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Review

Beneficial effects of tea and its polyphenols against prostate cancer

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Tea, next to water, is the most widely consumed beverage in the world. Depending upon the level of fermentation, tea can be categorized into three types: green (unfermented), oolong (partially fermented), and black (highly to fully fermented). In general, green tea has been found to be superior to black and oolong tea in terms of antioxidant and health promoting benefits owing to the higher content of (–)-epigallocatechin-3-gallate. Tea polyphenols comprise about one-third of the weight of the dried leaf, and they exhibit biochemical and pharmacological activities including antioxidant activities, inhibition of cell proliferation, induction of apoptosis, cell cycle arrest and modulation of carcinogen metabolism. Several studies demonstrate that most tea polyphenols exert their effects by scavenging reactive oxygen species (ROS) since excessive production of ROS has been implicated in the development of a variety of ailments including cancer of the prostate gland (CaP). Using cell culture and animal model systems, molecular targets for these remarkable beneficial effects of green tea drinking on CaP prevention and therapy have been defined. Geographical and case-control studies are showing that green tea drinking could afford CaP chemopreventive effects in human population. In this review we attempt to summarize the experimental as well as the epidemiological basis for the possible role of tea and its polyphenols for chemoprevention and chemotherapy of CaP.

Keywords: Chemoprevention / Chemotherapy / Epigallocatechin-3-gallate / Green tea polyphenols / Prostate cancer

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1 Introduction

The magnitude of the problem of cancer and unsatisfactory response of conventional strategies to affect a marked diminution in the total number of deaths from this disease demands that other preventive measures should be seriously considered. Many strategies are possible to reduce cancer-related deaths, four of which are noteworthy: (i) prevention, (ii) early diagnosis and intervention, (iii) successful treatment of localized tumor, and iv) improved management of nonlocalized cancer. Among these, preventing the occurrence of cancer(s) through chemoprevention has emerged as the most practical approach. Chemoprevention, by definition, is the means of cancer control in which the occurrence of the disease can be entirely prevented, slowed or reversed by the administration of one or more naturally occurring and/or synthetic compounds [1–5]. The concept of chemoprevention has matured to an extent that currently it is considered as a practical option to reduce the occurrence of the disease [3–5]. Thus, chemoprevention is often referred to as "prevention by delay". The ultimate aim of chemoprevention is to use the preventive substances in pills or in modified foods, as a prevention strategy for people at high risk for cancer. An ideal chemopreventive agent for human use should have (i) little or no toxicity, (ii) high efficacy in multiple sites, (iii) capability of oral consumption, (iv) a known mechanism of action, (v) low cost, and above all, (vi) human acceptance.

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Abbreviations: AR, androgen receptor; **BTE**, black tea extract; **CaP**, cancer of the prostate gland; **ECG**, (–)-epicatechin-3-gallate; **EGC**, (–)-epigallocatechin; **EGCG**, (–)-epigallocatechin-3-gallate; **Erk**, extracellular signal regulated kinase; **GTC, green tea catechins; GTP**, green tea polyphenols; **IGF**, insulin-like growth factors; **IGFBP-3**, insulin-like growth factors-binding protein 3; **MAPK**, mitogen activated protein kinase; **NF-κB**, nuclear transcription factor κB; **ODC**, ornithine decarboxylase; **PI3K**, phosphatidylinositol-3-kinase; **PKC-α**, protein kinase C-alpha; **PSA**, prostate specific antigen; **ROS**, reactive oxygen species; **TF**, theaflavins; **TRAMP**, transgenic adenocarcinoma of the mouse prostate; **uPA**, urokinase plasminogen activator; **VEGF**, vascular endothelial growth factor

Prostate cancer (CaP) is the most common nondermatological male malignancy and behind lung cancer is the second most common cause of cancer-related mortality among American men. According to a projection by the American Cancer Society, a total of 232 090 men are estimated to be diagnosed with CaP in the USA alone in the year 2005, and 30 350 CaP-related deaths are estimated [6, 7]. Despite the substantial morbidity and mortality associated with the disease, the advances in the techniques of radical prostactomy and external radiotherapy have not been fully effective in the treatment of the disease. Evidences from *in vitro* and *in vivo* experiments supported by geographic and epidemiological studies suggest that environmental carcinogenic factors and nutrition play important causative roles in the initiation, promotion, and progression stages of CaP [8 – 12]. Although clinical CaP incidence and mortality vary greatly among populations [8, 13] the frequency of latent CaP is evenly distributed. The rising incidence of CaP in several countries that previously were considered to have a low incidence rates appears to be coincidental with the adoption of western lifestyle in those populations, implicating factors such as low levels of physical activity, high relative body weight, and high dietary fat intake in patho-physiology of CaP [14]. An increase in the incidence of CaP has also been found in Asian populations migrating to the west possibly due to the same reasons.

One case-control study established a positive association of CaP risk with total energy intake as well as intake of total fat [15]. Also, there have been some studies suggesting the role of energy intake, body size, and physical activity in the progression and promotion of CaP [16] and the references therein. These facts and observations give numerous leads to explore testable CaP prevention strategies. Current treatment strategies such as hormone therapy, radiation, and surgery *etc.* are proving useful in reducing the mortality and morbidity associated with the disease, however, malignant and nonmalignant tumors continue to progress and become refractory. Besides, severe side effects associated with these treatments have also been reported [17]. Thus, novel approaches are urgently required to prevent and treat the mortality and morbidity associated with the disease. Because CaP is a complex disease involving different molecular events, blocking or inhibiting only one event will not be sufficient to prevent or delay the onset of the disease. Efforts are therefore undergoing for a better understanding of CaP, and for the development of novel approaches for its prevention and treatment.

Based on the *in vivo* and *in vitro* data indicating beneficial effects of tea against CaP and the epidemiological observation that the Japanese and Chinese populations which are habitual drinkers of several cups of tea have one of the lowest rate of CaP in the world [5, 18], it is important to establish if tea drinking could prevent CaP, at least in select groups of population. It will then be important to define such a population. CaP is an ideal disease for chemoprevention studies because of its high latency period and also it is typically diagnosed in men over the age of 50. Thus, even a slight delay in the progression of this disease by chemoprevention could result in a substantial reduction in the incidence of the disease and more importantly, improve the quality of life of the patients by simply delaying the onset of the disease [19, 20]. The identification of promising agents (and their molecular targets) for CaP chemoprevention is guided by data derived from a variety of sources *viz.* (i) epidemiological observations, (ii) CaP treatment trials, (iii) secondary analyses from large, randomized, controlled cancer prevention trials, (iv) an understanding of cancer biology and prostate carcinogenesis, and (v) experimental animal models.

