Protective Role of Pomegranate in ROS-Induced Prostate Cancer

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Abstract

In the developed countries, prostate cancer (PCa) is the second most common type of cancer in men, only after lung cancer. PCa grows slowly and remains localized for a considerable time. However, PCa may behave aggressively under certain circumstances. Administration of pomegranate juice (PJ) to PCa patients prolongs prostate-specific antigen (PSA) doubling time. It is now well established that cancer is associated with an increase in the level of ROS, which occurs due to down-regulation of cellular ROS scavengers and antioxidant enzymes level. ROS-induced DNA mutation may cause increase in the proliferation of cancer cells due to inactivation of the tumor suppressor genes such as p53 with eventual induction of pro-survival transcription factors like NF-κB and signal transduction

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S. Chakraborti (ed.), *Handbook of Oxidative Stress in Cancer: Therapeutic Aspects*,
https://doi.org/10.1007/978-981-16-5422-0_227

and activator of transcription (STAT). The unique chemical composition of PJ, rich in flavonoids, like ellagic acid and urolithin A showed their antioxidant potential and cancer therapeutic effects in many animal model systems. In addition to direct antioxidant role, the anti-cancer effects of PJ also involve the attenuation of cell growth, inhibition of the activity of protein kinases, stimulation of apoptosis, decrease in the activity of matrix metalloproteases, reduction of cell adhesion, and invasion. Upon treatment with PJ, the expression of Bax and Bak (pro-apoptotic) genes was increased, while Bcl-XL and Bcl-2 (anti-apoptotic) genes were decreased in PCa cells. Interestingly, PJ modulates the cyclin-dependent kinase (Cdk) activity and thereby inhibits the cell proliferation, which subsequently leads to apoptosis of highly aggressive human PCa cells.

Keywords

Prostate cancer · Pomegranate · Punicalagin · Ellagic acid · Urolithin A · Apoptosis · Signal transduction · Transcription factors · Oxidants · Antioxidants

Introduction

PCa is the second most common type of cancer in men especially in the western countries, where men live for a longer time compared to the under-developed and developing countries. In the developed countries, it is noticeably high among the African Americans in comparison to the Caucasian Americans. However, the occurrence of the disease is relatively low among the native Japanese and Chinese populations; the correlation can be attributed conceivably due to the fact that they have a different diet as compared to those residing in western countries (Chen et al. 2013).

Pomegranate (*Punica granatum*, Lythraceae), native to Persia, is cultivated mostly in modern Iran and Iraq, and also in some parts of Afghanistan, India, China, Japan, Russia, and the United States. Pomegranate has been designated in ancient Greek mythology as the "fruit of the dead." Pomegranate juice (PJ) contains two important types of polyphenols: anthocyanins (like delphinidin, cyanidin, and pelargonidin, which give the fruit and juice its characteristics red color) and hydrolyzable tannins (like punicalin, pedunculagin, punicalagin). Upon hydrolysis punicalagin produces urolithin A and ellagic acid, which contribute to significant antioxidant activity of the whole fruit (Malik et al. 2005). Supplementation of PJ in the diet has been shown to delay PSA doubling time in PCa patients. PJ has been shown to markedly decrease the proliferation of LnCap prostate cancer cells and a marked reduction in oxidative stress-induced oxidation, while PJ caused significant increase in apoptosis and serum nitric oxide level (Syed et al. 2013).

The anti-cancer role of PJ at the cellular and molecular levels is due to (i) attenuation of the growth of human PCa cells by modulating growth factor receptor signalling and the cell cycle progression; (ii) stimulation of the activities of hepatic xenobiotic metabolizing enzymes that contribute to additional defense mechanisms against oxidative stress and carcinogens; (iii) repression of the expression of the anti-apoptotic genes; (d) modulation of phosphatases and cyclooxygenase

pathways; and (e) inhibition in the activities of protein kinases and suppression of inflammation (Wang et al. 2011; Landete 2011).

Oral administration of PJ to patients with benign prostrate hyperplasia or PCa patients before surgery unravelled urolithin A and ellagic acid in the excised prostate (Landete 2011). They are produced from punicalagin by the action of gut microbiota (Fig. 1) (Livingstone et al. 2019; Paller et al. 2017; Coode-Bate et al. 2019). Ellagic acid and urolithin A can modulate cell cycle and signalling events associated with PCa initiation and progression (MdSaleem et al. 2020).

Localized prostate cancer can be effectively treated by surgery and/or radiation. However, in the locally advanced PCa, the situations develop ways to bypass androgen dependence, thereby becoming castration-resistant prostate cancer (CRPC), which is highly aggressive and metastatic (Albrecht et al. 2004). Although chemotherapy for treatment of refractory PCa is available for years, yet these are usually ineffective for advanced conditions of tumor because the cancer has already been metastasized to the bones by that time (Turrini et al. 2015). Researchers have,

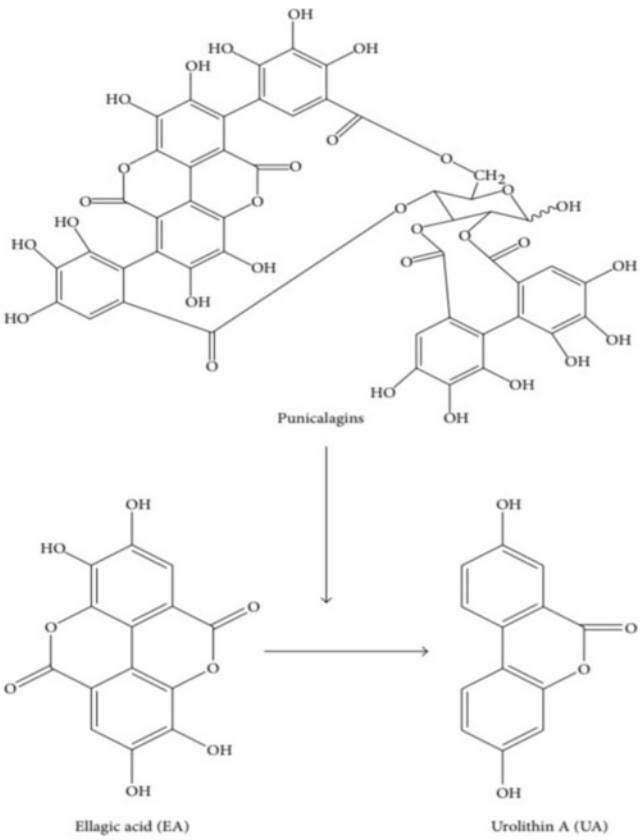

Fig. 1 Chemical structures of the major pomegranate-derived punicalagin metabolites. *EA* ellagic acid, *UA* urolithin

