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Green tea and prostate cancer: from bench to clinic

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1. ABSTRACT

Green tea, the most popular beverage next to water, is a rich source of tea catechins and has potential to be developed as a chemopreventive agent for prostate cancer. For centuries it has been used in traditional medicine in Far-East countries. Male populations in these countries where large quantities of green tea are consumed on regular basis have the lowest incidence of prostate cancer. In this review, after a description of prostate cancer and several risk factors associated with the disease, we evaluated studies reported with green tea or its major constituent, (-)-epigallocatechin-3-gallate in inhibition of prostate cancer. This review provides an in-depth overview of various biochemical and signaling pathways affected by green tea in in vivo and in vitro models of prostate cancer. This is followed by a comprehensive discussion of the epidemiological studies and some ongoing clinical trials with green tea catechins. The review concludes with a brief discussion of the future direction and development of clinical trials employing green tea catechins which could be developed for prevention and/or intervention of prostate cancer.

2. INTRODUCTION

Prostate cancer remains the second leading cause of cancer-death in men, in the United States and the most common non-skin, visceral cancer (1), yet it is highly curable if discovered while still confined to the prostate gland. In 2007 it is estimated that 218,890 new prostate cancer cases will be diagnosed, making it the commonest type of new cancer (1, 2). Prostate cancer develops from epithelial cells in the peripheral region of the male human prostate, which is a walnut-sized glandular organ located between the pubic bone and rectum, wrapped around the urethra. In most cases the disease grows very slowly; in fact, 89% of patients diagnosed with prostate cancer have a five-year survival rate and 63% live at least 10 years after the disease is detected and treated (1, 2). Eventually, cancer metastasizes from the prostate gland to the pelvic lymph nodes, following lymphatic channels, as well as to other sites in the body, particularly bones.

The glandular epithelium of the human prostate gland is made of three types of cells: basal, secretory luminal and neuroendocrine (3). The basal and secretory luminal cells are the two major types and can be

distinguished by their expression of specific markers. Basal cells express keratin 5, keratin 14 and p63 (4, 5) while the luminal cells express keratin 8, keratin 18, androgen receptor and secrete prostate-specific antigen (PSA) (4, 6). The sparsely present neuroendocrine cells express neuron-specific enolase and the neuropeptide chromogranin A (6). A very small stem cell population present in the basal layer is the progenitor of all the different epithelial types in the prostate (7). With the onset of pre-cancerous epithelial proliferation there is a gradual change in the expression profile and morphology of prostatic epithelial cells as they convert to the cancerous state, including shutting off of certain tumor suppressor genes and genes responsible for certain epithelial markers, and turning on the other proto-oncogenes and mesenchymal cell marker genes. This can occur both at the transcriptional as well as the translational levels. Very often transcriptional silencing of genes are secondary to epigenetic modifications resulting in a cancerous phenotype (8). Genes that have been identified as being epigenetically silenced in prostate cancer include androgen receptor (*AR*), glutathione-S-transferase *pi* (*GSTP1*), *E-cadherin*, retinoblastoma (*pRb*) and Ras association (RalGDS/AF-6) domain family 1 (*RASSF1*); silencing of these genes plays a critical role during the initiation and progression of prostate cancer (9).

Prostate cancer is associated with different pre-neoplastic stages namely (*i*) proliferative inflammatory atrophy (PIA), (*ii*) prostatic intraepithelial neoplasia (PIN) and (*iii*) high-grade prostatic intraepithelial neoplasia (HGPIN). Proliferative inflammatory atrophy of the prostate is a result of *de novo* proliferative lesions or regenerative lesions after chronic inflammation that leads to a reduction in the volume of preexisting glands and stroma (10). A 5-year follow up study conducted by our group suggests that chronic prostatic inflammation resulting in PIA may be a significant risk factor for prostatic adenocarcinoma (11). PIN, is a noninvasive neoplastic proliferation of epithelial cells, predominantly in the peripheral zone of the prostate; it is widely regarded as a precursor to invasive adenocarcinoma. Cell division in human prostate epithelium occurs in the basal cell compartment. In PIN, the cellular proliferation occurs within pre-existing ducts and glands, with preservation of their architecture, but with progressive disruption of the basal cell layer; however, stromal invasion is not established. Other histologic or biologic changes include loss of neuroendocrine and secretory differentiation, nuclear and nucleolar abnormalities, neovascularity, increased proliferative potential and genetic instability with variation of DNA content (12). Basal cell specific immunostaining for cytokeratin is present in PIN but is absent in areas of prostate cancer (13). PIN appears to predate the appearance of prostate adenocarcinoma by more than 5 years. With the passage of time, prostate cancer develops considerable histologic heterogeneity and is often multi-focal. To address this complexity, a grading system based on the predominant histologic pattern and secondary histologic pattern has been introduced to define various grades of prostate cancer. The Gleason score is the most frequently used grading system; scores range from 2 (1+1) to 10 (5+5). Advanced stage adenocarcinoma is generally correlated with a higher Gleason score (14).

Various factors contribute to the proclivity of a male individual to get prostate cancer. Age is the most important factor (15) with men over 65 years of age being the most likely to be diagnosed with the disease. Only 1 in 10,000 men under age 40 is diagnosed with the disease, while the rate shoots up to 1 in 39 for men aged 40 to 59 and 1 in 14 for men aged 60 to 69. In fact, about 65% of all prostate cancers are diagnosed in men over the age of 65. However, race and family history also play an important role in disease etiology (16). Men of African-American descent are 56% more likely to develop the disease as compared to Caucasian men and 2.5 times more likely to succumb to the disease. Men who have a single relative with prostate cancer are twice as likely to develop the disease, while those with two or more affected relatives are nearly four times as likely to be diagnosed with prostate cancer. The risk is even higher if the affected family members were diagnosed at a young age, with the highest risk seen in men whose family members were diagnosed before age 65. Genetics studies from several laboratories in the United States, Finland, and Israel, support the identification of the interferon-inducible, 2', 5'-oligoadenylate dependent Ribonuclease L gene (*RNase L*), as the hereditary prostate cancer 1-HPC1-allele (17, 18). R462Q mutations or variants that impair function of RNase L may contribute to the development of prostate cancer, and have been linked to infections with a virus identified as xenotropic MuLV-related virus (XMRV). This virus is closely related to xenotropic murine leukemia viruses (MuLVs), but its sequence is clearly distinct from all known members of this group (19).

In addition to genetics, social and environmental factors, particularly diet and lifestyle have a significant impact on this disease. While the exact relationship between obesity and prostate cancer remains unclear, there is no doubt that obesity has a negative effect on disease outcomes (20). Research has shown that PSA test results in obese men can be lower despite the presence of disease (21), potentially leading to a delay in diagnosis and treatment; recovery from surgery tends to be longer and more difficult; and the risk of mortality from prostate cancer can be higher. Improved detection and monitoring techniques and better treatment options have contributed to a reduction in the mortality rate from prostate cancer from its peak in 1993. The ability to diagnose prostate cancer earlier has resulted in the detection of a number of clinically insignificant prostate cancers, with the result that physicians often have to face the dilemma of having to decide whether to initiate attempts at curative treatment or to manage the disease with watchful waiting. Consequently, prevention strategies aimed at halting the initiation and development of prostate cancer are rapidly moving to the forefront of research, with increasing attention to identifying dietary and lifestyle changes that can influence the natural biological behavior of this malignancy.

Research conducted in the past few years has shown that certain bioactive dietary agents may reduce an individual's chances of developing prostate cancer, diminish the risk of disease recurrence, and inhibit disease progression. Within this class polyphenols from green tea and isoflavones from soy have come under increasing scrutiny by researchers and treating physicians, since the rate of prostate cancer development in Asian-born men regularly consuming soy products and green tea is considerably lower than that of American-born men, but this biologic advantage is lost in Asian men who live in Western countries and are exposed to Western-style diets, which are substantially higher in fat and carbohydrate contents.