Many types of natural agents have been reported to inhibit or delay various stage(s) of cancer including CaP [21, 22]. Epidemiological studies have observed a correlation between populations with higher consumption of selenium, vitamin E, fruits, and tomatoes, in lowering the risk of CaP [23, 24]. Consistent with this notion, currently several natural agents are under study for their assessment as preventive agents against CaP. The beverage tea derived from the plant *Camellia sinensis* has been studied extensively and it has emerged as an agent having antimutagenic and anticancer effects in animal tumor models [25, 26] and the references therein. In the year 1999, our laboratory initiated a program to assess whether tea consumption could afford chemopreventive effects against prostate carcinogenesis [27]. Our efforts have been subsequently followed up by the laboratories throughout the world. This review summarizes the laboratory, clinical trial and epidemiological observations on the use of the beverage tea or its constituent polyphenols for prevention and/or better management of CaP.

2 An overview of beverage tea

Tea, made from the leaves of *C. sinensis*, an evergreen shrub of the *Theaceae* family, is cultivated around the world in approximately 30 countries and is consumed at enormously varying levels. Although definite data is not available, it is generally accepted that tea is the most consumed beverage in the world next only to water; with a *per* capita worldwide consumption of approximately 120 mL *per* day [28] and the references therein.

Three main varieties of the commercial tea are available: green (unfermented), oolong (partially fermented), and black (fully fermented). Their composition varies according to the manufacturing process which differs in the degree of "enzymatic oxidation" or fermentation. Table 1 shows the principal polyphenolic components present in typical green

Table 1. Polyphenolic composition of green and black tea (%wt/wt)

Constituent	Green tea	Black tea
Catechins	30–42	3–10
Flavonols	5–10	6–8
Other flavanoids	2–4	–
Theogallin	2–3	–
Gallic acid	0.5	–
Quinic acid	2.0	–
Theanine	4–6	–
Methylxanthines	7–9	8–11
TF	–	3–6
Thearubigens	–	12–18

and black tea beverage but variations may be considerable. Oolong tea composition in general falls between that of green and black teas [29]. Black tea shares major part of world tea production accounting to 78% and is predominantly consumed in Western and some Asian countries. About 20% of the total tea manufactured is green tea and is produced in relatively few countries, and consumed in China, Japan, India, and a few countries in North Africa and Middle East. The rest 2% is oolong tea, mainly produced and consumed in southeastern China.

2.1 Tea and its polyphenolic constituents

Tea contains several polyphenolic components, which are antioxidant in nature, and in recent years, studies from ours and many laboratories around the world, conducted in various organ specific animal bioassay systems, have shown that tea and its polyphenolic constituents, are capable of affording protection against a variety of cancer types [22, 25, 26, 28, 30]. Green tea contains polyphenolic compounds, which include flavanols, flavandiols, flavonoids, and phenolic acids. These compounds account up to 30% of the dry weight of green tea leaves. Most of the polyphenols present in green tea are flavanols, commonly known as catechins like (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin-3-gallate (EGCG). The chemical structures of these compounds are shown in Fig. 1. In addition, caffeine, theobromine, theophylline, and phenolic acids such as gallic acids are also present in green tea (Table 1).

During the fermentation process involved in the manufacture of black and oolong teas, the monomeric flavan-3-ols undergo polyphenol oxidase-dependent oxidative polymerization leading to the formation of bisflavanols, theaflavins (TF), thearubigins, and some other oligomers. The thearubigen fraction is a mixture of substances, with a molecular weight distribution of 1000–40 000 and account for 15% of dry weight solids of black tea. TF (1–2%, on dry weight basis) contains benzotropolone rings with dihydroxy or trihydroxy substitution systems. About 10–20% of the dry weight of black tea is due to thearubigens, which are even more extensively oxidized and polymerized. The structures of TF and thearubigins are shown in Fig. 1. Oolong teas, on the other hand, are prepared by firing the leaves shortly after rolling to terminate the oxidation and drying the leaves. Oolong tea is considered to be about half fermented as compared to black tea. Oolong tea extracts contain catechins at a level of 8–20% of the total dry matter and contains monomeric catechins, TF, and thearubigins. In addition, epigallocatechin esters, theasinensins, dimeric catechins, and dimeric proanthocyanidins are also the characteristic components of oolong tea.

2.2 Tea polyphenols and their biochemical properties

Tea is consumed worldwide for a variety of reasons such as improving blood flow, eliminating toxins, improving resistance to various diseases and combating cancer and cardiovascular diseases. Studies have shown that oral consumption of green tea or EGCG to rats and human can lower serum cholesterol levels [31, 32], increase high-density lipoprotein cholesterol in rats [33] and decrease blood glucose in rats [34]. The health beneficial effects of tea and its polyphenols have been summarized in a review from our laboratory [28].

The most widely recognized biological properties of tea polyphenols are their antioxidant properties [35, 36]. Several studies have suggested that the polyphenols, present in tea possess high antioxidant activities, which protect cells against the adverse effects of damaging reactive oxygen species (ROS). Studies in cell culture systems have also shown that both green tea extract and EGCG are capable of inhibiting the growth of a variety of mouse and human cancer cell types without affecting normal cells. Ahmad *et al.* [37] initially reported these effects showing that green tea may protect against cancer by causing cell cycle arrest and inducing apoptosis in various human carcinoma cells.

Some of the effects of tea polyphenols may also be due to the chelation of metal ions as indicated by the fact that tea consumption lowers absorption of dietary iron in controlled feeding studies and decreases body iron balance [38]. This study also observed that EGCG may chelate the cations, which contribute to its ability to inhibit angiotensin-converting enzyme. Polyphenols of tea chelate copper ions and this mechanism has also been suggested to protect low-density lipoproteins from peroxidation [39]. Because of its chelating properties tea may additionally protect against toxicity due to heavy metals [38]. Catechins may also affect signal transduction pathways, modulate many endocrine systems, and alter hormones and other physiological processes

