a membrane-bound COMT (MB-COMT). A single nucleotide polymorphism (G to A) in codon 108 (S-COMT) or 158 (MB-COMT) results in a valine to methionine (Val to Met) substitution that encodes for a high- (Val/Val [H/H]), intermediate- (Val/Met [H/L]), or low-activity (Met/Met [L/L]) form of COMT [29]. There is a 3~4-fold difference in enzyme activity between the high- and low-activity expressed genes [30]. One group reported that women who carried at least one low-activity COMT allele and were tea drinkers had a significantly reduced risk of breast cancer compared with non-tea drinkers [31]. In contrast, risk of breast cancer did not differ between tea drinkers and non-tea drinkers among those who were homozygous for the high-activity COMT allele [31]. These results suggested that high COMT activity could methylate and inactivate EGCG and other green tea polyphenols, resulting in non-benefit of tea drinking in high COMT patients.

Recently, methylated EGCG and ECG analogs that are metabolites or potential metabolites of green tea polyphenols EGCG and ECG were synthesized and studied for their structure-activity relationships (SARs) using a purified 20S proteasome [32]. 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 [33]. These SARs were supported by our findings from *in silico* docking analysis, showing that methylation has no effect on nucleophilic susceptibility of EGCG and ECG, but it may disrupt the ability

of these polyphenols to interact with Thr 1 of the proteasome $\beta 5$ subunit [34]. *In silico* docking shows that methylation results in the ester carbon being moved away or blocked entirely from Thr 1 and that methylation impairs the ability of EGCG and ECG to dock in a consistent low energy pose. Such observations indicating no change in nucleophilic susceptibility upon moving or blocking the ester carbon from Thr 1, and lack of a consistent docking pose, suggest that methylation disrupts the ability of EGCG and ECG to bind to the proteasome $\beta 5$ subunit, which may then diminish their proteasomal chymotrypsin-inhibitory and, therefore, other biological activities [34].

Biological data support the *in silico* findings, which show that acetate-protected, monomethylated EGCG induces greater cellular proteasome inhibition and apoptosis than acetate-protected, trimethylated EGCG, consistent with the potencies of the parent methylated analogs against a purified 20S proteasome. Therefore, methylation on green tea polyphenols under physiological conditions could decrease their proteasome-inhibitory activity, contributing to decreased cancer-preventive effects of tea [32].

In a recent study, we hypothesized that suppression of COMT activity in human breast cancer cells could increase the proteasome-inhibitory potency of EGCG and therefore enhance its tumor cell growth-inhibitory activity. We first determined the COMT genotype and basal levels of COMT activity in various human breast cancer cell lines. Furthermore, when breast cancer MDA-MB-231 cells containing high COMT activity was tested, the diminished COMT activity with the potent COMT inhibitor dinitrocatechol (DNC) apparently increased the effectiveness of EGCG *via* augmented proteasome inhibition and apoptosis induction [35]. This study supplements the previous findings that methylated EGCG is less bioactive and supports the notion that COMT inhibition may increase the anti-cancer properties of green tea polyphenols and the combination may serve as a novel approach or supplemental treatment for breast cancer chemotherapy.

Toward the goal of targeting the problem of EGCG biological methylation *in vivo*, a novel EGCG analog devoid of the o-catechol structure (EGCG 7) (Fig. 1) that should not be a substrate of COMT [36] has been examined. While the proteasome-inhibitory activity of this EGCG analog 7 was not affected by the addition of DNC, proteasome inhibition by EGCG 7 partner, EGCG 5 (Fig. 1) as well as EGCG was enhanced. Similarly, addition of DNC potentially enhanced the antiproliferative activities of acetate protected EGCG, but not the acetate protected form of this novel synthetic compound in MDA-MB-231 breast cancer cells expressing high COMT activity [36].

Although EGCG 7, presumably resistant to COMT-mediated methylation and inactivation in cells, was able to inhibit the activity of purified 20S proteasome and cellular 26S proteasome [36], the involved molecular mechanism is unknown. Most recently, we applied computational solution to understand the possible interaction between EGCG analogs including EGCG 7 and the proteasome $\beta 5$ subunit [37] which is responsible for the chymotrypsin-like activity. We found that the ester carbonyls at C2 and C3 carbon atoms may be targets for nucleophilic attack in EGCG 7 and 5 (Fig. 1). The meta-diol structure in presumably COMT-resistant 7 gives more stable conformation and lower docked free energy than other EGCG analogs.

These studies suggest that COMT is a critical factor in mediating the antiproliferative activities of EGCG and EGCG analogs, especially in cells expressing high COMT activity, such as MDA-MB-231 breast cancer cells.

