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Pomegranate polyphenols down-regulate expression of androgen-synthesizing genes in human prostate cancer cells overexpressing the androgen receptor☆

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Abstract

Prostate cancer is dependent on circulating testosterone in its early stages and is treatable with radiation and surgery. However, recurrent prostate tumors advance to an androgen-independent state in which they progress in the absence of circulating testosterone, leading to metastasis and death. During the development of androgen independence, prostate cancer cells are known to increase intracellular testosterone synthesis, which maintains cancer cell growth in the absence of significant amounts of circulating testosterone. Overexpression of the androgen receptor (AR) occurs in androgen-independent prostate cancer and has been proposed as another mechanism promoting the development of androgen independence. The LNCaP–AR cell line is engineered to overexpress AR but is otherwise similar to the widely studied LNCaP cell line. We have previously shown that pomegranate extracts inhibit both androgen-dependent and androgen-independent prostate cancer cell growth. In this study, we examined the effects of pomegranate polyphenols, ellagitannin-rich extract and whole juice extract on the expression of genes for key androgen-synthesizing enzymes and the AR. We measured expression of the HSD3B2 (3β-hydroxysteroid dehydrogenase type 2), AKR1C3 (aldo-keto reductase family 1 member C3) and SRD5A1 (steroid 5α reductase type 1) genes for the respective androgen-synthesizing enzymes in LNCaP, LNCaP–AR and DU-145 human prostate cancer cells. A twofold suppression of gene expression was considered statistically significant. Pomegranate polyphenols inhibited gene expression and AR most consistently in the LNCaP–AR cell line (P=.05). Therefore, inhibition by pomegranate polyphenols of gene expression involved in androgen-synthesizing enzymes and the AR may be of particular importance in androgen-independent prostate cancer cells and the subset of human prostate cancers where AR is up-regulated.

Keywords: Pomegranate; Punicalagin; Ellagic acid; Prostate cancer; Androgen receptor; Androgen independence

1. Introduction

Pomegranate (*Punica granatum* L.) fruits are globally consumed fresh, in such processed forms as juice, jam, wine and oil and in extract supplements [1]. They contain high levels of a diverse range of phytochemicals of which polyphenols are part of, including punicalagin (PA), ellagic acid (EA), gallotannins, anthocyanins (cyanidin, delphinidin and pelargonidin glycosides) and other flavonoids (quercetin, kaempferol and luteolin glycosides) [1–6]. PA is the most abundant of these polyphenols, and EA has been previously shown to exhibit anticarcinogenic properties, such as induction of cell cycle arrest as well as apoptosis and inhibition of tumor formation and growth in animals [7–9]. Pomegranate juice (PJ) consumption has also shown potent anticarcinogenic properties in various cancers [4,10–15].

Abbreviations: AKR1C3, aldo-keto reductase family 1 member C3; AR, androgen receptor; DHT, dihydrotestosterone; CS-FBS, charcoal/dextran-stripped fetal bovine serum; DMSO, dimethyl sulfoxide; EA, ellagic acid; FBS, fetal bovine serum; HSD3B2, 3β-hydroxysteroid dehydrogenase type 2; IGF-1, insulin growth factor-1; RT, reverse transcription; PCR, polymerase chain reaction; SF, serum free; SRD5A1, steroid 5α reductase type 1; PA, punicalagin; PJ, pomegranate juice; POMx, pomegranate extract.

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Adenocarcinoma of the prostate is currently the most common malignancy in men in the United States, comprising 32% of all cancers, and prostate cancer remains the second most common cause of cancer death in men in the United States, accounting for 11% of all cancer deaths [16]. Both androgen and androgen receptor (AR) are recognized risk factors in the development of prostate cancer [17–20]. These observations are further corroborated by genetic evidence from transgenic mouse models suggesting that increased AR signaling in the prostate is linked to an increase in precancerous lesions [21]. Since an elevated level of androgen causes enhancement of prostate cancer, reduction of circulating levels of androgens is central to the treatment of prostate cancer [22,23]. The most effective treatment for early-stage prostate cancer includes suppression of AR function either by blocking androgen signaling with the antiandrogens bicalutamide (Casodex) and flutamide or by inhibiting the conversion of testosterone to the potent androgen dihydrotestosterone (DHT) with finasteride [22]. However, 30% of patients show relapse of the disease within 3 years as a result of the emergence of androgen-independent prostate cancer cells, which are either AR positive or AR negative [24,25]. Therefore, novel approaches are needed to treat advanced prostate cancer. AR can be activated despite androgen blockade therapy in AR-positive prostate cancer [26] via an increased sensitivity of AR to low concentrations of androgen.