therefore, engaged in developing novel approaches to treat prostate cancer. One avenue of treatment appeared to be use of vaccines against the PSA. FDA-approved therapeutic PCa vaccine, Sipuleucel-T (Provenges), has been observed to be effective in prolonging survival of PCa patients (Higano et al. 2010). Other anti-androgen agents, for example, Abiraterone, an irreversible inhibitor of CYP17A1lyase, that plays a crucial role in androgen synthesis, have shown potentiality in improving survival from PCa. Additionally, androgen receptor (AR) antagonist like MDV3100 binds with a greater affinity than bicalutamide (Casodex), an androgen receptor inhibitor used to treat Stage D2 metastatic carcinoma of the prostate (Higano et al. 2010; Schrijvers et al. 2010). Recently, there is a trend to identify active components from natural products like PJ to fight PCa.

Modulation of Inflammation and Proliferation by PJ

Like many cancers, chronic inflammation has been shown to have link with PCa (Kuper et al. 2000; Weitzman and Gordon 1990). Researches in the past have revealed that proliferation of the epithelial cells of human prostate increases by ROS-induced inflammation (Weitzman and Gordon 1990). There are several mechanisms by which arachidonic acid metabolites, for example, thromboxane A_2, induce increase in ROS generation, and among them NADPH oxidase derived $O_2^{\cdot -}$ production via activation of Arf-Cytohesin1-PLD-PKC axis plays a critical role in this scenario (Fig. 2) (Chakraborti et al. 2017).

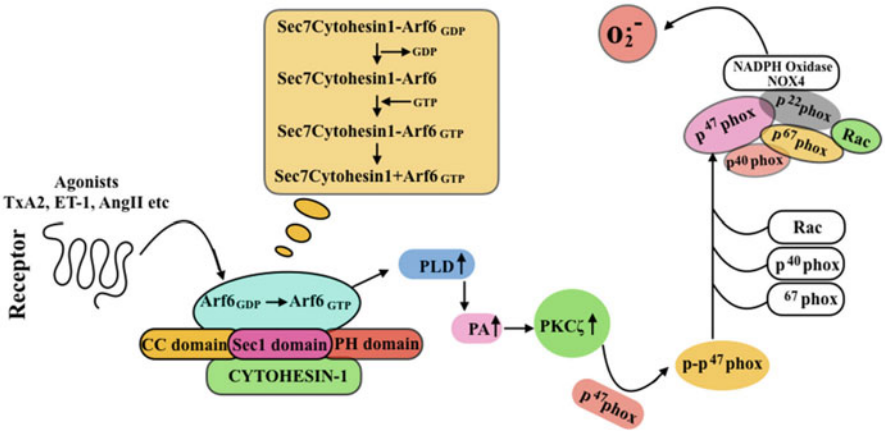

Fig. 2 Schematic representation depicting the signal transduction mechanism associated with Arf6-cytohesin1 axis on the activation of phospholipase D and subsequently increases in NADPH oxidase activity by stimulants like thromboxane A_2, endothelin-1, angiotensin II, IL-1β, and TNF-α. *CC domain:* coiled coil domain, *PH domain:* pleckstrin homology domain, *PA:* phosphatidic acid, *PKC :* protein kinase C, *MAPK:* mitogen activated protein kinase, *PAK:* phosphatidic acid dependent kinase. (Taken from S. Chakraborti et al. (2017) Archives of Biochemistry and Biophysics 633, 1–14 with permission)

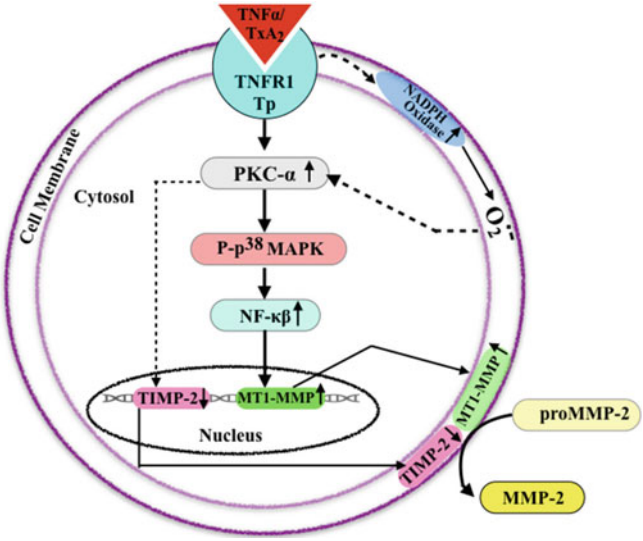

Fig. 3 Schematic representation of agonists induced activation proMMP-2 in cells. Stimulants such as thromboxane A_2, endothelin-1, angiotensin-II, IL-1β, and TNF-α bind to their cell surface receptors leading to activation of PKC-α. Upon activation, PKC-α phosphorylates IkB-α, which then ubiquitinated and subsequently degraded in the cytosol. The free NF-κB then translocates to the nucleus and increases the expression of MT1-MMP, which then accumulates on the cell surface. PKC-α, on the other hand, down-regulates TIMP-2 expression resulting in the activation of proMMP-2 with the involvement of MT-MMP. (Taken from S. Roy et al. (2013) J. Biochem. 153, 289–302 with permission).

$O_2^{\cdot -}$ has been shown to activate MT1MMP leading to stimulation of MMP-2 activity with the involvement of the transcription factor, NF-κB (Fig. 3) (Roy et al. 2013).

Upon interaction with inflammatory cytokines (such as TNFα/IL-1β)-mediated production of NADPH oxidase derived $O_2^{\cdot -}$, PJ reduces the expression and activities of MMPs in PCa cells (Toyokuni et al. 1995). $O_2^{\cdot -}$ also increases the generation of proinflammatory cytokines and subsequently the associated signalling pathways in the PCa microenvironment. Notably, inflammatory atrophy is currently recognized as the initial event of prostatic intraepithelial neoplasia that leads to PCa (Toyokuni et al. 1995). Inflammation may cause prolonged oxidative stress in cancer cells, and ROS have a survival advantage in PCa cells (Toyokuni et al. 1995). Moderate increase in oxidative stress has been shown to augment PCa cell proliferation and markedly increases mutation rates through DNA damage and/or epigenetic changes (Turrini et al. 2015).