EPIDEMIOLOGICAL AND CLINICAL STUDIES OF GREEN TEA

Green tea consumption may be promising as an activity against the development of cancer without inducing major toxicities. While a variety of studies shows that the risk of many types of cancers is reduced upon green tea consumption, highlighted herein are studies suggesting a reduced risk of prostate, breast, gastrointestinal, lung and skin cancer. It should be noted that cancer risk reduction is not conclusive in tea drinkers and the opposing evidence is also included.

Green Tea and Prostate Cancer

In a pilot study to investigate the effects of green tea on premalignant lesions before prostate cancer development, 60 patients with high-grade prostate intraepithelial neoplasia were administered a daily dose of 600 mg green tea in capsule form or placebo for one year. The results showed a significant reduction in the incidence of prostate cancer (3% incidence among the 30 green tea-treated subjects compared with 30% incidence among the 30 placebo-treated subjects). No significant side or adverse effects were documented [38].

While a number of case-control studies have indicated that general tea consumption was found to be associated with a decreased risk of prostate cancer [39–41], other case-control and prospective studies have concluded otherwise [42–46]. In fact, one study suggested that prostate cancer risk was increased with green tea consumption in a cohort of men of Japanese ancestry in Hawaii [47]. On the other hand, a recent large prospective study conducted in Japan showed that green tea consumption was associated with a decrease risk of advanced prostate cancer, but not localized prostate cancer [48]. These results suggest that some well-designed intervention studies could provide a clear demonstration of the cancer-preventive and anti-cancer activities of green tea polyphenols.

Green Tea and Breast Cancer

Similar to prostate cancer, epidemiologic studies have shown inconsistent results on association between green tea consumption and risk of breast cancer. For example, one meta-analysis included 13 papers that examined populations in eight countries and indicated a lower risk for breast cancer with green tea consumption [49]. Another meta-analysis indicated that consumption of 5 or more cups of green tea a day resulted in a non-statistically significant trend towards the prevention of breast cancer development [50]. However, recently, a large nested case-control study also demonstrated no overall association between plasma green tea polyphenols and the risk of breast cancer in Japan [51].

While some studies point toward no risk-reducing benefit of breast cancer in tea drinkers, numerous studies indicate quite the opposite. In fact, green tea protection against breast cancer was observed among carriers of low-activity COMT alleles but not among those who possessed high-activity COMT alleles in Asian-American women (discussed in section 2.3) [31]. Likewise, a low risk of breast cancer among women with higher green tea intake and the low-activity genotype of angiotensin-converting enzyme gene was observed among Singapore Chinese women [52] and regular consumption of green tea is associated with improved prognosis of breast cancer at early stages (I and II) [53, 54]. Other studies conducted in China indicated that drinking green tea is associated with a decreased risk for breast cancer compared to non-drinkers [55, 56]. Results showed that breast cancer risk was reduced in association with years of green tea drinking and with the amount of tea consumed per month [56].

Green Tea and Gastrointestinal Cancer

Green tea consumption has also been shown to be protective against the development of colon cancer and gastric cancer, yet some controversial data still persists. After the ingestion of a single dose of green tea, basal levels of prostaglandin E2 (PGE2), a biomarker of colorectal carcinogenesis, was reduced, suggesting green tea as a colorectal chemopreventive agent [57]. An analysis of six cohort studies that measured green tea consumption revealed a significantly decreased risk of gastric cancer in women whose intake was greater than or equal to five cups a day [58] and in women whose plasma levels of EGCG were the highest [59]. However, a large Phase II trial investigating the chemopreventive effects of decaffeinated green tea on esophageal squamous carcinogenesis in China concluded that decaffeinated green tea intervention for one year was not sufficient to alleviate esophageal precancerous lesions [60]. Furthermore, a meta-analysis to determine the association between the consumption of green tea and the risk of stomach cancer in cohort studies found no preventive effect [61].

Green Tea and Lung Cancer

Studies investigating the association of green tea consumption with lung cancer risk have reported inconsistent findings. In support of green tea consumption on reduced lung cancer risk, a meta-analysis was conducted by a literature search in PubMed from 1966 to 2008. The overall evaluation of 22 relevant studies suggests that high consumption of green tea but not black tea may be related to the reduction of lung cancer risk [62]. On the other hand, a large population-based prospective cohort study in Japan found no evidence that the consumption of green tea reduces lung cancer incidence [63].

