Molecular Targets for Green Tea in Prostate Cancer Prevention[1,2]

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ABSTRACT Prostate cancer (PCa) is the most frequently diagnosed malignancy and the second leading cause of cancer-related deaths in American males. For these reasons, it is necessary to intensify our efforts for better understanding and development of novel treatment and chemopreventive approaches for this disease. In recent years, green tea has gained considerable attention as an agent that could reduce the risk of several cancer types. The cancer-chemopreventive effects of green tea appear to be mediated by the polyphenolic constituents present therein. Based on geographical observations that suggest that the incidence of PCa is lower in Japanese and Chinese populations that consume green tea on a regular basis, we hypothesized that green tea and/or its constituents could be effective for chemoprevention of PCa. To investigate this hypothesis, we initiated a program for the chemoprevention of PCa by green tea. In cell-culture systems that employ human PCa cells DU145 (androgen insensitive) and LNCaP (androgen sensitive), we found that the major polyphenolic constituent (−)−epigallocatechin-3-gallate (EGCG) of green tea induces *1*) apoptosis, *2*) cell-growth inhibition, and *3*) cyclin kinase inhibitor WAF-1/p21–mediated cell-cycle dysregulation. More recently, using a cDNA microarray, we found that EGCG treatment of LNCaP cells results in *1*) induction of genes that functionally exhibit growth-inhibitory effects, and *2*) repression of genes that belong to the G-protein signaling network. In animal studies that employ a transgenic adenocarcinoma of the mouse prostate (TRAMP), which is a model that mimics progressive forms of human prostatic disease, we observed that oral infusion of a polyphenolic fraction isolated from green tea (GTP) at a human achievable dose (equivalent to 6 cups of green tea/d) significantly inhibits PCa development and metastasis. We extended these studies and more recently observed increased expression of genes related to angiogenesis such as vascular endothelial growth factor (VEGF) and those related to metastasis such as matrix metalloproteinases (MMP)-2 and MMP-9 in prostate cancer of TRAMP mice. Oral feeding of GTP as the sole source of drinking fluid to TRAMP mice results in significant inhibition of VEGF, MMP-2 and MMP-9. These data suggest that there are multiple targets for PCa chemoprevention by green tea and highlight the need for further studies to identify novel pathways that may be modulated by green tea or its polyphenolic constituents that could be further exploited for prevention and/or treatment of PCa. J. Nutr. 133: 2417S–2424S, 2003.

KEY WORDS: • *green tea* • *prostate cancer* • *chemoprevention* • *metalloproteinases* • *cell cycle* • *apoptosis*

Prostate cancer (PCa)[4] is a major public health concern and a leading cause of cancer-related deaths among males in the U.S. (1,2). According to estimates of the American Cancer Society, for the year 2002, ~189,000 new cases of PCa and 30,200 PCa-related deaths were predicted (2). It is therefore necessary to intensify our efforts to better understand this disease and develop novel approaches for its prevention and treatment. Chemoprevention involving the use of natural or synthetic agents to suppress, block or reverse the process of carcinogenesis could be an effective approach to reduce the incidence of PCa (3–13). Indeed, PCa represents an excellent candidate disease for chemoprevention, because it is typically diagnosed in elderly men; even a modest delay in the neoplastic development achieved through pharmacological or therapeutic intervention could result in substantial reduction in the incidence of the clinically detectable disease. Consistent with this assumption, there is intense activity in defining chemopreventive agents and molecular targets for PCa chemoprevention (14–26). Among the many such agents that are available, for a variety of reasons, naturally occurring nontoxic dietary substances are preferred. Also, the major targets of marketing such products are PCa patients. Among these dietary products

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[4] Abbreviations used: cdc2, cell-division cycle; Cdk, cyclin-dependent kinase; EGCG, (−)−epigallocatechin-3-gallate; GTP, green tea polyphenolic fraction; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; MMP, matrix metalloproteinase; ODC, ornithine decarboxylase; PCa, prostate cancer; PKC-α, protein kinase C–α; TRAMP, transgenic adenocarcinoma of the mouse prostate; Trk E, tyrosine receptor kinase type E; u-PA, urokinase-like plasminogen activator; VEGF, vascular endothelial growth factor.

are many formulations of green tea that are specially marketed for PCa patients.

Green tea, derived from the plant *Camellia sinensis*, is a popular beverage in some parts of the world. Studies conducted on cell-culture systems and animal models as well as human epidemiological studies show that the polyphenols that are present in green tea could afford protection against a variety of cancer types (14–26). Some studies also suggest that these polyphenols could be developed as therapeutic agents for some cancer types (27–28). An important fact in favor of the use of green tea against PCa is that the incidence of this disease is very low in the Asian population, which in addition to consuming low-fat and high-fiber diets regularly consumes green tea (29). Although these studies are correlative and like most nutritional epidemiological observations are inconclusive, laboratory data suggest that the polyphenols present in green tea may possess a chemopreventive effect against PCa in humans. At least two epidemiological studies (30,31) show that people who regularly consume tea have a lower incidence of PCa. Because of these encouraging facts, we initiated a program on chemoprevention of PCa by green tea and its major polyphenolic constituent (−)−epigallocatechin-3-gallate (EGCG). We provide evidence for the PCa chemopreventive effects of green tea that is based on a variety of studies on cell-culture systems and animal models. This article highlights our in vitro and in vivo findings. Based on the available evidence, it appears that there are multiple targets by which green tea could afford PCa chemopreventive effects.

Cell-culture studies

A variety of cell-culture studies show multiple targets as a mechanism of action of green tea polyphenols (GTP) or EGCG. The major studies that show the targets of PCa chemoprevention by EGCG are discussed below and summarized in **Table 1**.

Green tea, cell-cycle regulation and apoptosis. Androgen action is intimately associated with differentiation and proliferation of PCa (32). Therefore, PCa cells respond to androgen ablation and undergo rapid apoptosis (33–34). However, PCa is known to undergo a transition from an androgen-sensitive to a late (metastatic) androgen-insensitive form of cancer. At the time of clinical diagnosis, PCa represents a mixture of androgen-sensitive and -insensitive cells. Therefore, the key to effective control of PCa lies in the elimination of both androgen-sensitive and -insensitive cells. In a study from this laboratory, we demonstrate that EGCG treatment of prostate carcinoma DU145 cells results in the induction of apoptosis (35). Chung et al. (36) and Paschka et al. (37) confirm these observations using LNCaP, DU145 and PC-3 cells. In further studies, EGCG treatment of both androgen-sensitive LNCaP cells and androgen-insensitive DU145 cells is found to result in apoptosis (38). In this study, apoptosis is assessed by DNA fragmentation, flow cytometry and confocal microscopy. Also, EGCG treatment of LNCaP and DU145 cells is found to arrest cells in the G0/G1 phase of the cell cycle, which suggests that EGCG imposes an artificial cell-cycle checkpoint (38). An important observation of this study is that the G0/G1–phase arrest is independent of p53 status, because LNCaP cells carry a wild-type p53, and DU145 cells harbor mutant p53. It was also observed that EGCG treatment of both cell types induces the cyclin kinase inhibitor WAF1/p21, which suggests that the capability of EGCG to induce cell-cycle deregulation is independent of the p53 status in the cells.

