Utilizing Micronutrients as a Chemopreventive Strategy for Prostate Cancer

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Abstract

Prostate cancer (PCa) is the most common internal malignancy and the third most frequent cause of cancer death in men. It is estimated that in each week of 2009 over 490 Canadian men will be diagnosed with PCa, and 85 will die from the disease. The introduction of PSA (prostate-specific antigen) screening tests has contributed in part, to men being diagnosed and treated at an early stage. Chemoprevention is a strategy whereby patients are administered drugs, vitamins, or other agents to prevent/reduce the risk of cancer progression or even delay the development of recurrence thereby decreasing patient morbidity and mortality. Prostate cancer has a long latency period, thereby offering plenty of opportunity to intervene through the implementation of chemopreventive therapies. A large body of epidemiologic evidence, together with published data from in vivo and in vitro studies, strongly supports relationships between dietary constituents and the risk of prostate cancer. This article aims to review the diverse aspects of chemoprevention including the benefit of both macro- and micronutrients that have been implicated in prostate cancer prevention. In addition we discuss perspectives on the putative mechanisms of selected chemopreventive agents and highlight several clinical studies using specific chemopreventive interventions.
**Introduction**

In the Western world Prostate Cancer (PCa) is the most common internal malignancy in men, and the third most frequent cause of cancer death (1). An estimated 14% of Canadian men will be diagnosed with PCa at some point in their lives, and about 4% will die due to its complications (1). Although screening for PSA (Prostate Specific Antigen) remains a debatable issue, it has been a commonly used strategy that has aided in allowing many men to monitor the health of their prostate and facilitate early diagnoses of PCa. Early recognition of the disease has been advantageous toward allowing men to receive treatments at an early stage of the development of malignancy and is suggestive of reducing the morbidity and mortality associated with carcinogenesis of the prostate (3).

**Diet and Prostate Cancer**

Although the exact etiology of PCa still remains unknown, it is well established that the risk of developing PCa is largely influenced by environmental factors and lifestyle behaviors (4). Epidemiological studies have shown that the incidences of PCa are considerably higher in North America and Western Europe than they are in South Asian countries (5). Migratory studies have supported this trend by demonstrating a higher incidence of PCa in Chinese and Japanese men who migrate to the United States (6;7). Under such situations, it is evident, that modifications in diet and lifestyle are associated with an increased risk of PCa development. In a recent study, men following a three month nutrition and lifestyle intervention showed dramatic changes in their gene expression profiles in their prostate (8). Patients in this study were placed on an
intervention that included: a low-fat diet, with whole foods, stress management, moderate exercise and psychosocial group meetings (7). Microarray analysis was used for genetic profiling before and after the intervention (8).

Low-fat diets have also been shown to reduced the incidence of invasive carcinoma in Hi-Myc transgenic PCa mouse model as compared to a high-fat diet (9). Animals placed on this low-fat diet for several months resulted in higher Insulin-like growth factor binding protein (IGFBP)-1 levels and suppression of the Insulin-like growth factor (IGF)/Akt pathway, indicating a plausible mechanism that may be influencing progression of PCa (10). Considerable research has also been done assessing the consumption of different types of dietary fats. Using a validated food-frequency questionnaire Bidoli, et al.(11) assessed a group of 1294 men with histologically confirmed PCa and determined that higher dietary consumption of polyunsaturated fatty acids had a protective effect on PCa, whereas diets rich in monounsaturated fats and starch were directly associated with an increased risk. In vivo studies from our lab have reported that diets high in both fat and carbohydrates induce hyperinsulinemia and promote the growth of PCa xenografts in immune compromised animals (12).

In addition to studies on macronutrient intake, considerable research has been conducted on the impact of micronutrients and phytochemicals on PCa chemoprevention. Several studies have focused on dietary agents commonly consumed in the South Asian diet such as tea and soy which contain a considerable amount of flavonoids: epigallocatechin gallate and genistein respectively. Curcumin the active ingredient in the spice turmeric has also been studied as an anti-cancer agent, historically known to be one of the most highly consumed spices in India. Other studies have taken into consideration
the benefits of a “Mediterranean style” diet looking at particular phytochemicals including: lycopene, which is obtained in high concentrations in tomatoes and resveratrol, one of the active ingredients in red wine. More recent trends are studies examining the impact of vitamin D status and the incidences of cancers. This phenomenon has advanced in light of evidence that has looked at the significant global variation of sunlight exposure and lack of dietary consumption of this vitamin.

