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Molecular Targeted Therapies Using Botanicals for Prostate Cancer Chemoprevention

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Abstract

In spite of the large number of botanicals demonstrating promise as potential cancer chemopreventive agents, most have failed to prove effectiveness in clinical trials. Critical requirements for moving botanical agents to recommendation for clinical use include adopting a systematic, molecular-target based approach and utilizing the same ethical and rigorous methods that are used to evaluate other pharmacological agents. Preliminary data on a mechanistic rationale for chemoprevention activity as observed from epidemiological, *in vitro* and preclinical studies, phase I data of safety in suitable cohorts, duration of intervention based on time to progression of pre-neoplastic disease to cancer and using a valid panel of biomarkers representing the hypothesized carcinogenesis pathway for measuring efficacy must inform the design of clinical trials. Botanicals have been shown to influence multiple biochemical and molecular cascades that inhibit mutagenesis, proliferation, induce apoptosis, suppress the formation and growth of human cancers, thus modulating several hallmarks of carcinogenesis. These agents appear promising in their potential to make a dramatic impact in cancer prevention and treatment, with a significantly superior safety profile than most agents evaluated to date. The goal of this paper is to provide models of translational research based on the current evidence of promising botanicals with a specific focus on targeted therapies for PCa chemoprevention.

Keywords

Prostate cancer; Chemoprevention; Isoflavones; Green Tea Polyphenols (GTP); Decursin; Curcumin

Introduction

The disease: prostate cancer

Prostate Cancer (PCa) is the most frequently diagnosed malignancy in men with 241,740 new cases and 28,170 deaths estimated to occur in 2012 [1]. The initiation and progression of PCa may involve a complex array of both exogenous and endogenous factors [2-5]. Although it is clear that clinical PCa incidence and mortality vary greatly between populations, the frequency of latent PCa is evenly distributed among populations, suggesting that external factors such as diet, physical activity and other lifestyle factors are important in

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the transformation from latent into more aggressive, clinical cancer [2-5]. Although early screening and detection has been used historically as strategies for PCa prevention, recently these recommendations have been a subject of much debate. While screening using serum Prostate Specific Antigen (PSA) has not been shown to significantly reduce either PCa-specific or overall mortality, it has been linked to substantial overtreatment of clinically insignificant, potentially indolent tumors [6,7]. Taking into consideration all the evidence accumulated to date, the U.S. Preventive Services Task Force (USPSTF) recommended against PSA-based PCa screening in asymptomatic men (grade D recommendation) [8]. These features of PCa, namely, high prevalence in specific populations, the uncertainty with regard to effectiveness and value of early screening along with a concerned and eager cohort of men interested in reducing their risk for PCa provides an excellent opportunity and need to develop alternate PCa control strategies targeting these specific populations of men.

The goal of this review is to: 1. establish the rationale for use of botanicals for PCa chemoprevention; 2. provide a practical model using a systematic approach for evaluating botanicals, and; 3. provide examples of several botanicals that we have taken from bench to bedside using this approach, with a specific focus on the molecular pathways that these botanicals target.

Cancer chemoprevention

Chemoprevention refers to the inhibition of pre-invasive and invasive cancer and its progression or treatments of identifiable pre-cancers [9,10]. Chemoprevention efforts require a thorough understanding of the mechanism of carcinogenesis including signaling and metabolic pathways and genetic progression pathways. New technologies in genomics and proteomics have spurred this field of research. The use of this knowledge to develop pharmacologic agents (including botanicals/biologicals) to reverse or halt the process of carcinogenesis is called chemoprevention. Agents for chemoprevention include anti-promotion and anti-progression agents that prevent the growth and survival of cells that are already committed to become malignant [9,10].

Approach to identifying and evaluating safety and effectiveness of Botanicals for PCa chemoprevention

Although several targeted “smart” drugs have emerged over the past decade, it is clear that diseases like cancer have an etiology based on perturbations of multiple signaling pathways. Thus, targeting multiple pathways may represent a more effective approach to cancer control [11,12]. In addition, the mono-targeted “smart” drugs are associated with high cost, and produce numerous side effects. These drawbacks of mono-targeted drugs underscore the importance for the development of multi-targeted, innocuous, inexpensive, and readily available botanicals for the prevention of cancer [13]. Botanicals have been shown to influence multiple biochemical and molecular cascades that inhibit mutagenesis, proliferation, induce apoptosis, suppress the formation and growth of human cancers, thus modulating several hallmarks of carcinogenesis. Additionally, these agents appear promising in their potential to make a dramatic impact in cancer chemoprevention, with a significantly superior safety profile than most agents evaluated to date [14-20]. It is clear that although several botanicals have been characterized and used for hundreds of years in medicine [21,22], there have been several challenges and limitations towards progress in this field. Te slow pace of growth of several of these leads could be attributed to regulatory protection of classical formulation, lack of standardization, quality control, and molecular mechanism-based approach in evaluation, population-based normal range of bio-markers, laboratory practices and lack of translational scientists engaged in conducting well designed trials. However, several valuable lessons have been learnt from the chemoprevention trials of the past such as the Selenium and Vitamin E Cancer Prevention Trial (SELECT) [23], Alpha-