TABLE 1

Summary of targets affected by (−)−epigallocatechin-3-gallate in human prostate cancer cells[1]

Cell culture system	Target/outcome	Reference citation
DU145	Induction of apoptosis	(18)
LNCaP and DU145	Induction of apoptosis	(23)
LNCaP, DU145, PC-3	Induction of apoptosis	(23)
LNCaP and DU145	Induction of G0/G1 cell-cycle arrest	(23)
LNCaP and DU145	Induction of cyclin kinase inhibitor WAF1/p21	(23)
LNCaP	Induction of p53	(23)
LNCaP	Induction of protein kinase C-α and suppression of TrkE	(52)
LNCaP and PC-3	Inhibition of proteasome activity	(51)

[1] For more details, see the references cited.

Green tea and ornithine decarboxylase. Studies demonstrate that prostate contains some of the highest concentrations of polyamines and polyamine-metabolizing enzymes (39). We reasoned that ornithine decarboxylase (ODC), which is a rate-limiting enzyme of the polyamine pathway, could serve as the target for prevention and therapy of human PCa. In the prostate, ODC activity is regulated by androgens (40). When LNCaP cells are treated with testosterone, a significant increase in the level of ODC enzyme activity is observed (23). Pretreatment of LNCaP cells with GTP inhibits the testosterone-mediated increase in ODC activity and ODC mRNA, which suggests that ODC could be a target of green tea–mediated cell-growth inhibition. In the same study, the effects of testosterone and/or GTP on anchorage-independent growth of LNCaP cells by soft-agar colony-formation assay was ascertained. Researchers identified that testosterone induces a significant increase in the ability of the cells to form colonies, and this increase is inhibited by GTP in a dose-dependent fashion.

Green tea and proteasome activity. The 20S proteasome, which is a multicatalytic complex (700 kDa), constitutes the catalytic key component of the ubiquitous proteolytic machinery 26S proteasome (41–46). There are three major proteasomal activities: chymotrypsin-like, trypsin-like and peptidyl-glutamyl peptide hydrolyzing activities (41,46). The ubiquitin-proteasome system plays a critical role in the specific degradation of cellular proteins (47), and two of the proteasome functions are to allow tumor cell-cycle progression and to protect tumor cells against apoptosis (48). The chymotrypsin-like but not the trypsin-like activity of the proteasome is associated with tumor-cell survival (49,50). Many cell-cycle and cell-death regulators are identified as targets of the ubiquitin-proteasome–mediated degradation pathway. Proteasome inhibitors are able to induce tumor-growth arrest, and tea consumption is correlated with cancer prevention. Nam et al. (51) shows that an ester bond that contains tea polyphenols such as EGCG potently and specifically inhibits the chymotrypsin-like activity of the proteasome in vitro at concentrations that are found in the serum of green-tea drinkers. Atomic orbital energy analyses and high-performance liquid chromatography suggest that the carbon of the polyphenol ester bond is essential for targeting and thereby inhibiting the proteasome in cancer cells. This inhibition of the proteasome by EGCG in several tumor and transformed cell lines results in the accumulation of two proteasome substrates, p27/Kip1 and IκB-α, which is an inhibitor of transcription factor nuclear factor-κB (NF-κB), followed by growth arrest in the G1 phase of the cell cycle. Furthermore, compared with their simian virus–transformed counterparts,

the parental normal human fibroblasts are much more resistant to EGCG-induced p27/Kip protein accumulation and G1 arrest (51). This study suggests that proteasome is a cancer-related molecular target of tea polyphenols, and that inhibition of proteasome activity by ester bond–containing polyphenols may contribute to the cancer-preventive effects of tea.

Green tea and cDNA-array studies. To further identify the molecular targets of PCa chemoprevention by EGCG, we employed a cDNA microarray technique (52). We concentrated on a total of 250 genes associated with kinases and phosphatases that have biological functions associated with a variety of known signal transduction pathways such as cell cycle, apoptosis and metabolic biosynthesis. LNCaP cells were treated with or without 12 μmol EGCG/L for 12 h. EGCG-treated cDNA labeled with cyanine-3 and the control cDNA labeled with cyanine-5 were mixed and overlaid on the microarray, and the competitive binding for each gene was carried out. Analysis of the image from the cDNA microarray reveals a total of 25 genes (**Table 2**) that show a significant response to EGCG. Of these, the expression of 16 genes is found to be significantly increased as a result of EGCG treatment, and 9 genes are found to be significantly repressed (52) by EGCG. Intriguingly, all of these genes belong to different regulatory pathways, which suggests that EGCG affects multiple cellular events. The fact that 90% of the genes remain unaffected by EGCG indicates that these nonresponsive genes are not the transcriptional regulatory targets in the early stage of the EGCG-mediated effect. Also, EGCG was not found to affect genes such as cell-division cycle (cdc2) and cyclin-dependent kinase (Cdk) that regulate cell cycle, although EGCG is known to cause cell-cycle dysregulation, which suggests that posttranslational control of these proteins is more important than transcriptional regulation. Of the nine genes repressed by EGCG, six belong to the G-protein signaling network. Among these genes, the repression of protein kinase C (PKC)-α is most prominent. The expression of the PKC-α form is further validated by RNA slot blot hybridized with a PKC-α–specific probe. EGCG decreases PKC-α gene expression by approximately threefold. The repression of the PKC-α gene is interesting, because PKC are involved in diverse cellular functions such as differentiation, growth control, tumor promotion and cell death (53). PKC also are involved in the regulation of the cell cycle during G1 progression and G2/M transition (54). Recent studies suggest that inhibition of PKC-α gene expression could inhibit cell proliferation in the animal tumor model and in some human cancer-cell lines (53).