De Marzo et al. (De Marzo et al. 2003) have shown that inhibition of glutathione S-transferase activity is the primary event for the initiation of prostate tumors. ROS also increases PCa cell proliferation by enhancing growth factor receptor sensitivity, which leads to alteration of transcription factors activity. Inflammatory cells like macrophages and mast cells have been shown to produce angiogenic factors,

especially cytokines such as TNF-α, IL-1β, and VEGF that play a critical role in mediating signals for PCa cell growth and proliferation (Culig et al. 2005).

Pro-inflammatory secretory cytokines/chemokines especially IL-6, IL-12p40, IL-1β, and RANTES (regulated on activation of normal T cell expressed and secreted) play important roles in prostate tumor growth and PCa initiation, and these are inhibited by PJ treatment (Wang et al. 2011; Jurenka 2008; Wang et al. 2011). A marked increase in IL-6 has been observed in the tissues and serum of PCa patients and that was inhibited upon treatment with anti-IL-6 antibody (Culig et al. 2005). Additionally, some cytokines, for instance, IL-6 regulates angiogenesis and proliferation of PCa cells by modulating the effect of vascular endothelial growth factor (VEGF) (Turrini et al. 2015).

Interleukins, in addition to their primary role in inflammatory and immune responses, also elicit a critical role in different diseases including cancer. IL-12 has two subunits, and between them IL-12p40 subunit elicits the chemo-attractant role for inflammatory cells like macrophages, which in turn promotes migration of cells (Culig et al. 2005). Treatment with anti-IL-12p40-antibody has been shown to inhibit colon cancer metastasis (Steiner et al. 2004). Research in the recent past have indicated that IL-1β stimulates inflammation and thereby increases invasion of fibrosarcoma cells (Cooper and Khadar 2000; Apte et al. 2006). RANTES is a strong chemotactic factor for T cells, monocytes, and dendritic cells. Expression of RANTES and its receptor, CCR5, is positively correlated in PCa cells. Additionally, interplay of RANTES with CCR5 in cancer cells increases their invasiveness in PCa cells (Varday et al. 2006). PJ induced anti-metastatic effects on PCa are, at least partly, due to inhibition in the generation of pro-inflammatory cytokines and chemokines (Wang et al. 2011).

An increase in the proliferation of epithelial cells has been considered to be the hallmark for the initiation of benign prostate hyperplasia (BPH) (Varday et al. 2006). Importantly, dysregulation of the expression of cell proliferation associated genes plays an important role in the transition of BPH to PCa (Luo et al. 2002). Among them, cyclin-dependent kinase inhibitor 1A (CDKN1A: p21, Cip1) is a critical regulator of cell cycle progression at the G1 phase and is known to have an important role for promoting PCa (Sanchez and Dynlacht 2005). C-Myc, a strong positive regulator of cell proliferation, was shown to play a crucial role in androgen-independent PCa development (Bernard et al. 2013). The expression of MKi-67 gene (a marker of cell growth and proliferation) has been observed to be increased in PCa cells (Zhang et al. 2008). Thus, through investigation on the expression of genes associated with PCa initiation and progression may help to develop novel PCa therapeutics (Bernard et al. 2013; Zhang et al. 2008).

PJ-Induced Activation of SIRT3-SOD Pathway

Sirtuins (SIRTs) are NAD^+ dependent deacetylase, which regulate several diseases like cancer. In humans, seven sub-types of sirtuins (SIRT 1–7) have been identified. SIRT-3 has been observed to be localized in the mitochondria and modulates

oxidative stress responsiveness. Recent studies on the role of SIRT-3 to decrease mitochondria-modulated ROS generation by activating dismutase-2 (SOD-2) have gained much attention, especially among scientists, who are involved in cancer research (Zhao et al. 2016).

It has been demonstrated that PJ augments SIRT-3 and thereby markedly induces antioxidant properties, which are notably based on SIRT-3-mediated SOD2 activation and reduction of intracellular ROS level. Additionally, SOD2 and isocitrate dehydrogenase-2 are known to regulate the ratio of GSH and GSSG. PJ activates both SOD2 and the glutathione antioxidant system by activating SIRT-3 in the mitochondria (Zhao et al. 2016).

PJ in Modulating Cell Cycle Progression

In eukaryotes, the cell cycle is controlled by a group of protein kinase complexes (Luo et al. 2002). The essential features are that each complex consists of at least one catalytic subunit, the cdk, and its activator, the cyclins (Sanchez and Dynlacht 2005). Cyclin-D and cyclin-E were shown to be involved in G1–S phase of the cell cycle. In normal cell growth, combination of cyclin-D and cyclin-E with cdk2, cdk4, or cdk6 results in the progression of the cell cycle leading to cell proliferation (Sanchez and Dynlacht 2005). Dysregulation of this machinery can alter cell cycle leading to abnormal cell proliferation, resulting in the progression of cancer (Sanchez and Dynlacht 2005; Bernard et al. 2013). During the cell cycle progression, the cdk–cyclin complex is down-regulated by the inhibitors of cyclin-dependent kinases like WAF1 and KIP1 (Kim and Moon 2005). PJ has been shown to upregulate WAF1p21 and KIP1p27 during G1 phase arrest and apoptosis. In the absence of active p53, an increase in WAF1p21 and KIP1p27 by PJ was demonstrated in PCa (PC3) cells (Malik et al. 2005). Notably, exogenous stimuli may produce p53-dependent and independent stimulation of WAF1p21 and KIP1p27, which subsequently inhibit G1–S phase transition, and thereby leads to G1 phase cell cycle arrest and apoptosis (Kim and Moon 2005; Mukhtar and Ahmad 1999). WAF1p21 and KIP1p27 are well-known inhibitors of the cyclin–cdk complex. PJ treatment of the cells reduces the regulatory molecules of cyclins and cdks such as cyclins D1, D2, and E and cdk2, cdk4, and cdk6, which play important roles in regulating of G1 phase of the cell cycle (Kim and Moon 2005; Malik et al. 2005; Mukhtar and Ahmad 1999). The stimulation of apoptosis by PJ in PCa cells (PC3 cells) has been suggested to be an important aspect of tissue homeostasis, which is currently considered to be the probable mechanism to eliminate unwanted cells (Malik and Mukhtar 2006).