Tocopherol, Beta Carotene Cancer Prevention trial (ATBC) [24] and the Carotene and Retinol Efficacy Trial (CARET) [25]. Critical requirements for moving botanicals from bench to bedside include adopting a systematic, molecular-mechanism based approach and utilizing the same ethical and rigorous methods such as those used to evaluate other pharmacological agents. Preliminary data on a mechanistic rationale and molecular targets for chemoprevention activity as observed from *in vitro* and preclinical studies, phase I data of safety in suitable cohorts, duration of intervention based on time to progression of pre-neoplastic disease to cancer and using a valid panel of biomarkers, including safety markers representing the hypothesized carcinogenesis pathway for measuring efficacy must inform the design of phase I-II prior to embarking on phase III clinical trials. Chemoprevention trials using combinations of botanicals such as curcumin with piperine [26] have demonstrated that synergy between agents can lead to lower doses, improved efficacy and fewer or less severe toxicities. An assessment of endpoints in trials resulting in approval of an agent for cancer chemoprevention agent reveals that nearly all have been approved on the basis of intraepithelial neoplasia. Intermediate endpoint biomarkers must be identified, validated and must be conducive to be obtained using non-invasive techniques and without compromising safety to men in chemoprevention trials. To reduce patient burden, these markers must be obtained from accessible organs and during the normal course of clinical surveillance. Randomized, placebo-controlled design and the long-term follow-up and monitoring are critical to meet FDA requirements and promote acceptance in the marketplace [13,27,28]. Multiple botanicals have been identified and appear promising for PCa chemoprevention. Applying the lessons learnt from previous trials with botanicals to the design of future PCa chemoprevention trials should facilitate the translation of novel preventive agents from bench to bedside.

Target populations at high risk for PCa

Most chemoprevention trials of the past have demonstrated that there are significant benefits to targeting germline, familial, or increased-risk cohorts such as those with a family history or other risk based on race and ethnicity [13]. These trials can produce more power over a shorter time frame. In most epithelial tissues, including the prostate, genetic progression and loss of cellular control functions are observed as the cell and tissue phenotype changes from normal to dysplasia (prostatic intraepithelial neoplasia or PIN), then to increasingly severe dysplasia (High Grade PIN or HGPIN), superficial cancers and finally to invasive disease [3-5,29-31]. Recent studies have quantified the risk for invasive PCa in men with HGPIN, and it was suggested that the incidence of PCa was as high as 30% within 1 year after repeated biopsy [32,33]. Several lines of evidence derived from animal models, together with data obtained in epidemiological, morphological, genetic, and molecular studies, support HGPIN as the main premalignant lesion of PCa [3-5,29-33]. Thus, HGPIN is considered a possible pre-invasive precursor of PCa [3-5]. Isolated high-grade prostatic intraepithelial neoplasia has a 3% to 14% incidence and predicts cancer on repeat biopsy in 23% of cases [34-36]. More recently, Atypical Small Acinar Proliferation (ASAP) has emerged as a diagnosis of exclusion but with a greater association to prostatic carcinoma than HGPIN. ASAP is characterized by a focus of glands that do not contain sufficient cytologic or architectural atypia to establish a definitive diagnosis of cancer [34-36]. Atypical small acinar proliferation suspicious for malignancy designates foci that have either qualitative or quantitative limitations in atypia precluding a definite cancer diagnosis. Contemporary studies indicate that ASAP has a 39% predictive value for cancer on repeat biopsy. In studies reviewed in the literature, HGPIN/ASAP had a mean predictive value for cancer of 43.6%, much higher than isolated HGPIN but similar to ASAP [37-39]. Thus, HGPIN and ASAP are associated with progressive abnormalities of phenotype and genotype, which are intermediate between normal prostatic epithelium and cancer, indicating impairment of cell differentiation and regulatory control with advancing stages of

prostatic carcinogenesis. Due to the uncertainty with PCa screening and early detection strategies, especially in the high-risk populations, alternative cancer control strategies are needed. Importantly, PCa is an ideal malignancy for PCa chemoprevention due to the high prevalence, long latency, significant mortality and morbidity, and the availability of HGPIN and ASAP as intermediate predictive stages of progression. These estimates justify the rationale for selecting these groups of men with HGPIN and ASAP as a target high-risk population for evaluating promising chemopreventive agents for prevention of PCa.