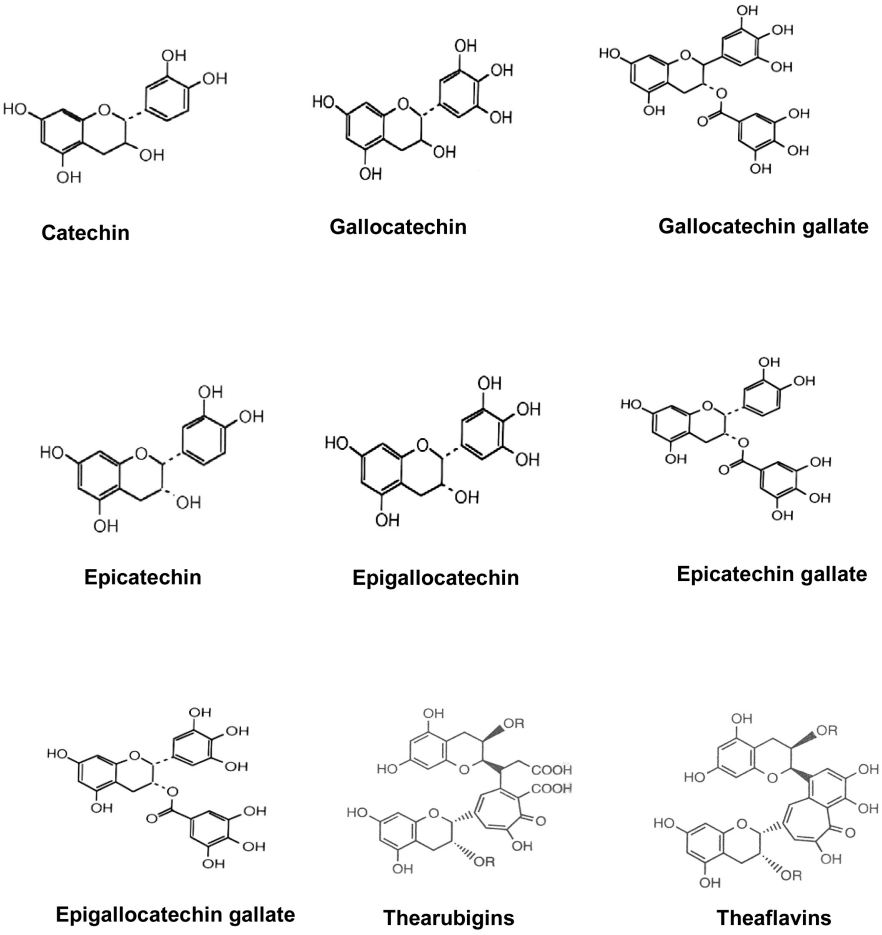

Figure 1. Chemical structures of major green and black tea polyphenols. R = galloyl group.

as a result of their binding to these metals/enzyme cofactors [40]. Tea polyphenols have been shown to inhibit the activities of transcription factors AP-1, nuclear transcription factor κB (NF-κB) and synthesis of nitric oxide [41 – 44]. Inhibition of cell transformation and cell growth by purified catechins and TF has been reported [45]. These activities have been attributed to the inhibition of AP-1 activity, possibly due to the inhibition of several steps of signal transduction pathways, *e.g.*, mitogen activated protein kinase (MAPK) activities by tea polyphenols [46]. It has been reported that tea and its polyphenolic constituents impart inhibitory effects on the activities of many enzymatic and metabolic pathways relevant to cancer development [47] and references therein.

2.3 CaP and the role of tea

The concept of reduction of CaP occurrence by means of dietary intervention is getting popularity and discussion in recent years as the use of botanical supplements is consistently increasing by CaP patients [48, 49]. This is based on the epidemiological studies that showed that Asian populations that consume tea on a regular basis have the lowest incidence of all types of cancer including CaP as compared to their western counterparts [8, 13, 18, 50]. It has been suggested that the low occurrence of CaP in Asian countries may be due, in part, to the consumption of green tea by these populations.

In the year 1999, we initiated a program to evaluate the relation between tea consumption and prevention of CaP. In our preliminary experiment we observed that ornithine decarboxylase (ODC) activity in CaP tissue was 2.7-fold higher as compared to the corresponding paired benign tissue of the same individual [51]. This exciting observation suggested that ODC could be studied as a target for prevention and therapy of CaP. Extending these studies we investigated the effect of the polyphenolic fraction isolated from green tea polyphenols (GTP) against testosterone-mediated induction of ODC in (i) LNCaP human CaP cells, (ii) Cpb : WU rats, and (iii) C57BL/6 mice and found that GTP resulted in a significant reduction in testosterone-mediated induction of ODC activity in the prostate [27]. We then reasoned that these preclinical studies should be conducted in transgenic adenocarcinoma of the mouse prostate (TRAMP), a model system that closely mimics progressive forms of human CaP [52]. In this model, we provided con-

Table 2. Summary of effect of EGCG in CaP cells

Cell culture system	Target/outcome	Reference
DU145	Induction of apoptosis	[37]
LNCaP and PC-3	Inhibition of proteosome activity	[89]
LNCaP	Modulation of kinases and phosphatases	[89]
LNCaP, DU145 and PC3	Induction of apoptosis	[93]
LNCaP and DU145	Induction of G_0/G_1 cell cycle arrest	[93]
LNCaP and DU145	Induction of cyclin kinase inhibitor WAF1/p21	[93]
LNCaP	Induction of p53	[93]
PNT1A and PC3	Inhibition of growth, caspase cascade activation and clusterin protein accumulation	[95]
LNCaP	Induction of PKC-α Supression of TrkE	[130]
LNCaP	Down regulate PSA and induce growth arrest	[133]
LNCaP	Accumulate p27 and IκBα proteins	[134]

Table 3. Summary of the effects of tea polyphenols in animal models of CaP

Animal studies	Chemopreventive agent	Target/outcome	References
Cpb:WU rats	0.2% GTP	Inhibition of ODC enzyme activity	[24]
C57BL/6	0.2% GTP	Inhibition of ODC enzyme activity	[24]
TRAMP	0.1% GTP	Increased tumor free and overall survival	[53]
TRAMP	0.1% GTP	Induction of apoptosis	[53]
TRAMP	0.1% GTP	Decrease in serum and tissue IGF-I	[53, 114]
TRAMP	0.1% GTP	Increase in serum and tissue IGFBP-3	[53, 114]
TRAMP	0.1% GTP	Decrease in proliferating cell nuclear antigen	[53]
TRAMP	GTC	Accumulation of clusterin mRNA and proteins, down-modulation of histone H3 and up-regulation of Gas 1 mRNA	[95]
TRAMP	0.1% GTP	Inhibition of MMP2 and MMP9	[114]
TRAMP	0.1% GTP	Inhibition of VEGF	[114]
TRAMP	0.1% GTP	Inhibition of uPA	[114]
Athymic nude mice	EGCG, ECG	Inhibition of tumor growth	[154]
Athymic nude mice	GTP, BTE, EGCG, TF	Inhibition of tumor growth, inhibition of serum PSA, inhibition of apoptosis, inhibition of angiogenesis	[155]

vincing evidence that oral infusion of green tea polyphenols (equivalent to six cups of green tea human consumption) inhibits prostate carcinogenesis [53]. Our subsequent studies have suggested that there are multiple targets for CaP chemoprevention by green tea and, therefore, highlight the need for further studies to identify novel pathways that may be modulated by tea or its polyphenolic constituents that could be further exploited for prevention and/or treatment of CaP. The effects of tea and its polyphenols in *in vitro* and *in vivo* situations have been summarized in Tables 2, 3.