3. GREEN TEA POLYPHENOLS

On a global basis, consumption of tea (*Camellia sinensis*) as a beverage is exceeded only by that of water. All types of tea (green, black, and oolong) are produced from the *Camellia sinensis* plant using different techniques. Fresh leaves from the *Camellia sinensis* plant are steamed to produce green tea, which contains several bioactive compounds, including catechins, a category of polyphenols that have chemopreventive properties (22, 23). Archaeological evidence indicates that tea has been consumed for almost 5000 years, with India and China being two of the first countries to cultivate it. Green tea has been used for centuries in China, Japan and Thailand as a traditional medicine with a variety of applications: controlling bleeding, enhancing wound healing, promoting digestion, regulating body temperature, and controlling blood sugar levels. Green tea catechins are flavan-3-ols found in green tea leaves. The four major catechins present in green tea leaves are (-)-epigallocatechin-3-gallate (EGCG), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epicatechin (EC), commonly referred to as green tea polyphenols and make up about 30% of the dry weight of green tea leaves (24). In this review, we present the results of recent research evaluating the role of green tea in the prevention of prostate cancer, and its biologic potential for other applications in the management of this very prevalent disease.

4. GREEN TEA POLYPHENOLS AND PROSTATE CANCER

Studies have shown that drinkers of green and black tea have lower risk of heart attacks and strokes (25). Epidemiological studies have associated both teas with health-promoting effects, such as antiviral, antibacterial, anti-mutagenic, antipyretic, diuretic and anti-carcinogenic activities (26-28). Number of studies has focused on the anti-carcinogenic effects of tea, such as inhibition of growth proliferation, induction of apoptosis, induction of phase II detoxifying enzymes and reduction of oxidative damage to DNA (29-32). In the next sections, we highlight the various signal transduction and biochemical pathways and processes through which green tea polyphenols appear to modulate the biologic behavior of prostate cancer demonstrated in *in vitro* and *in vivo* models, followed by epidemiological evidence to support these findings and review of clinical trials conducted with green tea polyphenols.

4.1. Studies in cell culture

Studies with green tea polyphenols in both androgen-responsive (22Rv1 and LNCaP cells) and androgen-refractory (DU145 and PC3 cells) human prostate cancer cell lines representing various stages of disease progression demonstrates cell growth inhibition by inducing apoptosis (33, 34). The *K-ras* proto-oncogene is frequently mutated at codon 61 in latent prostate cancer (35) allowing it to become the *Ras* oncogene. Green tea, green tea extracts and EGCG has been shown to decrease the expression of *k-ras* in LNCaP cells (36).

While androgen-sensitive prostate cancer cells undergo rapid apoptosis upon androgen ablation, the androgen-insensitive prostate cancer cells do not undergo apoptosis upon androgen blocking however maintain the molecular machinery of apoptosis. EGCG has been shown to impart anti-proliferative effects against both androgen-responsive LNCaP cells and androgen-refractory DU145 prostate cancer cells by deregulating cell cycle and inducing apoptosis. EGCG treatment has shown to result in a dose-dependent G0/G1-phase arrest of the cell cycle; increase of p53 in LNCaP cells (carrying wild-type p53), but not in DU145 cells (carrying mutant p53) and induction of cyclin kinase inhibitor WAF1/p21 in both LNCaP and DU145 cell types (37). Modulation of cyclin kinase inhibitor (cki)-cyclin-cyclin-dependent kinase (cdk) machinery in the induction of apoptosis by EGCG when further probed in LNCaP and DU145 cells revealed that treatment with EGCG resulted in a significant dose- and time-dependent up regulation of the protein expression of WAF1/p21, KIP1/p27, INK4a/p16, and INK4c/p18; down-modulation of the protein expression of cyclin D1, cyclin E, cdk2, cdk4, and cdk6, but not of cyclin D2; increase in the binding of cyclin D1 toward WAF1/p21 and KIP1/p27, and decrease in the binding of cyclin E toward cdk2 (38). Furthermore, EGCG-induced apoptosis in LNCaP cells was the result of p53 stabilization by phosphorylation of critical serine residues and p14ARF-mediated down-regulation of murine double minute 2 (MDM2) protein and negative regulation of NF-κB activity thereby causing a change in the Bax/Bcl-2 ratio thereby triggering apoptosis (39, 40). More recent studies have shown that ablation of either p21 or Bax can abrogate the 53-dependent apoptosis induced by green tea polyphenols in prostate cancer cells (41). In another study, induction of apoptosis in DU145 cells by green tea polyphenols and EGCG causes an increase in the formation of reactive oxygen species leading to mitochondrial depolarization (42). Furthermore, EGCG exposure to androgen-refractory DU145 and PC3 prostate cancer cell lines has been shown to potently and specifically inhibit the chymotrypsin-like activity of the proteasome *in vitro* resulting in the accumulation of two natural proteasome substrates, p27 (Kip1) and IκB-α, an inhibitor of transcription factor NF-κB, followed by growth arrest in the G1 phase of the cell cycle (43).

Green tea polyphenols have been shown to mimic chemical inhibitors of fatty acid synthase (FAS) while

inhibiting growth and inducing apoptosis in several cancer cell lines *in vitro* (44-46) and in tumor xenografts *in vivo* (47). EGCG inhibited the high levels of FAS activity in LNCaP cells in a dose-dependent manner and this was accompanied by a concomitant decrease in endogenous lipid synthesis, inhibition of cell growth and induction of apoptosis indicating that EGCG could inhibit FAS activity as efficiently as the presently known synthetic FAS inhibitors (48).

Levels of PSA are highly upregulated during prostate cancer progression (49). Pezzato *et al* report some new activities of PSA which include degradation of gelatin, degradation of type IV collagen in reconstituted basement membrane (Matrigel) and activation of progelatinase A (MMP-2), but not pro-MMP-9, in a cell-free system (50). EGCG can restrain these PSA activities in a dose-dependent manner, (51, 52).

During hypoxia, hypoxia-inducible factor-1 (HIF-1) activates transcription of a vast array of hypoxia-responsive genes, including those involved in angiogenesis, erythropoiesis, glucose metabolism, cell survival and tissue invasion (53). A study where the effect(s) of EGCG on normoxic HIF-1α expression in human prostate cancer cells were observed (54) concluded that while in the normal cells EGCG induced a dose-dependent increase in HIF-1-mediated transcription and HIF-1α protein levels, concomitant treatment of prostate cancer cells with EGCG and ferrous ions abolished the increase in HIF-1-mediated transcription, thereby indicating that EGCG may inhibit angiogenesis, at least in part, during prostate cancer progression.

Cyclooxygenase-2 (COX-2) is the inducible isoform of the rate-limiting enzymes that convert arachidonic acid to proinflammatory prostaglandins (55). It is also the primary target for nonsteroidal anti-inflammatory drugs. The enzyme has been shown to be up-regulated in PIA, but not in prostate cancer (56), though it promotes prostate cancer progression (57). EGCG is able to inhibit COX-2 without affecting COX-1 expression at both the mRNA and protein levels, in androgen-responsive LNCaP as well as in the androgen-refractory PC-3 prostate cells *in vitro* as well as in *in vivo* models (58, 59). A synergistic effect was observed on growth inhibition of prostate cancer cells both *in vitro* and *in vivo* when EGCG is used in a combination with COX-2 inhibitors (60).

Recently, researchers have focused considerable attention on one mechanism by which green tea polyphenols may contribute to chemoprevention in prostate cancer through its role in the reversal of epigenetically silenced genes that occurs during the transformation of normal prostate epithelial to neoplastic stage and its progression towards malignancy. This has been attributed to the inhibition of DNA methyltransferase 1 (DNMT1), the major enzyme involved in the hypermethylation of CpG islands which are present in the promoter region and exons of several genes (61, 62). This inhibition is strongly enhanced by Mg^{++} ions and computational modeling studies indicate that the gallic acid moiety of EGCG plays a crucial role in its high-affinity, direct inhibitory interaction with the catalytic site of the human DNMT1 and its binding with the enzyme is stabilized by Mg^{++} ions (63). Studies performed in our laboratory further addressed whether inhibition of DNMT1 translates into demethylation of the gene promoter(s) in prostate cancer by using androgen-responsive human prostate cancer LNCaP cells (64). We employed a recently developed promoter array to investigate whether green tea polyphenols had the potential to reverse the process of DNA hypermethylation. The promoter array consisted of 82 promoter sequences from various genes representing a variety of biological functions. DNA isolated from human prostate cancer LNCaP cells exhibited extensive promoter hypermethylation in 14 genes namely *CASP8, CD14, IL-4, IRF-7, Maspin, MYC L2, Nestin, NME2, PDGF-B, RPA2, Survivin, Tastin, TFF1 and VHL*. Treatment of LNCaP cells with green tea polyphenols at 10 μg/ml concentration up to 14 days significantly reversed DNA hypermethylation of promoter in these genes in a time-dependent manner. This increase in demethylated gene promoters in LNCaP cells after polyphenol treatment was further confirmed by methyl-specific (MS)-PCR. For 'proof of principle' these genes were demethylated by 5-aza-2'-deoxycytidine, a specific inhibitor of DNA methyltransferase. Furthermore, treatment of LNCaP cells with physiological relevant and non-toxic doses (1-10 μg/ml) of green tea polyphenols for 1, 3, 7 and 14 days resulted in subsequent inhibition of DNMT1 expression both at mRNA and protein levels which correlate with decreased promoter hypermethylation of these genes (64). These studies provide evidence that green tea polyphenols have the capability to reduce promoter hypermethylation of susceptible genes by inhibiting DNMT1 expression; this capability supports the potential usefulness of green tea polyphenols in the prevention of prostate cancer.