TABLE 2

Genes associated with kinases and phosphatases that are responsive to (−)−epigallocatechin-3-gallate in human prostate cancer LNCaP cells[1,2]

Gene induced	Gene repressed
Trk E mRNA	Protein kinase C-α
Phosphoglycerate kinase	41-kDa protein kinase related to rat ERK 2
Cdk8 protein kinase	Type 1b cGMP-dependent protein kinase
Ribosomal protein kinase B	Adenosine kinase short form
Prostatic acid phosphatase	Phosphatidylinositol 3-kinase homolog
Protein tyrosine phosphatase IC	Protein tyrosine phosphatase PIR1
STE-20–related kinase SPAK	Protein tyrosine phosphatase-ζ
IAR/receptor-like protein tyrosine phosphatase	*KIAA0369*
Glomerular epithelial protein-1	Leukocyte common antigen T200
Platelet-derived growth factor A-type receptor	—
Adenylate kinase 2A	—
Putative serine/threonine protein kinase	—
Mevalonate kinase	—
Receptor-type protein tyrosine phosphatase-γ	—
Protein tyrosine phosphatase	—
Pyrroline 5-carboxylate synthase	—

[1] Data are based on two independent cDNA-microarray hybridization experiments where similar results were observed. Total RNA was purified and converted to cDNA from LNCaP cells treated with or without 12 μmol (−)−epigallocatechin-3-gallate/L (EGCG) for 12 h. EGCG-treated cDNA was labeled with cyanine 3, to which a false red color overlay was assigned, whereas control cDNA was labeled cyanine 5, to which a false green color overlay was assigned. Image was analyzed using software from Perkin Elmer Life Sciences.

[2] Cdk, cyclin-dependent kinase; cGMP, cyclin gluanosine monophosphate; Erk 2, extracellular regulated kinase; IAR, islet-cell antigen-related; PIR1, phosphate that interacts with RNA/RNP complex 1; SPAK, Ste20-related proline-alanine–rich kinase; STE-20, sterile-20; Trk E, tyrosine receptor kinase type E.

The cDNA microarray also identified 16 genes with expression that is induced by EGCG (see Table 2). The induction of receptor-type protein tyrosine phosphatase-γ gene expression, which is a tumor-suppressor gene candidate that is frequently deleted in some human cancers (55,56), may have a role in PCa prevention mediated by EGCG. Prostatic acid phosphatase might help inhibit the growth rate by deactivation of erbB-2 and p38 mitogen-activated protein kinase (57). Pyrroline-5-carboxylate, an endogenous proline-derived metabolite, is shown (58) to inhibit cell proliferation and survival in some cancer cells, and an increase in the gene expression of pyrroline 5-carboxylate synthase by EGCG might display similar inhibitory effects in PCa cells.

Five genes whose regulatory relationship with cell-growth control is not well understood to date are found to be induced by EGCG (see Table 2); these include tyrosine receptor kinase type E (Trk E), adenylate kinase 2A, protein tyrosine phosphatase, JAR/receptor-like protein tyrosine phosphatase and glomerular epithelial protein-l. The prototype of TrkE, Trk type A, is a protooncogene and functions as a transmembrane receptor to which endogenous nerve-growth factor binds and signals the onset of cell proliferation (59). TrkE gene expression was increased by approximately twofold in our slot blotting analysis. Because neurotrophin receptors can functionally inhibit one another's actions to mediate the effects of neurotrophins (59), we speculated that EGCG-induced TrkE gene expression may negatively modulate the proliferation signal in prostate cells. The functional diversity of these responsive gene candidates illustrates the complex nature of molecular interaction during the early stage of PCa prevention by EGCG. These genes may prove useful as therapeutic targets for PCa.

Animal studies

Studies on animal models show the usefulness of green tea polyphenols on PCa chemoprevention. These studies identify the targets for PCa chemoprevention by green tea under in vivo situations; the important work in this direction is discussed below and summarized in **Table 3**.

Green tea, ODC activity and cellular proliferation. To validate in vitro findings and further understand the molecular targets of green tea, we undertook in vivo experiments on rats and mice. In our first approach, we used castrated and sham-

TABLE 3

Summary of effects of GTP/EGCG in animal models of prostate cancer[1,2]

Animal study	Chemopreventive agent	Target/outcome	Reference citation
Athymic nude mice	EGCG, (−)−epicatechin gallate	Inhibition of tumor growth	(61)
Cpb:WU rats	0.2% GTP	Inhibition of ODC enzyme activity	(60)
C57BL/6 mice	0.2% GTP	Inhibition of ODC enzyme activity	(60)
TRAMP mice	0.1% GTP	Increased tumor-free and overall survival rates	(26)
TRAMP mice	0.1% GTP	Induction of apoptosis	(26)
TRAMP mice	0.1% GTP	Increase in serum IGF-I	(26)
TRAMP mice	0.1% GTP	Increase in serum IGF binding protein-3	(26)
TRAMP mice	0.1% GTP	Decrease in proliferating cell nuclear antigen	(26)
TRAMP mice	0.1% GTP	Inhibition of MMP-2 and MMP-9	Unpublished data
TRAMP mice	0.1% GTP	Inhibition of VEGF	Unpublished data
TRAMP mice	0.1% GTP	Inhibition of u-PA	Unpublished data

[1] For more details, see the references cited.
[2] IGF-I, insulin-like growth factor-I; MMP, matrix metalloproteinase; ODC, ornithine decarboxylase; TRAMP, transgenic adenocarcinoma of mouse prostate; u-PA, urokinase-like plasminogen activator; VEGF, vascular endothelial growth factor.

operated Cpb:WU rats and C57BL/6 mice. This choice was based on the fact that carcinogenesis in the accessory sex glands of these animals is dependent on exposure to the chemical carcinogen and on chronic hormonal stimulation by testosterone. Our data demonstrate that androgen (testosterone) plays an important role in regulation of ODC in the prostate (60). Castration of Cpb:WU rats results in negligible enzyme activity as compared with sham-operated animals, and testosterone-mediated induction of ODC activity is much more pronounced in sham-operated Cpb:WU rat prostate than in castrated rats. Testosterone-mediated induction of ODC activity is found to be significantly inhibited by oral feeding of 0.2% GTP to these animals. These observations strengthen our hypothesis that androgen-mediated upregulation of ODC is an important contributor toward the development of PCa and demonstrate that GTP inhibits the androgen-mediated cell growth and ODC activity in prostate. Similar studies were conducted on CBL57/6 mice, because this model is generally considered tumor resistant (60). The data obtained with this model are similar to those from Cpb:WU rats. Liao et al. (61) subcutaneously inoculated the human PCa cell lines PC-3 (androgen insensitive) and LNCaP 104-R (androgen repressed) into athymic nude mice to produce prostate tumors. Intraperitoneal injection of EGCG but not structurally related catechins such as (−)epicatechin-3-gallate inhibits the growth and rapidly reduces the size of human prostate tumors in nude mice (61).