An estimated 35, 110 cases of PCa are expected to occur among African American men in 2011, accounting for 40% of all cancers diagnosed in that population. Between 2003 and 2007, the average annual PCa rate was 60% higher in AA men compared to white men [40]. In addition, AA men have the highest PCa-Specific mortality rate of any other racial or ethnic group in the US. Although the overall incidence of and mortality from PCa has been declining in Caucasian men since 1991, possibly due to improved diagnostic techniques, better screening and improved surgical and radiologic treatments, the decline in AA men lags behind Caucasian men. For AA men with a family history of hereditary PCa, the increased risk is even greater [41]. Autopsy studies and clinical findings support the argument that PCa exhibits more aggressive biological behavior in AA men than that observed in other populations. Interestingly, not only the prevalence of HGPIN is higher in the general population of AA men [42-44], but AA men with HGPIN are more likely to develop aggressive PCa [44]. Finally, HGPIN seems to be a risk factor for biochemical recurrence following the definitive treatment specifically in AA, but not in Caucasian, men [45]. These findings help delineate the cohorts of men under exceptionally high CaP risk. Importantly, such cohorts may represent ideal targets to evaluate botanicals for PCa chemoprevention.

Promising Agent for Chemoprevention of PCa

Isoflavones

Isoflavones in the diet are primarily derived from soy products, although Isoflavones are also found in other legumes, including peas, lentils, or other bean varieties [46]. Te primary Isoflavones in soybeans are genistein, daidzein, and glycitein. Epidemiological studies have consistently reported lower incidence of clinically evident disease in populations consuming Isoflavones. An inverse relationship between dietary intake, plasma [47-52] and prostatic fluid concentrations of Isoflavones and the incidence of PCa and Benign Prostatic Hyperplasia (BPH) has been observed in these populations, demonstrating the potential role of Isoflavones in mediating epigenetic effects. *In vitro* data have consistently shown that genistein, the most active and predominant isoflavone, modulates cell proliferation [53-57], angiogenesis [58,59], tumor cell invasion and tumor metastasis [53,60,61], cell cycle regulation [62], antioxidant [60,63] and induction of apoptotic cell death [64]. These data indicate that Isoflavones are promising chemopreventive agents, with several cellular effects which are both genomic and non-genomic. Specific anticarcinogenic activity of the isoflavone genistein include inhibition of protein-tyrosine kinase, which results in the alleviation of cancer growth via inhibition of PTK-mediated signaling mechanisms; inhibition of topoisomerases I and II and protein histodine kinase, which have antiproliferative or pro-apoptotic effects; antioxidant effects, through inhibition of the expression of stress-response related genes; inhibition of nuclear factor kappa B (NF-κB) and Akt signaling pathways, both of which are important for cell survival; inhibition of angiogenesis; down-regulation of transforming growth factor-beta; and the inhibition of Epidermal Growth Factor (EGF) [65]. Our computational docking and *in vitro* and *in vivo* proteasome activity studies confirmed that the isoflavone genistein is also a proteasome inhibitor [25,26]. In addition, we found that genistein at 1 μM could inhibit ∼30% of the chymotrypsin-like activity of purified 20S proteasome. It has been reported that plasma

levels of genistein are in a range of 0.5-2.5 μM and the concentrations of genistein vary in different tissues and organs. It is therefore possible that a partial inhibition of the proteasome activity by genistein at a physiological concentration might contribute to its reported cancer-preventative effects. Among different soy compounds, genistein was the most potent inhibitor of the proteasomal chymotrypsin-like activity. This is consistent with the previous reports that genistein is the most potent soy isoflavone. Inhibition of proteasome activity by genistein in PCa cells (LNCaP) was associated with increased levels of $p27^{Kip1}$, IκB-α (an important inhibitor of the tumor survival factor NFκB), Bax, and ubiquitinylated proteins, accompanied by induction of apoptotic cell death. We also found that genistein was the most potent of all the tested Isoflavones in terms of inducing Bax accumulation and PARP cleavage. However, daidzein and glycetin, in addition to genistein, were able to indice accumulation of the $p27^{Kip1}$ protein. These results suggest that accumulation of Bax and IκB-α is associated with apoptosis induction while $p27^{Kip1}$ accumulation is probably associated with G_1 arrest [24].

