Free Radical Research, October 2006; 40(10): 1095–1104

informa
healthcare

Polyphenols from green tea and pomegranate for prevention of prostate cancer

VAQAR M. ADHAMI & HASAN MUKHTAR

Department of Dermatology, University of Wisconsin-Madison, Madison, WI 53706, USA

Accepted by Professor H. Sies

(Received 24 March 2006; in revised form 21 April 2006)

Abstract
Prostate cancer (PCa) is the most common non-cutaneous cancer diagnosed in North America with similar trends in many Western countries. Geographic, epidemiological and laboratory studies suggest a role for dietary constituents in the etiology as well as prevention of PCa. The rising incidence of PCa in several countries appears to be coincidental with adoption of western lifestyle. Increase in the incidence of PCa has also been found in Asian populations migrating to the west. These facts give numerous leads to explore testable PCa prevention strategies. There is growing evidence in support of use of dietary ingredients in prevention and treatment of PCa. While substantial data exists in favor of use of polyphenols from tea as PCa chemopreventive agent, interest in anti-cancer properties of polyphenols from pomegranate has recently emerged. This review summarizes current literature on the effects of polyphenols from green tea and pomegranate against PCa.

Keywords: *Green tea, pomegranate, polyphenols, prostate cancer, chemoprevention*

Introduction

Cancer is caused by factors that include both external (such as tobacco, radiation, infectious agents) as well as internal (mutations, hormones and immune conditions) factors. These causal factors may act together or in succession to initiate or promote the process of carcinogenesis. It is usually too late when the disease is diagnosed and present strategies through surgery, chemotherapy and radiation offer minimal survival chances together with agonizing side effects. The time lapse between the initiation of the disease and the development of detectable tumors offers a window of opportunity to halt the march of cells to malignancy. Scientific evidence suggests that of the 564,830 cancer deaths predicted for the year 2006, one-third will be related to nutrition, obesity and lack of physical activity and therefore could be prevented [1]. These facts and observations give numerous leads to explore testable prevention strategies against the development of cancer. One way to reduce the occurrence of cancer is through chemoprevention, a means of cancer control in which the occurrence of the disease can be entirely prevented, slowed or reversed by the administration of one or more naturally occurring and/or synthetic compounds [2–10]. Chemoprevention also comprises chemotherapy of precancerous lesions [3–6] and includes such chemopreventive compounds that have (a) little or no toxic effects, (b) high efficacy in multiple sites, (c) capability of oral consumption, (d) a known mechanism of action, (e) low cost and (f) human acceptance. Chemoprevention thus is to intervene at the root of the disease, the process of carcinogenesis. This is entirely different from cancer treatment in that the ultimate objective of this approach is to lower the rate of cancer incidence.

Among all cancers, prostate cancer (PCa) offers an ideal candidate disease for chemoprevention because it is typically diagnosed in men over the age of 50 and has a high latency period. Therefore, even a slight delay in the carcinogenic pathway of progression of this

Correspondence: H. Mukhtar, Department of Dermatology, Medical Sciences Center, University of Wisconsin, 1300 University Avenue, Room B-25, Madison, WI 53706, USA. Tel: 1 608 263 3927. Fax: 1 608 263 5223. E-mail: hmukhtar@wisc.edu

ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/10715760600796498

disease by chemoprevention could result in a substantial reduction in the incidence of the disease and more importantly, improve the quality of life of the patients by simply delaying the onset of the disease [11–13]. The identification of promising agents (and their molecular targets) for PCa chemoprevention is guided by data derived from a variety of sources viz. (a) epidemiological observations, (b) PCa treatment trials, (c) secondary analyses from large, randomized, controlled cancer prevention trials, (d) an understanding of cancer biology and prostate carcinogenesis and (e) experimental animal models. Because PCa is a complex disease involving different molecular events, blocking or inhibiting only one event will not be sufficient to prevent or delay the onset of the disease. Efforts are therefore ongoing for a better understanding of this disease and for the development of novel approaches for its prevention and treatment.