3 Mechanisms for the positive association of tea against prostate carcinogenesis

3.1 Role of tea polyphenols in androgen metabolism

Androgen receptor (AR), a key nuclear transcription factor in the prostate gland, is expressed in all stages and types of CaP and at least one-third of advanced CaP contain amplified AR genes [54 – 56]. Our understanding of the biology of CaP etiology has changed since it was found that androgen action is intimately associated with proliferation and differentiation of CaP [10]. The role of androgen in prostate carcinogenesis can be elucidated from the fact that the populations with impaired androgen metabolism such as hereditary 5α-reductase deficiency do not develop CaP, while those with higher circulating levels of androgen are at higher risk of the disease [57]. Because androgens are capable of both stimulating proliferation as well as inhibiting the rate of the glandular epithelial cell death within the prostate, androgen ablation therapy is commonly suggested for men with this nonorgan confined disease [58]. In the year 1941, Huggins *et al.* [59] first demonstrated the utilization of androgen deprivation as a treatment for advanced CaP and since then removal of androgenic stimulation has been utilized to treat this metastatic disease. Kao *et al.* [40] reported that treatment with EGCG reduces the circulating testosterone levels by 70% in a murine model that can be associated with the lowering of CaP risk. Subsequent studies demonstrated that tea polyphenols might block the pathway that leads to the synthesis of androgen.

In tumor xenograft studies of CaP, the 5α-reductase inhibitors were found to slow the growth of previously established tumors [60]. It has been reported that green tea constituents EGCG and ECG, inhibit the activity of type 1 rat 5α-reductase [61]. These polyphenols also inhibited types 1 and 2 human 5α-reductase in microsomes from rat cells that expressed the human enzyme [62]. Several molecular mechanisms have been postulated to be accountable for the development of sporadic hormone-refractory tumors. Most of these involve alterations in the function of the AR and its complex signaling pathways [63]. Thus, it has been of great interest to seek more effective means of minimizing or eliminating its function in order to achieve preventive and/or therapeutic treatments for prostatic neoplastic disease. One study reported that tea polyphenols down-regulated the expression of the AR mRNA in androgen responsive LNCaP prostate carcinoma cells [64]. The basal activity of AR promoter is determined by a Sp1 binding site within the AR core promoter region [65, 66]. Sp1 not only regulates the basal expression of the AR but also acts as its coregulator [67]. Sp1 is involved in the expression of genes related to cell proliferation [68]. Since Sp1 regulates the expression of many critical genes, the decrease in this protein by tea polyphenols could somewhat decrease the growth rates of prostatic cells. EGCG has been reported to significantly decrease the Sp1 DNA binding activity [64]. This study also suggested that Sp1 is the target for tea polyphenols because treatment with EGCG decreased the expression, DNA binding activity, and transactivation activity of Sp1 protein. However, additional work is necessary to authenticate the effect of tea constituents on androgen synthesis or its receptor status in a manner that could lead to the protection against development of CaP.

3.2 Effect of tea polyphenols on prostate specific antigen (PSA)

PSA, a kallikrein-like serine protease glycoprotein secreted primarily by the epithelial cells that line the acini and ducts of the prostate gland, is the most clinically used marker for CaP [69, 70]. PSA is concentrated in prostatic tissue, and serum PSA levels are normally very low. Disruption of the normal prostatic architecture, such as by prostatic disease, pushes greater amounts of PSA to enter the general circulation. Elevated serum PSA levels have become an important marker of prostate pathologies, which include benign prostatic hyperplasia (BPH), prostatitis, and especially CaP. Prostatic intraepithelial neoplasia (PIN) does not appear to raise serum PSA levels. The most common cause for an elevated serum PSA is BPH, the incidence of which increases with age, similar to prostatic cancer. At present, the measurement of serum PSA levels is the most commonly employed biomarker for monitoring the progression of CaP and the response to therapy [71] and a reduction in serum PSA levels has been proposed as an endpoint biomarker for staging hormone-insensitive human CaP intervention. It has been suggested that serum PSA levels can be decreased by agents that lower serum testosterone levels such as leutinizing hormone releasing hormone (LHRH) agonists and antagonists, antiandrogens such as flutamide and bicalutamide and the 5α-reductase inhibitors such as finasteride [71]. In a study from our laboratory, we evaluated the effect of EGCG on the production of PSA in androgen-sensitive human prostate carcinoma LNCaP cells, which are known to produce PSA. This study demonstrated that EGCG treatment resulted in significant dose-dependent decrease in PSA level in the culture medium. Further, a significant time-dependent decrease in PSA production was also observed after EGCG treatment compared with the control [72]. The validity of these cell culture observations to human CaP patients could have implications in reducing CaP body burden. Therefore, *in vivo* and clinical data where PSA levels are being monitored after administration of green tea could be of significance.

3.3 Effects of tea polyphenols on polyamine synthesis

Polyamines *viz.* putrescine, spermidine, and spermine are present in all living cells and their biosynthesis is tightly regulated and involved in the control of many biological processes such as carcinogenesis, cell growth and differentiation, gene transcription and translation, and cell migration [73, 74]. Depletion of polyamines results in the inhibition of cell proliferation and migration and the failure of embryonic development, whereas accumulation of polyamines causes apoptosis [75, 76] and/or cell transformation [77, 78]. Cells maintain intracellular polyamines at optimal levels by regulating synthesis or degradation and by uptake or release of polyamines from or to the exterior. Prostate tissue is known to contain some of the highest concentrations of polyamines and polyamine-metabolizing enzymes in the body [79, 80] and prostate carcinoma has even greater elevated levels. Polyamine analogs (BE-4-4-4-4, BE-3-7-3, and BE-3-3-3) have been found to be effective inhibitors of CaP cell growth in *in vitro* and *in vivo* conditions [81]. ODC is a key regulatory enzyme for polyamine synthesis and the induction of its activity had been reported to be linked with various types of cancers including CaP [51, 82 – 84]. These observations suggest that ODC could be used as a biomarker for the chemopreventive studies [85] and the references therein. An induction in the ODC activity and ODC mRNA expression mediated by testosterone has been reported in prostate carcinoma cells [86, 87]. In a study we reported that green tea polyphenols significantly reverse the induction of ODC activity as well ODC mRNA expression in LNCaP cells. We also demonstrated that testosterone when administered to the C57BL/6 mice, caused a two-fold increase in ODC activity in the ventral prostrate while prior oral infusion of 0.2% w/v green tea polyphenol in drinking water resulted in 40% inhibition in this induction [27]. These data suggest that polyamines and ODC should be studied further for their roles in tea mediated effects on CaP.

