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REVIEWS: CURRENT TOPICS

Trapping of growth factors by catechins: a possible therapeutical target for prevention of proliferative diseases

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Abstract

The prevention of cancer through dietary intervention is currently receiving considerable attention. Several epidemiological studies substantiate that green tea has a protective effect against a variety of malignant proliferative disorders such as lung cancer, breast cancer and prostate cancer. This preventive potential of green tea against cancer is attributed to the biologically active flavonoids called catechins. Epigallocatechin 3-*o*-gallate, the major catechin found in green tea, mediates diverse physiological and pharmacological actions in bringing about the regression of the tumors and also lowers the risk of nonmalignant cardiovascular proliferative diseases. Much of the current research is being focused on how these catechins specifically bring about the regression of the experimentally induced tumors both in vitro and in vivo. These catechins exert diverse physiological effects against proliferative diseases by several mechanisms, most of which are not completely characterized. This review summarizes the mechanisms by which these catechins play an essential role in regulating the process of carcinogenesis, with a special emphasis on how these catechins antagonize the growth factor-induced proliferative disorders.
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Keywords: Growth factors; Catechins; Green tea; Proliferative disorders

1. Introduction

Tea, especially green tea, is the most consumed beverage in the world, next to water. Epidemiological studies demonstrated that the incidence of stomach cancer is low among tea drinkers in Shanghai [1]. It has also been reported that incidence of prostate cancer in China where green tea is consumed on a regular basis is the lowest in the world [2]. Similar studies reported that in Japan the onset of cancer in females who had consumed 10 cups of green tea per day was 8.7 years later and 3.0 years later among males, in comparison with patients who had consumed under three cups per day [3], and recurrence of stages I and II of breast cancer was reduced significantly by a daily consumption of four to five cups of green tea in Japan [4–6]. These findings prompted the world scientific community to unravel the startling anticancer properties attributed to green tea. Since then, much of the attention has been focused on finding a possible candidate against the proliferative diseases through the green tea extracts. For the last two decades, extensive investigations have been carried out to figure out the green tea factor(s) that contribute(s) to the observed antiproliferative activity of the green tea. Until now, the results coming out with the green tea extracts are promising and raising hopes for finding out a possible antiproliferative agent for the cure and prevention of several proliferative disorders, especially the cancer. To transform the hope into reality that the factors from green tea are the possible effective antiproliferative agent, much of the research is being carried out worldwide and some of them are already in clinical trials [4–6]. The mechanisms by which these green tea extracts control the process of carcinogenesis and in the cancer prevention are diverse and these extracts employ several mechanisms, all at a time, to bring about the regression of the tumors in an efficient way. The antiproliferative effects of green tea catechins are partially explained by their antioxidative properties [7,8]. Recent investigations reveal that the green tea catechins exhibit several novel mechanisms to ward off proliferative diseases. Hence, this review focuses and portrays the novel and update mechanisms by which these green tea catechins prevent carcinogenesis,

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with a special focus on the newly proposed and proven concept that these catechins are the scavengers of the growth factors that promote carcinogenesis and other proliferative diseases.

2. Green tea

Green tea, a lipid extract obtained from the plant *Camellia sinensis*, possesses more pharmacological benefits when compared to black and oolong tea, even though they all are derived from the same plant but produced according to the different processes of drying and fermentation. “Black tea” is fully fermented, “oolong tea” is partially fermented and “green tea” is not at all fermented but only steamed. To prepare black and oolong tea, the young leaves are picked, allowed to wilt and then rolled. The leaves are then allowed to ferment, converting the polyphenols to phlobaphenes and forming aromatic compounds. During the fermentation process, the enzymes from leaf including polyphenol oxidase react with tannins and catechins. For the green tea preparation, the young leaves are not allowed to oxidize by fermentation but are steamed to inactivate the enzymes thereby preserving as much as 90% of the polyphenols contained in fresh leaves from being degraded [9]. The preventive potential of the green tea against cancer as evidenced from the several experimental studies is mainly due to these polyphenols, which are found in green tea in a relatively higher concentration compared to black and oolong tea, and hence the green tea possesses more anticarcinogenic potential in comparison to black and oolong tea. Of the total amount of tea produced and consumed in the world, 78% is black tea, 20% is green tea and less than 2% is oolong tea. Green tea is consumed mostly in China, Japan, India and a few countries in North Africa and the Middle East while black tea is consumed primarily in Western countries and in some Asian countries and the oolong tea in southeastern China and Taiwan [10].

3. Catechins, general cellular mechanisms of action

Dried tea leaves are composed mainly of phytochemicals known as polyphenols (30–36%), mainly flavanols (including catechins), flavonoids and flavondiols. The majority of the polyphenols are flavanols, more commonly known as catechins [11]. The main catechins found in green tea are catechin epicatechin (EC), epigallocatechin (EGC), epicatechin-3 gallate (ECG), and epigallocatechin-3-gallate (Fig. 1). The catechin in green tea that has gained the most attention with respect to the anticarcinogenic activity is the potent antioxidant EGCG. Much of the anticarcinogenic effect of green tea is mainly attributed to EGCG. EGCG makes up about 10–50% of the total catechin content and appears to be the most powerful of all the catechins — with an antioxidant activity about 25–100 times more potent than vitamins C and E. EGCG has both