Experimental evidences supported by geographic and epidemiological data suggest that environmental carcinogenic factors and nutrition play important causative roles in the initiation, promotion and progression stages of PCa [14]. An increase in the incidence of PCa has been found in Asian populations migrating to the west probably due to adoption of western lifestyle [15]. One case-control study established a positive association of PCa risk with total energy intake as well as intake of total fat [16]. Also, there have been some studies suggesting the role of energy intake, body size and physical activity in the progression and promotion of PCa [17]. Epidemiological studies have observed a correlation between populations with higher consumption of selenium, vitamin E, fruits and tomatoes, in lowering the risk of PCa [18]. Consistent with this notion, currently several natural agents are under study for their assessment as preventive agents against PCa. The beverage tea has been studied extensively and it has emerged as an agent having anti-mutagenic and anticancer effects in animal tumor models [9]. Recently, interest has been generated in understanding the PCa chemopreventive properties of pomegranate, a mediterranean fruit rich in polyphenols [19–20]. Detailed below is a summary of the laboratory, clinical trial and epidemiological observations on the use of the polyphenols from green tea and pomegranate for prevention and management of PCa.

Polyphenols from green tea in the prevention of prostate cancer

Tea, the most popular beverage consumed by humans is derived from the leaves of *Camellia sinensis* and is a rich source of catechins, the water-soluble polyphenolic constituents that account for 30–42% of its dry weight. Of the estimated 2.5 million metric tons of tea produced annually, 78% is black tea, 20% is green tea and the rest 2% is oolong tea. The main catechins in green tea are epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG). EGCG constitutes the major catechin accounting for up to 50% of the total polyphenols and is also considered as the most active polyphenolic ingredient in tea [21]. Extensive studies from this laboratory and several laboratories around the world have demonstrated the cancer chemopreventive properties of polyphenols from green tea (Table I). Green tea has been shown to protect against all stages of carcinogenesis in several animal tumor bioassay systems such as of lung [22], skin [23], oesophagus [24], liver [25], stomach [26], breast [27] and our work has established that it possesses remarkable activity against PCa [9].

In vitro *cell culture studies with green tea*

The prostate is an androgen-regulated organ and androgens are the major stimulus for cell division in prostatic epithelium. Thus, androgens are strong candidates as major contributors to prostatic carcinogenesis

Table I. A summary of effects of green tea and its polyphenolic constituents against PCa.

Model system	Effect	Reference
DU145, LNCaP, PC-3	Induction of apoptosis	[37–43], [85,86]
LNCaP and DU145	Cell cycle arrest	[39,40]
LNCaP	Induction of p53	[39]
LNCaP and DU145	Induction of WAF1/p21	[39]
LNCaP and PC-3	Inhibition of proteasome activity	[46,47]
LNCaP	Upregulation of PKC and suppression of TrkE	[48]
LNCaP, PC-3	Inhibition of Cox-2	[82]
PC-3, PC-3ML	Inhibition of HIF-1α degradation	[81]
LNCaP, DU145, TRAMP	Inhibition of MMP-2 activation	[83,84,57]
LNCaP	Inhibition of fatty acid synthase	[85]
LNCaP and DU145	Induction PI3K/Akt and MAPK	[86]
LNCaP and PC-3	Inhibition of proteasome activity	[46,47]
LNCaP	Upregulation of PKC and suppression of TrkE	[48]
LNCaP, PC-3	Inhibition of Cox-2	[82]
TRAMP	Inhibition if IGF-I/IGFBP-3 signaling	[57]

and this fact is reinforced by the observation that PCa rarely occurs in eunuchs and in men with deficiency of 5-alpha reductase and is inhibited by androgen ablation [28,29]. Green tea catechins EGCG, and ECG were found to be potent inhibitors of 5-alpha reductase, the enzyme that converts testosterone to its active metabolite 5-alpha-dihydroxytestosterone [30]. EGCG inhibited LNCaP cell growth and the expression of androgen regulated PSA and hK2 genes. Moreover, EGCG also had a significant inhibitory effect on the androgenic inducibility of the PSA promoter. An Sp1 binding site in the androgen receptor gene promoter is an important regulatory component for its expression. This study suggested Sp1 binding site in the androgen receptor as the target for the tea polyphenols because treatments of EGCG decreased the expression, DNA binding activity and transactivation activity of Sp1 protein [31]. Production of PSA was significantly decreased in a dose as well as time-dependent manner when human PCa LNCaP cells were treated with EGCG [32]. These findings could have a direct relevance to a human situation and underscore the need for clinical data for screening PSA levels in patients being monitored after administration of green tea. Another androgen regulated molecule that is upregulated in PCa is ornithine decarboxylase (ODC) [33,34]. The authors observed a significant increase in the level of ODC enzyme activity when LNCaP cells were treated with testosterone. Pretreatment of LNCaP cells with green tea polyphenols (GTP), inhibited testosterone mediated increase in ODC activity and ODC mRNA. In addition GTP inhibited testosterone induced colony formation in a dose-dependent fashion [35].