Based on its structural and functional similarity to estrogen, genistein is considered a phytoestrogen. Although a role for the Estrogen Receptors (ERs), ERα and ERβ, has been implicated in prostate tumorigenesis, their role in mediating the chemo-preventive effect of genistein in prostate is not clear. Research led by Bai et al. [28,66,67] and others [68-70] showed that androgens and estrogens repressed the FOXO1 activity in PCa cells, a process that is independent of the PKB/AKT-mediated FOXO1 phosphorylation. Te repression is Androgen Receptor (AR) and ERα-dependent, respectively, and mediated through the formation of receptor-FOXO1 protein complex. These data demonstrate that FOXO1 as a novel target of genistein in PCa cells. Te mechanism of action of genistein signaling via the ER/AR-FOX01 pathway is relevant in specifically studying the effectiveness and safety of genistein in AA men. It has been shown that the AR activity is controlled by the length of poly-glutamine repeat in the N-terminal region and AA men, on an average, have been shown to have shorter poly-glutamine repeat and thus higher AR activity [70]. Based on these results that demonstrated that genistein down regulates AR expression and that the increase in FOX01 activity by genistein is mediated through AR down regulation, we suggest that genistein may have a stronger preventive effect in AA men [66-70].

Attempts to understand the cellular origin of cancer has advanced the theory of Cancer Stem Cells (CSCs). These rare cells have indefinite proliferative potential and are believed to be responsible for tumor invasiveness and heterogeneity [71]. Since Cancer Stem Cells (CSCs) are also involved in tumorigenesis and progression of PCa, Zhang et al. [72] reported that Tumorsphere (T) formation and colony formation of PCa cells were noticeably suppressed in the presence of genistein. Pretreatment of PCa Tumor Cells (TC) with genistein also suppressed tumorigenicity *in vivo* Additionally, genistein treatment inhibited growth of PCa TCs. Further studies showed that genistein treatment not only led to the down-regulation of PCa CSC markers CD44 *in vitro* and *in vivo*, but also inhibited Hedgehog-Gli1 pathway, which may contribute to the anti-CSC effect of genistein in PCa TCs. Their finding thus demonstrated that genistein may be a dietary phytochemical with the potential to target prostate CSCs.

Phase I trials have demonstrated the clinical characteristics and pharmacokinetics and safety of whole soy and purified Isoflavones with single and multiple-dose administration in healthy, early stage or treated cancer patient cohorts [73-75]. While the doses of purified soy Isoflavones ranged from 1-16 mgs/kg body weight, some of the doses were higher than those previously administered to humans as whole soy proteins, without significant clinical toxicity. A few pilot phase II clinical trials including our study, have demonstrated a trend towards stabilization or reduction of PSA with short-term isoflavone supplementation in PCa patient populations, without significant clinical toxicity [76-81], with the exception of

mild Gastrointestinal (GI) symptoms. In our phase II clinical trial of isoflavone supplementation in men with localized PCa, [78] we administered whole soy Isoflavones at a dose of 60 mgs in 60 grams soy protein. Fifty-nine patients completed the 12-week intervention. Serum free testosterone was reduced or showed no change in 61% of subjects in the isoflavone group compared to 33% in the placebo group. Serum total PSA decreased or was unchanged in 69% of the subjects in the isoflavone treated group compared to 55% in the placebo group and nineteen (19) percent of subjects receiving soy Isoflavones reduced total PSA by two points or more during the intervention period. Seventeen (17) subjects were unable to complete the study reporting constipation and GI symptoms such as bloating, discomfort, diarrhea and pain which were attributable to the protein content of these supplements and required early exclusion of these subjects from the study. Since the potent agent in these soy compounds are Isoflavones and not the protein, and as demonstrated by these earlier trials have few clinical symptoms attributable to them, Isoflavones preparations without the protein may be the most promising agent in clinical trials.