Green tea and 5α-reductase. The enzyme steroid 5α-reductase catalyzes the conversion of testosterone to 5α-dihydrotestosterone. In humans, 5α-reductase activity is critical for certain aspects of male sexual differentiation and may be involved in the development of benign prostatic hyperplasia, alopecia, hirsutism and PCa. Liao et al. (62) showed that EGCG is a potent inhibitor of 5α-reductase in cell-free assays but not in whole-cell assays, which suggests that it can regulate androgen action in target organs. A subsequent study by Hiipakka et al. (63) showed that replacement of the gallate ester in EGCG with long-chain fatty acids produces potent 5α-reductase inhibitors that are active in both cell-free and whole-cell assay systems.

Green tea and insulin-like growth factor and insulin-like growth factor binding protein-3. To have relevance to humans, we reasoned that PCa chemoprevention studies should be conducted on animal models that closely emulate human disease and possess surrogate endpoint biomarkers for rapid evaluation of chemopreventive and/or therapeutic agents. The transgenic adenocarcinoma of the mouse prostate (TRAMP) is one such model that mimics progressive forms of human disease and locations of cancer in natural tissue microenvironments without the need for any chemical or hormonal treatment (64). We conducted experiments in which TRAMP mice at 8 wk of age were given a 0.1% solution of GTP in tap water as the sole source of drinking fluid for 24 wk. An important observation of this work is that oral infusion of a human achievable dose of green tea (equivalent to 6 cups of green tea/d) results in significant inhibition in development and progression of PCa (26). In GTP-fed animals, no metastasis was detected, and most notably in these animals, increased tumor-free and overall survival rates were observed. High levels of circulating serum insulin-like growth factor (IGF-I) levels are associated with increased risk of several common cancers including PCa, and serum IGF-binding protein (IGFBP)-3 suppresses the mitogenic action of IGF-I (65). Because epidemiological studies implicate deregulation of the IGF axis in PCa progression (65), we measured the effect of GTP consumption by TRAMP mice on serum IGF-I and IGFBP-3 levels. GTP infusion to TRAMP mice causes significant inhibition of IGF-I and restoration of IGFBP-3 levels (26). These data suggest that the IGF-I and IGFBP-3 autocrine/paracrine loop is a target for PCa chemoprevention by green tea. Additionally, our studies demonstrate that feeding GTP to TRAMP mice results in increased apoptosis in the prostate (26). Based on these data, we suggest that GTP consumption by TRAMP mice causes significant apoptosis of PCa cells, which possibly results in reduced dissemination of cancer cells and thereby causes inhibition of PCa development. This observation validates our in vitro studies, which indicate that apoptosis is an endpoint target for chemoprevention of PCa by GTP.

Green tea and cDNA-array studies. Recently we investigated the expression of various genes with biological function that are associated with metastasis and angiogenesis in TRAMP. Using a gene-array technique (SuperArray, Bethesda, MD), a total of 96 genes were examined, each of which belongs to a metastasis- and/or angiogenesis-related pathway. Analysis of the metastasis-related gene array reveals a total of seven genes with expression that is more than fivefold higher and a total of five genes with expression that is more than fivefold lower (**Table 4**) in the dorsolateral prostate of TRAMP mice as compared with nontransgenic littermates. When the angiogenesis-related genes are examined, fivefold higher expression is observed in 10 genes, but four genes are found to be repressed (**Table 5**).

TABLE 4

Genes related to metastasis with expression that is at least fivefold higher or lower in the dorsolateral prostate of TRAMP mice compared with littermate nontransgenic mice[1,2,3]

Gene expression of TRAMP versus littermate nontransgenic mice	
Increase, more than fivefold	Decrease, more than fivefold
MMP-2	E-cadherin
MMP-9	Integrin-α3
TIMP-1	Integrin-α6
TIMP-2	Laminin-β2
VEGF	H-Ras
u-PA	—
u-PA receptor	—

[1] Total RNA was isolated from the dorsolateral prostate of 24-wk-old TRAMP mice bearing prostate cancer and littermate nontransgenic mice. RNA was reverse transcribed and hybridized onto a 3.8 × 4.8-cm nylon membrane that contained 96 cDNA fragments associated with metastasis-related pathways. After chemiluminescence detection, the raw image was extracted onto a computer and analyzed using GEArray analyzer (SuperArray, Bethesda, MD).
[2] Based on two independent experiments with similar results.
[3] TIMP-1, tissue inhibitor of MMP-1.

TABLE 5

Genes related to angiogenesis with expression that is at least fivefold higher or lower in the dorsolateral prostate of TRAMP mice compared with littermate nontransgenic mice[1,2]

Gene expression of TRAMP versus littermate nontransgenic mice	
Increase, more than fivefold	Decrease, more than fivefold
MMP-2	E-cadherin
MMP-9	Tenascin C
TIMP-1	Integrin-αV
VEGF	Laminin-β3
VEGF receptor	—
A disintegrin-like and metalloproteinase with thrombospondin motifs-1 and -8	—
u-PA	—
u-PA receptor	—
Epidermal growth factor	—
Angiopoietin-1	—

[1] Total RNA was isolated from the dorsolateral prostate of 24-wk-old TRAMP mice bearing prostate cancer and littermate nontransgenic mice. RNA was reverse transcribed and hybridized onto a 3.8 × 4.8-cm nylon membrane that contained 96 cDNA fragments associated with angiogenesis-related pathways. After chemiluminescence detection, the raw image was extracted onto a computer and analyzed using GEArray analyzer (SuperArray, Bethesda, MD).
[2] Based on two independent experiments with similar results.