Elucidation of the critical events associated with carcinogenesis provides the opportunity for dietary intervention to prevent cancer development through induction of apoptosis, particularly by bioactive agents [36]. Apoptosis is a form of programmed cell death and is a critical defense against the occurrence of cancer and is essential in maintaining tissue homeostasis. Many diet-related genes are involved in carcinogenesis as well as apoptosis, and thus are ultimately molecular targets for dietary chemoprevention. Thus, apoptosis is an emerging therapeutic target of bioactive agents of diet [36]. In a study aimed at investigating the inhibitory effects of green tea components, it was observed that EGCG treatment resulted in an induction of apoptosis in several human cancer cells including human PCa DU145 cells [37]. Similar effects were observed when tea catechins were tested on the PCa cell lines LNCaP, PC-3 and DU145 and it was observed that EGCG was the most potent catechin at inhibiting cell growth. The inhibition induced by EGCG was found to occur via apoptotic cell death as evidenced by changes in nuclear morphology and DNA fragmentation [38]. At the time of clinical diagnosis PCa represents a mixture of both androgen-sensitive and androgen-insensitive tumor cells and therefore androgen ablation alone is not sufficient to eliminate all cell types. Effect of EGCG treatment was studied on PCa cells line differing in their androgen and p53 status. A dose-dependent inhibition in cell growth was observed in both androgen-sensitive LNCaP and androgen-insensitive DU145 cells [39]. This cell growth inhibition was accompanied by a dose-dependent apoptosis of DUI45 and LNCaP cells as evident by DNA fragmentation. EGCG treatment resulted in a dose-dependent increase of p53 in LNCaP cells (carrying wild-type p53) but not in DUI45 cells (carrying mutant p53) suggesting cell cycle arrest produced by EGCG is dependent on p53 status in LNCaP cells but not in DUI45 cells. Subsequently, we provided molecular understanding of this effect and found that EGCG-mediated cell cycle dysregulation and apoptosis is mediated via modulation of cyclin kinase inhibitor (cki)-cyclin-cyclin-dependent kinase (cdk) machinery [40]. This was evident by an upregulation of the protein expression of WAF1/p21, KIP1/p27, INK4a/p16, and INK4c/p18, (i) down-modulation of the protein expression of cyclin D1, cyclin E, cdk2, cdk4 and cdk6, but not of cyclin D2, (ii) increase in the binding of cyclin D1 toward WAF1/p21 and KIP1/p27, and (iii) decrease in the binding of cyclin E toward cdk2. We further reported that EGCG-induced apoptosis in human prostate carcinoma LNCaP cells is mediated via modulation of two related pathways; one by stabilization of p53 by phosphorylation on critical serine residues and p14ARF-mediated downregulation of murine double minute 2(MDM2) protein, and the other negative regulation of NF-kappaB activity, thereby decreasing the expression of the anti-apoptotic protein Bcl-2 [41]. EGCG-induced stabilization of p53 caused an upregulation in its transcriptional activity, thereby resulting in activation of its downstream targets p21/WAF1 and Bax. Thus, EGCG had a concurrent effect on two important transcription factors p53 and NF-kappaB, causing a change in the ratio of Bax/Bcl-2 in a manner that favors apoptosis. This altered expression of Bcl-2 family members triggers activation of initiator capsases 9 and 8 followed by activation of effector caspase 3. Activation of the caspases was followed by poly (ADP-ribose) polymerase cleavage and induction of apoptosis. In contrast to DU145 and LNCaP cells, inactivation of p53 using small interfering RNA rendered p53 transfected PC-3 cells resistant to EGCG-mediated apoptosis [42]. Because p53 activation led to increase in p21 and Bax, ablation of p21 protein by siRNA prevented G1 arrest and apoptosis in PC3-p53 cells [43]. These studies demonstrated that EGCG activates growth arrest and apoptosis primarily via p53-dependent pathway that involves the function of both p21 and Bax such that down-regulation of either molecule confers a growth advantage to the cells. These data indicated

that EGCG induced apoptosis in human prostate carcinoma cells is accompanied by a shift in the balance between pro- and anti-apoptotic proteins in favor of apoptosis [43]. These observations seem to have a practical implication because EGCG was found to be effective against several PCa cells.