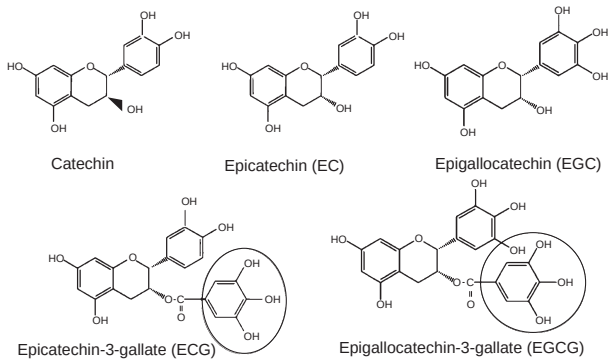


Fig. 1. Chemical structures of the green tea catechins. Only the catechins with the gallate group at 3′ position are the ones having high efficacy in inhibiting the proliferation of cells in in vitro tumor models. The gallate moiety in these catechins is rounded off.

antimatrix metalloproteinase and antiangiogenesis activities [12,13]. In black tea, the major polyphenols are theaflavin and thearubigens.

The mechanism of action of catechins on warding off the cancer is diverse and involves several modes of actions. EGCG, the primary catechin in green tea, appears to inhibit the growth of cancerous cells as well as play a role in stimulating apoptosis, both of which are crucial aspects of cancer prevention [14,15]. EGCG inhibits the cellular proliferation primarily by

1. acting as antioxidants and scavenging the free radicals [16–18];
2. inhibiting the enzymes involved in cell replication and DNA synthesis [19–22];
3. interfering with cell-to-cell contact adhesion [12,23–26] and
4. inhibiting some of the intracellular communication pathways required for cell division [27–30].

The recent studies about the inhibition of cancerous growth/proliferation by catechins are revealing the novel modes of actions of catechins.

4. Effect of catechins on the growth factors-induced cell proliferation and signal transduction pathways

Growth factors are the extracellular signal proteins that promote growth, proliferation, differentiation or survival of cells in animal tissues. These signal proteins, also called mitogens, mostly act through receptor tyrosine kinases (RTKs, the second major type of cell-surface receptors next to G protein-linked receptors) located on the cellular membrane. The notable growth factors playing an essential role in stimulating the proliferation of various cell types are platelet-derived growth factors (PDGF AA, BB, AB), epidermal growth factor (EGF), fibroblast growth factors (FGFs: FGF-1 to FGF-24), hepatocyte growth factor, vascular endothelial growth factor (VEGF), insulin-like

growth factors (IGF-1 and IGF-2), macrophage colony-stimulating factor and all the neurotrophins, including nerve growth factor. The RTKs are transmembrane proteins with their ligand-binding domain on the outer surface of the plasma membrane. Their cytosolic domain either has an intrinsic enzyme activity or associates directly with an enzyme. To activate a RTK, the ligand usually has to bind simultaneously to two adjacent receptor chains. Platelet-derived growth factor, for example, is a dimer, which cross-links two receptors together. This cross-linking enables the neighboring kinase domains of the receptor chains to cross-phosphorylate each other on multiple tyrosines, a process referred to as autophosphorylation.

Autophosphorylation of the cytosolic tail of RTKs contributes to the activation process in two ways. First, phosphorylation of tyrosines within the kinase domain increases the kinase activity of the enzyme. Second, phosphorylation of tyrosine residues outside the kinase domain creates high-affinity docking sites for the binding of a number of intracellular signaling proteins in the target cell. Each type of signaling protein binds to a different phosphorylated site on the activated receptor because it contains a specific phosphotyrosine-binding domain that recognizes surrounding features of the polypeptide chain in addition to the phosphotyrosine. Once bound to the activated kinase, the signaling protein may itself become phosphorylated on tyrosines and thereby activated. Alternatively, the binding alone may be sufficient to activate the docked signaling protein. Because different RTKs bind to different combinations of these signaling proteins, they bring about different cellular responses like cellular growth, proliferation and differentiation.

The growth factors usually act as local mediators at very low concentrations (about 10^{-9} to 10^{-11} M). The responses to them are typically slow (on the order of hours) and usually require many intracellular signaling events that eventually lead to changes in gene expression. These RTKs also have been found to mediate direct and rapid effects on the cytoskeleton, controlling the way a cell moves and changes its shape. These growth factors are not often

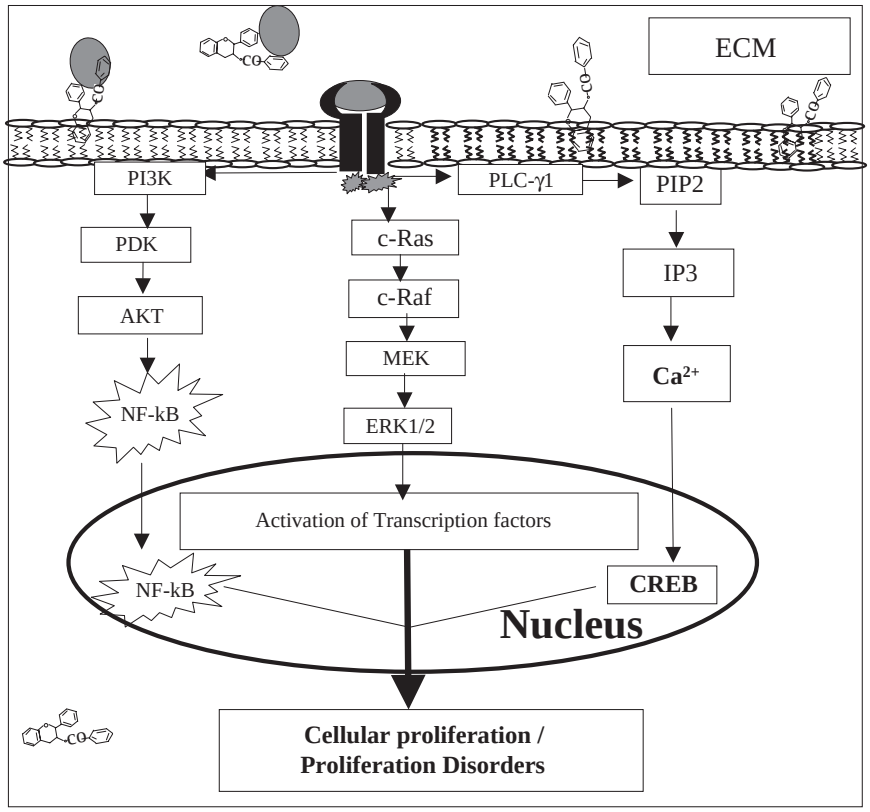
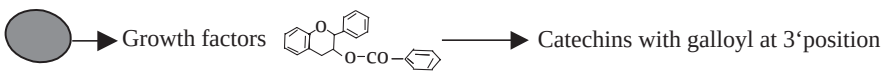