The matrix metalloproteinases (MMP; with expression that is induced in both arrays) are zinc-dependent metalloendopeptodases that belong to the collagenase supergene family and are involved in extracellular matrix regulation. MMP are frequently overexpressed in cancers (66). Studies indicate that synthetic inhibitors of MMP reduce tumor invasion and angiogenesis (67). Some synthetic MMP inhibitors are currently in clinical trials for cancer treatment but carry undesirable side effects (68). Using archival samples from our previous study (26), we demonstrate by immunoblot

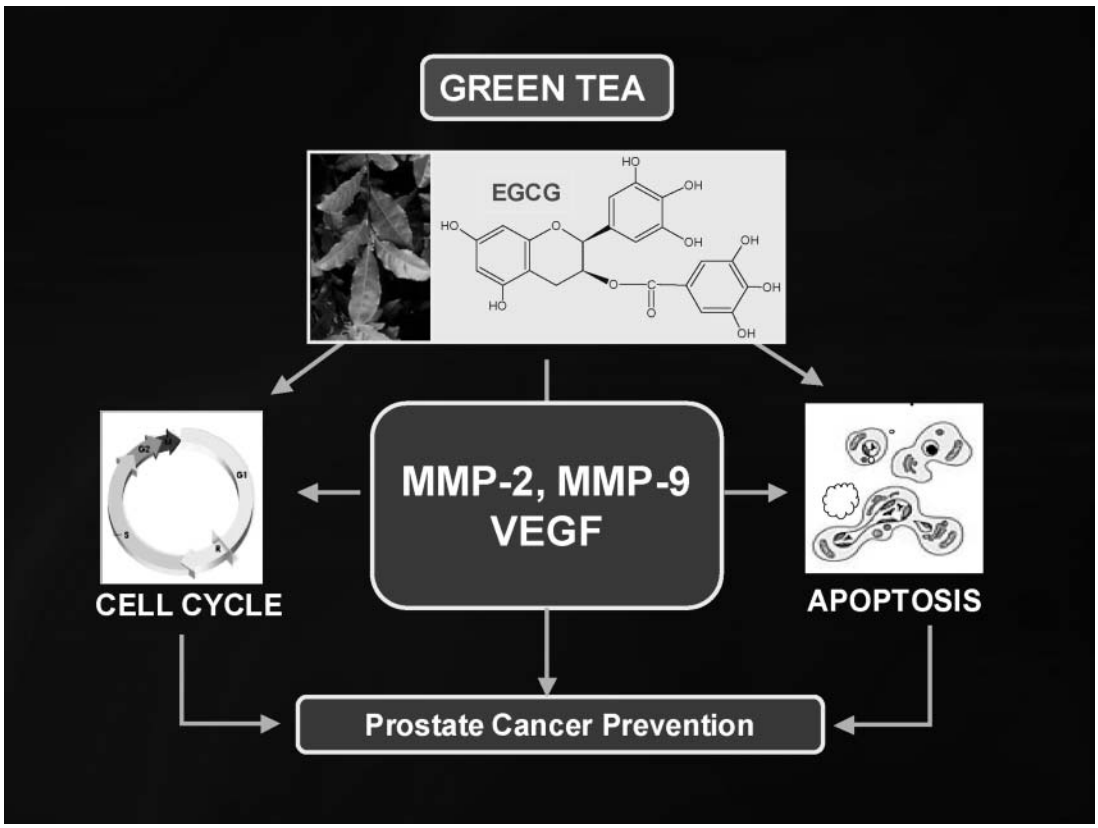

FIGURE 1 The targets by which green tea could afford prostate cancer chemopreventive effects are shown. EGCG, (−)−epigallocatechin-3-gallate; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor.

analysis that oral feeding of GTP significantly inhibits the expression of MMP-2, MMP-9, vascular endothelial growth factor (VEGF) and urokinase-like plasminogen activator (u-PA; see Table 3). Green tea and its constituents are found to inhibit tumor gelatinases and thereby prevent the invasion and angiogenesis that are associated with metastatic spread of cancer (69–71). EGCG, the most prevalent constituent of green tea, is identified as a direct inhibitor of MMP-2 and MMP-9 gelatinases (72).

EGCG is shown to inhibit tumor growth by inhibiting VEGF (73,74). One of the hydrolases that is implicated in the degradation of extracellular matrix and tumor invasion, u-PA is shown to be inhibited by EGCG (75,76). It is speculated that EGCG inhibits u-PA, which is upstream in the enzymatic cascade that ends with MMP-2 and MMP-9. However, because EGCG concentration is much lower in the plasma than that actually required to inhibit u-PA, it is likely that EGCG acts as a direct inhibitor of MMP-2 and MMP-9. It is known from studies with tumor cells that synthetic MMP inhibitors may induce cell-cycle arrest and even promote apoptosis. The MMP inhibitor batimastat (BB-94) is shown to enhance apoptosis and block ovarian cancer cells in the G0/G1 phase of the cell cycle. Another MMP inhibitor, AG3340, promotes apoptosis in human PCa and colon carcinoma models (77). The role of MMP and their inhibitors in the regulation of the cell cycle and apoptosis is still emerging.

Epidemiological studies

As yet, no detailed case-control study has been conducted to assess the effects of consumption of green tea for human PCa. All published data that seek an association between tea consumption and PCa risk have considered undefined tea preparations that are mostly black tea. At least two epidemiological studies show that people who regularly consume tea have a lower incidence of PCa (30,31). In a prospective cohort study that employed 7,833 men of Japanese ancestry living in Hawaii, Heilbrun et al. (30) observe a weak but significant negative association between black-tea intake (>1 cup/d) and PCa incidence ($P = 0.02$). In a case-control study conducted in three geographical areas of Canada, Jain et al. (31) observe a decrease in PCa risk with tea intake of >2 cup/d. Other epidemiological studies conducted in Italy (78), Utah (79) and Canada (80) do not find any difference of risk for PCa between tea drinkers and nondrinkers. However, most of these studies include populations that are predominantly black-tea drinkers. It should be noted that most of these studies lack parallels for comparisons in the categorization of tea consumption, type of tea consumed and ethnicity of the subjects, which weakens the overall impact of the studies. Epidemiological investigations that seek an association between green tea intake and presence of PCa should be undertaken to establish the validity of cell culture and animal data to human PCa patients.

Clinical trials with green tea

One recent phase II clinical trial explored the antineoplastic effects of green tea in patients with metastatic androgen-independent PCa (81). In this study, 48 patients were instructed to take 6 g of green tea/d orally in 6 divided doses. Patients were monitored monthly for response and toxicity. The study concludes that green tea carries limited antineoplastic activity among patients with androgen-independent PCa. It should be noted, however, that this study was conducted on patients with metastatic androgen-independent PCa and therefore, in principle, does not assess the chemopreventive effects of green tea. For an ideal study, a population with high risk for PCa development should be considered.

