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Extra View

Prostate Cancer Prevention Through Pomegranate Fruit

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KEY WORDS

prostate cancer, pomegranate, apoptosis, chemoprevention, chemotherapy

ABBREVIATIONS

CaP	prostate cancer
PFE	pomegranate fruit extract
PSA	prostate specific antigen
PARP	poly (ADP-ribose) polymerase

ABSTRACT

Prostate cancer (CaP) is the second leading cause of cancer-related deaths among U.S. males with a similar trend in many Western countries. CaP is an ideal candidate disease for chemoprevention because it is typically diagnosed in men over 50 years of age, and thus even a modest delay in disease progression achieved through pharmacological or nutritional intervention could significantly impact the quality of life of these patients. In this regard we and others have proposed the use of dietary antioxidants as candidate CaP chemopreventive agents. The fruit pomegranate derived from the tree *Punica granatum* has been shown to possess strong antioxidant and anti-inflammatory properties. In a recent study, we showed that pomegranate fruit extract (PFE), through modulations in the cyclin kinase inhibitor-cyclin-dependent kinase machinery, resulted in inhibition of cell growth followed by apoptosis of highly aggressive human prostate carcinoma PC3 cells. These events were associated with alterations in the levels of Bax and Bcl-2 shifting the Bax:Bcl-2 ratio in favor of apoptosis. Further, we showed that oral administration of a human acceptable dose of PFE to athymic nude mice implanted with CWR22Rv1 cells resulted in significant inhibition of tumor growth with concomitant reduction in secretion of prostate-specific antigen (PSA) in the serum. 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.

PERSPECTIVE

Prostate cancer (CaP) is the most common invasive malignancy and the second leading cause of cancer-related deaths among U.S. males with a similar trend in many Western countries.[1] For the year 2005, it has been estimated that 232,090 new cases of CaP will be diagnosed and 30,350 deaths related to CaP will occur in the U.S. alone.[1] In the absence of satisfactory treatment options, chemoprevention could be an effective approach to reduce the incidence of CaP. CaP is an ideal candidate disease for chemoprevention because it is typically diagnosed in men over 50 years of age, and thus even a modest delay in the duration of disease progression achieved through pharmacological or nutritional intervention could significantly impact the quality of life of these patients.[2] For a variety of reasons, the most important of which is human acceptance, chemoprevention through dietary intervention appears more practical. We and others have proposed the use of dietary antioxidants such as green tea (and many others) as candidate CaP chemopreventive agents. Many such naturally occurring antioxidants for chemoprevention of CaP are being evaluated in cell culture and in animal model systems.[2-6] It is noteworthy that some of these agents are showing promise in human CaP patients.[2,7-9] Almost all supplements sold to consumer with the promise for better prostate health contains several of these antioxidants. Consistent with this advocacy, many prostate cancer patients, in addition to their schedule treatments, following initial diagnosis extensively use dietary supplements. Thus, the news about newer fruit and common vegetable based agent for prostate cancer chemoprevention is generally received with much interest by CaP patients.

The fruit pomegranate derived from the tree *Punica granatum* is an edible fruit cultivated in Mediterranean countries, Afghanistan, India, China, Japan, Russia, and some parts of the United States. Pomegranate is believed to date back to the Garden of Eden has been used in folk medicine for centuries. Pomegranate has been shown to possess strong antioxidant,[10] anti-inflammatory,[11] antiatherogenic,[12] and some studies have suggested that it may possess antitumorigenic properties.[11] Infact the antioxidant activity of pomegranate fruit is shown to be higher than that of red wine and green tea,[10] two dietary substances, which are showing, promise in preclinical CaP models and in CaP patients.[2,7,13-19]

To explore whether pomegranate juice or its derived by products could be useful for prevention of CaP, we extracted edible portion of pomegranate fruit in 70% acetone -30% distilled water (1:20, w/v). The red extract was then filtered through (Whatman no.1) filter paper and the filtrate was condensed, freeze-dried and stored at 4°C. This extract, designated as PFE was analyzed by Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry, and found to contain six anthocyanins (pelargonidin 3-glucoside, cyanidin 3-glucoside, delphinidin 3-glucoside, pelargonidin 3,5-diglucoside, cyanidin 3,5-diglucoside, and delphinidin 3,5-diglucoside) and various ellagitannins and hydrolyzable tannins.[11] We recently showed that topical application of PFE prevents 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced tumor promotion in 7, 12-dimethybenz (a) anthracene (DMBA)-initiated CD-1 mouse skin.[11]