Fig. 2. Inhibition of growth factor-induced cell proliferation by EGCG and other catechins with a galloyl group at position 3. The growth factors transduce their mitogenic stimulus through RTK to activate the transcription factors such as ATF-2, Fos, Jun, Myc, and others. The catechins irreversibly bind with the growth factors. If the EGCG is already bound with membrane, then the EGCG-bound growth factors are immobilized on the membrane and thereby the growth factors are not available for binding with their respective cognate receptors. If the EGCG exists as free molecule, then EGCG-bound growth factor is free to move and may still mediate its mitogenic effect. Inhibition of intracellular signaling molecules by EGCG also is possible and reported.



diffusible but instead are found attached to surfaces over which the cell is crawling. Disorders of cell proliferation, differentiation, survival and migration are fundamental events that can give rise to cancer and abnormalities of signaling through RTKs have major roles in this class of disease. An increased activity of the RTKs such as the platelet-derived growth factor-α receptor (PDGF-Rα), the β-receptor (PDGF-Rβ) and EGFR contribute to the development of proliferative diseases such as cancer and atherosclerosis [31–34].

Platelet-derived growth factor is a dimeric protein composed of A and B chains. The A and B chains are able to form three isoforms: PDGF-AA, PDGF-AB and PDGF-BB [35,36]. All three isoforms bind with high affinity to the PDGF-Rα while only PDGF-BB binds with high affinity to PDGF-Rβ. PDGF-Rα and PDGF-Rβ transmit the mitogenic signals through ligand-induced receptor autophosphorylation. Tyrosine-phosphorylated PDGF-Rα or PDGF-Rβ induces tyrosine phosphorylation of different substrate proteins, including phospholipase C-γ1 (PLC-γ1), phosphatidylinositol 3′ -kinase and extracellular response kinases 1/2 (ERK1/2) via the p21ras/mitogen-activated protein kinase kinase pathway [37,38]. Activation of PLC-γ1 results in hydrolysis of phosphatidylinositol 4,5-bisphosphate to diacylglycerol (DG) and IP3. The DG remains embedded in the plasma membrane and activates the Ca^{2+} protein kinase C. IP3 triggers the release of Ca^{2+} from endoplasmic reticulum and thereby increases the concentration of Ca^{2+} in the cytosol [39]. The increased levels of the cytosolic Ca^{2+} activate Ca^{2+}/calmodulin-dependent protein kinases and eventually exert their effect on the transcription of the genes, thereby bringing about the cellular proliferation (mitogenesis). Autocrine activation of the PDGF-Rβ seems to be the initial cause of the development of the human A172 glioblastoma cells [40]. EGCG and other green tea catechins inhibit the proliferation of cells in several ways and their effects on the growth factor-induced signaling and subsequent cell proliferation are shown in (Figs. 2 and 3).

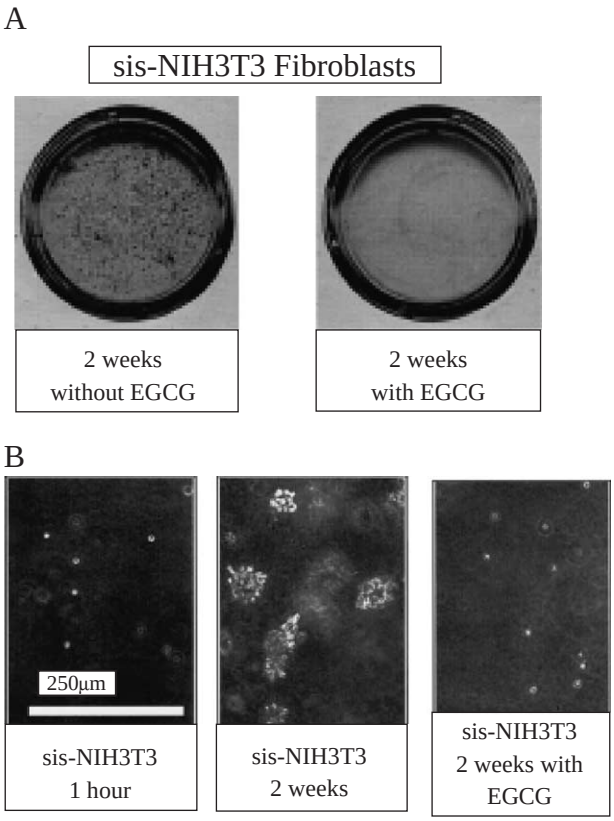


Fig. 3. (A–B) Anchorage-independent growth of *sis*-transfected NIH3T3 cells in the presence and absence of EGCG on semisolid agar. The oncogene v-*sis* of simian sarcoma virus is homologoues to the cellular gene encoding the PDGF-B chain. Transfection of NIH-3T3 cells with v-*sis* leads to their neoplastic transformation because of the persistent autocrine activation of PDGF-B. The colony formation of sis-NIH3T3 cells was completely inhibited by EGCG. Reprinted from *Molecular Biology of the Cell* (Mol. Biol. Cell 1999 10:1093–1104) with the permission of The American Society for Cell Biology.