The ubiquitin-proteasome system plays a critical role in the specific degradation of cellular proteins [44], and two of the proteasome functions are to allow tumor cell cycle progression and to protect tumor cells against apoptosis [45]. Proteasome inhibitors are able to induce tumor growth arrest and ester bond containing tea polyphenols, such as EGCG potently and specifically inhibit the chymotrypsin-like activity of the proteasome *in vitro* at concentrations found in the serum of green tea drinkers [46,47]. This inhibition of the proteasome by EGCG in several tumor and transformed cell lines results in the accumulation of two proteasome substrates, p27/Kip1 and IkB-α, an inhibitor of transcription factor NF-kappaB, followed by growth arrest in the G1 phase of the cell cycle [46]. This study suggests that proteasome is a cancer-related molecular target of tea polyphenols and that inhibition of the proteasome activity by ester bond-containing polyphenols may contribute to the cancer-preventive effects of tea.

To further characterize the molecular targets of PCa chemoprevention by green tea the authors employed a cDNA microarray technique and identified a total of 25 genes in LNCaP cells that showed a significant response to EGCG (12 μM, for 12 h). Of these, the expression of sixteen genes was found to be significantly increased as a result of EGCG treatment; and nine genes were found to be significantly repressed by EGCG [48]. Interestingly, all of these genes belonged to different regulatory pathways, suggesting EGCG affects multiple cellular events. Among these genes the repression of PKC-α was most prominent. Recent studies suggest that inhibition of PKC-α gene expression could inhibit cell proliferation in animal tumor model and in some human cancer cell lines [49,50]. The cDNA microarray also identified among several other the induction of receptor-type protein tyrosine phosphatase-γ gene expression, a tumor suppressor gene candidate frequently deleted in some human cancers [48].

In vivo *studies using prostate cancer xenograft mouse model*

To investigate the effect of GTP in an *in vivo* setting Liao et al. [51] implanted athymic nude with androgen-insensitive PC- 3 and androgen-sensitive LNCaP 104-R cells followed by treatment with EGCG (daily 1 mg/mouse, i.p.) starting at 2 weeks post-implantation of cells. EGCG treatment to mice resulted in a reduction in the initial tumor growth of both cell types by 20–30% [51]. Roomi et al. [52] made similar observations when they fed mice with a nutrient mixture containing green tea extract and further observed inhibition of MMP-9 and vascular endothelial growth factor (VEGF) secretion. Extending these studies, the authors employed androgen-responsive CWR22Rν1 PCa tumor xenografts implanted in athymic nude mice and investigated PCa chemopreventive effects of GTP, and its major constituent EGCG [53]. Data demonstrated that the treatment with GTP and EGCG resulted in significant inhibition in growth of implanted prostate tumors and reduction in the serum PSA levels. Furthermore, GTP (0.01 or 0.05% w/v) given after establishment of CWR22Rν1 tumors, caused a significant regression of tumors suggesting therapeutic effects of GTP at human achievable concentrations. The antiproliferative effects of green tea were found to be mediated by induction of apoptosis as observed by an upregulation in Bax, decrease in Bcl-2 proteins, and by the cleavage of poly (ADP-ribose) polymerase [53]. These data suggested that besides possessing chemopreventive properties green tea also possesses chemotherapeutic properties.

In vivo *studies using animal models of prostate cancer*

In order to obtain convincing evidence of the usefulness of green tea, it is recommended that chemoprevention studies must be conducted in animal models that emulate human disease and in which disease progression occurs without the administration of unrealistic amounts of carcinogens. The transgenic adenocarcinoma of the mouse prostate (TRAMP) is one such model in which progressive forms of human disease from prostatic intraepithelial neoplasia (PIN) to histologic cancer and from histologic to metastasizing prostate carcinoma occur spontaneously [54,55]. Using the TRAMP model the authors showed that oral infusion of GTP at a human achievable dose (equivalent to six cups of green tea per day) significantly inhibits PCa development and increases overall survival in these mice [56]. In two separate experiments, the cumulative incidence of palpable tumors at 32 weeks of age in 20 untreated mice was 100 and 95% of the animals exhibited distant site metastases to lymph nodes, lungs, liver and bone. However, 0.1% GTP (wt/vol) in drinking water from 8 to 32 weeks of age resulted in significant delay in primary tumor incidence and tumor burden as assessed by MRI and significant decrease in prostate and genitourinary weight. The striking observation of this study was that GTP infusion resulted in almost complete inhibition of distant site metastases. In a follow up study the authors examined the role of IGF/IGFBP-3 signaling and its downstream and other associated events during chemoprevention of PCa by GTP in TRAMP mice [57]. Data demonstrated an increase in the levels of IGF-I, phosphatidylinositol 3′-kinase, phosphorylated Akt (Thr-308), and extracellular signal-regulated kinase 1/2 with concomitant decrease in IGFBP-3 in