In a recent study,[20] employing highly aggressive human prostate cancer cells PC3 we first evaluated the antiproliferative properties of PFE. PFE treatment (10–100 μg/ml for 48 h) of PC3 cells was found to result in a dose-dependent inhibition of cell growth as assessed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium-bromide assay. Importantly, under similar conditions PFE did not result in cytotoxicity to normal human prostate epithelial cells. To assess whether PFE-induced growth inhibition of the cells is mediated through induction of apoptosis, we evaluated the effect of PFE on cleavage of PARP. Employing immunoblot analysis, we found that the full-size PARP protein (116 kDa) was cleaved to yield an 85-kDa fragment after treatment of cells with PFE at 10–100 μg/ml for 48 h. The induction of apoptosis by PFE was also evident from the morphology of cells, as assessed by fluorescence microscopy, after labeling the cells with an apoptosis detection kit containing annexin V and propidium iodide. We used this method because it identifies the apoptotic (green fluorescence) as well as necrotic (red fluorescence) cells.

Members of the Bcl-2 family of proteins are critical regulators of the apoptotic pathway.[21] Bcl-2 is an upstream effector molecule in the apoptotic pathway and is identified as a potent suppressor of apoptosis.[21] Bcl-2 is found at inappropriately high levels in more than half of all human tumors.[21] Bcl-2 has been shown to form a heterodimer complex with the proapoptotic member Bax, thereby neutralizing its proapoptotic effects. Therefore, the ratio of Bax/Bcl-2 is often considered as a decisive factor in determining whether cells will undergo death or survival. PFE treatment of PC3 cells for 48 h was found to result in a decrease in Bcl-2 protein expression with an increase in the protein expression of Bax. Importantly, in PFE-treated cells, the ratio of Bax to Bcl-2 was found to be altered in favor of apoptosis. These data suggest that upregulation of Bax and down-modulation of Bcl-2 may be a molecular mechanism through which PFE induces apoptosis of CaP cells (Fig. 1).

Others and we have shown the involvement of cell cycle regulation-mediated apoptosis as a mechanism of cell growth inhibition by many dietary antioxidants.[22-24] Therefore, we next investigated the involvement of the cyclin kinase inhibitor-cyclin-cdk machinery during the induction of cell cycle arrest and apoptosis by PFE in PC3 cells. In eukaryotes, passage through the cell cycle is orchestrated by a family of protein kinase complexes.[25] Each complex is composed minimally of a catalytic subunit, the cdk, and its essential activating partner, the cyclin.[25] Cyclins D and E are involved during G_1-S phase of the cell cycle. In controlled cell growth, association of cyclins D and E with cdk2, cdk4, or cdk6 leads to phosphorylation of Rb and its release from E2F, resulting in progression of the cell cycle and cellular proliferation. Any defect in this machinery results in an altered cell cycle regulation that may result in unwanted cellular

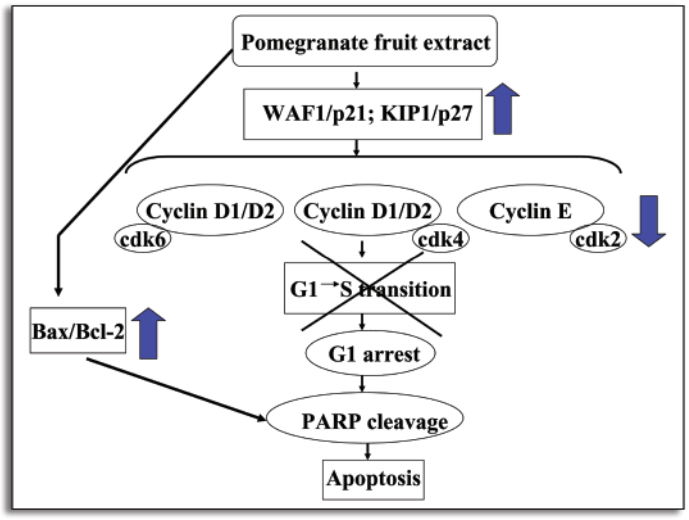

Figure 1. Proposed model for pomegranate fruit extract-mediated cell cycle dysregulation and apoptosis of human prostate carcinoma PC3 cells.

proliferation, ultimately culminating in the development of cancer.[25] During the progression of the cell cycle, the cdk-cyclin complexes are inhibited via binding to cyclin kinase inhibitors such as the WAF1 and KIP1 families of proteins.[26] We focused our efforts to examine the effect of PFE on cell cycle regulatory molecules operative in the G_1 phase of the cell cycle. A significant upregulation of the WAF1/p21 and KIP1/p27 during G_1 phase arrest and apoptosis of these cells by PFE treatment was observed (Fig. 1). In the absence of active p53 in PC3 cells, the observed induction of WAF1/p21 and KIP1/p27 by PFE appears to be independent of p53. It has been shown in many studies that exogenous stimuli may result in a p53-dependent, as well as a p53-independent, induction of WAF1/p21 and KIP1/p27, which may cause a blockade of G_1-S phase transition, resulting in a G_1 phase cell cycle arrest and apoptosis.[27] WAF1/p21 and KIP1/p27 are considered universal inhibitors of cyclin-cdk complexes; therefore, we assessed the effect of PFE treatment on the cyclins and cdks operative in the G_1 phase of the cell cycle (i.e., cyclins D1, D2, and E and cdk2, cdk4, and cdk6). We found that PFE treatment of the cells resulted in significant down-modulation of all of these regulatory molecules, although to a different extent (Fig. 1).