Bcl-2 is a proximal effector in the apoptotic pathway and is a well-known inhibitor of apoptosis. Bcl-2 has been observed at high levels in over half of all human tumors. Bcl-2 forms a heterodimer complex with the proapoptotic member, Bax. The ratio of Bax and Bcl-2 is a decisive factor in determining whether cells prefer death or survival. Upon treatment with PJ, PCa cells (PC3 cells) show

inhibition in Bcl-2 protein expression along with stimulation of the protein expression of Bax and, therefore, favor apoptosis (Oltersdorf et al. 2005).

PJ-Induced Modulation of NFκB

An important signal transduction mechanism that mediates inflammatory responses relevant to cancer is the nuclear factor-κB (NF-κB) pathway. Among the transcription factors, NF-κB received prime importance for studies on the modulation of gene expression of diverse pathophysiological conditions. Upon activation of NF-κB signalling pathway, phosphorylation of IκB ensues (Balswin Jr 2001). This results in nuclear translocation of NF-κB and thereby acts as a transcription factor. Notably, activation of NF-κB was found in androgen-independent prostate cancer and, therefore, considered to be a risk factor for reappearance of PCa after radical prostatectomy (Dominigo et al. 2005; Gupta et al. 2002; Rettig et al. 2008).

Compared with BPH, basal NF-κB activation is detected in low- and high-grade PCa specimens and is associated with the expression of NF-κB regulated gene products such as Bcl-2, cyclin D1, MMPs, and VEGF (Shukla et al. 2004). NF-κB activation has been observed in human PCa cells (Gasparian et al. 2002). Stimulation of NF-κB activity has been demonstrated during PCa oncogenesis in the transgenic adenocarcinoma of prostate cancer models (Shukla et al. 2004; Gasparian et al. 2002). Inhibition of NF-κB attenuates preclinical in vivo and in vitro models of PCa (Shukla et al. 2004; Gasparian et al. 2002), while activation of NF-κB in PCa specimens was positively correlated with intratumoral expression of Bcl-2 and cyclin D1, the regulators of cell survival and G1-S-phase progression, respectively (Kim and Moon 2005; Shukla et al. 2004; Gasparian et al. 2002). PJ has been demonstrated to inhibit NF-κB, which consequently induces apoptosis of the PCa cells (Turrini et al. 2015; Mouenchen et al. 2000).

Gene Expression and PJ

PJ modulates the mRNA quantities of CDKN1A (p21), MKi-67, and c-Myc (cancer-related markers that depend on their role on cell cycle and cell proliferation regulation) (Zhang et al. 2008; Suh et al. 2002). Stimulation in the expression of these genes was demonstrated in BPH and PCa specimens. Interestingly, in the PCa cells, a marked increase in c-Myc expression was observed in comparison to the benign prostate hyperplasia (BPH) group (Bernard et al. 2013). However, no difference in the levels of MKi-67 on PCa and BPH specimens has been observed, although MKi-67 gene expression was shown in PCa specimens (Zhang et al. 2008). An increase in CDKN1A expression has been demonstrated after prostatectomy and radiotherapy; however, down-regulation of the tumor suppressor genes, p21 and p27, elicits more virulent PCa phenotype. Importantly, p21 gene expression was shown to induce cell cycle arrest and inhibits proliferation of PCa cells (Rigaud et al. 2004; Roy et al. 2008; Birchmaier and Behrens 1994).

Modulation of Cell Adhesion, Migration, and Invasion by PJ

A study in the PCa cells demonstrated that the expression of genes that are involved in regulating cell adhesion machinery are increased upon PJ treatment; however, genes that cause increase in cell migration are down-regulated (Roy et al. 2008). Intercellular adhesion molecule-1 (ICAM-1) has been observed to play a crucial role in stabilizing cell–cell interactions. Additionally, E-cadherin and claudin 1 are assembled in the tight junction protein complexes, which congregate epithelial cells together. PJ increases cell adhesion by upregulating the cell junction proteins (Wang et al. 2011). PJ also stimulates the myristoylated alanine-rich protein kinase C substrate (MARCKS) that binds the cell membrane (Arbuzova et al. 2002). MARCKS has also been observed to be involved in cell adhesion and cell motility by regulating the actin cytoskeletal structure (Arbuzova et al. 2002; Aderem 1992). Interestingly, gene microarray studies revealed that PJ inhibits expression of the genes, which are associated with cell migration (Wang et al. 2011; Aderem 1992).

MicroRNAs, Prostate Cancer, and PJ

MicroRNAs (miRNAs) are established regulators of different genes at the post-transcriptional level. Altered miRNAs expression is absent in different types of human cancer (Wu et al. 2007). MiRNA-335 is known to function as a metastasis-suppressive miRNA in breast cancer by inhibiting type I collagen (COLA1) and tenascin C (TNC) gene expression. COLA1 is an extracellular matrix molecule and has been suggested to play a critical role in organizing and controlling the cytoskeleton, while TNC plays a crucial role in regulating cell migration (Tsunoda et al. 2003). Notably, PJ decreases the expression of COL1A1 and TNC genes in PCa cells by upregulating miRNA-335 (Tsunoda et al. 2003).

PKC-ε stimulates migration and invasion and promotes autocrine cell signaling events in PCa cells (Wu et al. 2002). MiRNA-205 is known to reduce PCa cell invasion by inhibiting PKC-ε and N-chimearin (CHN1) **(**Wu et al. 2007**)**. N-chimearin is a Rho GTPase activating protein, which upon down-regulation can cause a discernible reduction of cell migration (Wu et al. 2007; Tsunoda et al. 2003; Wu et al. 2002). Upon upregulation of miRNA205, PJ decreases the expression of PKC-ε and CHN1 in PCa cells, and thereby reduces cell migration and invasion (Wang et al. 2011; Wu et al. 2002).