3.4 Modulation of gene expression by tea polyphenols

The process of carcinogenesis involves modulation of several genes at various stages and at different levels. These include the genes that regulate growth, cell signaling, differentiation, cell death, cell division, and cell migration [88] and the references therein. Tea polyphenols have been shown to modulate the function of various genes. We, in a study identified nine genes, including six kinases and three phosphatases, whose expression was found to be down-regulated by EGCG [89]. Interestingly, the protein kinase C-alpha (PKC-α) form, whose inhibition of expression has been shown to inhibit cell growth in some cancer cells, was selectively repressed by EGCG while the expression of six other PKC isoforms (β, δ, ε, μ, η, and ζ) was unaffected. We have shown that EGCG inhibits the expression of the PKC-α gene, adenosine kinase and type I β cGMP-dependent protein kinase in LNCaP cells and hence is able to block the intracellular cyclic-nucleotide signaling cascade [89]. Inhibition of PKC-α gene expression is believed to inhibit cell proliferation in animal tumor model and in some human cancer cell lines [90].

The loss of tumor suppressor genes and the genes producing antigrowth factors is an important event in cancer development. Studies have shown that EGCG induces the expres-

sion of 16 kinases and phosphatase genes in prostate cells including tumor suppressor gene SHP-1 and the genes that produce pyrroline-5-carboxylate and prostatic acid phosphatase [89]. The p53 tumor suppressor gene is the most frequently mutated gene found in human malignancies, including Cap. Generally, no correlation between p53 mutation and early stage CaP has been noticed but p53 mutations are shown to be associated with 10 – 20% of advanced CaP patients [91, 92]. Our laboratory has reported that EGCG up-regulated p53 in LNCaP cells (with wild-type p53) but not in DU145 cells (with mutant p53) [93]. Recently, we have shown that EGCG-induced apoptosis in LNCaP cells occurs through the stabilization of p53 by phosphorylation on critical serine residues and $p14^{ARF}$-mediated down-regulation of MDM2 protein [94].

Caporali *et al.* [95] recently demonstrated that growth of SV40-immortalized human prostate epithelial cells (PNT1A) as well as PC-3 cells was potently inhibited by EGCG. EGCG also caused caspase cascade activation and clusterin protein accumulation in both cells lines but not in normal cells, in which clusterin remained undetectable. In the same study, consistent with our earlier work [53], they observed that while 100% of TRAMP mice developed CaP, only 20% of those receiving 0.3% green tea catechins (GTC) in drinking water developed the neoplasm. In TRAMP mice, the clusterin gene was dramatically down-regulated during onset and progression of CaP. In GTC-treated TRAMP mice in which tumor progression was chemoprevented, clusterin mRNA and protein progressively accumulated in the prostate gland. Clusterin dropped again to undetectable levels in animals in which GTC chemoprevention failed and CaP developed. Up-regulation of histone H3 and down-regulation of growth arrest-specific gene 1 (Gas1) mRNAs in CaP-developing TRAMP mice demonstrated a high proliferation rate in tumors, while the opposite occurred in the glands of GTC chemoprevented animals. Failure of GTC chemoprevention caused induction of both histone H3 and Gas1 and down-regulation of clusterin. Immunohistochemistry experiments confirmed clusterin down-regulation during CaP onset and progression, and clusterin sustained expression in chemoprevented TRAMP mice.

3.5 Effects of tea polyphenol on programmed cell death (apoptosis) in CaP

Programmed cell death (apoptosis) is a widespread phenomenon occurring normally at different stages of morphogenesis, growth and development, and during normal turnover in tissues. Apoptosis is initiated in specific cell types by both endogenous-tissue specific agents (generally hormones) and/or exogenous cell-damaging treatments (radiations, chemicals and viruses) [96]. Apoptotic cells are rapidly recognized, phagocitized, and digested by eithermacrophages or adjacent epithelial cells. In addition, apoptosis is a discrete way of cell death different from necrotic cell death and is regarded to be an ideal way of cell elimination [97, 98]. Under normal cellular situations a balance between cell growth and cell death is maintained. This balance is lost in favor of cell growth in the case of cancers including CaP. Correction of this imbalance could lead to the prevention and even ablation of CaP [99, 100] as apoptosis is closely involved in the initiation, progression, and metastasis of human CaP. Since human CaP is present as a heterogeneous mixture of androgen-dependent and -independent mixture of cells, surgery and chemotherapy have failed to address this problem. Hence, one potential strategy to eradicate this mixture of cells is to modulate the apoptotic machinery of the cells. Chemopreventive agents, which can modulate apoptosis, may be able to affect the steady-state cell population that can be useful in the management and therapy of cancer. In recent years, many cancer chemopreventive agents have been shown to induce apoptosis and conversely several tumor promoters have also been shown to inhibit apoptosis [101 – 103]. Therefore, it is reasonable to assume that the chemopreventive agents which have proven effects in animal tumor bioassay systems and/or human epidemiology on one hand and cause induction of apoptosis of cancer cells on the other hand may have wider implication for cancer control. Studies from ours and other laboratories have shown that tea polyphenol EGCG results in an induction of apoptosis in several human carcinoma cells [37, 93, 104]. A study from our laboratory has demonstrated that green tea constituent EGCG results in an induction of apoptosis in human CaP cells LNCaP and DU145 [93]. This observation was later verified by another laboratory [105]. We, recently demonstrated that EGCG-induced apoptosis in human prostate carcinoma LNCaP cells is mediated *via* modulation of two related pathways: (i) stabilization of p53 and down regulation of MDM2 protein, and (ii) negative regulation of NF-κB activity, thereby, decreasing the expression of the antiapoptotic protein Bcl-2 [94]. In another study we demonstrated that EGCG induces growth arrest and apoptosis primarily *via* p53-dependent pathway that involves the function of both p21 and Bax such that down-regulation of either molecule confers a growth advantage to the cells [106]. Yu *et al.* [107] demonstrated that the addition of EGCG and Cu^{2+} to the growth medium decreased the relative viability of androgen-sensitive and androgen-insensitive human CaP cells. However, the effects of EGCG on CaP cells was seen to be depended on (i) the relative concentrations of added EGCG and Cu^{2+} and (ii) their order of addition. These studies demonstrate the positive association of tea with the programmed cell death of human prostate carcinoma cells and their relevance to human population should be further explored to understand a cause and affect relationship.