To establish the relevance of these in vitro findings to in vivo situation, Twenty-four athymic nude mice were implanted with androgen-responsive CWR22Rv1 cells, which are known to secrete PSA in the bloodstream of the host and form rapid and reproducible tumors. These animals were then randomly divided into three groups consisting of 8 animals each. The first group of animals received normal drinking water and served as controls. The animals of group 2 and 3 received the same drinking water supplemented with 0.1% and 0.2% PFE (w/v), respectively. The 0.1 and 0.2% dose of PFE selected for feeding mice is 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. In our experimental protocol, we euthenized the animals when the implanted tumor reached to a volume of 1200 mm^3. Oral feeding of PFE significantly slowed the progression of CWR22Rv1 tumor growth in nude mice. Thus, as shown in Table 1, in water-fed animals the average tumor volume of 1200 mm^3 was reached in approximately 31 ± 3 days post tumor cell inoculation. The most effective tumor growth inhibitory response was observed in the 0.2%

Table 1 **Oral feeding of pomegranate fruit significantly delays the progression CWR22Rv1 tumor growth in athymic nude mice.**

Treatment	Number of Days to Reach a Tumor Volume	
Groups	600 mm^3	1200 mm^3
Water fed	23 ± 3	31 ± 3
0.1% PFE fed	30 ± 2	39 ± 3
0.2% PFE fed	35 ± 3	47 ± 4

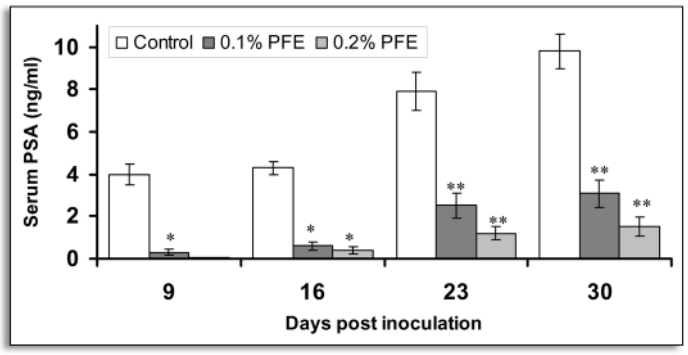

Figure 2. Effect of oral administration of PFE on PSA secretion in athymic nude mice. Serum PSA levels were analyzed by enzyme linked immunoabsorbant assay. Values represent mean ± SE of eight animals. *p < 0.01 vs. water-fed group of mice, **p < 0.001 vs. water-fed group of mice.

PFE -fed group where the targeted average tumor volume of 1200 mm^3 was reached at day 47 ± 4 post tumor cell inoculation. 0.1% PFE treatment was also found to be significantly effective where the average tumor volume of 1200 mm^3 was achieved in approximately 39 ± 3 days post tumor cell inoculation. Importantly, this tumor growth inhibition followed a significant decrease in the serum levels of PSA in PFE-fed groups at all time points as assessed by quantitative sandwich ELISA (Fig. 2). PSA is a clinical diagnostic serum marker for monitoring the presence and progression of CaP in human patients. Interestingly, the effects of PFE on PSA secretion were found to closely correlate with tumor growth inhibition.

In summary, we showed that PFE, through modulations in the cyclin kinase inhibitor-cyclin-cyclin-dependent kinase (cdk) machinery, resulted in inhibition of cell growth followed by apoptosis of highly aggressive human prostate carcinoma PC3 cells. These events were associated with alterations in the levels of Bax and Bcl-2 shifting the Bax:Bcl-2 ratio in favor of apoptosis. Further, we showed that oral administration of a human acceptable dose of PFE to athymic nude mice implanted with CWR22Rv1 cells resulted in significant inhibition of tumor growth with concomitant reduction in secretion of prostate-specific antigen (PSA) in the serum.

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. In summary, based on the present findings, we suggest that the effect of pomegranate and its byproducts should be evaluated in mouse models of human prostate cancer. Data from these studies will determine whether the need for trials of pomegranate-derived agents in human CaP patients.

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