4.2. Studies in preclinical models

Androgen action in the prostate is dependent upon conversion of testosterone to 5α-dihydrotestosterone (5α-DHT) by the enzyme, 5α-reductase (65). 5α-DHT then binds to androgen receptors to exert its cellular action (66). Inhibition of 5α-reductase limits the levels of 5α-DHT available to the prostate required for its growth and development, without affecting the levels of circulating testosterone, which can then be utilized for other testosterone-dependent testicular functions, sexual behavior and muscle growth. In one of the earliest reports on the beneficial effects of green tea polyphenols in prostate cancer (67) performed in rats demonstrated that EGCG and ECG constituents of green tea polyphenols but not EC and EGC were potent inhibitors of Type 1 but not Type 2, 5α-reductase. This decrease resulted in the inhibition of growth of the prostate in male Sprague Dawley rats indicating that green tea polyphenols were able to modify androgen action in the prostate. Another study with green tea constituents showed a significant reduction in tumor size and completely abrogate tumors in both androgen-repressed LNCaP 104-R and the androgen-refractory PC3 tumor xenograft in athymic nude mice (68). Similar results were observed in androgen-responsive LNCaP cells inoculated intraprostatically into SCID mice (69).

Studies from our group demonstrated that ornithine decarboxylase (ODC), an enzyme involved in the polyamine biosynthetic pathway, is over-expressed in human prostate adenocarcinoma and prostatic fluid (70). Testosterone-mediated induction of ODC was shown to be downregulated in LNCaP cells exposed to green tea polyphenols and in Cpb:WU rats and C57BL/6 mice (71). Another study demonstrated that PC-3 tumors implanted in athymic nude mice fed with regular diet supplemented with 0.5% of the nutrient mixture containing lysine, proline, arginine, ascorbic acid and green tea extract inhibited tumor growth. Histological studies of the tumors also revealed inhibition of matrix metalloproteinases (MMP)-9 and vascular endothelial growth factor (VEGF) secretion and mitosis (72). A recent study compared the cancer chemopreventive effects of EGCG and theaflavins in athymic nude mice implanted with androgen-sensitive CWR22Rnu1 cells (73). Results demonstrated that treatment with all the tea ingredients resulted in significant inhibition in growth of implanted prostate tumors; reduction in serum PSA levels; induction of apoptosis accompanied with up-regulation of Bax and decrease in Bcl-2 proteins and VEGF levels. In the same study when green tea polyphenols was provided to mice after establishment of CWR22Rnu1 tumor, a significant regression of tumors was observed.

In 1995, Greenberg and colleagues developed a transgenic mouse model for prostate cancer; the transgenic adenocarcinoma of the mouse prostate (TRAMP) (74). This animal model of prostate cancer was established on a genetic system to target heterologous gene expression specifically to the prostate epithelium and revolutionized prostate cancer research in preclinical trials. In one of the earliest studies conducted on male TRAMP mice in 2001, Gupta *et al*. reported that oral infusion of green tea polyphenols alone was sufficient to inhibit prostate carcinogenesis (75). TRAMP mice between 8 to 32 weeks of age received 0.1% oral infusion of a polyphenolic fraction isolated from green tea, which corresponds to drinking 6 cups of green tea per day by an adult individual. In two separate experiments, the cumulative incidence of palpable tumors at 32 weeks of untreated mice was 100% (20 of 20) with the mice developing distant site metastases to lymph nodes (95%), lungs (65%), liver (40%) and bone (25%). Oral infusion of green tea polyphenols resulted in significant delay in primary tumor incidence and tumor burden; decrease in prostate (64%) and genitourinary (GU) (72%) weight; inhibition in serum insulin-like growth factor-I (IGF-I); restoration of insulin-like growth factor binding protein-3 (IGFBP-3) levels and reduction in the protein expression of proliferating cell nuclear antigen (PCNA) in the prostate compared with control mice. Another striking observation of this study was that exposure with green tea polyphenols resulted in almost complete inhibition of distant site metastases and induction of apoptosis which possibly resulted in reduced dissemination of cancer cells, thereby causing inhibition of prostate cancer development and progression. Studies on the mechanistic action of green tea polyphenols in the TRAMP model showed downregulation of genes involved in angiogenesis *viz.* VEGF, and metastasis *viz.* MMP-2 and MMP-9 (76). Oral infusion with green tea polyphenols to TRAMP mice also resulted in significant inhibition of VEGF, MMP-2 and MMP-9 further elucidating the role of multiple target genes in prostate cancer (77). Another study elucidated the role of multiple pathways which are modulated by green tea in TRAMP mice. Evidence indicates that elevated levels of IGF-I with concomitant lowering of IGFBP-3 are associated with increased risk for prostate cancer development and progression (78). Increased levels of IGF-I, phosphatidylinositol 3'-kinase, phosphorylated Akt (Thr-308), and extracellular signal-regulated kinase (ERK)½ with concomitant decrease in IGFBP-3 in dorso-lateral prostate of TRAMP mice was observed during prostate cancer progression (79). Oral infusion with green tea polyphenols resulted in modulation of IGF/IGFBP-3 which was associated with inhibition of the protein expression of phosphatidylinositol 3'-kinase (PI3K), phosphorylated forms of Akt (Thr-308) and ERK½, VEGF, urokinase plasminogen activator, and matrix metalloproteinases-2 and -9. Another recent report on TRAMP mice has shown the involvement of metastasis-promoting Mts1 (S100A4) protein during prostate cancer progression in the dorsolateral prostate of TRAMP mice, but not in non-transgenic littermates (80). Oral infusion with green tea polyphenols to TRAMP mice resulted in marked inhibition of prostate cancer progression which was associated with reduction of S100A4 and restoration of E-cadherin.

In another study Caporali *et al*. reported that while 100% of TRAMP mice developed prostate cancer, only 20% of those fed 0.3% EGCG in drinking water developed neoplasm (81). This was attributed to the accumulation of clusterin (CLU) - a protein widely distributed in animal tissues and involved in many different processes, including apoptosis and neoplastic transformation. CLU is dramatically down-regulated with the onset and progression of prostate cancer. In EGCG-treated TRAMP mice where tumor progression was inhibited, CLU mRNA and protein progressively accumulated in the prostate gland. CLU levels were undetected in some animals which received EGCG and later showed emergence of prostate cancer. EGCG was also able to downregulate histone H3 and up-regulate growth arrest-specific gene 1 (Gas1) mRNAs levels in these mice.

Another study compared the efficacy of the anti-oxidants: natural antioxidant (NAO) from spinach extracts, EGCG from green tea polyphenols and synthetic antioxidant, N-acetylcysteine (NAC), and a vehicle, in slowing spontaneous tumorigenic progression in male TRAMP and wild-type mice after 5, 9, and 13 weeks of treatment (82). Prostatic histopathology, oxidative-stress blood markers and hyperplasia were evaluated. The effectivity of EGCG in reducing the severity and focalness of hyperplasia was highest in the ventral lobe. At 13 weeks EGCG also demonstrated a modest reduction in plasma peroxide levels.