Based on our experience and the results of these earlier phase I and II studies, we then hypothesized that supplementation with a constant dose of purified Isoflavones (*vs*. a placebo) will produce an increase in plasma levels of Isoflavones which will be correlated with stabilization or reduction in surrogate markers of proliferation (serum total PSA) and thereby contribute to a decrease or stabilization of disease progression in men diagnosed with early stage PCa. To test this hypothesis, we recently completed a pilot Phase II randomized, doubleblinded, placebo-controlled trial [79,81] of men with early stage PCa (Gleason 2-6) to receive purified Isoflavones, (Prevastein HC® 80 mgs/day, IND #61,949 Kumar) *vs*. a placebo, and observed the effectiveness of the study agent in producing an increase in plasma levels of Isoflavones (daidzein, glycitein and genistein) and a corresponding reduction/stabilization in serum total PSA. In addition, our aim was to evaluate compliance and toxicity. In this phase II trial, evaluation of the effectiveness of intervention was based on the magnitude of change in plasma levels of Isoflavones in the isoflavone-supplemented group compared to the placebo group and a corresponding stabilization or reduction in surrogate markers of proliferation (total PSA), increase in serum estradiol and reduction in free testosterone. Fifty subjects completed the 12-week intervention. Significant increases in plasma Isoflavones ($p \leq 0.001$) were observed from baseline to 4 and 12 weeks in the isoflavone-treated group compared to placebo, without significant clinical toxicity. Although greater mean reduction of serum free testosterone was observed in subjects in the isoflavone-treated group compared to the placebo group, these changes were not statistically significant for this duration of intervention ($p=0.3$). Increasing concentrations of plasma isoflavones diadzein ($p=0.02$) and genistein ($p=0.01$) in the isoflavone-treated group were inversely correlated to changes in serum PSA compared to the placebo arm. In a recently completed Phase II randomized-controlled trial [74] to evaluate the safe and effective dose of isoflavones to be used in future clinical trials for PCa prevention; forty-five eligible men were supplemented with 40, 60 and 80 mgs of purified isoflavones or no supplement from biopsy to prostatectomy. Compliance to study agent, toxicity, changes in plasma isoflavones, serum steroid hormones, Prostate Specific Antigen (PSA) and tissue Ki-67 were analyzed from baseline to completion of study. Forty-four subjects completed the study with duration of intervention of 30 (± 3) days. We observed significant increases in plasma Isoflavones with treatment for all doses compared to controls without producing any toxicity. A significant increase in serum total estradiol was observed in the 40 mgs and 60 mgs isoflavone-treated arms. However, significant increase in serum free testosterone was observed in the 60 mgs isoflavone-treated arm. Since only post-intervention tissue samples were available for staining, the difference between the treatment arms and control of percentage Ki-67 staining were estimated in these samples. Compared to the control group and other treatment arms, the 40 mgs isoflavone supplemented arm had a lowest percentage of cells expressing Ki-67, although this was not statistically significant for

this sample size and duration of intervention. We concluded that 40 mgs of purified isoflavones may be the best dose to be used in a future definitive, larger phase II clinical trial to evaluate purified Isoflavones in prostate carcinogesis. With prolonged consistent administration of purified isoflavones, we could potentially delay onset of the disease by interfering with the later stages of prostate carcinogenesis or growth and progression of pre-neoplastic and histologic cancer.

Based on the finding that genistein down regulates AR expression and produces an increase in FOX01 activity, a pathway that may be more relevant in African American (AA) men, we are now examining the comparative efficacy and safety of 40 mgs of isoflavones in AA and Caucasian men and validating the potential mechanisms by which Isoflavones modulate prostate carcinogenesis, specifically in AA men. In this clinical trial, we are testing the hypothesis that the pathway by which Isoflavones will suppress prostate tumorigenesis is mediated by the ERβ, which can be suppressed by ERα in PCa cells such that ERβ is decreased. In addition, genistein inhibits androgen signaling through FOXO1 by down regulating AR expression, resulting in apoptosis and leading to the suppression of prostate carcinogenesis. We additionally hypothesize that the effectiveness of isoflavones to modulate prostate carcinogenesis will be significantly higher in AA men compared to Caucasian men. This trial is scheduled for completion in (month and year), and we expect the results to be published by December 2013 [28].

Green Tea Polyphenols (GTP)

Similar to isoflavones, numerous reports have provided the epidemiological evidence suggestive of a protective effect of tea consumption against human cancers including PCa [82-85]. In contrast, a few studies have associated an increased risk potentially attributed to confounding factors that include consumption of salted or very hot tea, geographical location, tobacco and alcohol use, and other dietary differences [82-86]. Of all the tea produced worldwide, about 20% of green tea is consumed in Asian countries such as China, Japan, Korea and India. Interestingly, these populations consistently demonstrate lower risk of PCa [87-90].