4.1. Effect of EGCG on the angiotensin II-induced signal transduction pathway

Key events in the development of atherosclerosis-, hypertension- and angioplasty-induced restenosis are vascular smooth muscle cell (VSMC) hypertrophy and hyperplasia. Angiotensin II (Ang II) is a potent VSMC growth factor that is produced in higher levels during the pathogenesis of cardiovascular diseases [41]. Ang II-induced VSMC hypertrophy is defined as an increase in cell size and protein content without an increase in cell number and DNA replication. The signal transduction pathways underlying the Ang II-induced growth response involve the activation of mitogen-activated protein kinases, which activates AP-1. Emerging evidence suggests that endogenous reactive oxygen species (ROS) synthesis promotes VSMC hypertrophy and proliferation [42]. The inhibition of VSMC hypertrophy by EGCG is partly by inhibiting JNK signaling pathway by Ang II apart from the ROS scavenging activity of EGCG [43].

4.2. Effect of EGCG on the EGFR-mediated signal transduction pathway

EGFR's tyrosine kinase activation is believed to initiate multiple cellular responses associated with mitogenesis and cell proliferation. The overexpression of EGFR might produce a neoplastic phenotype. EGCG inhibits the autophosphorylation of EGFR by its ligand, EGF, and blocks the binding of EGF to its receptors [44,45]. EGCG also significantly inhibits DNA synthesis and protein kinase activities of EGFR [46].

4.3. Effects of catechins on the VEGF signal transduction pathway

There is a large body of evidence supporting a central role of angiogenesis in tumor growth and metastasis. Accordingly, the expression of VEGF, the most potent

angiogenic stimulus known, and its receptors was found to be up-regulated in most of the tumors [47]. EGCG has been shown to inhibit growth and to induce apoptosis in different human cancer cell lines. It has been proposed that an anti-angiogenic property of EGCG may be one of the mechanisms leading to the inhibition of VEGF-induced carcinogenesis [45,48]. VEGF is a mitogen for endothelial cells that is often associated with tumor-induced angiogenesis [49]. VEGF binds to VEGF receptor-1 and -2, the latter being responsible for most of its mitogenic and chemotactic effects [49,50]. VEGF is involved in the angiogenesis of many solid tumors including breast cancer, colon cancer, hepatoma, bladder cancer, brain tumors and prostate cancer [51–57]. Moreover, there is increasing evidence that neovascularization is one characteristic feature of atherosclerotic plaques [58,59]. The two VEGF receptors form a dimer to activate autophosphorylation of tyrosine residues on the cytoplasmic domain [49]. The study by Neuhaus et al. [60] showed that treatment with EGCG results in an inhibition of human umbilical arterial endothelial cell (HUAEC) mitogenesis (DNA synthesis and cell proliferation). In addition, the signal transduction pathways of VEGF in HUAEC, including autophosphorylation of VEGF-R1 and -R2, phosphorylation of ERK1/2, as well as the expression of EGR-1 mRNA, are also inhibited in EGCG-pretreated cells [60]. Similar to "PDGF trapping by EGCG" as described below, trapping of VEGF by soluble and by membrane-anchored EGCG is likely and might play an important role in inhibiting the neovascularization process at the tumor site. In addition, it is well established that catechins are able to interact with several proliferation-related proteins [61–63]. Thus, the inhibition of growth factor binding to its receptor may represent a general principle in modulation of growth factor activity by EGCG. Taken together, the inhibition of VEGF-induced endothelial cell mitogenesis by EGCG, which persists after removal of the compound in addition to possible direct binding of growth factors, would result in a sustained inhibition of endothelial cell proliferation even at low plasma concentrations between doses. Thus, EGCG or other galloyl group-containing plant-derived catechins are attractive candidates for prevention and/or treatment of proliferative disorders.

4.4. The proposed EGCG receptor

Using a subtraction cloning strategy involving cDNA libraries constructed from cells treated or untreated with all *trans*-retinoic acid (ATRA) which enhances the binding of EGCG to the cell surface of cancer cells, the single target that allows EGCG to bind to the cell surface was discovered to be a 67-kDa laminin receptor (67LR) [64]. The 67LR is expressed on a variety of tumor cells, and the expression level of this protein strongly correlates with the risk of tumor invasion and metastasis. Characterizing the mechanisms by which EGCG acts through this 67LR should help in the design of new strategies to prevent cancer. The mechanism by which EGCG inhibits tumor growth via this 67LR has to be speculated in future, even though it is not clear at present how EGCG mediates its antiproliferative effect via its receptor.