The role of antioxidants and cancer is well-established (13). Many antioxidants have been the focus as anti-cancer agents, since they have been highlighted for their ability to quench free radicals and prevent the formation of reactive oxygen species consequently preventing oxidative damage, thereby preventing the progression of carcinogenesis (13). As a result antioxidants like lycopene, vitamin E and selenium have been of particular interest in PCa chemoprevention. The antioxidant capacity of several foods has been of particular importance, particularly ellagic acid (present in pomegranates) due to their high antioxidant potential. However despite numerous studies some of these agents have not been able to translate into effective therapies in clinical trials. As PCa continues to escalate in North America, it is essential that emphasis is laid on improving dietary habits to reduce the morbidity and mortality that burdens society and the health care system. Numerous micronutrients and dietary agents including; lycopene, vitamin E, selenium, resveratrol, curcumin, vitamin D, pomegranate and flavonoids have all been highlighted as important components of the diet and major contributory factors in providing the chemopreventive potential for PCa (3;14). Understanding the modes of action by which PCa is affected with such dietary
manipulation may help better understand PCa and provide less harmful ways to manage and treat patients successfully.

**Current Strategies**

Current strategies used to treat PCa patients include surgery, chemotherapy, hormone-ablating, and radiation therapy (15). Although, all of these strategies are generally quite effective in treating PCa, they are not always appropriate for managing earlier stages disease(16). An alternative approach for men with low-risk, early-stage PCa (typically: Gleason score <6, a serum PSA level <10ng/mL, and a clinical stage T1c-T2a disease) would be active surveillance (AS), a regimen whereby PCa patients are cautiously and rigorously monitored, avoiding conventional treatments unless required (9;17). Recently Klotz (18) has shown AS to be safe option, as men on AS in several-Phase 2 trials had an 99% disease-free survival rate, and an 85% survival rate over a range of 2-11 years. AS allows for a patient and their health-care providers take an active approach to monitoring the status of their prostate (9;19). During this initiation phase of PCa development (duration ranging from 10 to 40 years) patients may be advised to adjust their diet and lifestyle to prolong or prevent progression of the disease (9;16). Several micronutrients have been studied for their chemopreventive potential in PCa, effective through several distinct mechanisms of action including reducing proliferation and/or inducing apoptosis of PCa cells. This mini review highlights a selected group of most promising dietary agents which have been attributed to possess chemopreventive potential on PCa. Investigating the mechanism of action of these micronutrients will help gain a better understanding of the natural history of PCa, and as to how therapeutic strategies and
interventions can be implemented to impose an optimal chemopreventive competence for patients thereby improving their quality of life (16;20).

**Dietary Agents: Antioxidants**

**Lycopene**

Lycopene is the lipophillic carotenoid and red pigment found most abundantly in tomatoes (21). It is a linear hydrocarbon, containing a series of conjugated and unconjugated double bonds as seen in Table 1, allowing for it to have a high affinity for quenching free radical singlet oxygen molecules (21;22). Unlike β-carotene, lycopene is acyclic and lacks the essential pro-vitamin A activity, and consequently is not physiologically required to be consumed in the diet (21). Several animal studies involving lycopene supplementation, have shown this agent to have a uniquely ability to deposit in androgen-sensitive regions of the body particularly the prostate (22;23). In 2005, a study by Herzog et al. (24) demonstrated that Copenhagen rats supplemented with lycopene for 8 weeks demonstrated a significant accumulation of lycopene in “all-trans form” in each of the four lobes of the prostate. This group also found that lycopene acted to reduce local androgen signaling, IGF-1 expression and basal inflammatory signals in normal prostate tissue (24). Several other in vivo and in vitro studies including our own have highlighted lycopene as a potential candidate for PCa chemoprevention (22;25;26). In vitro investigations have shown that lycopene has the ability to induce cell cycle arrest and apoptosis in a dose-dependent manner on several human PCa cells (27;28). Due to this lipophillic and unstable nature of lycopene, the optimal effective dose still remains a controversial and debatable amongst researchers (21;22;27-29).
addition to the ability of lycopene to quench free radicals, this compound has been shown to down-regulate IGF pathway, by increasing the concentration of IGFBP a critical factor that has been implicated to stimulate proliferation in PCa cells (29). Liu et al.(30) demonstrated the reversal of the stimulatory effect of DHT and IGF-1 mediated pathway with lycopene treatment in a co-culture system with human prostate stromal and epithelial cells. Using the Lady transgenic model Venkateswaran et al.(25), have shown a dramatic reduction in the incidence of PCa in vivo, when placed on a diet supplemented with lycopene in combination with vitamin E and selenium. More recently this group has also specifically highlighted the importance of lycopene as a key component of this combination study (26). It is important to note that although the favourable effects of lycopene have been well documented its chemopreventive nature remains highly controversial and should be reviewed with caution (31).