Epidemiological studies suggest that the risk for prostate cancer is reduced by consumption of a phytochemical-rich diet of fruits and vegetables [27]. Because pomegranates are widely consumed and have recently been shown to affect prostate cancer, the effects of pomegranate polyphenols (PA and EA), pomegranate extract (POMx) and PJ on cell proliferation and apoptosis were examined in androgen-dependent (LNCaP) and androgen-independent (LNCaP–AR and DU-145) human prostate cancer cell lines. Since androgen and AR play central roles throughout prostate cancer development, the effects of pomegranate polyphenols on the transcription of genes for androgen-synthesizing enzymes [HSD3B2 (3β-hydroxysteroid dehydrogenase type 2), AKR1C3 (aldo-keto reductase family 1 member C3) and SRD5A1 (steroid 5α reductase type 1)] and for AR were evaluated.

2. Materials and methods

2.1. Cell culture materials

The LNCaP and DU-145 prostate cancer cell lines were obtained from American Type Culture Collection (Manassas, VA, USA). The LNCaP–AR (a generous gift from Dr. C. Sawyers of the University of California, Los Angeles) cell line was developed at the University of California, Los Angeles, derived from LNCaP cells (American Type Culture Collection) [21]. LNCaP, LNCaP–AR and DU-145 prostate cancer cells were grown in RPMI-1640 media, and the media contained 10% fetal bovine serum (FBS) (Life Technologies, Grand Island, NY, USA) in the presence of 100 U/ml of penicillin and 0.1 g/L of streptomycin (Life Technologies). Cells were incubated at 37°C with 95% air and 5% CO_2. All cells were maintained below passage 20 and used in experiments during the linear phase of growth.

2.2. Confirmation of androgen dependence and independence

2.2.1. FBS treatment experiment

Cells were seeded into 96-well plates at a density of 10,000 cells/well for 24 h in 10% FBS–RPMI-1640 media and then treated with phenol-free RPMI media (MP Biochemical, Irvine, CA, USA) supplemented with either 2% FBS or 2% charcoal/dextran-stripped FBS (CS-FBS) (Invitrogen, Carlsbad, CA, USA) or without FBS [serum free (SF)] for 72 h. Then, cell proliferation was measured to test if LNCaP cells are androgen dependent and if LNCaP–AR and DU-145 cells are androgen independent. According to the manufacturer's data sheet, CS-FBS contains a very low concentration of steroid hormones (estradiol, progesterone, cortisol and testosterone) compared with normal FBS. SF media-treated cells were used as negative controls.

2.2.2. Hormone treatment experiment

Cells were seeded into 96-well plates at a density of 20,000 cells/well for 24 h in 10% FBS–RPMI-1640 media and stabilized in 2% CS-FBS phenol-free RPMI media for another 24 h. Then, the cells were treated with 10 nM DHT, 1 nM β-estradiol or 1 ng/ml of IGF-1 for 96 h to measure each hormone's effects on cell proliferation. The cells incubated with 2% CS-FBS were used as controls. All three hormones were purchased from Sigma Aldrich (St. Louis, MO, USA) and dissolved in ethanol (ethanol concentration not exceeding 0.001%). Three replicates per condition were assayed, and data averaged from four to six separate experiments are presented. Treatment media were refreshed every 24 or 48 h of treatment.

2.3. Preparation of POMx, PJ, PA and EA

POMx is a standardized extract (POM Wonderful, Los Angeles, CA, USA) of pomegranate fruit grown in California (*P. granatum* L., Wonderful Variety, Paramount Farms, Lost Hills, CA, USA). POMx contains monomeric and oligomeric ellagitannins as PA (37–40%) and 3.4% free EA but no anthocyanin as determined by high-performance liquid chromatography using previously described methods [28]. PJ (POM Wonderful) is commercially available for human consumption and was used in concentrate powder form of whole PJ. It contains 1 mg/ml of PA, 0.97 mg/ml of EA and anthocyanins [28]. Monomeric and oligomeric ellagitannins as PA were purified from fruit husk as previously reported and analyzed by high-performance liquid chromatography and liquid chromatography–electrospray ionization mass spectroscopy [22]. EA was purchased from Sigma Aldrich.