3.6 Effects of tea polyphenols on CaP angiogenesis

The process of carcinogenesis involves the movement of cancer cells from their original sites to surrounding and distant tissues, a phenomenon known as "metastasis." This is accompanied by formation of new blood vessel (neovascularization), a phenomenon known as "angiogenesis," a major event during later stages of carcinogenesis. The development of blood vessels is an essential step in the growth of a tumor. Without a proper blood supply tumors cannot grow to be large, so the tumor cells produce (or cause nearby cells to produce) growth factors that stimulate the formation of blood vessels. Studies have found that CaP tumors suffer from hypoxia, a condition where there is a lack of oxygen reaching the tissue despite the presence of oxygenated blood. Thus, the tumor must apparently create a greater blood supply (angiogenesis) to get more oxygen. Some hydrolases and matrix metalloproteases (MMP's) have been reported to be over-expressed during metastasis and angiogenesis [108, 109]. Jankun *et al.* [110] reported that EGCG inhibits urokinase, implicated in tumor invasion. EGCG was also found to inhibit tumor cell invasion and directly suppress the activity of two MMPs, MMP2 and MMP9, most frequently overexpressed in cancer and angiogenesis and essential in penetrating the basement membrane barriers [111 – 113]. Recently, we showed that green tea infusion in the TRAMP mice (an autochthonous mouse model that develops CaP spontaneously mimicking the human situation) resulted in marked inhibition of the markers of angiogenesis and metastasis most notably vascular endothelial growth factor (VEGF), urokinase plasminogen activator (uPA), and MMP2 and MMP9 [114]. It has been shown that tea components slow progression of LNCaP human prostate tumors in SCID mice, partly by inhibiting the formation of new blood vessels [115]. A recent study investigated the effect of EGCG on the tube formation of human umbilical vein endothelial cells (HUVEC) on matrigel. Tube formation was inhibited by treatment with EGCG both prior to plating and after plating endothelial cells on matrigel. EGCG treatment was also found to reduce the migration of endothelial cells in matrigel plug model. These findings suggest that EGCG also acts as an angiogenesis inhibitor by modulating protease activity during endothelial morphogenesis [112].

3.7 Insulin-like growth factor (IGF) signaling in CaP and its modulation by tea polyphenols

IGFs (IGF-I and IGF-II) are polypeptides functioning both as growth factors and endocrine hormones. IGF-I has autocrine and paracrine activities in addition to its endocrine activity on bone growth. Studies have established that elevated circulating levels of IGF-I are associated with increased risk of several common cancers, including those of the breast, prostate, lung, and colorectal [116]. Expression of an IGF-I transgene in mice has been demonstrated to cause the development of CaP [117]. IGF-I has mitogenic and antiapoptotic effects on normal and transformed CaP cells *in vitro* and *in vivo* and has been strongly implicated in the etiology of human CaP [118 – 121]. The level of IGF-binding protein 3 (IGFBP-3), a major IGF-I binding protein in serum that, in most situations, suppresses the mitogenic action of IGF-I, has been shown to be inversely associated with the risk of different cancers [122]. The elevated levels of IGF-I with concomitant lowering of IGFBP-3 in serum are an excellent predictor of CaP progression in humans, thus identification of agents that can inhibit the IGF-I signaling pathway could lead to development of highly successful prevention strategies for CaP. IGF-I has been implicated as an important factor in the initiation and progression of CaP in an autochthonous mouse model of CaP [123]. In a study, we observed that oral infusion of green tea polyphenols, significantly, lowers the IGF-I levels and restores the depleted levels of IGFBP-3 in TRAMP mice [53]. In one of our recent study, we observed that IGF/IGFBP-3 signaling is the prime pathway for GTP-mediated inhibition and metastasis of CaP in TRAMP mice that limits the progression of cancer through inhibition of metastasis and angiogenesis [114].

3.8 Proteasome activity in CaP and tea polyphenols

The ubiquitin-proteasome system has a critical role in the specific degradation of cellular proteins [124, 125], and two of the proteasome functions are to allow tumor cell cycle progression and to protect tumor cells against apoptosis [126]. In addition, the chymoptrypsin-like but not trypsin-like activity of proteasome is associated with tumor cell survival [127]. Inhibition of this enzyme has recently been acknowledged as a valid approach to cancer chemotherapy [128]. Many cell cycle and cell death regulators such as p53, pRB, p21/Cip1, p27/Kip1, IκB-α and Bax, have been identified as targets of the ubiquitin- proteasome-mediated degradation pathway [129]. Nam *et al.* [130] reported that EGCG and ECG, but not EGC and EC, potently inhibited the chymotryptic activity of 20s proteosome both in cell-free systems and in tumor cell lines at concentrations found in tea drinkers. Treatment of LNCaP cells with EGCG resulted in G0/G1 cell cycle arrest and accumulation of p27 and IκB, both of which are targets of proteosomes [130]. A recent study found that ubiquitin carboxyl extension protein 1 (UBCEP-1) is overexpressed in CaP. This study also suggested that UBCEP-1 mediated ubiquitin chain elongation may promote prostate carcinoma development by increasing *via* the proteasome pathway the degradation of proteins which are involved in growth inhibition or apoptosis [131].

Recently, ubiquitin-proteasome system was shown to be involved in the regulation of AR protein in CaP cells [132] and proteosome inhibitor was found to down-regulate PSA and induce growth arrest and apoptosis in LNCaP cells [133]. Treatment of LNCaP cells with either (−)-EGCG or (−)-GCG accumulated p27 and IκBα proteins, associated with an increased G1 population. EGCG treatment also accumulated the pro-apoptotic Bax protein and induced apoptosis in LNCaP cells expressing high basal levels of Bax, but not in CaP DU-145 cells with low Bax expression. The synthetic GTPs were also seen to significantly inhibit the colony formation by LNCaP cells [134].