**Vitamin E**

Vitamin E is a fat-soluble vitamin that is obtained from a variety of dietary sources including: vegetable oils, nuts and egg yolks to name a few (32). Vitamin E belongs to the family of tocopherols and tocotrienols, which can classified into α-,β-,γ- and δ-isoforms (32). Dietary isoforms of this antioxidant exist predominately as γ-tocopherol while supplementation forms are typically in the form of α-tocopherol as depicted in Table 1 which is the biologically active form of vitamin E in the body (32). Vitamin E has long been considered a potential candidate for PCa chemoprevention supported by numerous in vitro and in vivo animal studies and clinical trials. In vitro experiments have demonstrated that vitamin E in the form of α-tocopherol succinate can induced cell cycle
arrest in human PCa cells *in vitro* (33;34). Flow cytometry confirmed the effect of α-
tocopherol depicting cell cycle arrest, impeding cells in the G1 and G2/M phase in LNCaP and PC3 cells respectively (33). The mechanism by which vitamin E has taken
effect has not been clearly delineated however, *in vivo* studies have suggested that the
anti-cancer effects of PCa may be due to disruption of PI3K pathway and/or the
sphingolipid synthesis (35-37). Several *in vivo* studies using vitamin E in combination
with other micronutrients have supported *in vitro* studies. Fleshner et al. (38), have
shown that the promotional effect on tumor growth with a high-fat diet can be inhibited
with vitamin E in a nude mouse model. While Venkateswaran et al. (26) have shown that
a combination of vitamin E, with selenium, and lycopene is more effective in reducing
the incidence of PCa and liver metastasis, than either of these micronutrients alone. These
studies carried out in the *Lady* transgenic model that spontaneously develops localized
prostatic adenocarcinoma, demonstrated a 4-fold reduction in the incidence of PCa,
relative to the control with the administration of a combination of vitamin E, selenium
and lycopene (25;26).

**Selenium**

Selenium is a trace mineral which is biologically essential for the proper functioning of
various antioxidant enzymes and proteins in the body. Selenium exists naturally in foods
predominately in the organic form as selenomethionine, selenocystine, and
selenocysteine as shown in Table 1. Some vegetables and animal feed may also contain
the inorganic form of selenium as selenite and selenate (39). Many studies have
suggested selenium to play a beneficial role in the PCa (40;41). *In vitro* studies carried
out by Venkateswaran, et al. (40-42) have shown that selenium has significant effect on PCa cell lines containing a functional androgen-receptor. Cells were treated with seleno-DL-methionine for up to 72 hours exhibited a dose-dependent reduction in proliferation. Results from flow cytometry analysis of LNCaP cells demonstrated that selenium caused a G1 cell cycle arrest with an 80% reduction in the cells in the S phase, with no effect with PC3 cells (42). However, PC3 cells transfected with a functional androgen-receptor (PC3-AR2) regained sensitivity to selenium, arresting cells in the G2/M phase, suggesting that selenium chemopreventative effect may be mediated by the presence of a functional androgen receptor (42). In a similar in vitro experiment, treatment of LNCaP cells with sodium selenite resulted in apoptosis (43). It is suggested that selenite induced apoptosis through the production of superoxide which mediates the translocation of p53 to the mitochondria thereby increasing the production of superoxide eventually causing apoptosis (43). It has also been suggested that certain selenium metabolites may in part mediate the anticancer effect of selenium (44). Several mechanisms have been proposed in the induction of programmed cell death. A study by Wu, et al. (44) suggests that methylseleninic acid (MSA), a monomethylated metabolite of selenium, may stress the endoplasmic reticulum causing an accumulation of misfolded proteins triggering apoptosis in PC3 cells. The effect of MSA on PC3 cells have also been shown to alter the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, a pathway commonly affected in several cancers (45). It is presumed that the status of phospho-Akt is affected by selenium-mediated dephosphorylation of Akt through calcineurin (45). The anti-cancer effect of selenium supplementation in vivo has also been studied in depth in a variety of preclinical models (46;47). Studies have also assessed the effect of selenium in pre-
clinical models, whereby selenium has been shown to act optimally when combined with other micronutrients specifically lycopene and vitamin E (26).