2.4. Cell proliferation assay

Proliferation was measured using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI, USA). When added to cells, the assay reagent produces luminescence in the presence of ATP from viable cells. After seeding of cells for 24 h, they were treated with PA, EA, POMx and PJ (all normalized to PA content) for 72 h at a concentration of 3.125, 6.25, 12.5, 25 or 50 μg/ml. For hormone-treated cells, PA, EA, POMx and PJ were tested at 12.5-, 25- and 50-μg/ml concentrations. All stock solutions of samples were solubilized in dimethyl sulfoxide (DMSO), and the final concentration of DMSO in media was <0.1%. At the end of treatment, plates were equilibrated at room temperature for 30 min, 100 μl of the assay reagent was added to each well and cell lysis was induced on an orbital shaker for 2 min. Plates were incubated at room temperature for 10 min to stabilize the luminescence signal, and results were read on an Orion Microplate Luminometer (Bertholds Detection Systems, Pforzheim, Germany). All plates had control wells containing medium without cells to obtain a value for background luminescence. Data are expressed as the proportion of untreated cells, and at least three independent experiments were replicated.

2.5. Apoptosis assay

Apoptosis was assessed using the Cell Death Detection ELISA^{PLUS} Assay (Roche, Indianapolis, IN, USA). This assay is a photometric enzyme-linked immunosorbent assay that quantitatively measures the internucleosomal degradation of DNA, which occurs during apoptosis. Specifically, the assay detects histone-associated mononucleosomes and oligonucleosomes, which are indicators of apoptosis. Cells were plated in 60-mm dishes at a density of 100,000 cells/dish and allowed to attach for 24 h. Cells were treated with vehicle control (0.1% final concentration of DMSO), PA (50 μg/ml), EA (50 μg/ml), POMx (50 μg/ml) or PJ (100 μg/ml) for 48 h. Following treatments, nonadherent cells were collected and pelleted at 200×*g*. Adherent cells were washed with phosphate-buffered saline (Invitrogen, Carlsbad, CA, USA), trypsinized, collected and combined with nonadherent cells into a total of 1-ml medium. Live and dead cells were then counted via Trypan blue exclusion (Pierce, Rockford, IL, USA), and equal numbers of cells were added to the microtiter plate for all treatment groups; apoptosis assay was performed according to the manufacturer's instructions. Data are expressed as the percentage of control absorbance at 405 nm of each sample over vehicle controls, and at least three independent experiments were replicated.

2.6. RNA extraction and reverse transcription (RT)

Total RNA was extracted using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA). Sample RNA content was quantified by measuring the absorbance at 260 nm with a GeneQuant spectrophotometer (Amersham Pharmacia Biotech, Piscataway, NJ, USA). RT was performed on 3 μg of RNA using oligo(dT)$_{12–18}$ primers (Invitrogen) with SuperScript III Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions.

2.7. Quantitative real-time polymerase chain reaction (PCR)

Gene expression of HSD3B2, AKR1C3, SRD5A1 and AR each was determined using Taqman Universal PCR master mix and primers (Applied Biosystems, Foster City, CA, USA) by real-time PCR using an ABI 7900 HT sequence detection system (Applied Biosystems). The transcription level of target genes was normalized to r18S expression. The expression levels of r18S were similar among samples. Every other sample had the RT reaction repeated on a separate occasion, followed by PCR and quantitation to confirm the reproducibility of the assay. In addition, every set of RT reactions contains a minus-RT negative control to confirm that no contamination or anomaly has occurred.

2.8. Statistics

Data were analyzed by Student's *t* test or one-way analysis of variance followed by the Student–Newman–Keuls test with GraphPad PRISM 3.0 (GraphPad Software, San Diego, CA, USA).