5. Trapping of PDGF by EGCG

Catechins with a galloyl group in the 3-position of the catechin structure, such as ECG, CG and EGCG, are able to suppress the PDGF-BB-induced stimulation of the PDGF-Rβ-mediated signal transduction pathways and mitogenesis of vascular smooth muscle cells [65]. The inhibitory effects of EGCG on PDGF-induced cell signaling and mitogenesis are due to the incorporation of EGCG into different cellular compartments, including cell surface membranes which leads to an irreversible and nondisplaceable binding of PDGF to nonreceptor binding sites, most likely resulting in a reduced PDGF binding to the respective receptors. Although only about 2% of the incorporated EGCG is found in cell surface membranes, the morphological analysis of EGCG-treated human VSMC revealed that most of the cytoplasmic EGCG is stored in vesicle-like inclusion bodies. The EGCG incorporated into these structures apart from the membranes is not likely to inhibit PDGF-R kinase or other signaling molecules [66]. This favors a surface membrane-linked mechanism of action of this catechin. In addition, the inhibitory actions of EGCG occur at a very early step in the PDGF signal transduction, namely, at the site of PDGF-R autophosphorylation. The incorporation of the EGCG onto the membrane has not altered the physical properties of the membrane as evidenced in terms of the membrane fluidity and hence the membrane embedded with the EGCG is intact without any distortion of the membrane structure [66]. Moreover, no inhibition by EGCG is encountered when soluble EGCG binds directly with the PDGF-BB. Hence, the inhibitory effect of EGCG on the PDGF-Rβ-mediated mitogenesis is mainly by "trapping" of the PDGF ligand by only the EGCG catechin incorporated or adsorbed onto the plasma membrane, which might have prevented the specific binding of PDGF-BB to its respective receptors. This means that the PDGF bound to free EGCG (i.e., non-membrane-bound EGCG) is still freely moving and in fact is able to cross-link and activate the specific receptors in the normal way as the free PDGF does. When the EGCG adsorbed on to the membrane binds irreversibly to the PDGF ligand, the resulting ligand–EGCG complex is immobilized on the membrane. Since the EGCG is anchored firmly on/in the membrane, the transducing potential of the bound ligand is abolished since the bound ligand cannot move freely to get in to contact with the specific receptors and cross-link them. That is, the ligands are "entrapped." This mode of molecular action of EGCG provides an attractive model to explain the antiproliferative effects of the catechins [66]. It has been reported that all *trans*-retinoic acid (ATRA) enhances the binding of EGCG to the surface of the cancer cells [64].

An interesting aspect of the inhibition of PDGF-induced proliferation by EGCG is the preferential inhibition of PDGF-BB- as compared to PDGF-AA-induced cell signaling and mitogenesis. Some specificity for PDGF-BB is observed in human VSMC [66]. For example, EGCG only partially inhibits PDGF-AA-induced phosphorylation of PDGF-Rα, as opposed to an almost complete inhibition of PDGF-BB-induced receptor phosphorylation. This apparent selectivity is supported by experiments with porcine aortic endothelial cells (AEC) stably transfected with PDGF-Rα [66]. In these cells, EGCG did not inhibit PDGF-AA-induced phosphorylation of PDGF-Rα, PLC-γ1 or ERK-1/2. Similarly, PDGF-AA-induced $[Ca^{2+}]_i$ transients were not affected by EGCG. In contrast, EGCG markedly inhibited PDGF-BB-induced receptor phosphorylation and intracellular signaling events. In porcine AEC stably transfected with PDGF-Rβ, EGCG inhibited both PDGF-BB- and PDGF-AB-induced phosphorylation of PDGF-Rβ and PLC-γ1. Thus, the specificity of the inhibitory effects of EGCG for the PDGF-BB-induced cell signaling is not absolute and not necessarily restricted to PDGF. Some degree of specificity for the PDGF-BB isoform is also observed for the inhibition of cell proliferation by EGCG. Although more pronounced effects are seen with PDGF-BB as a stimulus, cell proliferation induced by any PDGF isoform is inhibited by EGCG. Furthermore, in PDGF-Rα-transfected porcine AEC, EGCG significantly inhibited PDGF-AA-induced mitogenesis without affecting PDGF-AA-induced signaling events (phosphorylation of PDGF-Rα and of PLC-γ1, $[Ca^{2+}]_i$ transients). Thus, the inhibitory effect of EGCG must be mediated by additional mechanisms independent of the early signal transduction pathway of the PDGF-Rα, for example, by modulation of the expression/activity of cell cycle components [67].

Furthermore, a binding of PDGF to membrane components, such as gangliosides GM1 or GM2, has been reported [68]. These gangliosides have also been shown to bind to other growth factors, such as basic FGF (bFGF) [69]. Thus, trapping of growth factors by nonreceptor binding sites may represent a general principle in modulation of growth factor activity. Also, it is interesting to note that catechins not only bind to PDGF but are also able to interact with several other proliferation-related proteins [39,70–75].

6. Conclusion

Recently, extensive investigations have been made to develop synthetic RTK inhibitors as drug targets for therapy of cancer and cardiovascular diseases [76–78]. In this context, there are currently a large number of small-molecule RTK antagonists directed to PDGF-R, EGF-R and VEGF-R in phase I–III clinical development stages. In addition to direct binding of PDGF (and possibly other growth factors), the noncompetitive inhibition of PDGF-induced (also other growth factors like VEGF, EGF etc.) mitogenesis by membrane-bound EGCG results in a sustained inhibition of cell proliferation even at low plasma concentrations. The mechanism of the inhibitory actions of EGCG, as proposed from our present studies, will help to better understand the beneficial effects of catechins in human epidemiological studies and in animal atherosclerosis models. Thus, EGCG or other galloyl group-containing plant-derived catechins are attractive candidates for the prevention of proliferative disorders.