The miRNA-200 has been observed to inhibit the expression of ZEB1 and ZEB2 genes that are known transcriptional repressors of the E-cadherin gene (Yang and Kazanietz 2007; Korpal et al. 2008; Birchmaier and Behrens 1994). Loss of E-cadherin has been suggested to be a molecular hallmark that initiates epithelial-mesenchymal transition (EMT) and thereby plays a crucial role in the initiation of PCa (Mouenchen et al. 2000). PJ treatment stimulates the expression of E-cadherin gene by inhibiting its transcriptional repressor ZEB by upregulating miRNA-200 (Yang and Kazanietz 2007). MiRNA-126 has been demonstrated to

reduce prostate cancer cell invasiveness by attenuating prostein (Korpal et al. 2008; Birchmaier and Behrens 1994), a novel prostate-specific protein whose function is not clearly known. miRNA-21, known to be a pro-invasive miRNA, down-regulates the expression of tropomyosin 1 (TMP1: an actin-binding protein, whose over-expression suppresses cell invasion), programmed cell death 4 (Pdcd4) protein (a tumor suppressor protein known to interact with eukaryotic initiation factor 4A: EIF4A to inhibit protein synthesis), and MARCKS in PCa specimens (Wang et al. 2011; Aderem 1992). In prostate cancer cells, the mRNA levels of Pdcd4 and MARCKS were found to be markedly decreased, which is in agreement with the inhibition by miRNA-21. PJ treatment to the PCa cells upregulates expression of TMP1, Pdcd4, and MARCKS by inhibiting miRNA-21 (Wang et al. 2011; Aderem 1992; Wu et al. 2007). miRNA-373, a pro-invasive micro-RNA, targets CD44, a transmembrane glycoprotein that is known to be involved in cell adhesion and cell-stromal interactions (Huang et al. 2008), and thereby acts as a hyaluronan receptor in addition to hyaluronan-mediated motility receptor (HMMR) (Lin et al. 2007). HMMR is known to act as a hyaluronan (HA) receptor, and association of HA with HMMR may increase the RhoA-activated protein kinase (ROCK) signal transduction pathway, and subsequently tumor cell migration and invasion in prostate cancer (Turrini et al. 2015). Notably, PJ decreases the expression of miRNA-373 and HMMR indicating that the anti-metastatic effect of PJ seems, at least in part, to be due to inhibition of the hyaluronan signalling pathway (Wang et al. 2011; Turrini et al. 2015). Additionally, PJ markedly attenuate the concentrations of secreted pro-inflammatory cytokines/chemokines like IL-6, IL-12p40, IL-1β, and RANTES, which decrease inflammation and, thereby, elicit its effectiveness in inhibiting cancer progression (Malik et al. 2005; Wang et al. 2011). The chemokine, SDF1a, and its receptor CXCR4 are known to play a critical role in metastasis of cancer cells to the bone. PJ has been demonstrated to decrease the chemoattractant ability of SDF1a to PCa cells. Understanding the mechanisms by which PJ increases adhesion, and thereby inhibits migration, may shed light for effective therapeutics of the metastasis in PCa (Wang et al. 2011; Turrini et al. 2015).

Role of PJ in PLA_2-MMP Signalling Pathway Induced Prostate Cancer

Oxidants have been shown to activate phospholipase A_2 (PLA_2), the enzyme that is primarily involved for generation of arachidonic acid and its metabolites such as prostaglandins, thromboxane, and leukotrienes upon stimulation of cyclooxygenase (COX) and lipoxygenase (LOX) (Chakraborti et al. 1989; Chakraborti et al. 2005). These arachidonic acid metabolites play an important role in generating inflammatory cytokines and chemokines, for example, TNF-α, which stimulate MMPs such as MM1-MMP and MMP-2 activities (Roy et al. 2013) **(**Fig. 4**).** Inhibitors of PLA_2, COX, or LOX are able to attenuate the production of cytokines and chemokines, thereby inhibiting MMPs activities in PCa cells (Nie et al. 2001). PJ has been shown

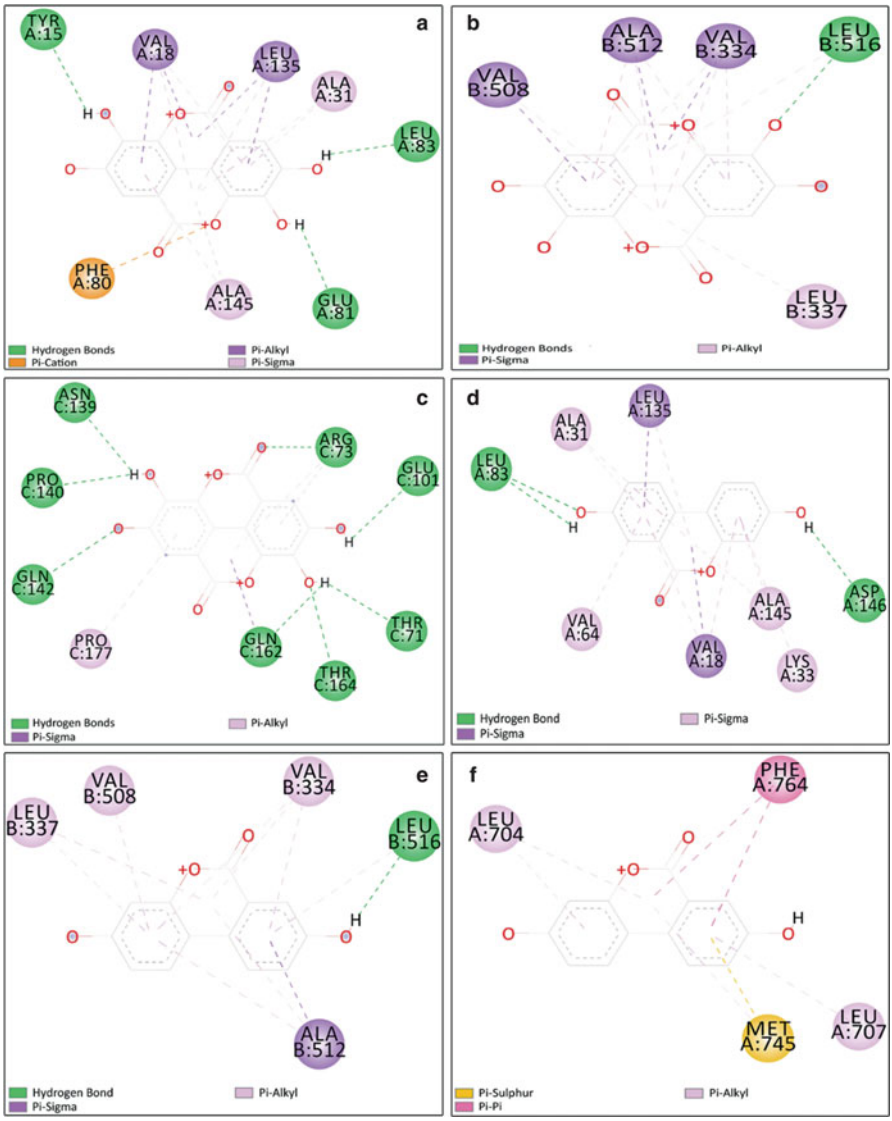

Fig. 4 Inhibitory interaction of PJ ingredients. Ellagic acid complexes with (**a**) CDK1 (PDB ID 4Y72), (**b**) COX-2 (PTGS2, PDB ID 5F19), and (**c**) NF-κβ (RELA, PDB ID 1NFI). Urolithin A complexes with (**d**) CDK1 (PDB ID 4Y72), (**e**) COX-2 (PTGS2, PDB ID 5F19), and (**f**) androgen receptor (NR3C4, PDB ID 5CJ6)

to inhibit PLA_2 and thereby attenuates MMPs-mediated signalling events leading to decrease in the production of inflammatory cytokines in PCa cells (Malik et al. 2005; Attiga et al. 2000).