Although antioxidants have been generally supported for their chemopreventive potential by preventing DNA damage and carcinogenesis it has been suggested to avoid using them during chemotherapy and radiotherapy. A recent paper by D’Andrea (48) proposes that the protective effect of antioxidants may in fact reduce the effectiveness of conventional therapies for PCa. As chemotherapy and radiotherapy patients may often improve their antioxidant intake to prevent toxicity to their body, these antioxidants may play a role in diminishing the cytotoxic effect on cancerous cells (49). This data is still uncertain more studies should be carried out to determine the interaction between conventional therapies and antioxidant intake.

**Polyphenols**

*Flavonoids*

Flavonoids refer to a wide class of structurally related polyphenols, (over 4000), found in a wide variety of plants. As flavonoids are consumed in high concentrations in South-eastern countries where risk for PCa development is low, there has been considerable interest in assessing their chemopreventive potential in PCa (15). Biologically, flavonoids have been shown function with antioxidant, anti-inflammatory, anti-thrombogenic, and antiangiogenic properties (15). Although flavonoids have similar chemical structure their biological function are reported to vary. We have chosen to discuss a selected set of commonly studied flavonoids displayed in Table 1.
Genistein

Genistein is a soy isoflavone regularly consumed in Asian countries. Although genistein has been actively studied, its chemopreventive potential has not been clearly defined (50). Several *in vivo* studies have found that genistein can inhibit the carcinogenesis in animal models via the modulation of cell cycle regulatory genes and apoptosis (51). Supportive mechanistic studies have shown have proposed that genistein may act by the inhibition of NFκB and AKT signaling pathways (51). A recent study by El Touny et al (50), it is suggests that genistein may have a biphasic role in PCa progression. They have shown that 12-week old transgenic adenocarcinoma mouse prostate (TRAMP)-FVB mice given high doses of genistein (250mg/kg) developed more aggressive PCa, demonstrated as an increase in proliferation, invasion and matrix metalloproteinase-9 (MMP-9) (49). Corresponding *in vitro* studies using PC3 cells treated with high concentrations of genestein caused an increase in proliferation, invasion and MMP-9 whereas treatment with a lower pharmacologic dose exhibited a reduction in proliferation, invasion and MMP-9 (50). Here it was suggested that phosphatidylinositol 3-kinase and the estrogenic properties and genistein may play a mechanistic role of genistein treatment and warrant further investigation on genistein and hormone-dependent cancers (50). Combinatorial studies have also found that genistein may enhances the anti-proliferative effect of vitamin D by up regulating vitamin D receptor and p21 protein levels (52). These studies suggest that the role of genistein on PCa must be further investigated to determine its potential chemotherapeutic potential.
EGCG-epigallocatechin

Green tea catechins are produced by inactivating oxidative enzymes through steaming or pan-frying fresh tea leaves. A brewed tea may contain upwards of 500-700mg of extractable materials of which 30-40% are catechins. Epigallocatechin-3-gallate (EGC) is one of the most common catechin found in green tea. In vitro analysis by Gupta et al. (53), has shown that infusing green tea polyphenols significantly inhibited the incidence of tumors as well as the metastasis in the TRAMP model. Yet another study using the TRAMP model suggests that the chemopreventive effect of green tea polyphenols decreases as PCa progresses, stressing the importance of intervening with green tea polyphenols early on in the development of PCa (54).