Taken together, our studies and the data from other laboratories suggest that green tea and its constituents induce apoptosis, inhibit cell growth, arrest the progression of the cell cycle, inhibit angiogenesis and metastasis and importantly, inhibit prostate tumor growth in an animal model in which PCa progresses as in humans. Based on studies in cell-culture systems and cDNA-microarray analysis, it is apparent that there are multiple targets by which green tea and its constituent EGCG can afford PCa chemopreventive effects. We propose that there are at least three major molecular targets by which green tea could afford PCa chemopreventive effects. As depicted in **Figure 1**, the targets by which green tea affords PCa chemopreventive effects could work either independently or in concert with one another. It is noteworthy that green tea is one of the few agents known to inhibit tumorigenesis that is generally devoid of toxicity. PCa represents an excellent candidate disease for studying chemoprevention by dietary agents such as green tea, because it is typically diagnosed in elderly men; even a modest delay in the neoplastic development achieved through pharmacological or therapeutic intervention could result in a substantial reduction in the incidence of the clinically detectable disease. If our animal data could be validated in additional PCa models, then consideration could be given for developing green tea as a chemopreventive agent against PCa. However, because of very large gaps in our knowledge of the mechanisms of PCa prevention by green tea and discrepancies between epidemiological, laboratory and clinical studies, more extensive studies are needed to obtain conclusive evidence.

LITERATURE CITED

1. Greenlee, R. T., Hill-Harmon, M. B., Murray, T. & Thun, M. (2001) Cancer statistics. CA Cancer J. Clin. 51: 15–36.

2. American Cancer Society (2002) Cancer Facts and Figures. Available on the Internet at http://www.cancer.org/downloads/STT/CancerFacts&Figures2002TM.pdf.

3. Mukhtar, H. & Ahmad, N. (1999) Cancer chemoprevention: future holds in multiple agents: contemporary issues in toxicology. Toxicol. Appl. Pharmacol. 158: 207–210.

4. Katiyar, S. K. & Mukhtar, H. (1996) Tea in chemoprevention of cancer: epidemiological and experimental studies. Int. J. Oncol. 8: 221–238.

5. Kelloff, G. J., Lieberman, R., Steele, V. E., Boone, C. W., Lubet, R. A., Kopelovitch, L., Malone, W. A., Crowell, J. A. & Sigman, C. C. (1999) Chemoprevention of prostate cancer: concepts and strategies. Eur. Urol. 35: 342–350.

6. Mukhtar, H. & Ahmad, N. (1999) Green tea in chemoprevention of cancer. Toxicol. Sci. 52(suppl. 2): 111–117.

7. Ahmad, N. & Mukhtar, H. (1999) Green tea polyphenols and cancer: biologic mechanisms and practical implications. Nutr. Rev. 57: 78–83.

8. Kelloff, G. J., Crowell, J. A., Steele, V. E., Lubet, R. A., Boone, C. W., Malone, W. A., Hawk, E. T., Lieberman, R., Lawrence, J. A., Kopelovich, L., Ali, I., Viner, J. L. & Sigman, C. C. (1999) Progress in cancer chemoprevention. Ann. N. Y. Acad. Sci. 889: 1–13.

9. Weisburger, J. H. (1999) Tea and health: the underlying mechanisms. Proc. Soc. Exp. Biol. Med. 220: 271–275.

10. Yang, C. S. & Wang, Z. Y. (1993) Tea and cancer. J. Natl. Cancer Inst. 85: 1038–1049.

11. Mukhtar, H., Katiyar, S. K. & Agarwal, R. (1992) Green tea and skin anticarcinogenic effects. J. Invest. Dermatol. 18: 3–7.

12. Katiyar, S. K., Agarwal, R. & Mukhtar, H. (1993) Protection against malignant conversion of chemically induced benign skin papillomas to squamous cell carcinomas in SENCAR mice by a polyphenolic fraction isolated from green tea. Cancer Res. 53: 5409–5412.

13. Katiyar, S. K., Agarwal, R. & Mukhtar, H. (1993) Inhibition of both stage I and stage II skin tumor promotion in SENCAR mice by a polyphenolic fraction isolated from green tea: inhibition depends on the duration of polyphenol treatment. Carcinogenesis 14: 2641–2643.

14. Khan, S. G., Katiyar, S. K., Agarwal, R. & Mukhtar, H. (1992) Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention. Cancer Res. 52: 4050–4052.

15. Gensler, H. L., Timmermann, B. N., Valcic, S., Wachter, G. A., Dorr, R., Dvorakova, K. & Alberts, D. S. (1996) Prevention of photocarcinogenesis by

topical administration of pure epigallocatechin gallate isolated from green tea. Nutr. Cancer 26: 325–335.

16. Record, I. R. & Dreosti, I. E. (1998) Protection by tea against UV-A + B-induced skin cancers in hairless mice. Nutr. Cancer 32: 71–75.

17. Wang, Z. Y., Huang, M. T., Ho, C. T., Chang, R., Ma, W., Ferraro, T., Reuhl, K. R., Yang, C. S. & Conney, A. H. (1992) Inhibitory effect of green tea on the growth of established skin papillomas in mice. Cancer Res. 52: 6657–6665.

18. Ahmad, N., Feyes, D. K., Nieminen, A. L., Agarwal, R. & Mukhtar, H. (1997) Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. J. Natl. Cancer Inst. 89: 1881–1886.

19. Ahmad, N., Gupta, S. & Mukhtar, H. (2000) Green tea polyphenol epigallocatechin-3-gallate (EGCG) differentially modulates nuclear factor kappa B (NF-κB) in cancer cells vs. normal cells. Arch. Biochem. Biophys. 376: 338–346.

20. Chen, Z. P., Schell, J. B., Ho, C. T. & Chen, K. Y. (1998) Green tea epigallocatechin gallate shows a pronounced growth inhibitory effect on cancerous cells but not on their normal counterparts. Cancer Lett. 129: 173–179.

21. Xu, Y., Ho, C. T., Amin, S. G., Han, C. & Chung, F. L. (1992) Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and major polyphenol as antioxidants. Cancer Res. 52: 3875–3879.

22. Ahmad, N., Feyes, D. K., Nieminen, A. L., Agarwal, R. & Mukhtar, H. (1997) Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. J. Natl. Cancer Inst. 89: 1881–1886.

23. Gupta, S., Ahmad, N., Nieminen, A. L. & Mukhtar, H. (2000) Growth inhibition, cell-cycle dysregulation, and induction of apoptosis by green tea constituent (−)−epigallocatechin-3-gallate in androgen-sensitive and androgen-insensitive human prostate carcinoma cells. Toxicol. Appl. Pharmacol. 164: 82–90.

24. Setiawan, V. W., Zhang, Z. F., Yu, G. P., Lu, Q. Y., Li, Y. L., Lu, M. L., Wang, M. R., Guo, C. H., Yu, S. Z., Kurtz, R. C. & Hsieh, C. C. (2001) Protective effect of green tea on the risks of chronic gastritis and stomach cancer. Int. J. Cancer 92: 600–604.