In Silico Investigation of the Possible Molecular Interactions of Ellagic Acid and Urolithin A with CDK1, COX2, and NF-κB

We have determined the direct molecular association of key PJ ingredients, ellagic acid (EA, PubChem ID 5281855) and urolithin A (UA, PubChem ID 5488186) with the known molecular targets of PCa (Fig. 4) through molecular docking using the CB-Dock server (Kim et al. 2016; Liu et al. 2020). Both the ligands came up as excellent modulators of cell cycle progression by determining their highest inhibitory affinity toward the CDKs (CDK1, CDK2, CDK4, CDK6). EA and UA also registered similar inhibitory potential against PLA_2–MMP axis in docking studies, by binding with MMP2 and COX-2 (PTGS2) with similar affinity (Fig. 4). Both ligands also appear to be excellent anti-androgens that modulate the NF-κβ pathway by binding to p65 (RELA) subunit and cyclin D1 (CCND1). The ligands illustrate almost similar affinity toward the molecular targets, but EA elicits a broader inhibitory spectrum exhibiting additional affinity toward p52 subunit of NF-κβ (NFKB2) and apoptosis regulator Bcl-2 (BCL2). The in silico analyses corroborate with the present knowledge of possible manifold anti-cancer effects of PJ ingredients and further document the possibility of direct interference of PJ ingredients with multiple biological processes through direct molecular interaction.

Conclusion and Future Perspectives

There is no effective treatment for inhibition of PCa progression, especially when it recurs after hormone ablation therapy. Several clinical trials have demonstrated that PJ inhibits PCa progression and significantly prolongs PSA doubling time. PJ interferes with multiple biological processes in PCa cells such as inhibition of cancer cell growth by modulating growth factor receptor signalling and cell cycle progression, and regulating the activities of kinases, phosphatases, cyclooxygenases, and lipoxygenases, which are involved in decreasing inflammation along with stimulation of cell adhesion and also inhibition of cell migration, and suppression of the production of pro-inflammatory cytokines and chemokines (Malik et al. 2005; Wang et al. 2011; Varday et al. 2006).

The epithelial cells of PCa may lead to hyperplasia during ROS overload, which succeed to prostatic intraepithelial neoplasm and invasive adenocarcinoma, which eventually becomes metastatic leading to spread of the cancer cells primarily to the lymph nodes, bone marrow, and then in the lung. Metastatic transition of cells involves loss of adhesion and rearrangement of cytoskeletal elements that allow the cells to move. PJ has been shown to increase expression of the cell adhesion properties and thereby inhibits expression of the molecules that facilitate cell migration (Malik et al. 2005; Wang et al. 2011).

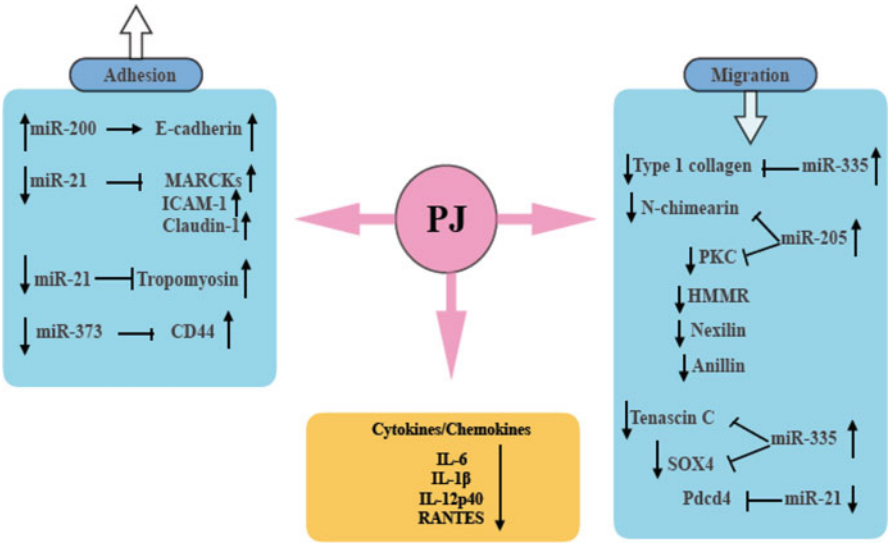

Fig. 5 Schematic summary of the effects of pomegranate juice (PJ) on hormone-independent prostate cancer cells. E-cadherin is a calcium-dependent cell–cell adhesion glycoprotein and one of the important components in adherens junctions. Myristoylated alanine-rich protein kinase C substrate (MARCKS) is localized in the plasma membrane and is an actin filament cross-linking protein. Intercellular adhesion molecule-1 (ICAM-1) is an endothelial transmembrane protein known for its importance in cell adhesion and in stabilizing cell–cell interactions. Claudin-1, a transmembrane protein, is an important component of tight junctions. Type I collagen is an important component of ECM with both structural and signalling functions that mediate cell migration and survival. N-chimearin is known as a Rho GTPase-activating protein (GAP). Studies showed that N-chimearin cooperated with Rac1 in inducing changes in cytoskeletal morphology. Inhibition of PKC-ε reduces the invasiveness of prostate cancer cells. Hyaluronan-mediated motility receptor (HMMR) or CD168 functions as hyaluronan receptor. Anillin and nexilin are actin-binding proteins involved in the regulation of cytoskeleton structure. Tenascin C is an extracellular molecule known to promote cell migration. Programmed cell death 4 (Pdcd4) has been identified as a tumor suppressor with the property to suppress cancer cell invasion through JNK signaling pathway. MiR-335 represses the expression of type I collagen and tenascin C. MiR-205 represses the expression of N-chimearin and PKC-ε. N-chimearin is a GTPase-activating protein that upon down-regulation results in loss of filopodia and reduction of migration. The miR-200 family represses E-cadherin transcriptional repressor ZEB1 and ZEB2, resulting in upregulation of E-cadherin. MiR-21 represses expression of MARCKS, tropomyosin 1 (TPM1), and Pdcd4. MARCKS and TPM1 are actin-filament binding proteins that are involved in the regulation of cell adhesion and cancer cell invasion, respectively. MiR-373 represses transmembrane adhesion glycoprotein CD44 expression, a glycoprotein that mediates cell–cell and cell–stromal interactions, binds HA and several other matrix molecules, and controls cell shape through the cytoskeleton. PJ significantly upregulates miR-335, miR-205, and miR-200 family. MiR-126 previously identified as metastasis suppressor microRNAs and significantly down-regulated miR-21 and miR-373 previously identified as pro-invasive microRNAs. (Taken from Wang et al. (2011) Cellular and molecular mechanisms of pomegranate juice-induced anti-metastatic effect on prostate cancer cells. Integr. Biol., 2011, 3, 742–754 with permission)