Silibinin/Silymarin

Silibinin is the active compound in silymarin which can be isolated from dried fruits of the milk thistle flavones (55). Recent literature has shown that silibinin can induce cell cycle (G1) arrest with upregulation of cell cycle regulatory proteins p21 and p27 in DU145 as well as 22Rv1 PCa cells (androgen-independent and androgen-dependent respectively) in vitro (55). In a preclinical study using a 4 week old TRAMP animals with palpable prostate tumor placed on a 0.5% or 1% silybin-phytosome diet for 11 weeks, there was significant inhibition of tumor growth, progression, local invasion, and distant metastasis involving the suppression of angiogenesis (56).

Resveratrol
Resveratrol is a polyphenolic phytochemical synthesized predominately by the leaf epidermis and skin of red grapes, peanuts and several other plants (56). Consumption of red wine has been noted as a good source containing about 1-10mg of resveratrol per liter (57). This compound has been implicated to have a multitude of beneficial effects including longevity, cardiovascular health as well as preventing the initiation, progression, and promotion of several cancer cells (57-59). Further examination of resveratrol by Bishayee (60) explains that this micronutrient can exert its anticancer effect at several organ sites and therefore be looked at as a strategy to relieve the burden of cancer. Further studies specifically examining resveratrol and carcinogenesis of the prostate have been looked at in more recent literature (57;61). Cell culture studies examining several androgen sensitive PCa cell lines have shown a reduction in DNA synthesis and cell growth in addition to the induction of apoptosis with resveratrol treatment at various concentrations (57). Using concentrations of as low as 5µM, Wang et al. (57), have shown a significant reduction in the growth of LNCaP cells (57). Modulation of both androgen- and estrogen-mediated events, seem to be responsible for inhibition of LNCaP cells. In parallel studies, global gene analysis of LNCaP cells treated with resveratrol showed significant changes in androgen-responsive genes (ARG), in addition to protein changes relating to the Akt-mediated pathway including; those of IGF-1R, PIK3R3, FRAP/Mtor and FOX3A (57;57). In a separate study using androgen independent PCa cells (PC-3 and DU-145), there was an observed reduction in the growth of cells that only occurred only at significantly higher concentrations of resveratrol (59). Unlike the events that occurred in vitro, xenograft studies in nude mice, demonstrated delayed tumor development in an androgen-dependent manner mediated by
apoptosis and angiogenesis (57). Conversely in a study by Harper et al. (58) using TRAMP males administered either dietary resveratrol for 12 weeks, resulted in reduced cellular proliferative rates and IGF-1 levels, as well as a 8 fold reduction in the incidence of poorly differentiated prostatic adenocarcinoma compared to a placebo treated group. This inconsistency in data warrants further investigation to accurately assess the plausible chemopreventive potential of resveratrol.

**Ellagic acid**

Ellagic acid is a polyphenol found in the pomegranate fruit and several varieties of berries. This polyphenol shown in Table 1 has antioxidant and consequently anti-cancer properties (61). The pomegranate fruit contains a high concentration of ellagic acid in addition to many other of polyphenolic compounds, giving it a high antioxidant potency comparable to blueberries and cranberries (62). A review by Bell and Hawthorne (62) highlight the polyphenol ellagic acid to be the main compound responsible for the much of the exhibited chemopreventive potency. Ellagic acid was shown to have biological effects on PCa cells similar to pomegranate extract *in vitro* by initiating cell cycle arrest as well as inducing apoptosis (62;63). Ellagic acid is thought to act through a series of mechanisms including, decreasing NFκB, cyclooxygenase-2, cyclin D1 levels as well as promoting p53 and p21 expression and reducing vascular endothelial growth factor (VEGF) (62;64-66). Bell and Hawthorn et al., stress the importance of combining ellagic acid combined with other phytochemicals to observe an additive or even synergistic effect, targeting multiple pathways for heightened effectiveness as therapies for PCa (62). It has been shown that elevated levels of androgen signaling promote the
development of PCa. A study by Hong et al. (67), has looked at the effects of several components of pomegranate viz., pomegranate juice (PJ), pomegranate polyphenols, [punicalagin (PA) and ellagic acid (EA)] and pomegranate extract (POMx) on androgen dependent LNCaP, LNCaP-AR (LNCaP cells designed to over express AR) cells as well as DU145 cells in vitro. They found that all of these treatments inhibited the proliferation of all cell lines tested in a dose-dependent manner and shown to induced apoptosis, with the strongest effect exhibited with PJ and POMx (67). It was also reported that PJ and POMx significantly down-regulated gene expression of several enzymes involved in androgen synthesis in PCa cells in vitro (67). In an in vivo study using severe combined immunodeficient (SCID) mice injected with LAPC4 PCa cells, POMx administered for four weeks decreased tumor size, tumor vessel density, VEGF peptide levels and hypoxia-inducible factor 1α (HIF1α) (68) suggesting that pomegranate could be used as a potential chemopreventive strategy in the prevention and/or treatment of PCa.