4.3. Epidemiologic studies

Demographic studies indicate that the worldwide incidence and mortality of prostate cancer differs greatly,

with the highest incidence in the African-American population in the United States and the lowest incidence and mortality rates in Asian populations (Singapore, China and Japan) (83). Comparative geographic-pathologic autopsy studies on the frequency and histologic findings in patients with latent prostate carcinomas reveal that both populations have similar number of foci of prostate cancer, but the size of the foci and pattern types differ greatly (84). In countries with low incidence and mortality rate, the foci of the latent prostate carcinoma are small and show only limited tendency to proliferate, while in subjects from countries with high incidence and mortality rate, cancer foci are typically larger and histologically more aggressive in appearance, suggesting that the difference in clinical incidence of prostate cancer is not based on different initiation factors leading to malignant transformation, but on different promoting factors after malignancy has been established (85). A recent study published on cancer incidence, mortality, risk factors and screening for five of the largest Asian American ethnic groups in California in order of population size (Chinese, Filipino, Vietnamese, Korean, and Japanese) indicated that prostate cancer incidence and mortality were lowest among the Chinese Americans (86). Filipinos followed by Japanese Americans had the highest incidence and death rate from prostate cancer. Variations in risk factors were observed for prostate cancer and for most part consistent with variations in cancer incidence and mortality. Understanding the genetic and/or environmental basis for this difference with changing demographics may provide valuable information on suspected environmental factors for prostate cancer.

Epidemiological studies or the study of factors affecting the health and illness of populations has served as the foundation and logic of interventions made in the interest of public health and preventive medicine. Several epidemiological studies have been reported with green tea in the human population (87-90). Earlier studies indicate that people who consume tea regularly may have a decreased risk of prostate cancer (87, 88). These studies were carried out in men of Japanese ancestry where an inverse association of tea intake and prostate cancer incidence was reported (88). It has been suggested that the low occurrence of prostate cancer in Asian countries may be due, in part, to the consumption of green tea by these populations. A recent case-control study demonstrated the protective effect of green tea against prostate cancer (89). The study included 130 incident patients with histologically confirmed adenocarcinoma of the prostate while the controls were 274 hospital inpatients without prostate cancer or any other malignant diseases and matched to the age of prostate cancer cases. The adjusted odds ratio (OR), relative to non-tea drinkers, was 0.28 for tea drinking, 0.12 for those drinking tea over 40 years, 0.09 for those consuming more than 1.5 kg of tea leaves yearly, and 0.27 for those drinking more than 3 cups (1 liter) daily. The study concluded that the risk of prostate cancer declined with increasing frequency, duration and quantity of green tea consumption and that the dose response relationships were also significant when suggesting that green tea was protective against prostate cancer. Further continuation of the case-control study on prostate cancer and probing the possible joint effect of lycopene and green tea on prostate cancer risk was conducted (90). Interaction analysis showed that the protective effect from tea and lycopene consumption was synergistic ($p<0.01$) and their study concluded that in Chinese men regularly drinking tea and consuming vegetables and fruits rich in lycopene led to a reduced risk of prostate cancer.

4.4. Clinical trials

Cancer prevention clinical trials are biomedical-related research studies in humans that use a systematic and phased approach along pre-defined protocols to investigate the efficacy of natural and/or synthetic agents (91). This phased approach includes: phase I studies on dose-related safety and toxicity; phase II studies on efficacy in a small subset of individuals at high-risk for either a specific cancer or the presence of biomarkers; and phase III studies on large, randomized, double-blinded, placebo-controlled trials conducted in a large population. Agents, including both food constituents and drugs, that proceeds successfully through each of the first two phases represent the best hope for use in large phase III prevention clinical trials. To date, strategies for developing prevention clinical trials for prostate cancer have focused primarily on prevention by hormonal modulation and through the use of natural and synthetic bioactive food components (92).

Studies have shown that polyphenols and theaflavins from both green as well as black tea are bio-available and bioactive in human serum and human tissues (93). In this study 20 men scheduled for surgical prostatectomy were randomly assigned to consume 1.42 liters daily of green tea, black tea, or a caffeine-matched soda control for 5 days before radical prostatectomy. Higher concentration of tea polyphenols were observed in prostate specimens from men consuming tea than in men consuming caffeine-matched soda. However, no detectable levels of polyphenols were found in serum. Consumption of decaffeinated black tea or green tea also resulted in the accumulation of these polyphenols in various organs including the small and large intestine, liver, and prostate in conjugated and free forms (93, 94).

Previous studies on phase I clinical trials with green tea polyphenols include dose-escalation studies on the bioavailability and toxicity of green tea in humans (95-98). The oral bioavailability of tea catechins was found to be low in humans, resulting in plasma concentrations 5 to 50 times less than concentrations shown to exert biological activities in *in vitro* systems (95). Number of factors may affect the oral bioavailability of green tea catechins and subsequently their biological responses. A Phase I pharmacokinetic study to determine the systemic availability of green tea polyphenols after a single oral dose administration of EGCG and Polyphenon E (71% EGCG, 7.8% EGC, 14% EC, and 7.2% ECG) on participants randomly assigned to one of the dose levels (200, 400, 600, and 800 mg based on EGCG content) concluded that there were no significant differences in the pharmacokinetic characteristics of EGCG between the two study medications. The study participants had similar plasma EGCG levels and the single oral dose over the dose range

studied was well tolerated (96). A subsequent study on the pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of EGCG and polyphenon E (800 mg EGCG once/day, 400 mg EGCG twice/day, 800 mg EGCG as Polyphenon E once/day, 400 mg EGCG as Polyphenon E twice/day, or a placebo once/day) in healthy individuals concluded that it was safe for healthy individuals to take green tea polyphenol products in amounts equivalent to the EGCG content in 8-16 cups of green tea once a day or in divided doses twice a day. There was a >60% increase in the systemic availability of free EGCG after long term green tea polyphenol administration at a high daily bolus dose of 800 mg EGCG or Polyphenon E once daily (97). Studies on the dosing effects and oral bioavailability of green tea polyphenols after single-dose administration of Polyphenon E (400, 800, or 1200 mg) concluded greater oral bioavailability of free catechins could be achieved by taking Polyphenon E capsules on an empty stomach after an overnight fasting. Polyphenon E up to a dose that contains 800 mg EGCG was shown to be well-tolerated when taken under fasting condition. This dosing condition optimizes the biological effects of tea catechins (98).

One of the earliest phase II clinical trials in which 6 g/day of tea was administered to 42 patients with asymptomatic, androgen-independent prostate cancer demonstrated that a single patient achieved a PSA response of >50% that lasted for approximately one month. These patients suffered with side-effects that include diarrhea, nausea and fatigue (99). Another clinical study used 250 mg dose of green tea polyphenols twice daily. In this study, 6 out of 19 patients had disease control for 3 to 5 months and only one patient whose PSA rise was affected by green tea supplementation. The dose used in this study did not discernibly alter the course of hormone-refractory prostate cancer (100). These results suggest that green tea possibly possess cancer chemopreventive properties and minimal anti-neoplastic activity against advance-stage prostate cancer.

An interesting year long clinical trial conducted in Italy, studied the effects of green tea polyphenols in males with high-grade prostate intraepithelial neoplasia, HGPIN (101). Sixty volunteers with HGPIN received three green tea polyphenol capsules of 200 mg, three times per day constituting a total polyphenol of 600 mg/day. Following one year of treatment, only 3% of patients that received the green tea polyphenols were diagnosed with cancer compared with 30% in the placebo group. Furthermore, patients that received green tea capsules exhibited a longer latency to tumor detection and exhibited an improved quality of life. This was the first study showing that green tea polyphenols were safe and effective for treatment of pre-malignant lesions before progression of cancer and could be developed as a chemopreventive agent for prostate cancer. Since then a number of ongoing clinical trials on prostate cancer have been documented in the United States.