dorso-lateral prostate of TRAMP mice during the course of cancer progression. Continuous GTP infusion for 24 weeks to these mice resulted in substantial reduction in the levels of IGF-I and significant increase in the levels of IGFBP-3 in the dorso-lateral prostate. This modulation of IGF/IGFBP-3 was found to be associated with an inhibition of protein expression of phosphatidylinositol 3′-kinase, phosphorylated forms of Akt (Thr-308) and extracellular signal-regulated kinase 1/2. Furthermore, marked inhibition of markers of angiogenesis and metastasis most notably VEGF, urokinase plasminogen activator, and matrix metalloproteinases 2 and 9 were also observed by GTP infusion. These data suggested that IGF-I/IGFBP-3 signaling pathway is a prime pathway for GTP-mediated inhibition of PCa that limits the progression of cancer through inhibition of angiogenesis and metastasis [57]. The effect of green tea on the development of PCa in TRAMP was corroborated is a study by Caporali et al. [58]. They reported that while 100% of TRAMP mice developed PCa, only 20% of those receiving 0.3% green tea catechins (GTC) in drinking water developed neoplasm. Further, in the TRAMP mice clusterin gene was dramatically down-regulated during onset and progression of PCa and in mice that received GTC tumor progression was inhibited with progressive accumulation of clusterin mRNA and protein in the prostate gland suggesting a possible role for clusterin as a novel tumor-suppressor gene in the prostate. These data further demonstrate that green tea may be an effective chemopreventive agent against PCa.

Green tea consumption and epidemiologic studies

Evidences collected from geographic, epidemiologic and migration studies suggest that diet and nutrition play an importance in the incidence and risk of PCa [14]. Data indicate that frequent consumption of green tea is inversely associated with the risk of several types of human cancers and the lower frequencies of PCa in Asian population in general, compared to those in Western societies [59]. Most reports on Asians who predominantly drink green tea have shown positive cancer-preventive effects [60]. In contrast, the high-fat diet typical of Western countries is associated with high incidence rates (>40 cases per 100,000 men) and with a higher risk for PCa [61,62]. These associations are further strengthened by observations that suggest Asian men migrating to the USA and their subsequent US born generations acquire a higher clinical incidence of PCa [63]. Two epidemiologic studies have shown that persons who regularly consume tea have a lower PCa incidence; however, these studies include populations that are predominantly black-tea drinkers and lack proper controls for comparisons [64,65]. Recently a case-control study was conducted in Hangzhou, south-east China to investigate whether green tea consumption had an etiological association with PCa [66]. One hundred and thirty incident patients with histologically confirmed adenocarcinoma of the prostate were compared with 274 hospital inpatients without PCa or any other malignant diseases. Among the cases, 55.4% were tea drinkers compared to 79.9% for the controls. Almost all the tea consumed was green tea. The PCa risk declined with increasing frequency, duration and quantity of green tea consumption. The adjusted odds ratio (OR), relative to non-tea drinkers, were 0.28 (95% CI = 0.17–0.47) for tea drinking, 0.12 (95% CI = 0.06–0.26) for drinking tea over 40 years, 0.09 (95% CI = 0.04–0.21) for those consuming more than 1.5 kg of tea leaves yearly, and 0.27 (95% CI = 0.15–0.48) for those drinking more than threee cups (1 l) daily. The dose response relationships were also significant, suggesting that green tea is protective against PCa [66]. A better understanding of how dietary factors interact to cause or prevent PCa through further studies will facilitate design of appropriate public health strategies in order to reduce the incidence of PCa.

Clinical trials with green tea

A phase II clinical trial explored green tea's anti-neoplastic effects in patients with androgen independent prostate carcinoma [67]. Forty two patients who were asymptomatic and had manifested progressive and rising PSA levels with hormone therapy were instructed to take 6 g of green tea extract per day orally in six divided doses. Patients were monitored monthly for response and toxicity. Significant decrease in the baseline PSA value occurred in a single patient or 2% of the cohort (95% confidence interval) and this response was not sustained beyond 2 months. Green tea toxicity was reported in 69% of patients the study concluded that green tea carries limited anti-neoplastic activity as defined by a decline in PSA levels among patients with androgen independent prostate carcinoma [67]. Another clinical study evaluated the efficacy and toxicity of green tea, prescribed as an alternative complementary formulation on hormone refractory PCa [68]. Nineteen patients were inducted into the study and prescribed green tea extract capsules at a dose level of 250 mg twice daily. Of the fifteen patients that completed at least 2 months of therapy, nine had progressive disease within 2 months of starting therapy, six developed progressive disease after additional 1–4 months of therapy. The study concluded that green tea as a complementary alternative therapy had minimal clinical activity against hormone refractory PCa [68]. It is important to mention here that these studies, in principal, do not qualify as a chemopreventive studies since they were conducted in patients with androgen-independent and hormone refractory PCa. An ideal study should consider a population with a high risk for PCa development.