3.9 Effects of tea polyphenols on regulation of cell cycle in CaP

Mammalian cell cycle is a set of events that governs the self-replication of the cell. A normal cell cycle progression is dependent on the ability of the cell to translate extracellular signals such as mitogenic stimuli and intact extracellular matrices in order to efficiently replicate DNA and divide. Loss of those controls in cells can result in unrestrained cell division which ultimately leads to cell proliferation, tumorigenesis, and cancer. A controlled cell cycle progression is an important physiological event that is regarded to be essential for normal tissue homeostasis [97]. One or more cell-cycle checkpoint defects are involved in most of the cancer types including CaP [135–137]. Tea polyphenols have been shown to arrest the cell division of cancer cells and enhance the expression of cdk inhibitors [138]. In our laboratory, EGCG was found to increase the expression of p16, p18, p21, and p53, which are associated with negative regulation of cell cycle progression in prostate carcinoma cells [93, 139]. Recently, we elucidated the molecular mechanism involved in the cell cycle arrest in human prostate carcinoma cells [139]. We observed that EGCG treatment of LNCaP and DU-145 cells resulted in significant dose- and time-dependent (i) up-regulation of the protein expression of WAF1/p21, INK4a/p16, and INK4c/p18, (ii) down-modulation of the protein expression of cyclin D1, cyclin E, cdk2, cdk4, and cdk6, but not of cyclin D2, (iii) increase in the binding of cyclin E toward cdk2. This series of events could impose a blockade of G1 to S transition, causing a G0/G1 phase arrest of the cell cycle. The effect of EGCG on the interbinding between the different components of the cki-cyclin-cdk network, however, needs further investigation.

3.10 Modulation of phosphatidylinositol-3-kinase (PI3K)/protein kinase B (PKB) and MAPK by tea

PI3K/PKB and MAPK signaling pathways have critical roles in cellular biochemistry and defects in these signaling pathways have been found to result in the development of cancer [140]. Increase in 3′-phosphoinositides, which are produced by PI3K, leads to membrane translocation of downstream effectors such as the serine/threonine protein kinase Akt resulting in increased cellular proliferation and protection from apoptosis [141]. Likewise, the MAPKs are also critical for cellular proliferation as the transcription of many early genes is mediated *via* the sequential activation of MAPK. The MAPK family such as extracellular signal regulated kinase (Erk) 1 and 2 are shown to be constitutively active in CaP in humans, and possibly play causative roles in the progression of this malignancy from an androgen-sensitive phenotype to an advanced and androgen-insensitive metastatic disease. It has been shown that the activation of MAPK by green tea might be a potential signaling pathway in the regulation of antioxidant-responsive element mediated phase II enzyme gene expression [142]. Potent activation of Erk2 and JNK1 was observed by treatment with GTP and the treatment also increased mRNA levels of the immediate-early genes c-jun and c-fos, as determined by reverse transcriptase-coupled PCR. Exposure of normal human epidermal keratinocytes (NHEK) to UVB radiation induces oxidative stress and phosphorylation of MAPK cell signaling pathway. We demonstrated that pretreatment of normal NHEK with EGCG inhibits UVB-induced H_2O_2 production and H_2O_2-mediated phosphorylation of MAPK signaling pathway [143].

We studied the role of black and green tea polyphenols on the modulation of constitutive levels of PI3K/PKB and MAPK pathways in human prostate carcinoma cells differing in androgen status. We observed that tea polyphenols down modulated the protein expression of PI3K/PKB proteins and an up-regulation of Erk1/2 was also observed [140]. The up-regulation of Erk1/2 was possibly due to oxidative stress induced by EGCG because studies have suggested that this up-regulation of Erk could be blocked by the use of antioxidants such as glutathione and *N*-acetyl-L-cysteine [144]. We have also observed that tea polyphenols down-modulate EGF induced protein expression of various signal transduction pathways (unpublished data). More recently in an *in vivo* study, we observed that oral infusion of GTP resulted in a significant inhibition in (i) protein expression of PI3K, (ii) phosphorylation of Akt at Thr-308, and (iii) phosphorylation of Erk1/2 in an autochthonous mouse model of CaP [114].

3.11 Modulation of NF-κB by tea polyphenol EGCG

The NFκB plays an important role in inflammation [145], autoimmune response, cell proliferation and apoptosis [146] by regulating the expression of genes involved in these processes and thus is widely recognized as a critical mediator of immune and inflammatory responses. Constitutive activation of the transcription factor NFκB has been

observed in a high proportion of androgen-independent CaP [147 – 149]. In addition to suppressing apoptosis, NFκB promotes malignant behavior in other ways like stimulating transcription of cell cycle progression factors (c-myc, cyclin D1), proteolytic enzymes (MMP9, uPA), and angiogenic factors VEGF and IL-8 [149, 150]. Thus, it is not surprising that nuclear localization of NF-κB in CaP biopsies has been shown to correlate with poor clinical prognosis [151]. A mystifying array of natural products, some of which have putative chemopreventive activity, are reported to inhibit constitutive and/or stimulated NF-κB activity in human CaP. We demonstrated that EGCG had a concomitant effect on p53 and NF-κB, causing a change in Bax/Bcl-2 ratio in a way that favors apoptosis in LNCaP prostate carcinoma cells [94]. Although it has been suggested that ROS are involved in the activation of NF-κB and that its inhibition by EGCG is due to the antioxidant activity, direct evidences are still lacking [152, 153], thus further investigations are required to elucidate the mechanism of action of tea on transcriptional factor NF-κB.

3.12 Effects of tea and its polyphenols on the growth of prostate tumor xenografts

In vivo preclinical models like athymic nude mice implanted with tumor xenografts are a widely accepted model to assess the effect of drugs/synthetic agents and dietary substances on the development and progression of a variety of cancer types including CaP. One study demonstrated the effects of tea polyphenols on growth inhibition and regression of prostate tumors in athymic nude mice. They showed that *intraperitoneal* injection of EGCG but not structurally related catechins, such as ECG, inhibited the growth and progression of androgen-insensitive PC-3 and androgen-repressed LNCaP 104-R human prostate carcinoma tumors [154]. In a recent study [155], we observed that GTP, black tea extract (BTE), EGCG, and TF resulted in significant inhibition in the growth of CWR22Rv1 (androgen responsive) implanted tumors in athymic nude mice. We also observed that treatment of mice with all tea preparations resulted in significantly reduced serum PSA secretion measured at 3 and 4 wk postinoculation of CWR22Rv1 cells. Tumor growth inhibitory response of all tea preparations was accompanied with (i) decrease in Bcl-2 (antiapoptotic) with a concomitant increase in Bax (proapoptotic), (ii) activation of Caspase-3, (iii) cleavage of PARP, and (iv) decrease in VEGF. This study concluded that GTP and BTE as well as their major polyphenolic constituents EGCG and TF significantly inhibited the development of CaP *via* (i) induction of apoptosis and (ii) inhibition of angiogenesis in tumors. Furthermore, in an independent experiment, we found that GTP, at physiologically achievable doses (0.1 and 0.05% in water) resulted in a significant regression of tumors in athymic nude mice when given after CWR22Rv1 tumors were established to a volume of approximately 400 mm^3. These data point to a potential therapeutic benefit of black as well as green tea against CaP. In a recent study green tea supplementation was found to strongly suppress the growth of PC3 tumors without any adverse effects in nude mice, suggesting strong potential of tea polyphenols as anticancer agents [156].