A recent phase II study is ongoing which designed to determine the effects of short-term supplementation with the active compounds in green tea on c-Met and other signaling pathways in tissue and serum biomarkers in patients with prostate cancer (102). Men with positive prostate biopsies scheduled for surgery were provided daily doses of Polyphenon E, which contained 800 mg of EGCG and lesser amounts of EC, EGC and ECG and treatment was continued until time of radical prostatectomy. Records from the first 14 available subjects were used after an average of 5 weeks. Levels of serum hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) were significantly lower after treatment, $p=0.02$ and $p=0.03$, respectively. PSA levels decreased ($p=0.07$) and 90% of the men had decreasing PSA levels ($p=0.021$). No significant differences in IGF-I, IGFBP-3, or IL-8 levels were observed. Tissue specimens from treated patients stained by immunohistochemistry were compared against untreated retrospective control prostate cancer patients for p-AKT and p-Met. Results demonstrate that the patients treated with Polyphenon E had lower tissue levels of p-Met ($p<0.04$) and p-AKT ($p<0.02$) staining, compared to the retrospective untreated control patients. In conclusion, a significant decrease in HGF, VEGF and PSA in serum and p-AKT and p-Met in tissue was observed in patients who were maintained on Polyphenon E (containing 800 mg of EGCG) per day, from the time of biopsy to resection. Despite small patient numbers, these observations suggest that green tea polyphenols inhibit cellular signaling which contributes to prostate cancer progression.

Another ongoing interventional, randomized, double-blind, placebo-controlled phase Ib study of green tea polyphenols (Polyphenon E) in a pre-prostatectomy prostate cancer study, funded by National Cancer Institute, is currently recruiting patients at the University of Arizona (103). The study being led by Dr. Frederick R. Ahmann, aims to determine the bioavailability of green tea catechins and changes in clusterin, MMP-2 and MMP-9 in prostate tissue, serum IGF-I and IGFBP-3 and oxidative DNA damage in peripheral blood leukocytes.

Another phase II randomized, double-blinded placebo-controlled study with green tea polyphenols (Polyphenon E) in men with localized prostate cancer scheduled for radical prostatectomy is being conducted by Dr. Sanjay Gupta and colleagues at Case Western Reserve University and University Hospitals Case Medical Center which is currently recruiting patients for the study (104). Based on the results of phase I studies, patients with localized prostate cancer will receive 4 capsules of 200 mg each of Polyphenon E or placebo (800 mg total polyphenol/day) in the interval between prostate biopsy (pre-treatment) and radical prostatectomy (approximately 6 weeks, post-treatment). The study is designed to compare changes in levels of cellular proliferation, survival, angiogenesis markers *viz.* Ki-67, Bcl-2, cyclin D, KiP1/p27, VEGF, and CD31, in biopsy (pre-treatment) and prostatectomy (post-treatment) specimens to evaluate disease characteristic and develop biomarkers which could predict the efficacy of green tea polyphenols. Through this clinical trial the bioavailability of catechins from Polyphenon E in tissues and plasma as well as catechins

metabolites in urine samples will be determined. This study will also assess the safety and tolerability of Polyphenon E in these patients.

5. CONCLUSION AND FUTURE DIRECTIONS

A decade of research with green tea polyphenols in cell culture systems, pre-clinical models *viz.* mouse tumor xenograft and transgenic mouse models (especially the TRAMP) have proven that green tea polyphenols can influence a number of molecular pathways that has relevance to tumor growth, proliferation and metastasis. Polyphenols from green tea has been shown to modulate some critical targets in the prostate which include p53, WAF1/p21, KIP1/p27, INK4a/p16, INK4c/p18, cyclin D1 and cyclin E, cyclin-dependent kinases cdk2, cdk4 and cdk6, COX-2, MDM2, IGF-I, IGFBP-3 and NF-κB. Green tea polyphenols can increase reactive oxygen species production causing mitochondrial depolarization, inhibit proteasome and fatty acid synthase leading to induction of apoptosis. Although green tea polyphenols inhibit and reverse tumorigenesis in the post-initiation stages, they also hold therapeutic potential through their ability to specifically kill cancer cells without affecting the growth of normal cells. It is only now with the development of sophisticated investigational techniques that the various molecular signaling pathways modulated by them are being understood. A relatively new aspect of the effect of green tea polyphenols which is gradually gaining the attention of both researchers and clinicians is their ability to reverse epigenetic gene silencing during cancer initiation and progression.

The need of the hour for chemoprevention studies in prostate cancer to continue is the development and validation of biomarker(s). These biomarkers then need to be integrated with ongoing, mechanistic, *in vitro* and *in vivo* studies to evaluate the chemopreventive efficacy of green tea in prostate cancer and later validated in clinical settings. DNA methylation assays show potential to be developed into sensitive and diagnostic tests for prostate cancer, however the appropriate biomarker to be used still needs to be ascertained. *GSTP1* is a gene that is hypermethylated and transcriptionally silenced in HGPIN and prostate cancer but not in PIA and may potentially be developed as a biomarker for certain stages of prostate cancer. Studies in our laboratory indicate that green tea polyphenols are capable to reversing epigenetically silenced *GSTP1*gene in human prostate cancer cells. Despite the evidence of its potential beneficial effects in prostate cancer, incorporation of green tea polyphenols into clinical trials of prostate cancer chemoprevention has not been widely accepted. No appropriate biomarker of the various pathways that are influenced by green tea polyphenols has been developed. Measurement of serum PSA levels still remains the gold standard for detecting and monitoring prostate cancer even though deaths from prostate cancer have marginally decreased. In addition, PSA testing fails to identify a small but significant proportion of aggressive cancers and only about 30% of men with a 'positive' PSA have a positive biopsy, 25% of men treated for prostate cancer require additional treatment due to disease recurrence, while in some men prostate cancer remains indolent. In these individuals, prostate cancer treatment is unnecessary and harmful as these men do not benefit from treatment and are at a risk of treatment-related side-effects and complications. Thus, the challenge now is to develop improved diagnostic and prognostic biomarkers for prostate cancer that need to be validated so that the use of these markers can ultimately translate into increased life-span and quality of life.

Phase I clinical studies have concluded that a daily dose of 800 mg EGCG is well tolerated in humans with bioactive quantities of EGCG being available in the prostate. However, results from two completed clinical trials indicate that green tea polyphenols do not have a significant effect on androgen-refractory prostate cancer. Phase II clinical trials that address this are currently ongoing and preliminary results from these trials suggests that green tea polyphenols may be more effective in hormone-responsive prostate cancer, when prostatic lesions are in still in the pre-malignant stages. It is important to identify and validate biomarkers for prostate cancer that are modified by consumption of green tea polyphenols and correlate them with pathological changes in the prostate. It would be ideal if these biomarkers were specific for various biologic stages of prostate cancer: initiation, progression, metastasis, and development of the hormone refractory state. Overall, recent attention on green tea and prostate cancer through phase II clinical trials will be to address issues related to compliance, bioavailability, identification of stage-specific biomarkers and above all safety evaluations. Completion of these trials will provide useful information and will aid in moving forward to phase III clinical trials leading to the development of green tea polyphenols as chemopreventive agent for prostate cancer and its translation from bench to clinic.

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7. REFERENCES

1. Prostate cancer incidence, at www.prostatecancerfoundation.org (2007)

2. American Cancer Society. Cancer Statistics, (2007)

3. A. van Bokhoven, M. Varella-Garcia, C. Korch, W. U. Johannes, E. Smith, H. L. Miller, S. K. Nordeen, G. J. Miller and M. Scott Lucia: Molecular Characterization of Human Prostate Carcinoma Cell Lines. *The Prostate* 57, 205-225 (2003)

4. S. Signoretti, D. Waltregny, J. Dilks, B. Isaac, D. Lin, L. Garraway, A. Yang, R. Montironi, F. McKeon and M.

Loda: p63 is a prostate basal cell marker and is required for prostate development. *Am. J. Pathol* 157, 1769–1775 (2000)

5. G. J. van Leenders, H. Dijkman, C. A. Hulsbergen-van de Kaa, D. J. Ruiter and J. A. Schalken: Demonstration of intermediate cells during human prostate epithelial differentiation *in situ* and *in vitro* using triple-staining confocal scanning microscopy. *Lab Invest* 80, 1251–1258 (2000)

6. H. Bonkhoff and K. Remberger: Differentiation pathways and histogenetic aspects of normal and abnormal prostatic growth: A stem cell model. *Prostate* 28, 98–106 (1996)

7. J. S. Lam and R. E. Reiter: Stem cells in prostate and Prostate Cancer development. *Urol Oncol* 24, 131-140 (2006)

8. W. G. Nelson, S. Yegnasubramanian, A. T. Agoston, P. J. Bastian, B. H. Lee, M. Nakayama and A. M. De Marzo: Abnormal DNA methylation, epigenetics, and prostate cancer. *Front Biosci* 12, 4254-4266 (2007)