25. Inoue, M., Tajima, K., Mizutani, M., Iwata, H., Iwase, T., Miura, S., Hirose, K., Hamajima, N. & Tominaga, S. (2001) Regular consumption of green tea and the risk of breast cancer recurrence: follow-up study from the Hospital-Based Epidemiologic Research Program at Aichi Cancer Center (HERPACC), Japan. Cancer Lett. 167: 175–182.

26. Gupta, S., Hastak, K., Ahmad, N., Lewin, J. S. & Mukhtar, H. (2001) Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols. Proc. Natl. Acad. Sci. U S A 98: 10350–10355.

27. Gupta, S. & Mukhtar, H. (2002) Green tea and prostate cancer. Urol. Clin. North Am. 29: 49–57.

28. Gupta, S., Ahmad, N. & Mukhtar, H. (1999) Prostate cancer chemoprevention by green tea. Semin. Urol. Oncol. 17: 70–76.

29. Muir, C., Waterhouse, J., Mack, T., Powell, J. & Whelan, S. (1987) Cancer Incidence in Five Continents, vol. V, IARC Scientific Publications no. 88. International Agency for Research on Cancer, Lyon, France.

30. Heilbrun, L. K., Nomura, A. & Stemmermann, G. N. (1986) Black tea consumption and cancer risk: a prospective study. Br. J. Cancer 54: 677–683.

31. Jain, M. G., Hislop, G. T., Howe, G. R., Burch, J. D. & Ghadirian, P. (1998) Alcohol and other beverage use and prostate cancer risk among Canadian men. Int. J. Cancer 78: 707–711.

32. Wilding, G. (1995) Endocrine control of prostate cancer. Cancer Surv. 23: 43–62.

33. Huggins, C. & Hodges, C. V. (1972) Studies on prostatic cancer: I. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. CA Cancer J. Clin. 22: 232–240.

34. Lu, S., Tsai, S. Y. & Tsai, M. J. (1997) Regulation of androgen-dependent prostatic cancer cell growth: androgen regulation of CDK2, CDK4 and CKIp16 genes. Cancer Res. 57: 4511–4516.

35. Ahmad, N., Feyes, D. K., Nieminen, A. L., Agarwal, R. & Mukhtar, H. (1997) Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. J. Natl. Cancer Inst. 89: 1881–1886.

36. Chung, L. Y., Cheung, T. C., Kong, S. K., Fung, K. P., Choy, Y. M., Chan, Z. Y. & Kwok, T. T. (2001) Induction of apoptosis by green tea catechins in human prostate cancer DU145 cells. Life Sci. 68: 1207–1214.

37. Paschka, A. G., Butler, R. & Young, C. Y. (1998) Induction of apoptosis in prostate cancer cell lines by the green tea component, (−)−epigallocatechin-3-gallate. Cancer Lett. 130: 1–7.

38. Gupta, S., Ahmad, N., Nieminen, A. L. & Mukhtar, H. (2000) Growth inhibition, cell-cycle dysregulation, and induction of apoptosis by green tea constituent (−)−epigallocatechin-3-gallate in androgen-sensitive and androgen-insensitive human prostate carcinoma cells. Toxicol. Appl. Pharmacol. 164: 82–90.

39. Danzin, C., Jung, M. J., Grove, J. & Bey, P. (1979) Effect of α-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase, on polyamine levels in rat tissues. Life Sci. 24: 519–524.

40. Betts, A. M., Waite, I., Neal, D. E. & Robson, C. N. (1997) Androgen regulation of ornithine decarboxylase in human prostatic cells identified using differential display. FEBS Lett. 405: 328–332.

41. Groll, M., Ditzel, L., Lowe, J., Stock, D., Bochtler, M., Bartunik, H. D. & Huber, R. (1997) Structure of 20S proteasome from yeast at 2.4 A resolution. Nature 386: 463–471.

42. Maupin-Furlow, J. A. & Ferry, J. G. (1995) A proteasome from the methanogenic archaeon Methanosarcina thermophila. J. Biol. Chem. 270: 28617–28622.

43. Goldberg, A. L. (1995) Functions of the proteasome: the lysis at the end of the tunnel. Science 268: 522–523.

44. Baumeister, W., Walz, J., Zuhl, F. & Seemuller, E. (1998) The proteasome: paradigm of a self-compartmentalizing protease. Cell 92: 367–380.

45. Heinemeyer, W., Fischer, M., Krimmer, T., Stachon, U. & Wolf, D. H. (1997) J. Biol. Chem. 272: 25200–25209.

46. Loidl, G., Groll, M., Musiol, H. J., Huber, R. & Moroder, L. (1999) Bivalency as a principle for proteasome inhibition. Proc. Natl. Acad. Sci. U.S.A. 96: 5418–5422.

47. Hochstrasser, M. (1995) Ubiquitin, proteasomes, and the regulation of intracellular protein degradation. Curr. Opin. Cell Biol. 7: 215–223.

48. Dou, Q. P. & Li, B. (1999) Proteasome inhibitors as potential novel anticancer agents. Drug Resistance Updates 2: 215–223.

49. An, B., Goldfarb, R. H., Siman, R. & Dou, Q. P. (1998) Novel dipeptidyl proteasome inhibitors overcome Bcl-2 protective function and selectively accumulate the cyclin-dependent kinase inhibitor p27 and induce apoptosis in transformed, but not normal, human fibroblasts. Cell Death Differ. 5: 1062–1075.

50. Lopes, U. G., Erhardt, P., Yao, R. & Cooper, G. M. (1997) p53-Dependent induction of apoptosis by proteasome inhibitors. J. Biol. Chem. 272: 12893–12896.

51. Nam, S., Smith, D. M. & Dou, Q. P. (2001) Ester bond–containing tea polyphenols potently inhibit proteasome activity in vitro and in vivo. J. Biol. Chem. 276: 13322–13330.

52. Wang, S. I. & Mukhtar, H. (2002) Gene expression profile in human prostate LNCaP cancer cells by (−) epigallocatechin-3-gallate. Cancer Lett. 182: 43–51.

53. Livneh, E. & Fishman, D. D. (1997) Linking protein kinase C to cell-cycle control. Eur. J. Biochem. 248: 1–9.

54. Fishman, D. D., Segal, S. & Livneh, E. (1998) The role of protein kinase C in G1 and G2/M phases of the cell cycle. Int. J. Oncol. 12: 181–186.