4 Epidemiological studies

Till date very few case-control studies have been conducted to evaluate the effect of consumption of tea and human CaP. All published data seeking an association between tea consumption and the risk of CaP considered undefined tea preparations, mostly black tea, with most of these studies conducted in Asian populations. Two epidemiological studies have shown that people who regularly consume tea have a lower incidence of CaP [157, 158]. Heilbrun *et al.* [157], in a prospective cohort study employing 7833 men living in Hawaii with Japanese ancestry, observed a weak but significant negative association between black tea intake (more than one cup *per* day) and CaP incidence ($p = 0.02$). Jain *et al.* [158], in a case-control study conducted in three geographical areas of Canada, observed a decrease in CaP risk with tea intake of more than two cups *per* day. Other epidemiological studies conducted in Italy [159], Utah [160], and Canada [161] did not find any difference in the risk for CaP between tea drinkers and nondrinkers. It should be noted that most of these studies lacked appropriate controls for comparison in categorization of tea consumption, the type of tea consumed and the ethnicity of the subjects, which weakens the overall impact of the study. Recently in a case-control study [162] the protective effect of green tea against CaP was studied. The cases were 130 incident patients with histologically confirmed adenocarcinoma of the prostate. The CaP risk was observed to decline with increasing frequency, duration, and quantity of green tea consumption. The adjusted odds ratio (OR), relative to nontea drinkers, were 0.28 (95% CI = 0.17 – 0.47) for tea drinking, 0.12 (95% CI = 0.06 – 0.26) for drinking tea over 40 years, 0.09 (95% CI = 0.04 – 0.21) for those consuming more than 1.5 kg of tea leaves yearly, and 0.27 (95% CI = 0.15 – 0.48) for those drinking more than three cups (1 L) daily. The dose response relationships were also significant, suggesting that green tea is protective against CaP. A better understanding of the association between green tea and CaP through further studies with a multiethnic perspective will facilitate appropriate health strategies to minimize high risk factors and maintain the protective factors that will keep CaP at bay.

5 Clinical trials with green tea

Progress in cancer prevention has been accelerated as prevention clinical trials are completed and reported. A pro-

mising strategy is the identification of cancer risk factors through epidemiologic and experimental research with lifestyle and medical approaches that allow translation of clinical trial results to clinical practice. A green tea extract phase I trial in patients with solid tumors was conducted to ascertain the maximum-tolerated dose, toxicity, and pharmacology of oral green tea extract. This study concluded that 1.0 g/m^2 of green tea extract three times a day (equivalent to seven to eight Japanese cups (120 mL) of green tea) is well-tolerated and is not associated with any side effects [163]. One recent phase II clinical trial explored the antineoplastic effects of green tea in patients with metastatic androgen-independent CaP [164]. In this study, forty-eight patients were instructed to take 6 g of green tea/day orally in six divided doses. Results showed that only one patient within this 42-patient cohort manifested a 50% decline in PSA values from baseline but this reduction was not sustained beyond 2 months. Although, green tea was tolerated well, a notable percentage of patients experienced toxicity such as insomnia, fatigue *etc.*, presumably from caffeine present in the tea. It should however, be noted that this study was conducted in patients with metastatic androgen-independent CaP and therefore, in principle, is not representation of the chemopreventive effects of green tea. However, this study could not be conclusive as, the median time on study was only 1 month, and whereas prior preclinical data have been suggesting that green tea requires prolonged exposure to exert its antitumor activity [113, 114]. Taken into consideration the drawbacks of this trial, the negative findings of this study do not contradict the results of earlier epidemiological studies which suggest that green tea may confer antitumor effect in relatively healthy population. Greenwald [165] stated the position of the NCI that GTP is being investigated for CaP prevention and that future prevention clinical trial will rely on multidisciplinary medical approaches that bring together expertise in many fields to address disease across the cancer spectrum. In a recent clinical trial [166], 19 patients were enrolled in the study and prescribed with 250 mg green tea extract capsules twice daily. The outcome of the study concluded that green tea as alternative complementary therapy has minimal clinical activity against hormone-refractory CaP. Thus, for an ideal prospective study, a population with high risk for CaP development should be considered and the length of exposure of green tea to the subjects should be taken into consideration. An ideal population to establish the effectiveness of tea polyphenols as cancer chemopreventive and/or chemotherapeutic agents will be to evaluate the effect of tea drinking in patients selected for prostatectomy.

6 Future perspectives

CaP is a disease of many etiological factors and involves several mechanisms in its progression, therefore, the modulation of a single mechanism alone by tea polyphenols may not completely impede the progression of CaP. However, tea polyphenols have been shown to modulate various molecular targets involved in progression of CaP as previously discussed in this review. Till date, there is no information available that links various molecular pathways involved in the progression of CaP, to each other.

Based on our and other *in vitro* and *in vivo* studies, tea and its constituents have been observed to cause cell cycle dysfunction, induce apoptosis, inhibit enzymes and signaling pathways associated with cellular proliferation, inhibit tumor growth and delay onset and progression to tumorigenesis. Because tea is relatively inexpensive, simple to use, perhaps nontoxic, and shown to have inverse association in various rodent tumor bioassay system, studies to assess its role as CaP chemopreventive agent will be of interest. Although studies on tea polyphenols demonstrate its efficacy as a potent chemopreventive agent against CaP, there are still many gaps in our current awareness. There are certain factors such as bioavailability, tissue level of tea constituents, tea type, drinking habit, race, *etc.* that need to be taken care of before a recommendation could be made for tea polyphenols as preventive or therapeutic agents in humans. The bioavailability of the active polyphenolic constituents after tea consumption by laboratory animals and humans, is poorly defined. Yang *et al.* [167–169] and others have measured the concentration of tea polyphenols in human plasma, saliva, feces, and urine after consuming decaffeinated green tea and found that EGCG was in lesser concentration than EGC. Therefore, a great deal of laboratory research and many more epidemiological studies are needed for obtaining conclusive evidence. The increase or decrease of PSA, IGF-I, and IGFBP-3 in relation to tea consumption and levels of tea polyphenols in urine samples may be used as biomarkers in CaP chemoprevention. This type of study could answer the question of how much tea should be consumed by humans for CaP chemoprevention and explain the mechanism involved.

Also, a strategy of acting on multiple signaling pathways using a combination of chemopreventive agents should be explored for CaP chemoprevention in preclinical studies. Agents should be selected in such a way that they in combination will target two or more signaling pathways to impart a synergistic response against CaP.

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