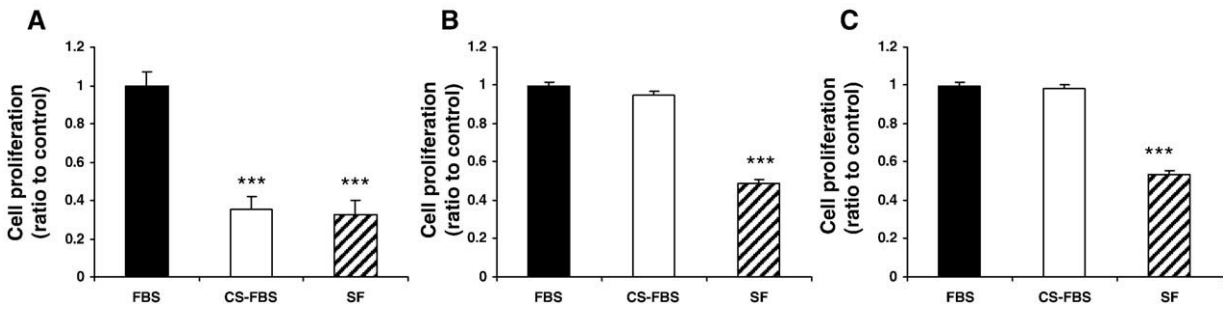

Fig. 1. Effects of FBS, CS-FBS and SF on cell proliferation in (A) LNCaP, (B) LNCaP–AR and (C) DU-145 human prostate cancer cells. CS-FBS decreased cell proliferation in LNCaP cells but not in LNCaP–AR and DU-145 cells. As a negative control, SF treatment decreased cell proliferation in all cell lines. Mean±S.E.M.; n=4–6. The triple asterisk indicates significant difference from FBS at P<.001.

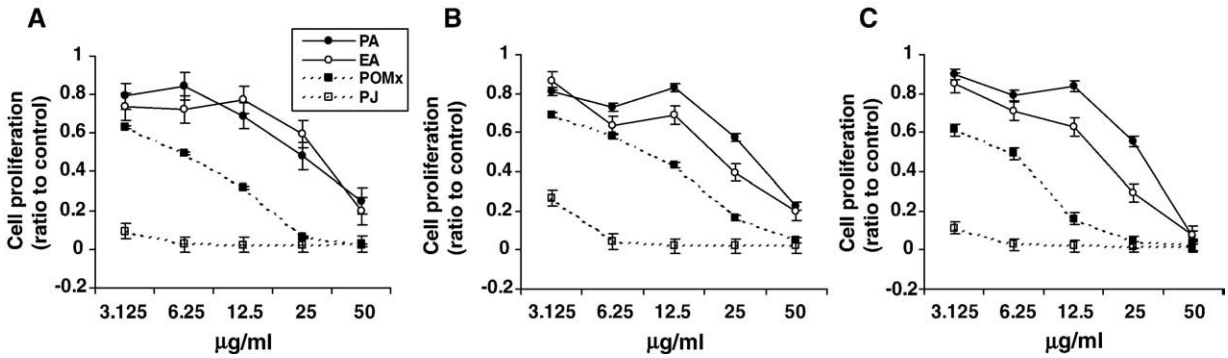

Fig. 2. Antiproliferative effects of PA, EA, POMx and PJ in (A) LNCaP, (B) LNCaP–AR and (C) DU-145 cells. Exposure to PA, EA, POMx or PJ for 72 h inhibited prostate cancer cell growth in a dose-dependent manner ($P<.01$). Mean±S.E.M.; $n=4–6$.