Acknowledgments

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References

[1] Yu GP, Hsieh CC, Wang LY, Yu SZ, Li XL, Jin TH. Green-tea consumption and risk of stomach cancer: a population-based case-control study in Shanghai. China Cancer Causes Control 1995;6:532–8.
[2] Gupta S, Ahmad N, Mohan RR, Husain MM, Mukhtar H. Prostate cancer chemoprevention by green tea: in vitro and in vivo inhibition of testosterone-mediated induction of ornithine decarboxylase. Cancer Res 1999;59:2115–20.
[3] Fujiki H, Suganuma M, Okabe S, et al. Cancer inhibition by green tea. Mutat Res 1998;402:307–10.
[4] Fujiki H, Suganuma M, Okabe S, et al. Mechanistic findings of green tea as cancer preventive for humans. Proc Soc Exp Biol Med 1999;220:225–8.
[5] Fujiki H. Two stages of cancer prevention with green tea. J Cancer Res Clin Oncol 1999;125:589–97.
[6] Nakachi K, Suemasu K, Suga K, Takeo T, Imai K, Higashi Y. Influence of drinking green tea on breast cancer malignancy among Japanese patients. Jpn J Cancer Res 1998;89:254–61.
[7] Yang CS, Yang GY, Chung JY, Lee MJ, Li C. Tea and tea polyphenols in cancer prevention. Adv Exp Med Biol 2001;492:39–53.
[8] Yang CS, Chung JY, Yang G, Chhabra SK, Lee MJ. Tea and tea polyphenols in cancer prevention. J Nutr 2000;130:472S–8S.
[9] Chen ZY, Yan RQ, Qin GZ, Qin LL. Effect of six edible plants on the development of AFB1-induced gamma-glutamyltranspeptidase-positive hepatocyte foci in rats. Zhonghua Zhong Liu Za Zhi 1987;9:109–11.
[10] Katiyar SK, Agarwal R, Wang ZY, Bhatia AK, Mukhtar H. (−)-Epigallocatechin-3-gallate in *Camellia sinensis* leaves from Himalayan region of Sikkim: inhibitory effects against biochemical events and tumor initiation in Sencar mouse skin. Nutr Cancer 1992;18:73–83.
[11] Ahmad N, Mukhtar H. Green tea polyphenols and cancer: biologic mechanisms and practical implications. Nutr Rev 1999;57:78–83.
[12] Garbisa S, Biggin S, Cavallarin N, Sartor L, Benelli R, Albini A. Tumor invasion: molecular shears blunted by green tea. Nat Med 1999;5:1216.
[13] Cao Y, Cao R, Brakenhielm E. Antiangiogenic mechanisms of diet-derived polyphenols. J Nutr Biochem 2002;13:380–90.
[14] Ahmad N, Feyes DK, Nieminen AL, Agarwal R, Mukhtar H. Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. J Natl Cancer Inst 1997; 89:1881–6.
[15] Ahn WS, Huh SW, Bae SM, et al. A major constituent of green tea, EGCG, inhibits the growth of a human cervical cancer cell line, CaSki cells, through apoptosis, G(1) arrest, and regulation of gene expression. DNA Cell Biol 2003;22:217–24.