Green Tea and Skin Cancer

Epidemiological evidence and laboratory studies demonstrate that solar ultraviolet (UV) radiation is the major reason for the initiation of skin cancer [64]. In animal models, oral administration or topical treatment of green tea provided significant protection against UV radiation-induced skin tumorigenesis (reviewed in [65]). Green tea administration in the drinking water to SKH-1 hairless mice was found to protect against UVB radiation-induced tumorigenesis [66, 67]. Green tea was also shown inhibitory effects at the stage of skin cancer progression; for example, it caused partial regression of established skin papillomas in mice [68].

In human, green tea drinking or topical treatment were shown to protect against the harmful effects of solar UV radiation. The topical application of green tea on the skin prior to UV irradiation apparently reduced the UV-induced erythema response, the number of sunburned cells as well as UV-induced DNA damage [69]. The topical treatment of human skin with green tea or EGCG resulted in inhibition of UVB-induced myeloperoxidase activation and prostaglandin metabolites production, which play a critical role in tissue infiltration and inflammatory disorders [70].

For each of the various types of cancers described above some controversy exists for risk reduction in association with green tea consumption. However, the overwhelming evidence suggests that the purified form of the most prevalent green tea polyphenol, EGCG, acts as an anticancer agent in tumor cells (for prostate cancer alone: [71–75]). Finally, it should be noted that consumption of green tea products should probably be contraindicated during therapy for multiple myeloma with bortezomib [76]. It appears that EGCG antagonizes the cell-killing efficacy of bortezomib by reacting directly with it. Therefore the potential nutrition–drug interactions should also be kept in mind when investigating the combinational therapies with natural compounds.

MOLECULAR MECHANISMS OF GREEN TEA POLYPHENOL EGCG

Green tea polyphenols exert chemopreventive effects through induction of cell cycle arrest and apoptosis or suppression of angiogenesis and metastasis.

Green Tea Polyphenols Induce Tumor Cell Cycle Arrest

Green tea polyphenol EGCG inhibits cell proliferation, blocks cell cycle progression, and induces apoptosis in a variety of cancer cell lines [77–81]. EGCG significantly increases the expression of tumor-suppressor proteins p53, p21, Fas/APO-1, and Bax in p53-positive Hep G2 cells [78] and similar results were observed in human prostate cancer cells [82]. In fact, when LNCaP cells with wild-type p53 were used as a model to study the role of p53 and NF- κ B in EGCG-induced growth arrest and apoptosis, it was found that EGCG could stabilize p53 protein *via* phosphorylation of critical serine residues on p53 and modulation of MDM2-p14^{ARF} pathway. Furthermore the results demonstrated that activation of p53-dependent downstream targets p21 and Bax and down-regulation of NF- κ B-dependent Bcl-2 resulted in growth arrest and apoptosis [82]. Another study showed that in Hep G2 cells, EGCG blocked cell cycle progression at G₁ by p53 expression and p21 up-regulation [83].

Green Tea Polyphenols Induce Tumor Cell Apoptosis

Regarding to apoptosis induction, we have found that growth-arrested prostate cancer cells express high levels of hyperphosphorylated Bcl-X_L in mitochondria and that treatment with green tea polyphenols or EGCG block expression of the hyper-, but not hypo-phosphorylated Bcl-X_L, accompanied by cytochrome c release, caspase activation, and apoptosis [84]. Furthermore, EGCG has been shown to induce cell death *via* activation of caspase-8 and down-regulation of Bid in MIA PaCa-2 cells while a dominant negative caspase-8 variant of the same cells abrogates EGCG-induced apoptosis [85]. A similar analysis of acute myeloid leukemia cells showed an increase of death-associated protein kinase 2 (DAPK2) levels and cell death after treatment with EGCG while cell death was significantly reduced in DAPK2 silenced cells [86].

Green Tea Polyphenols Inhibit Tumor Angiogenesis

The process of tumor vessel formation, migration, and metastasis is required for tumor cell survival in which vascular endothelial growth factor (VEGF) plays a critical role. EGCG exhibits anti-angiogenic activities in various experimental studies [87, 88], which is associated with a decrease of VEGF production [89–92], expression [93–95], binding activity [96] and phosphorylation [97]. VEGF is a downstream target of hypoxia-inducible factor-1 (HIF-1), which is a transcription factor of oxygen-regulated genes that are involved in cell proliferation, cell survival, and angiogenesis [98]. EGCG significantly inhibits the protein level of transcription factor HIF-1 α , and the mechanism may involve an increase in HIF-1 α protein degradation [94]. Additionally, signal transducer and activator of transcription 3 (STAT3) regulates the transcriptional activation of genes involved in angiogenesis such as VEGF [99] and EGCG treatment has been shown to markedly reduce activation of STAT3, resulting in reduced VEGF protein expression both *in vitro* and *in vivo* [95].