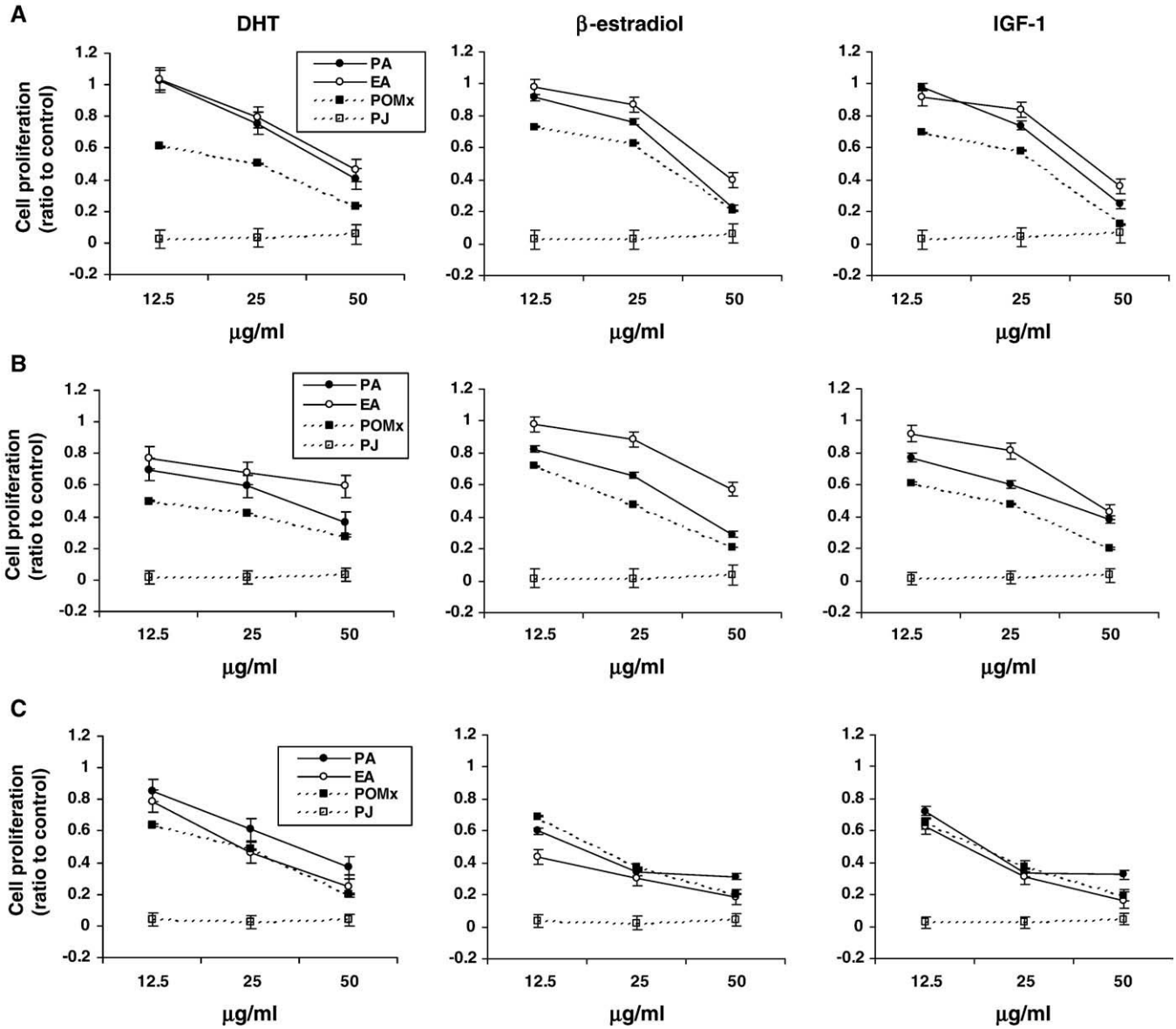

Fig. 3. Antiproliferative effects of PA, EA, POMx and PJ in hormone (DHT, β-estradiol or IGF-1)-treated (A) LNCaP, (B) LNCaP–AR and (C) DU-145 cells. PA, EA, POMx and PJ inhibited hormone-exposed prostate cancer cell growth in a dose-dependent manner ($P<.01$). Mean±S.E.M.; $n=4–6$.

3. Results

The androgen dependence of the LNCaP prostate cancer cell line and the androgen independence of the LNCaP–AR and DU-145 cell lines were confirmed by incubation of cells with FBS or CS-FBS (without androgen) and then by treatment with DHT, β-estradiol and insulin growth factor-1 (IGF-1). CS-FBS and SF treatments decreased cell growth by more than 60% in LNCaP cells ($P<.001$) (Fig. 1A). In contrast, the growth of both LNCaP–AR and DU-145 cells with CS-FBS treatment was similar to that of the FBS-treated cells (Fig. 1B and C). SF media incubation as a negative control inhibited the growth of LNCaP–AR and DU-145 cells ($P<.001$) (Fig. 1B and C). Tumor cell growth with treatment using DHT, β-estradiol and IGF-1 was induced by 2.7-, 2.4- and 1.5-fold, respectively, in LNCaP cells ($P<.05$), but the growth of LNCaP–AR cells and that of DU-145 cells were not affected (data not shown).

PA, EA, POMx and PJ treatments inhibited cell proliferation in a dose-dependent manner in the androgen-dependent LNCaP and androgen-independent LNCaP–AR and DU-145 human prostate cancer cell lines ($P<.001$) (Fig. 2). In the LNCaP cell line, all pomegranate polyphenols inhibited cell proliferation even in the presence of DHT, β-estradiol or IGF-1 (Fig. 3). The antiproliferative effects of PJ and POMx (normalized to PA content) were much stronger than the effect of the same amount of PA under all hormone treatment conditions (Figs. 2 and 3). The antiproliferative effect of PJ was greater than that of POMx with normalization to PA content.