Other Dietary Agents

Vitamin D

The relationship between vitamin D and PCa is poorly understood and currently under intense investigation. Vitamin D (1, 25 hydroxyvitamin D) depicted in Table 1 is commonly referred to as the ‘sunshine vitamin’ as it is produced photochemically by the skin from 7-dehydrocholesterol when exposed to solar ultraviolet (UV) radiation (69). It is estimated that 80-100% of the vitamin D requirements in the body is produced endogenously by the skin with exposure to UV solar radiation (69). In the diet small amounts of vitamin D can also be obtained in foods such as fish, eggs, fortified milk and
dairy products, or through supplementation (68). Emissions of UV radiation are subject to a substantial amount of variation based on the impact of latitude, season, time of the year, and ozone to name a few, impacting the subcutaneous synthesis of vitamin D in individuals all around the world (69). Several studies examining the increased sun exposure has shown to have a protective effect on prostate health (70-72). An insufficient vitamin D status may in fact predispose men to a higher risk of PCa (69). Vitamin D is a key player in the tight regulation of calcium and phosphorus levels in the blood, acting to promote their absorption in the intestines, in addition to the re-absorption of calcium in the kidneys, for mineralization of bones. It is biologically active as 1, 25 hydroxyvitamin D [1,25(OH)D] whereby it can bind to vitamin D receptors (VDR) exhibiting its effect (71). Normal and malignant epithelial cells of the prostate contain VDR and well as enzymes necessary for vitamin D metabolism, which have been shown to respond to the 1,25(OH)D in vitro, by inhibiting proliferation and promoting differentiation (71;73). It has also been found that men with higher circulating levels of 1,25 (OH)D have a reduced risk of clinically advanced PCa (74). By association, high intake of calcium has also been suggested to suppress 1,25 (OH)D production and elevate the risk of advanced and fatal PCa (74). Recent literature suggests that vitamin D deficiency is associated with an elevated risk of PCa, whereas the affect of vitamin D supplementation still remains to be a controversial issue (71).

**Curcumin**

Curcumin as shown in Figure 1, is the active ingredient found in the turmeric, *(Curcuma longa)* a spice commonly consumed in South Asia (74). Curcumin has been suggested to
have anti-tumor, anti-inflammatory, and antioxidant properties, in addition to a host of benefit in wound-healing, anti-viral, anti-infectious and anti-amyloid properties (75). In both in vitro and in vivo studies using pre-clinical animal models, curcumin has been shown to have anti-cancer properties in several cancer cells including breast, cervical, colon, gastric, hepatic, leukemia, oral epithelial, ovarian, pancreatic, and prostate (76). An in vitro study by Shankar et al. (77), has revealed that curcumin was able to inhibit proliferation and induce apoptosis in both androgen dependent and independent PCa cell lines, with no outcome on human prostate epithelial cells. It has been proposed that the Akt pathway may be modified by curcumin changing the direct action of p53 on the caspase-dependent mitochondrial death pathway (77). Curcumin has been shown to down-regulate the expression of Bcl-2 and Bcl-xl and up regulate the expression of p53, Bax, Bak, PUMA, Noxa, and Bim (77). Using mouse embryonic fibroblasts (MEF) cells deficient in the Bax and Bak genes, mice protect themselves from the pro-apoptotic effect of curcumin, where as normal mice were able to show elevated levels of Bax and Bak and have mitochondrial translocation causing the release of downstream apoptogenic molecules (75). Thus proposing that the roles of Bcl-2 family members (Bax and Bak) may be essential for curcumin-induced apoptosis (75). A recent in vivo study examined the chemopreventive combinatorial effect of curcumin and phenethylisothiocyanate (PEITC), a naturally occurring isothiocyanate found in a variety of cruciferous vegetables (78). The TRAMP model was placed, on a diet supplemented with curcumin or PEITC alone or in combination for 16 weeks, here they found a statistically significant decrease in the incidence of prostate tumor formation ($P = 0.0064$) and a significant inhibition of high-grade PIN (78). Immunoblotting analysis revealed decrease cell proliferation to be
attributed to the down regulation of the Akt signaling pathway (78), implicating curcumin to be a promising chemopreventive agent for PCa.