Recently, a proof-of-principle clinical trial was conducted to assess the safety and efficacy of GTCs

for the chemoprevention of PCa in volunteers with high grade prostatic intraepithelial neoplasia (HGPIN) [69]. Sixty volunteers with HGPIN were given daily three GTCs capsules of 200 mg each. After 1 year, only one tumor was diagnosed among the 30 GTCs-treated men (incidence, approximately 3%), whereas nine cancers were found among the 30 placebo-treated men (incidence, 30%). Total prostate-specific antigen did not change significantly between the two arms, but GTCs-treated men showed values constantly lower with respect to placebo-treated ones. This is the first study that showed GTCs are safe and very effective for treating premalignant lesions before PCa develops [69].

Polyphenols from pomegranate in the prevention of prostate cancer

Pomegranate from the tree *Punica granatum* possesses strong antioxidant and anti-inflammatory properties. Previous studies have demonstrated the anti-carcinogenic activity of pomegranate extracts in a series of human cancer cells and pomegranate has been described as nature's power fruit [70]. Antioxidant activity of flavonoids extracted from pomegranate fermented juice showed strong activity close to that of butylated hydroxyanisole and green tea and significantly greater than that of red wine [71]. The antioxidant activity of pomegranate juices was evaluated and compared to those of red wine and a green tea infusion. Commercial pomegranate juices showed an antioxidant activity (~18–20 TEAC) as determined by trolox equivalent antioxidant capacity (TEAC) three times higher than those of red wine and green tea (6–8 TEAC). The activity was higher in commercial juices extracted from whole pomegranates than in experimental juices obtained from the arils only (12–14 TEAC) [72]. Noda et al. [73] evaluated antioxidant activities of freeze-dried preparations of a 70% acetone extract of pomegranate and its three major anthocyanidins (delphinidin, cyanidin and pelargonidin). Free radical scavenging activities were examined using an electron spin resonance technique with spin trapping; DMPO for hydroxyl ($^{\cdot}OH$) and superoxide ($O_2^{\cdot-}$) radicals; and [$MGD_2Fe_2^{+}$] for nitric oxide (NO). Pomegranate extract exhibited scavenging activity against $^{\cdot}OH$ and $O_2^{\cdot-}$. Anthocyanidins inhibited a Fenton reagent $^{\cdot}OH$ generating system possibly by chelating with ferrous ion. Anthocyanidins scavenged $O_2^{\cdot-}$ in a dose-dependent manner. The ID_{50} values of delphinidin, cyanidin and pelargonidin were 2.4, 22 and 456 μM, respectively. In contrast, anthocyanidins did not effectively scavenge NO. Anthocyanidins inhibited H_2O_2-induced lipid peroxidation in the rat brain homogenates and ID_{50} values of delphinidin, cyanidin and pelargonidin were 0.7, 3.5 and 85 μM, respectively. These findings suggested that anthocyanidins present in pomegranate contribute to the antioxidant activity of these fruits.

The anti-cancer properties of pomegranate have only recently been identified. Polyphenol-rich fractions from pomegranate fruit were assessed *in vitro* for possible chemopreventive or adjuvant therapeutic potential in human breast cancer [74]. Polyphenols from fermented juice at concentration ranging from 100 to 1000 μg/ml inhibited aromatase activity by 60–80% and 17-β-hydroxysteroid dehydrogenase Type 1 activity by 79%. In two breast cancer cell lines MCF-7 and MB-MDA-231 cells, fermented pomegranate juice polyphenols consistently showed about twice the anti-proliferative effect as fresh pomegranate juice polyphenols. Pomegranate seed oil effected 90% inhibition of proliferation of MCF-7 at 100 μg/ml of medium, 75% inhibition of invasion of MCF-7 across a Matrigel membrane at 10 μg/ml, and 54% apoptosis in MDA-MB-435 estrogen receptor negative metastatic human breast cancer cells at 50 μg/ml. In a murine mammary gland organ culture, fermented juice polyphenols effected 47% inhibition of cancerous lesion formation induced by the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA). The anti-angiogenic potential of polyphenols from pomegranate was tested by measuring VEGF, interleukin-4 (IL-4) and migration inhibitory factor (MIF) in the conditioned media of estrogen sensitive MCF-7, estrogen resistant MDA-MB-231 human breast cancer cells and immortalized normal human breast epithelial cells MCF-10A [75]. VEGF was strongly downregulated in MCF-10A and MCF-7, and MIF upregulated in MDA-MB-231, overall showing significant potential for downregulation of angiogenesis by pomegranate fractions. A significant decrease in new blood vessel formation was observed using a chicken chorioallantoic membrane (CAM) model. These observations provided an evidence for an anti-angiogenic potential of pomegranate fractions rich in polyphenols.