The apoptotic effects of PJ and POMx and their purified polyphenols (PA and EA) on the LNCaP, LNCaP–AR and DU-145 prostate cancer cell lines were evaluated to ascertain whether the observed reductions in viable cell numbers were due to the induction of apoptosis. PA, EA as well as POMx at a 50-μg/ml concentration and PJ at a 100-μg/ml concentration all induced apoptosis in LNCaP cells by 2.5-, 3.3-, 1.7- and 3.2-fold, respectively ($P<.05$) (Fig. 4A). PA, EA, POMx and PJ induced apoptosis in LNCaP–AR cells by 1.8- 1.9-, 1.7- and 1.8-fold, respectively ($P<.05$) (Fig. 4B), and in DU-145 cells by 1.9-, 3.3-, 1.9- and 1.7-fold, respectively ($P<.05$) (Fig. 4C).

Table 1
Differential expression of HSD3B2, AKR1C3 and SRD5A1 in LNCaP, LNCaP–AR and DU-145 prostate cancer cells

Cell line	HSD3B2	AKR1C3	SRD5A1	AR
LNCaP	+	−	+	+
LNCaP–AR	++	−	++	++
DU-145	−	+	+	−

(+) and (++) indicate the degree of gene expression in the cell line, with (++) indicating more than fivefold greater expression compared with (+) ($P<.01$). (−) indicates that very little amount was expressed.

The effects of PA, EA as well as POMx at a 50-μg/ml concentration and PJ at a 100-μg/ml concentration on the expression of androgen-synthesizing enzymes and AR were examined in LNCaP, LNCaP–AR and DU-145 prostate cancer cells. Interestingly, HSD3B2 was expressed in LNCaP–AR in the greatest amounts and to some extent in LNCaP cells. However, there was very little expression of HSD3B2 in DU-145 ($P<.01$) (Table 1). While AKR1C3 was highly expressed in DU-145, it was expressed at very low levels in LNCaP and LNCaP–AR cells ($P<.01$) (Table 1). SRD5A1 expression was the greater one in LNCaP–AR cells compared with LNCaP and with DU-145 cells ($P<.01$) (Table 1). Finally, AR expression was up-regulated in LNCaP–AR cells compared with that in LNCaP cells ($P<.01$) (Table 1), but DU-145 cells expressed the AR gene to a very small degree (Table 1).

POMx reduced expression of HSD3B2 by fourfold in LNCaP cells compared with control ($P<.05$) (Table 2). In LNCaP–AR cells, PA, EA, POMx and PJ down-regulated the gene expression more than twofold compared with control ($P<.05$) (Table 2). EA decreased AKR1C3 gene expression by twofold in DU-145 cells ($P<.05$) (Table 2). POMx suppressed the transcriptional level of SRD5A1 by more than twofold in LNCaP, LNCaP–AR and DU-145 cells ($P<.05$) (Table 2). AR expression was also reduced by POMx treatment by more than threefold in LNCaP cells

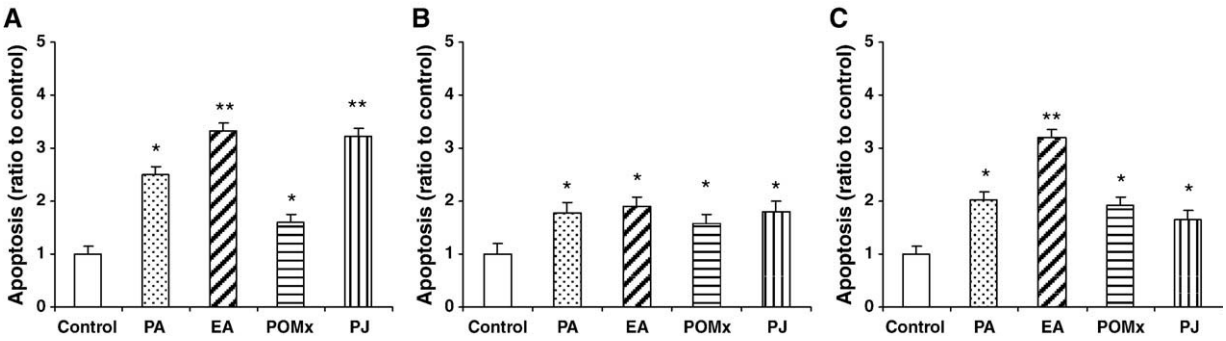

Fig. 4. Proapoptotic effects of PA, EA, POMx and PJ in (A) LNCaP, (B) LNCaP–AR and (C) DU-145 cells. Cells exposed to PA, EA, POMx or PJ for 48 h enhanced apoptosis. Cells were treated with PA (50 μg/ml), EA (50 μg/ml), POMx (50 μg/ml) or PJ (100 μg/ml). Mean±S.E.M.; $n=4–6$. The single asterisk and double asterisk indicate significant difference from control at $P<.05$ and at $P<.01$, respectively.