Several published preclinical studies using green tea, green tea leaves, green tea extracts, GTP mixtures, Green Tea Catechin (GTC) mixtures, and individual catechins have demonstrated chemopreventive efficacy in PCa [91-95]. Using the TRAMP mice model, Gupta et al. [91] were able to demonstrate that oral infusion of GTP extract at a human achievable dose (equivalent to six cups of green tea per day) significant ly delayed primary tumor incidence and tumor burden as assessed sequentially by Magnetic Resonance Imaging (MRI), decreased prostate (64% of baseline) and Genitourinary (GU) (72%) weight, inhibited serum insulin-like growth factor-I (IGF-I) and restoration of insulin-like growth factor binding protein-3 levels (IGFBP-3), and produced marked reduction in the protein expression of Proliferating Cell Nuclear Antigen (PCNA) in the prostate compared with water-fed TRAMP mice. Furthermore, GTP consumption caused significant apoptosis, which possibly resulted in reduced dissemination of cancer cells, thereby causing inhibition of development, progression and metastasis to distant organ sites. However, in another similar animal model, Epigallocatechin Gallate (EGCG) only slightly reduced occurrence of these endpoints [91]. These disparate observations may be attributed to the pharmacokinetic properties of EGCG, which has relatively low oral bioavailability, possibly due to slow absorption as well as high metabolic clearance by the liver [27]. Other potential confounders may include doses, method of infusion, duration of intervention and timing of castration, all of which may infuence the markers of progression and the antioxidant property of EGCG. Oral administration of GTPs (*vs*. pure EGCG) at 500 mg/kg/day in drinking water to TRAMP mice is expected to cause a higher systemic exposure compared to gavage and may

explain the protective effects observed by Gupta et al. and other groups [91,93-95] compared with Suttie et al. [92]. In the authors' opinion, the animal data demonstrating the chemopreventive efficacy of GTP in PCa appear promising, although additional research is needed to resolve the aforementioned concerns before a controlled phase II/III human trial could be initiated.

Tea and tea compounds reduce growth and/or induce apoptosis in several human cancer cell lines *in vitro*, including the prostate. Among the constituents of Green Tea Extracts (GTE), laboratory studies have identified Epigallocatechin Gallate (EGCG) as the most potent chemopreventive agent which appears to affect a number of molecular processes including induction of apoptosis and inhibition of tumor growth and angiogenesis [96-99]. More recently, EGCG has been found to affect several cancer-related proteins including p27, Bcl-2 or Bcr-Abl oncoproteins, Bax, matrix metalloproteinases (MMP-2 and MMP-9) [100], the androgen receptor, EGF receptor, Activator proteins 1(AP1), and some cell cycle regulators [101-103]. Based on these studies of GTP in cell culture systems, Adhami et al. [100] were able to demonstrate that EGCG in GTP induces apoptosis, cell growth inhibition and cyclin kinase inhibitor WAF-1/p21-mediated cell cycle-dysregulation. Using cDNA microarrays, they also observed the EGCG treatment of LNCaP cells results in induction of genes that exhibit the growth-inhibitory effects and repression of genes that belong to the G-protein signaling network [100]. These data confirm that GTPs exert potent and selective *in vitro* and *in vivo* pro-apoptotic activity on PCa cells.

By using various proteasome inhibitors, several recent studies have suggested that the ubiquitin/proteasome pathway plays an essential role in the regulation of apoptosis, and activation of the cellular apoptotic program is a current strategy for treatment of human cancers. Although there are several mechanisms by which EGCG may operate in prostate carcinogenesis, our group has demonstrated that EGCG potently and selectively inhibits the proteasome activity in intact human cells leading to the accumulation of IkB-α and p27 proteins, and growth arrest [32-35,40]. This inhibition of proteasome activity by EGCG occurred at or near physiological concentrations similar to that found in the body fluids of green tea drinkers. We have observed that Polyphenon E (a mixture of tea catechins) specifically inhibits the proteasomal chymotrypsin-like activity with an IC50 value of 7 μM [27]. Te IC50 value for trypsin-like activity was above 100 μM, demonstrating that Polyphenon E preferentially inhibits the proteasomal chymotrypsin-like activities. Our data strongly suggest that the proteasome is a PCa-related molecular target of EGCG and Polyphenon E, and that inhibition of the proteasome activity by EGCG in Polyphenon E, and subsequent apoptosis, may contribute to the PCa preventative effect of GTP. Several Phase I studies and a single phase II pilot trial have compared the pharmacokinetics and safety of oral green tea, Polyphenon E and EGCG [104-109] demonstrating safety in single and multi dose studies of doses ranging from 200-1200 mgs per day administered for up to 12 months in both healthy men and men at high risk for PCa. A significant increase in plasma catechins was observed in association with all Adverse Events (AEs); however, AEs were related to the cafeine in the Polyphenon E preparation and not to the catechins. Based on the promising results of our studies and those of others as well as the relatively safety, we are currently completing a phase II clinical trial, powered to examine the effects of a standardized green tea preparation (Polyphenon E) in inhibiting the progression to PCa in a cohort diagnosed with HGPIN lesions or ASAP, while validating the molecular targets observed in the laboratory. Te results of these studies can inform the design of well powered phase III clinical trials in the coming years.