The effects of pomegranate on inhibition of cell proliferation and induction of apoptosis in human PCa cells have only recently been investigated (Table II). Pomegranate in the form of oils, fermented juice polyphenols and pericarp polyphenols was tested on human PCa cell growth both *in vitro* and *in vivo* [76–78]. Each form of pomegranate inhibited *in vitro* proliferation of LNCaP, PC-3, and DU 145 human cancer cell lines whereas normal prostate epithelial cells were significantly less affected. These effects were mediated by changes in both cell cycle distribution and induction of apoptosis. Androgen-independent DU145 cells treated with pomegranate cold pressed oil (35 μg/ml) showed a significant increase in G_2/M cells with only a modest induction of apoptosis. This arrest in cell cycle was associated with a significant up-regulation of the cyclin-dependent kinase inhibitor p21 and down-regulation of c-myc. In PC-3, cell proliferation was inhibited predominantly by induction

Table II. A summary of effects of pomegranate against PCa.

Model system	Effect	Reference
LNCaP, PC-3 and DU145	Induction of apoptosis	[20,78]
PC-3, DU145	Inhibition of invasion	[76,77]
DU145, PC-3	WAF1/p21 up-regulation	[78,20]
DU145	*c-myc* down-regulation	[78]
RWPE-1, 22Rv1, PC-3	Inhibition of proliferation	[20,80]

of apoptosis partially through a caspase 3-mediated pathway and rapid changes in mRNA levels of gene targets. In parallel all forms of pomegranate preparations potently suppressed PC-3 invasion through Matrigel and potently inhibited growth of PC-3 xenograft in athymic mice. Overall a significant anti-proliferative and anti-tumor activity of pomegranate-derived fractions against human PCa was observed [76–78]. Components from pomegranate fruit each belonging to different representative chemical classes and showing known anti-cancer activities were tested as potential inhibitors of *in vitro* invasion of human PC-3 PCa cells in an assay employing Matrigel artificial membranes. All compounds significantly inhibited invasion when employed individually at 4 μg/ml and when equally combined at the same dose showed a supra-additive inhibition of invasion as measured by the Kruskal-Wallis non-parametric test [79].