[16] Hakim IA, Harris RB, Brown S, et al. Effect of increased tea consumption on oxidative DNA damage among smokers: a randomized controlled study. J Nutr 2003;133:3303S–9S.
[17] Kumari MV, Yoneda T, Hiramatsu M. Scavenging activity of "beta catechin" on reactive oxygen species generated by photosensitization of riboflavin. Biochem Mol Biol Int 1996;38:1163–70.
[18] Leanderson P, Faresjo AO, Tagesson C. Green tea polyphenols inhibit oxidant-induced DNA strand breakage in cultured lung cells. Free Radic Biol Med 1997;23:235–42.
[19] Katiyar SK, Agarwal R, Mukhtar H. Green tea in chemoprevention of cancer. Compr Ther 1992;18:3–8.
[20] Han J. Highlights of the cancer chemoprevention studies in China. Prev Med 1993;22:712–22.
[21] Agarwal R, Katiyar SK, Zaidi SI, Mukhtar H. Inhibition of skin tumor promoter-caused induction of epidermal ornithine decarboxylase in SENCAR mice by polyphenolic fraction isolated from green tea and its individual epicatechin derivatives. Cancer Res 1992;52:3582–8.
[22] Bachrach U, Wang YC. Cancer therapy and prevention by green tea: role of ornithine decarboxylase. Amino Acids 2002;22:1–13.
[23] Kinashi T, Escobedo JA, Williams LT, Takatsu K, Springer TA. Receptor tyrosine kinase stimulates cell-matrix adhesion by phosphatidylinositol 3 kinase and phospholipase C-gamma 1 pathways. Blood 1995;86:2086–90.
[24] Liu JD, Chen SH, Lin CL, Tsai SH, Liang YC. Inhibition of melanoma growth and metastasis by combination with (−)-epigallocatechin-3-gallate and dacarbazine in mice. J Cell Biochem 2001;83:631–42.
[25] Sazuka M, Imazawa H, Shoji Y, Mita T, Hara Y, Isemura M. Inhibition of collagenases from mouse lung carcinoma cells by green tea catechins and black tea theaflavins. Biosci Biotechnol Biochem 1997;61:1504–6.
[26] Vayalil PK, Katiyar SK. Treatment of epigallocatechin-3-gallate inhibits matrix metalloproteinases-2 and -9 via inhibition of activation of mitogen-activated protein kinases, c-jun and NF-kappaB in human prostate carcinoma DU-145 cells. Prostate 2004;59:33–42.
[27] Wang ZY, Huang MT, Ho CT, et al. Inhibitory effect of green tea on the growth of established skin papillomas in mice. Cancer Res 1992;52:6657–65.
[28] Jain AK, Shimoi K, Nakamura Y, Kada T, Hara Y, Tomita I. Crude tea extracts decrease the mutagenic activity of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine in vitro and in intragastric tract of rats. Mutat Res 1989;210:1–8.
[29] Liao S, Umekita Y, Guo J, Kokontis JM, Hiipakka RA. Growth inhibition and regression of human prostate and breast tumors in athymic mice by tea epigallocatechin gallate. Cancer Lett 1995;96:239–43.
[30] Isemura M, Saeki K, Kimura T, Hayakawa S, Minami T, Sazuka M. Tea catechins and related polyphenols as anti-cancer agents. Biofactors 2000;13:81–5.
[31] Chen C, Yu R, Owuor ED, Kong AN. Activation of antioxidant-response element (ARE), mitogen-activated protein kinases (MAPKs) and caspases by major green tea polyphenol components during cell survival and death. Arch Pharm Res 2000;23:605–12.
[32] Ingredient in green tea kills prostate cancer cells, study finds. Mayo Clin Health Lett 1999;17:4.
[33] Shibata K, Moriyama M, Fukushima T, Kaetsu A, Miyazaki M, Une H. Green tea consumption and chronic atrophic gastritis: a cross-sectional study in a green tea production village. J Epidemiol 2000;10:310–6.
[34] Bernstein BJ, Grasso T. Prevalence of complementary and alternative medicine use in cancer patients. Oncology (Huntingt) 2001;15:1267–72.
[35] Fredriksson L, Li H, Eriksson U. The PDGF family: four gene products form five dimeric isoforms. Cytokine Growth Factor Rev 2004;15:197–204.
[36] Li X, Eriksson U. Novel PDGF family members: PDGF-C and PDGF-D. Cytokine Growth Factor Rev 2003;14:91–8.
[37] Bernstein BJ, Grasso T. Prevalence of complementary and alternative medicine use in cancer patients. Oncology (Huntingt) 2001;15:1267–72.
[38] Benzie IF, Szeto YT, Strain JJ, Tomlinson B. Consumption of green tea causes rapid increase in plasma antioxidant power in humans. Nutr Cancer 1999;34:83–7.
[39] Ahn HY, Hadizadeh KR, Seul C, Yun YP, Vetter H, Sachinidis A. Epigallocathechin-3 gallate selectively inhibits the PDGF-BB-induced intracellular signaling transduction pathway in vascular smooth muscle cells and inhibits transformation of sis-transfected NIH 3T3 fibroblasts and human glioblastoma cells (A172). Mol Biol Cell 1999;10:1093–104.
[40] Ahn SC, Kim GY, Kim JH, et al. Epigallocatechin-3-gallate, constituent of green tea, suppresses the LPS-induced phenotypic and functional maturation of murine dendritic cells through inhibition of mitogen-activated protein kinases and NF-kappaB. Biochem Biophys Res Commun 2004;313:148–55.
[41] Nakajima M, Hutchinson HG, Fujinaga M, et al. The angiotensin II type 2 (AT2) receptor antagonizes the growth effects of the AT1 receptor: gain-of-function study using gene transfer. Proc Natl Acad Sci U S A 1995;92:10663–7.
[42] Dimmeler S, Zeiher AM. Reactive oxygen species and vascular cell apoptosis in response to angiotensin II and pro-atherosclerotic factors. Regul Pept 2000;90:19–25.
[43] Zheng Y, Song HJ, Kim CH, et al. Inhibitory effect of epigallocatechin 3-*O*-gallate on vascular smooth muscle cell hypertrophy induced by angiotensin II. J Cardiovasc Pharmacol 2004;43:200–8.
[44] Liang YC, Lin-Shiau SY, Chen CF, Lin JK. Suppression of extracellular signals and cell proliferation through EGF receptor binding by (−)-epigallocatechin gallate in human A431 epidermoid carcinoma cells. J Cell Biochem 1997;67:55–65.
[45] Masuda M, Suzui M, Lim JT, Deguchi A, Soh JW, Weinstein IB. Epigallocatechin-3-gallate decreases VEGF production in head and neck and breast carcinoma cells by inhibiting EGFR-related pathways of signal transduction. J Exp Ther Oncol 2002;2:350–9.
[46] Masuda M, Suzui M, Weinstein IB. Effects of epigallocatechin-3-gallate on growth, epidermal growth factor receptor signaling pathways, gene expression, and chemosensitivity in human head and neck squamous cell carcinoma cell lines. Clin Cancer Res 2001;7:4220–9.
[47] McMahon G. VEGF receptor signaling in tumor angiogenesis. Oncologist 2000;5(Suppl 1):3–10.
[48] Jung YD, Kim MS, Shin BA, et al. EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells. Br J Cancer 2001;84:844–50.
[49] Ferrara N. Vascular endothelial growth factor as a target for anticancer therapy. Oncologist 2004;9(Suppl 1):2–10.
[50] Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669–76.
[51] Takahashi Y, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res 1995;55:3964–8.
[52] Yoshiji H, Kuriyama S, Hicklin DJ, et al. KDR/Flk-1 is a major regulator of vascular endothelial growth factor-induced tumor development and angiogenesis in murine hepatocellular carcinoma cells. Hepatology 1999;30:1179–86.
[53] Yoshiji H, Kuriyama S, Ways DK, et al. Protein kinase C lies on the signaling pathway for vascular endothelial growth factor-mediated tumor development and angiogenesis. Cancer Res 1999;59:4413–8.
[54] Kurebayashi J, Otsuki T, Kunisue H, et al. Expression of vascular endothelial growth factor (VEGF) family members in breast cancer. Jpn J Cancer Res 1999;90:977–81.
[55] Droller MJ. Vascular endothelial growth factor is a predictor of relapse and stage progression in superficial bladder cancer. J Urol 1998;160:1932.