9. W. A. Schulz and J. Hatina: Epigenetics of prostate cancer: beyond DNA methylation. *J Cell Mol Med* 10, 100-125 (2006)

10. A. M. De Marzo, V. L. Marchi, J. I. Epstein and W. G. Nelson: Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. *Am J Pathol* 155, 1985-1992 (1999)

11. G. T. MacLennan, R. Eisenberg, R. L. Fleshman, J. M. Taylor, P. Fu, M. I. Resnick and S. Gupta: The influence of chronic inflammation in prostatic carcinogenesis: a 5-year followup study. *J Urol* 176, 1012-1016 (2006)

12. F. Algaba, I. Trias and Y. Arce: Natural history of prostatic carcinoma: the pathologist's perspective. *Recent Results Cancer Res* 175, 9-24 (2007)

13. K. A. Iczkowski: Current prostate biopsy interpretation: criteria for cancer, atypical small acinar proliferation, high-grade prostatic intraepithelial neoplasia, and use of immunostains. *Arch Pathol Lab Med* 130, 835-843 (2006)

14. D. F. Gleason: Histologic grading of prostate cancer: a perspective. *Hum Pathol* 23, 273-279 (1992)

15. G. Sanchez-Visconti, L. Herrero, M. Rabadan, I. Pereira and A. Ruiz-Torres: Ageing and prostate: age-related changes in androgen receptors of epithelial cells from benign hypertrophic glands compared with cancer. *Mech Ageing Dev* 82, 19-29 (1995)

16. O. W. Brawley, A. B. Jani and V. Master: Prostate cancer and race. *Curr Probl Cancer* 31, 211-225 (2007)

17. J. Carpten, N. Nupponen, S. Isaacs, R. Sood, C. Robbins, J. Xu, M. Faruque, T. Moses, C. Ewing, E. Gillanders, P. Hu, P. Bujnovszky, I. Makalowska, A. Baffoe-Bonnie, D. Faith, J. Smith, D. Stephan, K. Wiley, M. Brownstein, D. Gildea, B. Kelly, R. Jenkins, G. Hostetter, M. Matikainen, J. Schleutker, K. Klinger, T. Connors, Y. Xiang, Z. Wang, A. De Marzo, N. Papadopoulos, O. P. Kallioniemi, R. Burk, D. Meyers, H. Gronberg, P. Meltzer, R. Silverman, J. Bailey-Wilson, P. Walsh, W. Isaacs and J Trent: Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. *Nat Genet* 30, 181-184. (2002)

18. E. A. Klein, G. Casey and R. Silverman: Genetic susceptibility and oxidative stress in prostate cancer: integrated model with implications for prevention. *Urology* 68, 1145-1151 (2006)

19. A. Urisman, R. J. Molinaro, N. Fischer, S. J. Plummer, G. Casey, E. A. Klein, K. Malathi, C. Magi-Galluzzi, R. R. Tubbs, D. Ganem, R. H. Silverman and J. L. DeRisi: Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. *PLoS Pathog* 2, 0211-0225 (2006)

20. S. Halabi, S. S. Ou, N. J. Vogelzang and E. J. Small: Inverse correlation between body mass index and clinical outcomes in men with advanced castration-recurrent prostate cancer. *Cancer* 110, 1478-1484 (2007)

21. D. M. Werny, T. Thompson, M. Saraiya, D. Freedman, B. J. Kottiri, R. R. German and M. Wener: Obesity is negatively associated with prostate-specific antigen in U.S. men, 2001-2004. *Cancer Epidemiol Biomarkers Prev* 16, 70-76 (2007)

22. A. H. Lee, M. L. Fraser, X. Meng and C. W. Binns: Protective effects of green tea against prostate cancer. *Expert Rev Anticancer Ther* 6, 507-513 (2006)

23. R. L. Thangapazham, A. K. Singh, A. Sharma, J. Warren, J. P. Gaddipati and R. K. Maheshwari: Green tea polyphenols and its constituent epigallocatechin gallate inhibits proliferation of human breast cancer cells *in vitro* and *in vivo*. *Cancer Lett* 245, 232-241 (2007)

24. S. Gupta and H. Mukhtar: Green tea and prostate cancer. *Urol Clin North Am* 29, 49-57 (2002)

25. M. G. L. Hertog, P. M. Sweetnam, A. M. Fehily, P. C. Elwood and D. Kromhout: Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. *Am J Clin Nutr* 65, 1489- 1494 (1997)

26. M. L. Brandi: Flavonoids: biochemical effects and therapeutic applications. *Bone Miner* 19, S3-S14 (1992).

27. A. Bu-Abbas, M. N. Clifford, R. Walker and C. Ioannides: Marked antimutagenic potential of aqueous green tea extracts: mechanism of action. *Mutagenesis* 9, 325-331 (1994)

28. B. Stavric, T. I. Matula, R. Klassen and R. H. Downie: The effect of teas on the *in vitro* mutagenic potential of

heterocyclic aromatic amines. *Food Chem Toxicol 34*, 515-523 (1996)

29. J. K. Lin, Y. C. Liang and S. Y. Lin-Shiau: Cancer chemoprevention by tea polyphenols through mitotic signal transduction blockade. *Biochem Pharmacol* 58, 911-915 (1999)

30. H. Hibasami, Y. Achiwa, J. Fujikawa and T. Komiya: Induction of programmed cell death (apoptosis) in human lymphoid leukemia cells by catechin compounds. A*nticancer Rex* 16, 1943-1950 (1996)

31. R. Yu, J. J. Jiao, J. L. Duh, K. Gudehithlu, T. H. Tan and A. N. Kong: Activation of mitogen-activated protein kinases by green tea polyphenols: potential signalling pathways in the regulation of antioxidant-responsive element-mediated phase I1 enzyme gene expression. *Carcinogenesis* 18, 451-456 (1997)

32. A. Rietveld and S. Wiseman: Antioxidant effects of tea: evidence from human clinical trials. *J Nutr* 133, 3285S-3292S (2003)

33. N. Ahmad, D. K. Feyes, A. L. Nieminen, R. Agarwal and H. Mukhtar: 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 (1997)

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

35. K. Anwar, K. Nakakuki, T. Shiraishi, H. Naiki, R. Yatani and M. Inuzuka: Presence of ras oncogene mutations and human papillomavirus DNA in human prostate carcinomas. *Cancer Res* 52, 5991-5996 (1992)

36. B. D. Lyn-Cook, T. Rogers, Y. Yan, E. B. Blann, F. F. Kadlubar and G. J. Hammons: Chemopreventive effects of tea extracts and various components on human pancreatic and prostate tumor cells *in vitro*. *Nutr Cancer* 35, 80-86 (1999)

37. S. Gupta, N. Ahmad, A. L. Nieminen and H. Mukhtar: 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 (2000)

38. S. Gupta, T. Hussain and H. Mukhtar: Molecular pathway for (-)-epigallocatechin-3-gallate-induced cell cycle arrest and apoptosis of human prostate carcinoma cells. *Arch Biochem Biophys* 410, 177-185 (2003)

39. K. Hastak, S. Gupta, N. Ahmad, M. K. Agarwal, M. L. Agarwal and H. Mukhtar: Role of p53 and NF-kappaB in epigallocatechin-3-gallate-induced apoptosis of LNCaP cells. *Oncogene* 22, 4851-4859 (2003)

40. S. Gupta, K. Hastak, F. Afaq, N. Ahmad and H. Mukhtar: Essential role of caspases in epigallocatechin-3-gallate-mediated inhibition of nuclear factor kappa B and induction of apoptosis. *Oncogene* 23, 2507-2522 (2004)

41. K. Hastak, M. K. Agarwal, H. Mukhtar and M. L. Agarwal: Ablation of either p21 or Bax prevents p53-dependent apoptosis induced by green tea polyphenol epigallocatechin-3-gallate. *FASEB J* 19, 789-791 (2005)

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

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

44. D. Vergote, C. Cren-Olive, V. Chopin, R. A. Toillon, C. Rolando, H. Hondermarck and X. Le Bourhis: (-)-Epigallocatechin (EGC) of green tea induces apoptosis of human breast cancer cells but not of their normal counterparts. *Breast Cancer Res Treat* 76, 195-201 (2002)