Green Tea Polyphenols Suppress Cancer Metastasis

Matrix metalloproteinases represent a class of proteases that are involved in tumor cell invasion and metastasis [100] and active matrix metalloproteinase (MMP), such as MMP-2 and MMP-9, are generated through cleavage of inactive forms by membrane-type 1 (MT1-MMP). EGCG has been shown to reduce MMP-2 and MMP-9 expression and enzymatic activities [101, 102] *via* inhibition of MT1-MMP [103]. Reversion-inducing, cysteine-rich

protein with Kazal motifs (RECK) is a newly identified tumor suppressor gene that negatively regulates MMPs. Treatment of oral cancer cells with EGCG partially reversed the hypermethylation status of the RECK gene and significantly enhanced the expression level of RECK mRNA and correlated with the inhibition of MMP-2 and MMP-9 levels after treatment with EGCG [104]. Finally, EGCG also inhibits the activation of β 1 integrin expression and activation of its downstream molecules such as focal adhesion kinase (FAK), AKT and extracellular signal-regulated kinase (ERK), resulting in inhibition of invasion and migration in salivary gland carcinoma cells [102].

CONCLUSION

Green tea or its active constituent is widely investigated in various cancers and multiple investigations indicate that green tea consumption is associated with reduced cancer risk although human epidemiological studies still remain inconsistent. Well-controlled clinical studies are required to clarify which populations will reap the greatest health benefits from green tea consumption and the amount of green tea to be consumed. Green tea polyphenols target a wide range of molecules that influence cell proliferation, cell death, angio-genesis and metastasis. While the chemopreventive mechanism of green tea cannot be limited to a specific pathway, protein, or gene, its use as a therapeutic agent has limitless possibilities in its natural and analog forms and should continue to be pursued in future studies.

Acknowledgments

This work is supported in part by research grants from the National Cancer Institute-National Institutes of Health (1R01CA120009, 3R01CA120009-04S1, to QPD).

ABBREVIATIONS

Boc	<i>tert</i> -butoxycarbonyl
C	catechin
CG	catechin-3-gallate
COMT	catechol-O-methyltransferase
DAPK2	death-associated protein kinase 2
DNC	3,5-dinitrocatechol
EC	epicatechin
ECG	epicatechin-3-gallate
EGC	epigallocatechin
EGCG	epigallocatechin-3-gallate
ERK	extracellular signal-regulated kinase
FAK	focal adhesion kinase
GC	galocatechin
GCG	galocatechin-3-gallate
MMP	matrix metalloproteinase
Para-amino EGCG	EGCG analog possessing a <i>p</i> -NH ₂ on the D ring
PGPH	peptidylglutamyl peptide hydrolyzing

PGE2	prostaglandin E2
Pro-EGCG	the acetate protected EGCG analog
Pro-F- EGCG2	acetate protected fluoro-substituted benzoates of EGCG at the meta position
Pro-F- EGCG4	acetate protected 3,4-difluorobenzoate EGCG
RECK	reversion-inducing, cysteine-rich protein with Kazal motifs
STAT	signal transducer and activator of transcription
SAR	structure-activity relationship
Thr 1	N-terminal threonine
UV	ultraviolet
VEGF	vascular endothelial growth factor

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Fig. 1.
Chemical structures of EGCG and its synthetic analogs and prodrugs.

Fig. 2. Docking of EGCG to the chymotrypsin site of the yeast 20S proteasome

A stick figure of EGCG with a transparent surface is used to show the proximity between EGCG and Thr 1, represented by a space-filling model (**A**). The dotted line represents a distance of 3.18 Å from the hydroxyl of Thr 1 to the carbonyl carbon of EGCG. EGCG filled most of the binding cleft, which was seen by drawing a water-accessible mesh surface around EGCG (**B**) when docked into the binding site as depicted by a ribbon structure of the $\beta 5$ subunit (**C**). The S1 pocket of the $\beta 5$ subunit is defined by the hydrophobic residues, Ala 20, Val 31, Ile 35, Met 45, Ala 49, and Gln 53. The hydrophobic portion of A ring on EGCG is oriented in the middle of the S1 pocket between the side-chains of Ala 49, Ala 20, and Lys 33 (**D**). This conformation would allow the hydrophilic hydroxyls of the A ring to project out of the two sides of the S1 hydrophobic pocket and participate in H-bonding. In addition, the sidewalls of the S1 pocket that interact with EGCG are created by Met 45 and Val 31 (**D**). Reproduced with permission from *Proteins: Structure, Function, and Bioinformatics*.