Table 2
Effects of PA, EA, POMx and PJ on gene expression of HSD3B2, AKR1C3, SRD5A1 and AR in LNCaP, LNCaP–AR and DU-145 prostate cancer cells

Cell line/Treatment	HSD3B2	AKR1C3	SRD5A1	AR
LNCaP				
PA	NS	ND	NS	NS
EA	NS	ND	NS	NS
POMx	↓	ND	NS	↓
PJ	NS	ND	NS	NS
LNCaP–AR				
PA	↓	ND	↓	↓
EA	↓	ND	↓	NS
POMx	↓	ND	↓	↓
PJ	↓	ND	↓	↓
DU-145				
PA	ND	NS	NS	ND
EA	ND	↓	NS	ND
POMx	ND	NS	↓	ND
PJ	ND	NS	NS	ND

Cells were incubated with PA (50 μg/ml), EA (50 μg/ml), POMx (50 μg/ml) or PJ (100 μg/ml) for 48 h. n=4–6. (↓) indicates more than twofold reduction at P<.05; NS, not significantly different compared with control (0.1% DMSO) at P<.05; and ND, not detected, with very low levels of gene expression in these cells.

(P<.05) and by PA, POMx or PJ treatment by twofold in LNCaP–AR cells (P<.05) (Table 2).

4. Discussion

Pomegranate fruits, juices and extracts have been used extensively in ancient cultures for various medicinal purposes [29]. Pomegranate polyphenols are potent antioxidants that have been shown both in vitro and in vivo to inhibit the growth of prostate and some other forms of cancer [10–15]. Our group has demonstrated that 8 oz. of PJ consumed daily following primary treatment of prostate cancer significantly prolonged prostate-specific antigen doubling time from 15 to 54 months [30]. We have also shown that pomegranate ellagitannins suppressed prostate tumor formation in a SCID mouse xenograft model [31].

Androgens are critical to the normal development, proliferation and differentiation of prostate epithelial cells, and signaling occurs via intracellular AR [23,32]. Recurrent prostate cancer after the primary treatment is typically androgen dependent, and so androgen deprivation therapy is effective in causing a remission of prostate cancer. However, later in the course of the disease, with the development of androgen independence, AR expression is increased. Increased AR expression has been demonstrated both in animals with xenografts following castration and in about one third of human prostate cancers, implicating AR in the development of androgen-independent prostate cancer [22,23]. Furthermore, in androgen-independent cancer cells, testosterone is produced intracellularly by well-described enzymes to maintain tumor growth in the absence of significant circulating concentrations of testosterone [22,26]. These tumors are more difficult to treat therefore they arise with an eventual fatal outcome [23–26,33–35]. For this reason, the prevention of the development of androgen-independent prostate cancer is a critical target for the reduction of prostate cancer mortality. In this study, we tested the hypothesis that pomegranate polyphenols can inhibit the development of androgen-independent prostate cancer growth via inhibition of the expression of genes involved in androgen synthesis and the AR in human prostate cancer cell lines.

There are several enzymes that are involved in intracellular testosterone synthesis in the prostate cancer cell [25,36–39]. HSD3B2 catalyzes the conversion of dehydroepiandrosterone to androstenedione [25,36]. In addition, AKR1C3 converts androstenedione to testosterone, and increased amounts of AKR1C3 have been demonstrated in prostatic adenocarcinoma and carcinoma [37]. Testosterone is converted to DHT by 5α-reductase (SRD5A1) [38]. Since DHT has a higher affinity for AR than testosterone, it has been proposed that DHT is critical to prostate cancer development [36]. Inhibitors of SRD5A1, such as finasteride, reduce prostate size and have been shown to reduce the development of prostate cancers by 25% but to increase the numbers of advanced cancers found [40,41].

We measured the gene expression of androgen-synthesizing enzymes in all three cell lines. It has previously been shown that messenger RNA levels of the enzymes are correlated with protein amounts and activities in other studies [37,42–45], so we did not repeat these studies.