Lycopene

Lycopene is a red-colored carotene with no recognized vitamin A activity and a potent antioxidant, found in certain red-colored vegetables and fruits, such as tomatoes (the main dietary source for the most people), red peppers and watermelon [110]. Epidemiological studies have demonstrated that populations with high intake of dietary lycopene have lower risk of PCa [111-116]. While prospective and case control studies have shown lycopene to be significantly lower in serum and tissue of cancer patients than in controls [111,117-120], results of a large nested case-control study, found no association between serum lycopene and PCa [121]. This variability in the experimental data obtained in these epidemiological studies may be related to lycopene source, exposure misclassification, lack of a dose response and other confounding lifestyle factors such as obesity, use of tobacco and alcohol, other dietary differences, varying standardization of quantities and compositions of lycopene, geographical location and genetic risk factors. Given these caveats, result based on epidemiological evidence should be interpreted with caution [122].

Biological PCa protective mechanisms of lycopene appear to be related either to the antioxidative and anti-inflammatory or apoptosis-inducing properties, such as ability to induce G0/G1 cycle arrest, apoptosis and delayed *in vivo* growth in different PCa cell lines. Other mechanisms mediated by steroid hormones may also be involved. *In vitro* data have consistently shown that lycopene modulates cell cycle progression, proliferation [123], has an inhibitory effect on DNA synthesis [124], initiating up-regulation of gap-junction proteins and a reduction of local androgen signaling [125], impacts IGIF-1 signaling [126], Antioxidant [127] and induction of apoptotic cell death [128]. These data indicate that lycopene is a promising chemopreventive agent, with several cellular effects. On the other hand, lycopene has also been observed to up-regulate the expression of urokinase plasminogen activator that is known to facilitate metastasis to the bone [129]. Several laboratories have examined the effects of lycopene in prostate carcinogenesis in rodent models, [129,130-133] suggesting that lycopene metabolism was modulated by androgens [130,133], as castrated rats accumulated twice the liver lycopene as compared to intact controls, [130] interfering with local testosterone activation. Prostatic IGF-I and IL-6 expression was also found to be down-regulated by lycopene [131]. A few clinical trials have reported reduction of tumor volume, [134,135] and lower prostate Specific antigen [136,137] with lycopene supplementation. To date, the results of the initial early clinical trials appear promising, although they have included various lycopene preparations and relatively short and varying duration of interventions (ranged from 12 mg/day for 8 weeks to 150 mg/day for 7 days) and men at various stages of PCa, utilizing both intermediate and surrogate biomarkers to evaluate chemoprevention efficacy. In a Phase II randomized-controlled trial [74] to evaluate the safety and effect of administering several doses of lycopene to men with clinically localized PCa, on intermediate endpoint biomarkers implicated in prostate carcinogenesis, forty-five eligible men with clinically localized PCa were supplemented with 15, 30 or 45 mg of lycopene or no supplement from biopsy to prostatectomy. Compliance to study agent, toxicity, changes in plasma lycopene, serum steroid hormones, PSA and tissue Ki-67 were analyzed from baseline to completion of intervention. Forty-two of forty-five subjects completed the intervention in approximately 30 days from the time of biopsy until prostatectomy. Plasma lycopene increased from baseline to post treatment in all treatment groups with greatest increase observed in the 45 mg lycopene-supplemented arm compared to the control arm without producing any toxicity. Overall, subjects with PCa had lower baseline levels of plasma lycopene similar to those observed in previous studies in men with PCa. Serum free testosterone decreased with 30 mg lycopene supplementation and total estradiol increased significantly with 30 mg and 45 mg supplementation from baseline to end of treatment, with no significant increases in serum PSA or tissue Ki-67. These changes were not significant compared to the control arm

for this sample size and duration of intervention. Although antioxidant properties of lycopene have been hypothesized to be primarily responsible for its beneficial effects, our study suggests that other mechanisms mediated by steroid hormones may also be involved [74]. Because PCa in AA men may demonstrate decreased apoptosis [138,139], lycopene may be more potent in that population. Unfortunately, the number of AA participants in the major lycopene studies was small thus precluding a separate sub-analysis for that racial group. Smaller studies have shown that blood lycopene levels are generally lower in AA men compared to White men [138], and that lycopene administration leads to increased plasma lycopene concentrations in AA men [139]; however, the value of the aforementioned observations for PCa prevention remains to be established. Lycopene is generally well tolerated and is considered safe in either its natural or synthetic form [140,141]. Collectively, these earlier findings support a hypothesis that lycopene may play a role in the modulation of prostate carcinogenesis, warranting further well powered and well-designed phase II clinical trials.