45. P. A. Townsend, T. M. Scarabelli, E. Pasini, G. Gitti, M. Menegazzi, H. Suzuki, R. A. Knight, D. S. Latchman and A. Stephanou: Epigallocatechin-3-gallate inhibits STAT-1 activation and protects cardiac myocytes from ischemia/reperfusion-induced apoptosis. *FASEB J* 18, 1621-1623 (2004)

46. M. H. Pan, C. C. Lin, J. K. Lin and W. J. Chen: Tea polyphenol (-)-epigallocatechin 3-gallate suppresses heregulin-beta1-induced fatty acid synthase expression in human breast cancer cells by inhibiting phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase cascade signaling. *J Agric Food Chem* 55, 5030-5037 (2007)

47. M. A. Shammas, P. Neri, H. Koley, R. B. Batchu, R. C. Bertheau, V. Munshi, R. Prabhala, M. Fulciniti, Y. T. Tai, S. P. Treon, R. K. Goyal, K. C. Anderson and N. C. Munshi: Specific killing of multiple myeloma cells by (-)-epigallocatechin-3-gallate extracted from green tea: biologic activity and therapeutic implications. *Blood* 108, 2804-2810 (2006)

48. K. Brusselmans, E. De Schrijver, W. Heyns, G. Verhoeven and J. V. Swinnen: Epigallocatechin-3-gallate is a potent natural inhibitor of fatty acid synthase in intact cells and selectively induces apoptosis in prostate cancer cells. *Int J Cancer* 106, 856-862 (2003)

49. M. Yashi, O. Muraishi, Y. Kobayashi, A. Tokue and H. Nanjo: Elevated serum progastrin-releasing peptide (31-98) in metastatic and androgen-independent prostate cancer patients. *Prostate* 51, 84-97 (2002)

50. E. Pezzato, M. Dona, L. Sartor, I. Dell'Aica, R. Benelli, A. Albini and S. Garbisa: Proteinase-3 directly

activates MMP-2 and degrades gelatin and Matrigel; differential inhibition by (-) epigallocatechin-3-gallate. *J Leukoc Biol* 74, 88-94 (2003)

51. I. Dell'Aica, M. Dona, L. Sartor, E. Pezzat and S. Garbisa: (-) Epigallocatechin-3-gallate directly inhibits MT1-MMP activity, leading to accumulation of nonactivated MMP-2 at the cell surface. *Lab Invest* 82, 1685-1693 (2002)

52. E. Pezzato, L. Sartor, I. Dell'Aica, R. Dittadi, M. Gion, C. Belluco, M. Lise and S. Garbisa: Prostate carcinoma and green tea: PSA-triggered basement membrane degradation and MMP-2 activation are inhibited by (-)epigallocatechin-3-gallate. *Int J Cancer* 112, 787-792 (2004)

53. G. L. Semenza: Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* 3, 721–732 (2003)

54. R. Thomas and M. H. Kim: Epigallocatechin gallate inhibits HIF-1alpha degradation in prostate cancer cells. *Biochem Biophys Res Commun* 334, 543-548 (2005)

55. T. Hussain, S. Gupta and H. Mukhtar: Cyclooxygenase-2 and prostate carcinogenesis. *Cancer Lett* 191, 125-135 (2003)

56. S. Zha, W. R. Gage, J. Sauvageot, E. A. Saria, M. J. Putzi, C. M. Ewing, D. A. Faith, W. G. Nelson, A. M. De Marzo and W. B. Isaacs: Cyclooxygenase-2 is up-regulated in proliferative inflammatory atrophy of the prostate, but not in prostate carcinoma. *Cancer Res* 61, 8617-8623 (2001)

57. H. Fujita, K. Koshida, E. T. Keller, Y. Takahashi, T. Yoshimito, M. Namiki and A. Mizokami: Cyclooxygenase-2 promotes prostate cancer progression. *Prostate* 53, 232-240 (2002)

58. T. Hussain, S. Gupta, V. M. Adhami and H. Mukhtar: Green tea constituent epigallocatechin-3-gallate selectively inhibits COX-2 without affecting COX-1 expression in human prostate carcinoma cells. *Int J Cancer* 113, 660-669 (2005)

59. C. E. Harper, B. B. Patel, J. Wang, I. A. Eltoum, and C. A. Lamartiniere: Epigallocatechin-3-Gallate suppresses early stage, but not late stage prostate cancer in TRAMP mice: Mechanisms of action. *Prostate* 67, 1576-1589 (2007)

60. V. M. Adhami, A. Malik, N. Zaman, S. Sarfaraz, I. A. Siddiqui, D. N. Syed, F. Afaq, F. S. Pasha, M. Saleem and H. Mukhtar: Combined inhibitory effects of green tea polyphenols and selective cyclooxygenase-2 inhibitors on the growth of human prostate cancer cells both *in vito* and *in vivo*. *Clin Cancer Res* 13, 1611-1619 (2007)

61. M. Z. Fang, Y. Wang, N. Ai, Z. Hou, Y. Sun, H. Lu, W. Welsh and C. S. Yang: Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Cancer Res* 63, 7563-7570 (2003)

62. M. Fang, D. Chen and C. S. Yang: Dietary polyphenols may affect DNA methylation. *J Nutr* 137, 223S-228S (2007)

63. W. J. Lee, J. Y. Shim and B. T. Zhu: Mechanisms for the inhibition of DNA methyltransferases by tea catechins and bioflavonoids. *Mol Pharmacol* 68, 1018-10130 (2005)

64. M. Pandey and S. Gupta: Green tea polyphenols inhibit promoter hypermethylation through downregulation of DNMT expression in prostate cancer LNCaP cells. Late Breaking Abstract-LB212 *Proc Amer Assoc Cancer Res* 98, 53 (2007)

65. S. Liao: The medicinal action of androgens and green tea epigallocatechin gallate. *Hong Kong Med J* 7, 369-374 (2001)

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

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

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

69. J. R. Zhou, L. Yu, Y. Zhong and G. L. Blackburn: Soy phytochemicals and tea bioactive components synergistically inhibit androgen-sensitive human prostate tumors in mice. *J Nutr* 133, 516-521 (2003)

70. R. R. Mohan, A. Challa, S. Gupta, D. G. Bostwick, N. Ahmad, R. Agarwal, S. R. Marengo, S. B. Amini, F. Paras, G. T. MacLennan, M. I. Resnick and H. Mukhtar: Overexpression of ornithine decarboxylase in prostate cancer and prostatic fluid in humans. *Clin Cancer Res* 5, 143-147 (1999)

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

72. M. W. Roomi, V. Ivanov, T. Kalinovsky, A. Niedzwiecki and M. Rath: *In vivo* anti-tumor effect of ascorbic acid, lysine, proline and green tea extract on human prostate cancer PC-3 xenografts in nude mice: evaluation of tumor growth and immunohistochemistry. *In vivo* 19, 179-183 (2005)

73. I. A. Siddiqui, N. Zaman, M. H. Aziz, S. R. Reagan-Shaw, S. Sarfaraz, V. M. Adhami, N. Ahmad, S. Raisuddin and H. Mukhtar: Inhibition of CWR22Rnu1 tumor growth and PSA secretion in athymic nude mice

by green and black teas. *Carcinogenesis* 27, 833-839 (2006)

74. N. M. Greenberg, F. DeMayo, M. J. Finegold, D. Medina, W. D. Tilley, J. 0. Aspinall, G. R. Cunha, A. A. Donjacour, R. J. Matusik and J. M. Rosen: Prostate cancer in a transgenic mouse. *Proc Natl Acad Sci USA* 92, 3439-3443 (1995)

75. S. Gupta, K. Hastak, N. Ahmad, J. S. Lewin and H. Mukhtar: Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols. *Proc Natl Acad Sci USA* 98, 10350-10355 (2001)

76. V. M. Adhami, N. Ahmad and Mukhtar, H: Molecular targets for green tea in Prostate Cancer prevention. *J Nutr* 133, 2417S-2424S (2003)

77. M. Saleem, V. M. Adhami, I. A. Siddiqui and H. Mukhtar. Tea beverage in chemoprevention of Prostate Cancer: a mini-review. *Nutr Cancer* 47, 13-23 (2003)