In examining the effects of PJ and POMx on the various cell lines and taking a twofold suppression of gene expression as significant, it is clear that the most consistent suppression of both androgen-synthesizing enzymes and AR was seen in the LNCaP–AR cell line. There are other means by which prostate cancer cells can develop androgen independence, including the bypass of pathways that involve other growth signals. However, overexpression of AR is considered a significant pathway, and these results suggest that pomegranate polyphenols may be particularly helpful in the subgroup of patients with androgen-independent prostate cancer and AR up-regulation. Clinical studies to test this hypothesis are needed to confirm the importance of this observation.

Our study showed that pomegranate protects against prostate cancer by down-regulation of genes involved in androgen synthesis. However, the mechanism of the down-regulation is not known. One study [46] implicated the involvement of the cAMP–protein kinase signal transduction pathway in regulating lymphocyte expression of HSD17B1. Therefore, the cAMP–protein kinase signal transduction pathway may be one of the mechanisms for down-regulation of androgen-synthesizing enzymes. However, our data are premature to establish the hypothesis, and this requires further study.

In this study, we confirmed observations by other laboratories [47–50] demonstrating that LNCaP cells are androgen dependent and that LNCaP–AR and DU-145 cells are androgen independent. LNCaP–AR cells are

engineered to overexpress AR, which models a mechanism of androgen independence seen in about one third of androgen-independent prostate cancers clinically. It is likely that the mechanisms underlying androgen-independent growth are different in LNCaP–AR cells compared with DU-145 cells. LNCaP–AR cells are androgen independent by overexpressing AR, while DU-145 cells are androgen independent with low levels of AR expression.

This study showed that pomegranate products and their polyphenols reduced tumor cell growth and induced apoptosis in both androgen-dependent and androgen-independent prostate cancer cells. These antiproliferative effects were also consistent in hormone-treated cells. This implies the potential possibility that pomegranate and its polyphenols are used as novel dietary supplements with maximum potential for androgen-dependent and androgen-independent prostate chemoprevention. In addition, more studies are needed to determine how the alteration of cell proliferation and apoptosis is related to the expression of androgen-synthesizing enzymes and AR.

It was observed that there were differences in the apoptosis between cell lines and test compounds. For example, the POMx was relatively not effective in all cell lines and the LNCaP–AR cells were more resistant than the other two cell lines. EA treatment showed a higher apoptosis response in LNCaP and DU-145 than LNCaP–AR cells. Further studies are needed to explain the cell type-specific and test compound-specific responses on apoptosis.

In this study, PJ at a 100-μg/ml concentration compared with POMx, PA and EA at 50 μg/ml was applied to measure its effect on apoptosis. Our preliminary data showed that PJ did not show the proapoptotic effect at the concentration of 50 μg/ml but did so at 100-μg/ml concentration. This may occur because PJ is a diluted form compared with the POMx and the pure compounds.

The biological properties associated with pomegranate fruits prompted us to evaluate their major phytochemical ingredients both as extracts and as single purified compounds, PA and EA. We demonstrated that PJ and POMx were more potent inhibitors of cell growth than isolated individual polyphenols in all three cell lines, suggesting synergistic and/or additive effects of several phytochemicals present in PJ and POMx, including proanthocyanidins, anthocyanins (glycosides of delphinidin, peonidin and cyanidin) and flavonoid glycosides [1–6]. All of these have been shown to have antioxidant and antiproliferative activities [1,51]. These observations are also consistent with those of our previous studies on the effects of pomegranate polyphenols on the RWPE-1 and 22RV1 prostate cancer cell lines [52].

Our recent study [31] showed that EA was detected in serum (134 ng/ml) and prostate (676 ng/ml) after intraperitoneal administration of POMx in a mouse model. It is a significant observation that EA is more targeted to prostate tissue, which implies the beneficial role of pomegranate against prostate cancer. The level of EA in prostate tumor in vivo is not known. More data from in vitro and in vivo studies should be obtained to decide the application amount for clinical studies.

In short, PJ and POMx and their polyphenols showed a capability to arrest proliferation and stimulate apoptosis in human androgen-dependent and androgen-independent prostate cancer cells. Inhibition of gene expression involved in androgen-synthesizing enzymes and the AR may contribute to the growth-inhibitory effects of pomegranate polyphenols and may provide a molecular target for the inhibition of the emergence of androgen-independent prostate cancer.

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