The authors recently demonstrated that pomegranate fruit extract (PFE) possesses remarkable anti-proliferative and pro-apoptotic properties against human PCa cells both *in vitro* and *in vivo* [20]. Treatment of human PCa PC-3 cells with an extract of pomegranate fruit (PFE, 10–100 μg/ml; 48 h) resulted in a dose-dependent inhibition of cell growth/cell viability and induction of apoptosis. This treatment of PC3 cells was associated with an induction of proapoptotic Bax and Bak, down-regulation of anti-apoptotic Bcl-XL and Bcl-2, induction of WAF1/p21 and KIP1/p27, a decrease in cyclins D1, D2, and E and decrease in the protein expression of cyclin-dependent kinase-2, -4 and -6. These data strongly suggested an involvement of the cyclin kinase inhibitor-cyclin-cdk network during the anti-proliferative effects of PFE. To establish the relevance of these *in vitro* findings to *in vivo* situation, athymic nude mice were implanted with androgen-responsive CWR22Rv1 cells, which are known to secrete PSA in the bloodstream of the host and given 0.1 and 0.2% (wt/vol) PFE in drinking water *ad libitum* starting at day 1 after tumor cell implantation.. The 0.1 and 0.2% doses of PFE selected for feeding mice were based on the assumption that a typical healthy individual (~70 kg) may be persuaded to drink 250 or 500 ml of pomegranate juice extracted from one or two fruits, respectively. Oral administration of PFE to athymic nude mice implanted with androgen-sensitive CWR22Rv1 cells resulted in a significant inhibition in tumor growth. Eight days after cell inoculation, the appearance of small solid tumors was observed in animals receiving water as a drinking fluid. This latency period was prolonged to 11–14 days in animals receiving PFE in drinking fluid. Tumor growth, as inferred by computed tumor volume, was significantly inhibited in mice receiving both 0.1 and 0.2% PFE, with higher inhibitory effects in animals receiving 0.2% PFE than in those receiving 0.1% PFE. In water-fed animals the average tumor volume of 1200 mm^3 was reached in ~31 ± 3 days after tumor cell inoculation. At this time point, average tumor volumes of the 0.1 and 0.2% PFE-fed groups were 776 and 558 mm^3, respectively. The most effective tumor growth inhibitory response was observed in the 0.2% PFE-fed group, where the targeted average tumor volume of 1200 mm^3 was reached at day 47 ± 4 after tumor cell inoculation. PFE treatment of 0.1% was also found to be significantly effective where the average tumor volume of 1200 mm^3 was achieved in ~39 ± 3 days after tumor cell inoculation. Tumor data were analyzed for survival probability by Kaplan-Meier analysis, which indicated that continuous PFE infusion to athymic nude mice resulted in increased survival ($P < 0.0001$, log-rank test), with a median survival of 39 and 47 days (0.1 and 0.2% PFE, respectively), compared with 31 days in water-fed mice ($P < 0.0001$, log-rank test). Concomitant with inhibition of tumor growth a significant decrease in serum prostate-specific antigen levels was observed [20]. In PFE-fed animals a significant inhibition of PSA secretion was observed at all time points examined. Twenty-three days after cell inoculation, secreted PSA levels were 7.9 ± 0.82, 2.5 ± 0.58 and 1.2 ± 0.97 ng/ml in water-fed, 0.1% PFE-fed and 0.2% PFE-fed animals, respectively. At 30 days after inoculation of tumor cells in 0.1 and 0.2% PFE-fed animals, 70% ($P < 0.001$) and 85% ($P < 0.001$) reduced levels of PSA were observed as compared with the water-fed group, respectively. The reduction in tumor growth with concomitant reduction in PSA levels observed in the xenograft model may have human clinical relevance. The outcome of this study could have a direct practical implication and translational relevance to CaP patients, because it suggests that pomegranate consumption may retard CaP progression, which may prolong the survival and quality of life of the patients.

While epidemiological, clinical and case-control studies have not been undertaken with pomegranate, it is however noteworthy to mention results from a recent phase II clinical trial in patients with rising

PSA. Pantuck et al. [80] conducted a phase II study of pomegranate juice in men with rising PSA following surgery or radiation for PCa. This study indicates positive and significant beneficial effects on PSA parameters suggesting a potential of pomegranate derived products for prevention of human PCa.

Conclusions and future directions

PCa management represents a formidable challenge being a complicated malignancy with heterogeneity of androgen-dependent and androgen-independent forms and exists in either a clinically insignificant form or an aggressive form that metastasizes to various sites in the body. There is also evidence of variation in PCa incidence based on geographical location and ethnicity of the population. While substantial improvements in diagnosis and treatment have improved overall survival, PCa continues to remain a leading cause of death in men. There is growing support in favor of use of non-toxic dietary ingredients for cancer management. Indeed, many such agents are under investigation for their possible use as cancer chemopreventive agents. These agents are proving to be unique based on their targeted action on cancer cells and their ability to spare normal cells. Based on the laboratory studies as outlined in this review, there is a pressing need for more in depth clinical studies to categorically identify the need for development of natural plant based polyphenols for PCa management. Also, as previously advocated by the authors cancer chemoprevention studies should be carried out in combination with agents with complementary mechanisms [87]. The agents in combination would produce either synergistic or additive effects. While appropriate clinical trials with green tea have recently underscored the importance of tea as a chemopreventive agent, there is a need to undertake similar studies with polyphenols from pomegranate fruit. In spite of the availability of large amount of data there are gaps in our knowledge on the mechanisms of chemoprevention of PCa by various polyphenols and inconsistencies between epidemiological, laboratory and clinical studies stipulate more extensive studies for obtaining conclusive evidence.

Acknowledgements

The original work from the author's (H.M.) laboratory outlined in this review was supported by United States Public Health Service Grants R01 CA 78809, R01 CA 101039, P50 DK065303-01 and by a grant from the Lynda and Stewart Resnick Revocable Trust.

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