55. Panagopoulos, I., Pandis, N., Thelin, S., Petersson, C., Mertens, F., Borg, A., Kristoffersson, U., Mitelman, F. & Aman, P. (1996) The FHIT and PTPRG genes are deleted in benign proliferative breast disease associated with familial breast cancer and cytogenetic rearrangements of chromosome band 3p14. Cancer Res. 56: 4871–4875.

56. Wary, K. K., Lou, Z., Buchberg, A. M., Siracusa, L. D., Druck, T., LaForgia, S. & Huebner, K. (1993) A homozygous deletion within the carbonic anhydrase-like domain of the Ptprg gene in murine L-cells. Cancer Res. 53: 1498–1502.

57. Zhang, X. Q., Lee, M. S., Zelivianski, S. & Lin, M. F. (2001) Characterization of a prostate-specific tyrosine phosphatase by mutagenesis and expression in human prostate cancer cells. J. Biol. Chem. 276: 2544–2550.

58. Maxwell, S. A. & Davis, G. E. (2000) Differential gene expression in p53-mediated apoptosis-resistant vs. apoptosis-sensitive tumor cell lines. Proc. Natl. Acad. Sci. U.S.A. 97: 13009–13014.

59. Kaplan, D. R. & Miller, F. D. (2000) Neurotrophin signal transduction in the nervous system. Curr. Opin. Neurobiol. 10: 381–391.

60. Gupta, S., Ahmad, N., Mohan, R. R., Husain, M. M. & Mukhtar, H. (1999) Prostate cancer chemoprevention by green tea: in vitro and in vivo inhibition of testosterone-mediated induction of ornithine decarboxylase. Cancer Res. 59: 2115–2120.

61. Liao, S., Umekita, Y., Guo, J., Kokontis, J. M. & Hiipakka, R. A. (1995) Growth inhibition and regression of human prostate and breast tumors in athymic mice by tea epigallocatechin gallate. Cancer Lett. 96: 239–243.

62. Liao, S. & Hiipakka, R. A. (1995) Selective inhibition of steroid 5 alpha-reductase isozymes by tea epicatechin-3-gallate and epigallocatechin-3-gallate. Biochem. Biophys. Res. Commun. 214: 833–838.

63. Hiipakka, R. A., Zhang, H. Z., Dai, W., Dai, Q. & Liao, S. (2002) Structure-activity relationships for inhibition of human 5alpha-reductases by polyphenols. Biochem. Pharmacol. 63: 1165–1176.

64. Greenberg, N. M., DeMayo, F., Finegold, M. J., Medina, D., Tilley, W. D., Aspinall, J. O., Cunha, G. R., Donjacour, A. A., Matusik, R. J. & Rosen, J. M. (1995) Prostate cancer in a transgenic mouse. Proc. Natl. Acad. Sci. U.S.A. 92: 3439–3443.

65. Yu, H. & Rohan, T. (2000) Role of the insulin-like growth factor family in cancer development and progression. J. Natl. Cancer Inst. 92: 1472–1489.

66. Egelbad, M. & Werb, Z. (2002) New functions for the matrix metalloproteinases in cancer progression. Nat. Rev. Cancer 2: 161–174.

67. O-charoenrat, P., Rhys-Evans, P. & Eccles, S. (2002) A synthetic matrix metalloproteinase inhibitor prevents squamous carcinoma cell proliferation by interfering with epidermal growth factor receptor autocrine loops. Int. J. Cancer 100: 527–533.

68. Coussens, L. M., Fingleton, B. & Matrisian, L. M. (2002) Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 295: 2387–2392.

69. Cao, Y., Cao, R. & Brakenhielm, E. (2002) Antiangiogenic mechanisms of diet-derived olyphenols. J. Nutr. Biochem. 13: 380–390.

70. Singh, A. K., Seth, P., Anthony, P., Husain, M. M., Madhavan, S., Mukhtar, H. & Maheshwari, R. K. (2002) Green tea constituent epigallocatechin-3-gallate inhibits angiogenic differentiation of human endothelial cells. Arch. Biochem. Biophys. 401: 29–37.

71. Jung, Y. D. & Ellis, L. M. (2001) Inhibition of tumour invasion and angiogenesis by epigallocatechin gallate (EGCG), a major component of green tea. Int. J. Exp. Pathol. 82: 309–316.

72. Garbisa, S., Sartor, L., Biggin, S., Salvato, B., Benelli, R. & Albini, A. (2001) Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. Cancer 91: 822–832.

73. Sartippour, M. R., Shao, Z. M., Heber, D., Beatty, P., Zhang, L., Liu, C., Ellis, L., Liu, W. & Go, V. L. (2002) Green tea inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells. J. Nutr. 132: 2307–2311.

74. Jung, Y. D., Kim, M. S., Shin, B. A., Chay, K. O., Ahn, B. W., Liu, W., Bucana, C. D., Gallick, G. E. & Ellis, L. M. (2001) EGCG, a major component of green tea, inhibits tumour growth by inhibiting VEGF induction in human colon carcinoma cells. Br. J. Cancer 84: 844–850.

75. Garbisa, S., Sartor, L., Biggin, S., Salvato, B., Benelli, R. & Albini, A. (2001) Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. Cancer 91: 822–832.

76. Jankun, J., Selman, S. H., Swiercz, R. & Skrzypczak-Jankun, E. (1997) Why drinking green tea could prevent cancer. Nature 387: 561.

77. Daniel, C., Duffield, J., Brunner, T., Steinmann-Niggli, K., Lods, N. & Marti, H. P. (2001) Matrix metalloproteinase inhibitors cause cell cycle arrest and apoptosis in glomerular mesangial cells. J. Pharmacol. Exp. Ther. 297: 57–68.

78. La Vecchia, C., Negri, E., Franceschi, S., D'Avanzo, B. & Boyle, P. (1992) Tea consumption and cancer risk. Nutr. Cancer 17: 27–31.

79. Slattery, M. L. & West, D. W. (1993) Smoking, alcohol, coffee, tea, caffeine, and theobromine: risk of prostate cancer in Utah (United States). Cancer Causes Control 4: 559–563.

80. Ellison, L. F. (2000) Tea and other beverage consumption and prostate cancer risk: a Canadian retrospective cohort study. Eur. J. Cancer Prev. 9: 125–130.

81. Jatoi, A., Dakhil, P. B., Mattar, B., Sloan, J., Fitch, T., Novotny, P., Camoriano, J., Young, C., Rowland, K., Quevedo, F. & Tan, W. (2002) A phase II trial of green tea for androgen-independent prostate cancer: a north central cancer treatment group (NCCTG) trial. Proc. of the 93rd Annual Meeting of the American Association for Cancer Research 43: 492 (Abs.).