Other Promising Botanicals in the Pipeline

Other than Isoflavones green tea catechins and lycopne, other botanicals that appear promising for PCa chemoprevention include curcumin and Dercusin.

Decursin

Decursin is a novel coumarin compound, which inhibits the growth of human PCa cells. A coumarin compound decursin (C19H20O5; molecular weight 328) was isolated from angelica (Angelica gigas) root. Singh et al. [142,143] observed that decursin (25-100 μmol/L) treatment strongly inhibits growth and induces death in human prostate carcinoma DU145, PC-3, and LNCaP cells. Decursinol, in which (CH3)2-C=CH-COO- side chain of decursin is substituted with –OH, shows lesser effects as compared to decursin, suggesting for a possible structure-activity relationship. Decursin induced a strong G1 arrest in DU145 and LNCaP cells, and G1 as well as G2-M arrest in PC-3 cells. Further, decursin was nontoxic to human prostate epithelial PWR-1E cells and exhibited only moderate growth inhibition and G1 arrest [142,143]. With cell cycle effect on G1 phase, decursin strongly increased Cip1/p21 but showed a moderate increase in Kip1/p27 with a decrease in cyclin-dependent kinases CDK2, CDK4, CDK6, and cyclin D1, and inhibited CDK and cyclin-associated kinase activity. Decursin-caused cell death was associated with an increase in apoptosis and cleaved caspase-9, caspase-3, and poly(ADP-ribose) polymerase. Pan-caspases inhibitor only partially reverses decursininduced apoptosis, suggesting the involvement of both caspase-dependent and caspase-independent pathways [142]. Furthermore, decursin significantly decreased human umbilical vein endothelial cell (HUVEC) proliferation concomitant with G1 phase cell cycle arrest in biologically relevant growth (with serum) conditions. Decursin also inhibited HUVEC-capillary tube formation and invasion/migration in which was associated with the suppression of matrix metalloproteinase (MMP) -2 and -9 activities. Decursin suppressed angiogenesis in *ex vivo* rat aortic ring angiogenesis model where it inhibited blood capillary-network sprouting from rat aortic sections [144]. These findings suggested anti-angiogenic activity of decursin in biologically relevant condition, and warrants further pre-clinical studies for its potential clinical usefulness. Taken together, these findings revealed the novel anticancer efficacy of decursin mediated via induction of cell cycle arrest and apoptosis selectively in human prostate carcinoma cells. Anti-angiogenic activity of decursin could also contribute to its *in vivo* anticancer efficacy. Further studies are needed to explore the pan-efficacy and mechanisms of decursin or angelica root extract in different stages of PCa.

Curcumin

Curcumin is a naturally occurring plant-derived phenol that is a component of a popular Indian spice turmeric and a powerful antioxidant, that has been extensively studied because of its beneficial health effects including antimicrobial/antifungal [145,146], hepatoprotective [147], neuro-protective [148], cardio-protective [149], anti-inflammatory [150] and anticancer properties [151]. Mechanistic effects of curcumin on PCa *in vitro* and *in vivo* have been extensively studied and are both antiproliferative (down regulated AR, EGFR and cyclin D expression, inactivated NFkB) and proapoptotic (down regulated bcl-xl, bcl-2 and surviving expression) [152]. Importantly, curcumin was shown to inhibit PCa growth (50% inhibition) and induce the caspase-dependent apoptosis and reduce lung metastases by 89% *in vivo* [153]. Taken together, this evidence indicates that curcumin exhibits robust multi targeted anticancer activity against PCa, [154-156]. Curcumin is generally considered safe even at the very high doses of up to 12 grams per day [157]. Despite convincing and very encouraging preclinical data and established safety, prostate chemoprevention clinical trials of curcumin are lacking.

Conclusions

Based on the promising trends observed preclinical and early clinical trials by our group and others, including the relatively safety compared to currently available agents for PCa chemoprevention [47-49] the current research using a systematic approach to identify molecular targets of botanicals and translating the findings to design and implement clinical trials for chemoprevention provides an alternate to strategies other than screening. Although, currently there are no chemopreventive strategies that are standard of care in medical practice that have resulted from over 2 decades of research, it is clear that several valuable lessons have been learnt from earlier studies that continue to inform the design and approach of current chemoprevention trials using botanicals. With a better understanding of the promiscuous targeting of botanicals and a clear understanding of the synergistic effects of these agents present as whole mixtures or compounds based on evidence from *in vitro*, cutting-edge pre-clinical informing design of clinical studies and selection of intermediate endpoint biomarkers, the path has been paved to move several botanicals from bench to bedside.

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