[56] Samoto K, Ikezaki K, Ono M, et al. Expression of vascular endothelial growth factor and its possible relation with neovascularization in human brain tumors. Cancer Res 1995;55:1189–93.
[57] Weidner N, Carroll PR, Flax J, Blumenfeld W, Folkman J. Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. Am J Pathol 1993;143:401–9.
[58] Chen YX, Nakashima Y, Tanaka K, Shiraishi S, Nakagawa K, Sueishi K. Immunohistochemical expression of vascular endothelial growth factor/vascular permeability factor in atherosclerotic intimas of human coronary arteries. Arterioscler Thromb Vasc Biol 1999;19: 131–9.
[59] Kumamoto M, Nakashima Y, Sueishi K. Intimal neovascularization in human coronary atherosclerosis: its origin and pathophysiological significance. Hum Pathol 1995;26:450–6.
[60] Neuhaus T, Pabst S, Stier S, et al. Inhibition of the vascular-endothelial growth factor-induced intracellular signaling and mitogenesis of human endothelial cells by epigallocatechin-3 gallate. Eur J Pharmacol 2004;483:223–7.
[61] Jankun J, Selman SH, Swiercz R, Skrzypczak-Jankun E. Why drinking green tea could prevent cancer. Nature 1997;387:561.
[62] Kazi A, Smith DM, Daniel K, et al. Potential molecular targets of tea polyphenols in human tumor cells: significance in cancer prevention. In Vivo 2002;16:397–403.
[63] Nam S, Smith DM, Dou QP. Ester bond-containing tea polyphenols potently inhibit proteasome activity in vitro and in vivo. J Biol Chem 2001;276:13322–30.
[64] Tachibana H, Koga K, Fujimura Y, Yamada K. A receptor for green tea polyphenol EGCG. Nat Struct Mol Biol 2004;11:380–1.
[65] Sachinidis A, Hescheler J. Are catechins natural tyrosine kinase inhibitors? Drug News Perspect 2002;15:432–8.
[66] Weber AA, Neuhaus T, Skach RA, et al. Mechanisms of the inhibitory effects of epigallocatechin-3 gallate on platelet-derived growth factor-BB-induced cell signaling and mitogenesis. FASEB J 2004;18: 128–30.
[67] Weber AA, Neuhaus T, Skach RA, et al. Mechanisms of the inhibitory effects of epigallocatechin-3 gallate on platelet-derived growth factor-BB-induced cell signaling and mitogenesis. FASEB J 2004;18:128–30.
[68] Gouni-Berthold I, Seul C, Ko Y, Hescheler J, Sachinidis A. Gangliosides GM1 and GM2 induce vascular smooth muscle cell proliferation via extracellular signal-regulated kinase 1/2 pathway. Hypertension 2001;38:1030–7.
[69] Rusnati M, Tanghetti E, Urbinati C, et al. Interaction of fibroblast growth factor-2 (FGF-2) with free gangliosides: biochemical characterization and biological consequences in endothelial cell cultures. Mol Biol Cell 1999;10:313–27.
[70] McCarty MF. Natural antimutagenic agents may prolong efficacy of human immunodeficiency virus drug therapy. Med Hypotheses 1997; 48:215–20.
[71] Erba D, Riso P, Colombo A, Testolin G. Supplementation of Jurkat T cells with green tea extract decreases oxidative damage due to iron treatment. J Nutr 1999;129:2130–4.
[72] Chung JH, Han JH, Hwang EJ, et al. Dual mechanisms of green tea extract (EGCG)-induced cell survival in human epidermal keratinocytes. FASEB J 2003;17:1913–5.
[73] Fang MZ, Wang Y, Ai N, et al. Tea polyphenol (−)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. Cancer Res 2003;63:7563–70.
[74] Conney AH, Wang ZY, Huang MT, Ho CT, Yang CS. Inhibitory effect of green tea on tumorigenesis by chemicals and ultraviolet light. Prev Med 1992;21:361–9.
[75] Tang FY, Nguyen N, Meydani M. Green tea catechins inhibit VEGF-induced angiogenesis in vitro through suppression of VE-cadherin phosphorylation and inactivation of Akt molecule. Int J Cancer 2003; 106:871–8.
[76] Yang CS, Chung JY, Yang G, Chhabra SK, Lee MJ. Tea and tea polyphenols in cancer prevention. J Nutr 2000;130:472S–8S.
[77] Tosetti F, Ferrari N, De Flora S, Albini A. 'Angioprevention': angiogenesis is a common and key target for cancer chemopreventive agents. FASEB J 2002;16:2–14.
[78] Levitzki A, Gazit A. Tyrosine kinase inhibition: an approach to drug development. Science 1995;267:1782–8.