Overall, PJ (i) stimulates PCa cells to adhere strongly; (ii) attenuates the migratory potentiality of PCa cells; (iii) stimulates expression of the genes involved in cell adhesion, but reduces expression of the genes associated with cell migration; and (iv) stimulates adhesion-enhancing miRNAs level, while it decreases expression of pro-invasive miRNAs level and (v) decreases the concentration of pro-inflammatory cytokines/chemokines such as IL-6, IL-12p40, IL-1β, and RANTES (Wang et al. 2011). Schema depicting the role of PJ on hormone-independent PCa is presented in Fig. 5.

PJ has the capacity to prevent or at least able to delay the metastasis of ROS-induced PCa. Understanding the details of the molecular mechanisms by which PJ increases cell adhesion and inhibition of cell migration and decreases the expression of pro-inflammatory molecules will shed light for the avenues for effective treatment strategies of PCa.

References

Aderem A (1992) The MARCKS brothers: a family of protein kinase C substrates. Cell 71:713–716

Albrecht M, Jiang W, Kumi-Diakes J et al (2004) Pomegranate extracts potentially suppress proliferation, xenograft growth and invasion of human prostate cancer cells. J Med Food 7: 274–283

Apte RN, Dotan S, Elkabets M et al (2006) The involvement of IL-1 in tumorigenesis, tumor invasiveness, metastasis and tumour host interactions. Cancer Metastsis Rev 25:387–408

Arbuzova A, Schmitz AP, Vergeres G (2002) Cross-talk unfolded: MARCAS proteins. Biochem J 362:1–12

Attiga FA, Fernandez PM, Weeraratna AT et al (2000) Inhibitors of prostaglandin synthesis inhibit human prostate tumour cell invasiveness and reduce the release of matrix metalloproteinases. Cancer Res 60:4629–4637

Balswin AD Jr (2001) Series introduction: the transcription factor NF-κB and human disease. J Clin Invest 107:3–6

Bernard D, Pourtier-Manzanedo A, Gil J et al (2013) Myc confers androgen independent prostate cancer cell growth. J Clin Invest 112:1724–1731

Birchmaier W, Behrens J (1994) Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. Biochim Biophys Acta 1198:11–26

Chakraborti S, Gurtner GH, Michael JR (1989) Oxidant –mediated activation of phospholipase A_2 in pulmonary endothelium. Am J Phys 257:L430–L437

Chakraborti S, Das S, Chakraborti T (2005) Proteolytic activation of protein kinase Cα by peroxynitrite in stimulating cytosolic phospholipase A_2 in pulmonary endothelium: role of a pertuissis toxin sensitive protein. Biochemistry (USA) 44:5246–5257

Chakraborti S, Sarkar J, Chowdhury A, Chakraborti T (2017) Role of ADP ribosylation factor6-cytohesin1-phosdpholipase D signalling axis in U46619 induced activation of NADPH oxidase in pulmonary smooth muscle cell membrane. Arch Biochem Biophys 638:1–14

Chen J, Song Y, Zhang L (2013) Lycopene/tomato consumption of prostate cancer: a systemic review. J Nutr Sci Vitaminol (Tokyo) 59:213–223

Coode-Bate JST, Melchini A, Saha S et al (2019) Accumulation of dietary S-methyl cysteine sulfoxide in human prostate tissue. Wiley-VCH. Verlag GmBH Co. KGaA, Weinheim

Cooper AM, Khadar SA (2000) IL-12p40: an inherently agonistic cytokine. Trends Immunol 28: 33–38

Culig Z, Steiner H, Bartsch G, Hobisch A (2005) Interleukin-6 regulation of prostate cancer cell growth. J Cell Biochem 95:497–505

De Marzo AM, Meeker AK, Zha S et al (2003) Human prostate cancer precursor and pathobiology. Urology 62:55–62

Dominigo DJ, Mellado B, Ferrer B et al (2005) Activation of nuclear factor κB in human prostate carcinogenesis and association to biochemical relapse. Br J Cancer 93:1285–1394

Gasparian AV, Yao YJ, Kowalezyk D et al (2002) The role of IKK in constitutive activation of NF-κB transcription factor in prostate carcinoma cells. J Cell Sci 115:141–151

Gupta S, Afaq F, Mukhtar H (2002) Involvement of nuclear factor-kappa B, Bax and Bcl-2 in induction of cell cycle arrest and apoptosis by apigenin in human prostate cancer. Oncogene 21: 2727–3738

Higano CS, Small EJ, Schellhamner P et al (2010) Sipuleucel-T. Nat Rev Drugs Discov 9:513–514

Huang Q, Gumireddy K, Schrier M, IeSage C et al (2008) The micrornas mir-373 and mir-520c promote tumour migration, invasion and metastasis. Proc Am Assoc Cancer Res Annual Meeting 49:1196–1197

Jurenka JS (2008) Therapeutic applications of pomegranate (Punica granatum L.): a review. Altern Med Rev 13:128–144

Kim CH, Moon SK (2005) Epigallocatechin-3-gallate causes the p53WAFI–mediated G1 phase arrest of cell cycle and inhibit matrix metalloproteinase-9 expression in TNF-α induced vascular smooth muscle cells. Arch Biochem Biophys 435:264–272

Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J, Yu B, Zhang J, Bryant SH (2016) PubChem substance and compound databases. Nucleic Acids Res 44(D1):D1202–D1213