Clinical Trials

Although there are numerous clinical trials that are important we have preferred to highlight recent studies that involve selected compounds that have been discussed in this review.

Lycopene: The effect of lycopene has been reviewed in several clinical trials. As lycopene is most abundant in tomatoes, many clinical trials use tomatoes and/or tomato products as an indicator of lycopene consumption. Several epidemiological studies have seen that there is an indirect relationship with tomato consumption and the risk of developing PCa (22;74;79;80). Whether or not the lycopene alone can be attributed to the observed effect is still debatable (31). Patients with BPH (Benign Prostate Hyperplasia) have shown to have lower levels plasma lycopene and higher accumulation of the antioxidant in their prostate tissues suggesting that the body may be using lycopene as a protective mechanism (81). In a whole-food intervention study, 32 men with localized prostate adenocarcinoma were told to consume tomato-sauce based pasta dishes three weeks prior to a scheduled radical prostatectomy. Seven randomized patients were then assessed and shown to have experienced significantly higher prostate and serum lycopene concentrations and have significant reduction in oxidative damage in prostate tissue, in addition to decreased PSA levels relative to baseline (82). In a small pilot study Schwarz et al. (81) looked at the chemopreventive ability of lycopene supplementation on
men with BPH. Forty patients with BPH free of clinical PCa were included in the randomized, placebo-controlled, double-blind study, and were given a dietary supplement with 15mg of lycopene or a placebo (81). It was reported that men receiving lycopene experienced a significant reduction in PSA (p<0.05) with no prostate enlargement, compared to the placebo group who experienced no significant change in PSA and demonstrated prostate enlargement (81). These clinical trials emphasize the need for more clinical studies to better understand the chemopreventive mechanism of lycopene and further support the need for early interventions particularly at the stage of PCa diagnosis.

**Ellagic Acid:** As previously eluted to the pomegranate has been suggested to have a potent anti-cancer effects due to its high concentration of polyphenolic compounds, particularly, ellagic acid. In 2003, the first clinical trial involving PCa patients and pomegranate juice was reported (82;83). Here eligible patients with rising PSA levels after surgery or radiotherapy consumed 8 ounces of pomegranate juice daily for 15 months. Men on this study experienced a statistically significant increase in PSA doubling time (83;84). Further laboratory investigations analyzing serum of these men with androgen-sensitive PCa cells *in vitro* showed alterations in cell proliferation, apoptosis and oxidative stress (83).

**Flavonoids:** The strong evidence for the use of silibinin as a chemopreventive strategy has gained substantial attention and has progressed into a phase II trial as the phase I clinical trial has been completed (84). The phase I trial looked at associated toxicities
with high consumption of the commercially available oral formulation of silibinin, silybin-phytosome to determine a proper dose for the phase II trial (84). Here it was noted that asymptomatic liver toxicity was the most commonly seen adverse event with high consumption of silybin-phytosome suggesting the use of a 13 g of oral silybin-phytosome daily for a phase II trial (84). Currently a phase II trial is ongoing, with the objective to uncover clinical relevance with silibinin as a potential chemopreventive agent (56;85).

**Selenium:** One of the most promising studies undermining the anti-carcinogenic effect of selenium was a randomized controlled trial study by the Nutritional Prevention of Cancer Study Group. Between 1983 and 1991 over 1300 patients with a history of basal cell or squamous cell carcinomas were randomized to receive 200µg/day of selenium or the placebo (86). In this study there was no significant impact on non-melanoma skin cancer however there were significant reductions in prostate cancer (63%), lung cancer (46%), and colorectal cancer (58%) as well as an overall reduced the incidence of cancer (53%) (86). Conversely the SELECT trial discussed in further detail below, found that men supplemented with selenium alone did not have any effect on PCa warranting further examination of this dietary agent (87;88).