78. J. V. Silha, P. C. Sheppard, S. Mishra, Y. Gui, J. Schwartz, J. G. Dodd and L. J. Murphy: Insulin-like growth factor (IGF) binding protein-3 attenuates prostate tumor growth by IGF-dependent and IGF-independent mechanisms. *Endocrinology* 147, 2112-2121 (2006)

79. V. M. Adhami, I. A. Siddiqui, N. Ahmad, S. Gupta and H. Mukhtar: Oral consumption of green tea polyphenols inhibits insulin-like growth factor-I-induced signaling in an autochthonous mouse model of Prostate Cancer. *Cancer Res* 64, 8715-8722 (2004)

80. M. Saleem, V. M. Adhami, N. Ahmad, S. Gupta and H. Mukhtar: Prognostic significance of metastasis-associated protein S100A4 (Mts1) in Prostate Cancer progression and chemoprevention regimens in an autochthonous mouse model. *Clin Cancer Res* 11, 147-153 (2005)

81. A. Caporali, P. Davalli, S. Astancolle, D. D'Arca, M. Brausi, S. Bettuzzi and A. Corti: The chemopreventive action of catechins in the TRAMP mouse model of prostate carcinogenesis is accompanied by clusterin over-expression. *Carcinogenesis* 25, 2217-2224 (2004)

82. A. Nyska, A. Suttie, S. Bakshi, L. Lomnitski, S. Grossman, M. Bergman, V. Ben-Shaul, P. Crocket, J. K. Haseman, G. Moser, T. L. Goldsworthy and R. R. Maronpot: Slowing tumorigenic progression in TRAMP mice and prostatic carcinoma cell lines using natural anti-oxidant from spinach, NAO--a comparative study of three anti-oxidants. *Toxicol Pathol* 31, 39-51 (2003)

83. C. J. Mettlin and G. Murphy. The National Cancer Data Base report on prostate cancer. *Cancer* 74, 1640-1648 (1994)

84. W. A. Sakr and D. J. Grignon: Prostatic intraepithelial neoplasia and atypical adenomatous hyperplasia: Relationship to pathologic parameters, volume and spatial distribution of carcinoma of the prostate. *Anal Quant Cytol Histol* 20, 417-423 (1998)

85. I. Cheng, M. C. Yu, W. P. Koh, M. C. Pike, L. N. Kolonel, B. E. Henderson and D. O Stram: Comparison of prostate-specific antigen and hormone levels among men in Singapore and the United States. *Cancer Epidemiol Biomarkers Prev* 14, 1692-1696 (2005)

86. M. McCracken, M. Olsen, M. S. Chen Jr, A. Jemal, M. Thun, V. Cokkinides, D. Deapen and E. Ward: Cancer incidence, mortality, and associated risk factors among Asian Americans of Chinese, Filipino, Vietnamese, Korean, and Japanese ethnicities. *CA Cancer J Clin* 57, 190-205 (2007)

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

88. L. J. Kinlen, A. N. Willows, P. Goldblatt and J. Yudkin: Tea consumption and cancer. *Br J Cancer* 58, 397-401 (1988)

89. L. Jian, L. P. Xie, A. H. Lee and C. W. Binns: Protective effect of green tea against Prostate Cancer: a case-control study in southeast China. *Int J Cancer* 108, 130-135 (2004)

90. L. Jian, A. H. Lee and C. W. Binns: Tea and lycopene protect against prostate cancer. *Asia Pac J Clin Nutr* 16, 453-457 (2007)

91. I. M. Thompson, C. M. Tangen, E. A. Klein and S. M. Lippman: Phase III prostate cancer prevention trials: are the costs justified? *J Clin Oncol* 23, 8161-8164 (2005)

92. P. Greenwald: Clinical trials in cancer prevention: current results and perspectives for the future. *J Nutr* 134, 3507S-3512S (2004)

93. S. M. Henning, W. Aronson, Y. Niu, F. Conde, N. H. Lee, N. P. Seeram, R. P. Lee, J. Lu, D. M. Harris, A. Moro, J. Hong, L. Pak-Shan, R. J. Barnard, H. G. Ziaee, G. Csathy, V. L. Go, H. Wang and D. Heber: Tea polyphenols and theaflavins are present in prostate tissue of humans and mice after green and black tea consumption. *J Nutr* 136, 1839-1843 (2006)

94. M. J. Lee, P. Maliakal, L. Chen, X. Meng, F. Y. Bondoc, S. Prabhu, G. Lambert, S. Mohr and C. S. Yang: Pharmacokinetics of tea catechins after ingestion of green tea and (-)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. *Cancer Epidemiol Biomarkers Prev* 11, 1025-1032 (2002)

95. K. Nakagawa, S. Okuda and T. Miyazawa: Dose-dependent incorporation of tea catechins, (-)-epigallocatechin-3-gallate and (-)-epigallocatechin, into human plasma. *Biosci Biotechnol Biochem* 61, 1981-1985 (1997)

96. H. H. Chow, Y. Cai, D. S. Alberts, I. Hakim, R. Dorr, F. Shahi, J. A. Crowell, C. S. Yang and Y. Hara: Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. *Cancer Epidemiol Biomarkers Prev* 10, 53-58 (2001)

97. H. H. Chow, Y. Cai, I. A. Hakim, J. A. Crowell, F. Shahi, C. A. Brooks, R. T. Dorr, Y. Hara and DS Alberts: Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. *Clin Cancer Res* 9, 3312-3319 (2003)

98. H. H. Chow, I. A. Hakim, D. R. Vining, J. A. Crowell, J. Ranger-Moore, W. M. Chew, C. A. Celaya, S. R. Rodney, Y. Hara and D. S. Alberts: Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. *Clin Cancer Res* 11, 4627-4633 (2005)

99. A. Jatoi, N. Ellison, P. A. Burch, J. A. Sloan, S. R. Dakhil, P. Novotny, W. Tan, T. R. Fitch, K. M. Rowland, C. Y. F. Young and P. J. Flynn: A phase II trial of green tea in the treatment of patients with androgen independent metastatic prostate carcinoma. *Cancer* 97, 1442–1446 (2003)

100. E. Choan, R. Segal, D. Jonker, S. Malone, N. Reaume, L. Eapen and V. Gallant: A prospective clinical trial of green tea for hormone refractory Prostate Cancer: an evaluation of the complementary/alternative therapy approach. *Urol Oncol* 23, 108-113 (2005)

101. S. Bettuzzi, M. Brausi, F. Rizzi, G. Castagnetti, G. Peracchia and A. Corti: Chemoprevention of human Prostate Cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. *Cancer Res* 66, 1234-1240 (2006)

102. J. McLarty, R. L. Bigelow, B. J. Williams, D. Elmajian, M. Ankem, M. Smith, C. Yang and J. A. Cardelli: Prostate cancer patients administered green tea polyphenols demonstrate a reduction in serum levels of HGF and VEGF, and tissue levels of phosphorylated c-Met and phosphorylated Akt. *Proc Amer Assoc Cancer Res* 98, 2657 (2007)

103. Prostate Cancer Clinical Trials on the web: ClinicalTrials.gov identifier: NCT00459407

104. Clinical Trials on the web: CASE13805, http://henge.case.edu/sip/SIPControlServlet

Abbreviations: PIN, prostatic intraepithelial neoplasia; PIA, proliferative inflammatory atrophy; XMRV, xenotropic MuLV-related virus; PSA, prostate-specific antigen; cki, cyclin kinase inhibitor; cdk, cyclin-cyclin-dependent kinase; MDM2, murine double minute 2 protein; NF-κB, Nuclear Factor-kappaB; FAS, fatty acid synthase; HIF, hypoxia-inducible factor; COX, Cyclooxygenase; DNMT, DNA methyltransferase; ODC, ornithine decarboxylase; PCNA, proliferating cell nuclear antigen; TRAMP, transgenic adenocarcinoma of the mouse prostate; MMP, matrix metalloproteinases; VEGF, vascular endothelial growth factor; PI3K, phosphatidylinositol 3'-kinase; ERK, extracellular signal-regulated kinase; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; CLU, clusterin; Gas, growth arrest-specific gene; NAC, N-acetylcysteine; GSTP, glutathione-S-transferase pi; EGCG, epigallocatechin-3-gallate; ECG, epicatechin gallate; EGC, epigallocatechin; EC, epicatechin; RASSF1, Ras association (RalGDS/AF-6) domain family 1

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