Korpal M, Lee EC, Hu G, Kang Y (2008) The miR-200 family inhibits epithelial mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcrtiptional repressor ZEB1 and ZEB2. J Biol Chem 283:14910–14915

Kuper H, Adami HO, Trichopoulos D (2000) Infections as a major preventable cause of human cancer. J Intern Med 248:171–183

Landete JM (2011) Ellagitannins, ellagic acid and their derived metabolites. A review about source, metabolism, functions and health. Food Res Int 44:1155

Lin SL, Chang D, Ying S-Y (2007) Hyaluronan stimulates transformation of androgen independent prostate cancer. Carcinogenesis 28:310–320

Liu Y, Grimm M, Dai WT, Hou MC, Xiao ZX, Cao Y (2020) CB-Dock: a web server for cavity detection-guided protein–ligand blind docking. Acta Pharmacol Sin 41:138–144

Livingstone TL, Beasy G, Mills RD, Plumb J et al (2019) Plant bioactives and their prevention of prostate cancer: evidence from human studies. Nutrients 11(2245):1–30

Luo J, Dunn T, Ewing C, Sawvageot J et al (2002) Gene expression signature of benign prostatic hyperplasia revealed by cDNA microarray analysis. Prostate 51:189–200

Malik A, Mukhtar H (2006) Prostate cancer prevention through pomegranate fruit. Cell Cycle 5: 371–373

Malik A, Aftaq F, Sarfaraz S, Adhami VM, Syed DN, Mukhtar H (2005) Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. Proc Natl Acad Sci U S A 102:14813–14818

MdSaleem YI, Albassam H, Selim M (2020) Urolithin a induces prostate cancer cell death in p53 dependent and in p53 independent manner. Eur J Nutr 59:1607–1618

Mouenchen HJ, Lin DL, Walsch MA et al (2000) Tumour necrosis factor-α induced apoptosis in prostate cancer cells through inhibition of nuclear factor-κB by an IκBα "superrepressor". Clin Cancer Res 6:1969–1977

Mukhtar H, Ahmad N (1999) Cancer chemoprevention: future hold in multiple agents. Toxicol Appl Pharmacol 158:207–210

Nie D, Che M, Gringnon D, Tyang K, Honn KV (2001) Role of eicosanoid in prostate cancer progression. Cancer Metastasis Rev 20:195–206

Oltersdorf T, Elmore SW, Shomaker AR et al (2005) An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 435:677–681

Paller CJ, Pantuck A, Carducci MA (2017) A review of pomegranate in prostate cancer. Prostate Cancer Prostatic Dis 20:265–270

Rettig MNO, Heber D, An J et al (2008) Pomegranate extract inhibits androgen-independent prostate cancer growth through a nuclear factor-κB dependent mechanism. Mol Cancer Ther 7:2662–2671

Rigaud J, Tiguert R, Decobert M et al (2004) Expression of p21 cell cycle protein is an independent predictor of response to salvage radiotherapy after radical prostectomy. Prostate 58:269–276

Roy S, Singh RP, Agarwal C et al (2008) Down regulation of both p21/ Cip1 and p27/Kip1 produces a more aggressive prostate cancer phenotype. Cell Cycle 7:1828–1835

Roy S, Chakraborti T, Chowdhury A, Chakraborti S (2013) Role of PKC-α in NFκB-MT1MMP mediated activation of pro-MMP-2 by TNF-α in pulmonary artery smooth muscle cells. J Biochem 153:289–302

Sanchez I, Dynlacht BD (2005) New insights into cyclins, CDKs, and cell cucle control. Semin Cell Dev Biol 16:311–321

Schrijvers D, Van Erps P, Cortvriend J (2010) Castration-refractory prostate cancer: new drugs in the pipeline. Adv Ther 27:285–296

Shukla S, MacLennan GT, Fu P et al (2004) Nuclear factor-κB/p65 (RelA) is constitutively activated in human prostate adrenocarcinoma and correlates with disease progression. Neoplasia 6:390–400

Steiner H, Berger AP, Godoy-Tundidov SM, Bjartell HL et al (2004) An autocrine loop for vascular endothelial growth factor is established in prostate cancer cells generated after prolonged treatment with interleukin-6. Eur J Cancer 40:1066–1072

Suh J, Payrandi F, Edelstein LC et al (2002) Mechanism of constitutive NF-κB activation in human prostate cancer cells. Prostate 52:183–200

Syed DN, Chamcheu JC, Adhami VM, Mukhtar H (2013) Pomegranate extracts and cancer prevention: molecular and cellular activities. Anti Cancer Agents Med Chem 13:1149–1161

Toyokuni S, Okamoto K, Yodoi J, Hiai H (1995) Persistent oxidative stress in cancer. FEBS Lett 16: 1–3

Tsunoda T, Inada H, Kaleimbeyi I et al (2003) Involvement of large tenascin-C splice variants in breast cancer progression. Am J Pathol 162:1857–1867

Turrini E, Ferruzzi L, Fimognari C (2015) Potential effects of pomegranate polyphenols in cancer prevention and therapy. Oxidative Med Cell Longev 2015:1–19. Article ID 938475

Varday GG, Peehl DM, Kadam PA et al (2006) Expression of CCL5 (RANTES) and CCR5 in prostate cancer. Prostate 66:124–134

Wang L, Alvon A, Yuan H et al (2011) Cellular and molecular mechanisms of pomegranate juice-induced anti-metastatic effect on prostate cancer cells. Integr Biol 3:742–754

Weitzman SA, Gordon LI (1990) Inflammation and cancer: role of phagocyte generated oxidants in carcinogenesis. Blood 76:655–663

Wu D, Foreman TL, Gregory CW et al (2002) Protein kinase C epsilon has the potential to advance the recurrence of human prostate cancer. Cancer Res 62:2423–2429

Wu W, Sun M, Zou GM, Chen J (2007) MicroRNA and cancer: current status and perspective. Int J Cancer 120:953–960

Yang C, Kazanietz MG (2007) Chimaerins: GAPs that bridge diacetylglycerol signalling and the small G protein, Rac. Biochem J 403:1–12

Zhang W, Peng J, He H et al (2008) MKi67 and PCNA expression in prostate cancer and benign prostate hyperplasia. Clin Invest Med 31:E8–E15

Zhao C, Sakaguchi T, Fujita K, Ito H et al (2016) Pomegranate derived polyphenols reduce reactive oxygen species production via SIRT-3 mediated SOD2 activation. Oxidative Stress Cell Longev 2016:1–9, Article ID 2927131