**The Alpha-Tocopherol and Beta-Carotene (ATBC) Cancer Prevention Study:** This was a controlled trial conducted in Finland on male smokers, aimed at assessing the impact of supplementation of vitamin E (α-tocopherol-50mg) of and/or β-carotene (20mg) alone or in combination on PCa (87). Over 29 000 smokers aged 50-69 were recruited and
randomly placed on the supplement or placebo for a period of 5-8 years (87). It was found that male smokers supplemented with α-tocopherol experienced a significant reduction in the incidence and mortality from clinical PCa, whereas supplementation of β-carotene slightly increased both the incidence and mortality of PCa in men who were smokers (87).

**Selenium and Vitamin E Cancer Prevention Trial (SELECT):** One of the most ambitious cancer prevention studies stemming from the large body of evidence supporting the chemopreventive properties of vitamin E and selenium was the SELECT (87;88). In this randomized, placebo-controlled trial over 35,000 men were recruited from Canada, United States, and Puerto Rico, to test the efficacy of selenium and vitamin E in a clinical setting (88;89). The study was initiated in 2001, and was anticipated to last a duration of 7-12 years. Men with a PSA reading of 4ng/mL or lower and a nonsuspicious digital rectal exam (DRE) were recruited in a randomized fashion, and given either selenium (200µM in the form of L-selenomethionine) and/or vitamin E (400 IU in the form of All-rac-α-tocopheryl acetate) supplements alone or in combination (88;90). Despite the hopeful outcomes of the SELECT trial, subjects were asked to discontinue taking supplements in 2008, after an estimated 5.46 years, based on evidence from the independent data and safety monitoring committee (89). The trial found that patients consuming vitamin E and/or selenium supplements alone or in combination did not show any observed benefit on prostate cancer prevention. On the other hand participants taking vitamin E alone showed increasing trends (not-significant) for prostate cancer and
patients on selenium supplementation alone showed a non-significant increase in the risk of type-2 diabetes (89).

With the substantial support of many clinicians and scientists the SELECT trial has stirred much debate with respect to intensely investigated micronutrients selenium and vitamin E. A recent article by Hatfield et al. (91), attributes the ineffectiveness of selenium to the form of L-selenomethionine as well as high levels of selenium in the participants prior to the start of the trial. This study also stressed the need for further investigation into the biology of selenium or specifically targeting a subset population that would most likely benefit from such a supplementation. In addition, Venkateswaran et al. (26), has recently published data that highlights the importance of combinatorial treatments in appropriate pre-clinical models to assess the effectiveness of certain pre-clinical therapies. This newly released data is supportive in the ineffectiveness of selenium and vitamin E alone or in combinations in the Lady transgenic model, in addition to showing a significant reduction in PCa and liver metastasis when treated with a combination of lycopene, selenium, and vitamin E in the same model system (26). It is indisputable that additional studies are essential looking at the specific forms and biology of vitamin E and selenium, as well as alternative combinations are warranted to delineate, in appropriate pre-clinical models before initiating further clinical trials.

**Translating in vitro to in vivo studies into clinical trials**

With the large body of existing in vitro and corresponding in vivo data, the translation into clinical trials is the next logical step in the development of effective chemopreventive strategies. Unfortunately based on unsuccessful results of several large
scale clinical trials, it seems necessary to re-examine the approach that is required on the use of appropriate pre-clinical studies and/or model systems. Likewise, many studies translating \textit{in vitro} results into \textit{in vivo} model systems has been far from ideal, re-enforcing the need for additional rigorous investigation using appropriate pre-clinical models (26;60). One such proposal by Scott et al. (92) sketch a logical design for the development of dietary chemopreventive agents, which includes a series of steps that begin with several \textit{in vitro, in vivo} studies and later translating them into clinical pilot studies and clinical trials. Here Scott et al. (92) has examined a strategy to identify appropriate clinical doses of micronutrients through the use of suitable long-term preclinical studies with attainable and realistic concentrations achievable in humans (92). Furthermore this proposal highlights the importance of devising sophisticated strategies that include the utilization of appropriate preclinical models required to successfully implement the use of chemopreventive agents in PCa.
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