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The anti-proliferative and anti-androgenic activity of different pomegranate accessions

Ola Orgil [a,b], *Limor Spector* [a], *Doron Holland* [c], *Jamal Mahajna* [a,b], *Rachel Amir* [a,b,*]

[a] *Migal Galilee Technology Center, P.O. Box 831, Kiryat Shmona 11016, Israel*
[b] *Tel Hai College, Upper Galilee, Israel*
[c] *Institute of Plant Sciences, Agricultural Research Organization, Newe Ya'ar Research Center, Ramat Yishay 30095, Israel*

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ABSTRACT

This study monitored the anti-proliferative activity of pomegranate (*Punica granatum* L.) peel homogenates against breast (MCF7 and MDA-MB-453) and prostate (LNCaP and PC-3) cancer cell lines using 29 different accessions to study the natural diversity in these accessions. Using MTT method, it was revealed that high anti-proliferative activity was observed against MCF7, MDA-MB-453 and LNCaP (androgen-dependent) cell lines for most accessions, while the androgen-independent prostate cancer cell line, PC-3, exhibited relatively high resistance. Peel extracts displayed the highest LNCaP inhibition activity compared to aril juice and seed homogenates. Eight accessions exhibiting the highest anti-proliferative activity were studied further using the LNCaP and PC-3 cancer cells. IC_{50} and clonogenicity inhibition were determined. Several accessions were found to inhibit androgen receptor activity but not NF-κB transcription function. This study illustrated the importance of exploiting natural pomegranate variation to identify accessions exhibiting potential selective activity for the treatment of specific types of cancer.

1. Introduction

Studies have shown that juice from pomegranate (*Punica granatum* L.) (PJ) has high benefit to human health. The juice has high polyphenol and total phenols content (TPC) (Seeram, Zhang, Reed, Krueger, & Vaya, 2006), which act as scavengers of free radicals and reactive oxygen species (ROS) (Aviram et al., 2008). Phenols antioxidative activities have been associated with the reduction of stress-related chronic diseases and age-related disorders, such as cardiovascular diseases (Aviram et al., 2008), neurodegeneration and skin deterioration (Quideau, Deffieux, Douat-Casassus, & Pouysegu, 2011). Studies have also shown that PJ exhibits anti-carcinogenic and anti-proliferative activities (Adhami, Khan, & Mukhtar, 2009; Orgil et al., 2014), as well as other health-promoting abilities (Ismail, Sestili, & Akhtar, 2012).

Detailed studies have revealed that most of the phenols and polyphenols in PJ are derived from the non-edible sections of

* *Corresponding author.* Laboratory of Plant Science, Migal Galilee Technology Center, P.O. Box 831, Kiryat Shmona 11016, Israel. Fax: +972 4 6944980.
E-mail address: Rachel@migal.org.il (R. Amir).
Abbreviations: AR, androgen receptor; ETs, ellagitannins; PJ, pomegranate juice; TPC, total phenols content
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the fruit (peels and inner lamellas) (Gil, Tomas-Barberan, Hess-Pierce, Holcroft, & Kader, 2000; Orgil et al., 2014). Analysis of PJ compounds revealed that the polyphenol group of ellagitannins (ETs) contributes significantly to the health beneficial effects of PJ (e.g., Aviram et al., 2008). PJ contains unique ETs that include punicalagins, pedunculagin I, punicacortein, punigluconin and galloylpunicalin (Fischer, Carle, & Kammerer, 2011). Some of these compounds such as the isomers of punicalagins are most abundant in pomegranate peels and are responsible for more than 50% of the juice's potent antioxidant activity (Adams et al., 2006; Gil et al., 2000).

It has been reported in recent years that ETs are hydrolysed in mammals to ellagic acid under physiological conditions, and that ellagic acid is then gradually metabolised by the intestinal microbiota to produce different types of urolithins (hydroxy-6H-dibenzopyran-6-one derivatives) and dimethylellagic acid glucuronide (Bialonska et al., 2010; Landete et al., 2015; Seeram et al., 2006). Consumption of ETs and ellagic acid-rich food is proposed to be protective against certain chronic diseases (Landete et al., 2015). Disposition studies reveal that urolithins are enriched in prostate, intestinal and colon tissues in mice, which could explain why urolithins inhibit prostate and colon cancer cell growth (Kasimsetty et al., 2010; Sanchez-Gonzalez, Ciudad, Noe, & Izquierdo-Pulido, 2014). Moreover, anti-proliferative and apoptosis-inducing activities of ellagic acid and urolithins have been demonstrated to inhibit cancer cell growth (Bialonska et al., 2010; Landete et al., 2015).

In addition to the ETs, many other phenolic compounds, such as dihydrokaempferol, caffeic, ferulic, coumaric, gallagic, gallic, vanillic, protocatechuic, and hydrocinnamic acids, as well as non-phenolic compounds were detected in PJ (Fischer et al., 2011). However, there are several other metabolites that have not yet been identified and annotated (Fischer et al., 2011). The small phenolic compounds can be absorbed from PJ to the plasma and contribute *in vivo* to antioxidant activity (Aviram et al., 2008; Gil et al., 2000), anti-proliferative activities (Adhami et al., 2009), and health beneficial properties, as shown by *in vitro* and *in vivo* studies (Adams et al., 2006; Manasathien, Indrapichate, & Intarapichet, 2012).

Most studies on the health benefits of pomegranates were performed mainly on the "Wonderful" accession (e.g., Adams et al., 2006; Aviram et al., 2008; Gil et al., 2000). However, many other accessions exist that differ in peel and aril colour, size, taste and other traits (e.g., Dafny-Yalin et al., 2010; Tzulker et al., 2007). In this study, we used 29 pomegranate accessions that differed significantly in these traits, representing the accessions in the collection at Newe Ya'ar (Holland, Hatib, & Bar-Ya'akov, 2009). These 29 accessions were tested previously for their antioxidant capacity (Tzulker et al., 2007). In the current study, these accessions were monitored for their anti-proliferative activity against hormone-dependent and independent breast cancer cell lines, MCF7 and MDA-MB-453, and androgen-dependent and independent prostate cancer cell lines, LNCaP and PC-3, respectively. These four types of cancer cell lines are considered to be "classical" (Choi, Lee, & Kim, 2012) and are known to be inhibited by PJ that is produced from the whole fruit, including extract from the peels (Adhami et al., 2009; Kim et al., 2002). These cancer lines were chosen because epidemiological and basic studies have shown that in addition to genetic susceptibility and environmental factors, diet plays a pivotal role in the initiation and development of these types of cancer (Shishir, Adams, Bhatt, & Aggarwal, 2006). Moreover, it was suggested that reduced risk to these types of cancer is associated with the consumption of a phytochemical-rich diet (Adhami et al., 2009).

2. Materials and methods

2.1. *Plant materials and fruit processing*

In this study, we used 29 pomegranate accessions grown at the Newe Ya'ar Research Center, ARO [registered in the Israel Gene Bank for Agriculture Crops (IBG, website: http://igb.agri.gov.il)] (Holland et al., 2009). These accessions differed in peel and aril colour, size and taste (Dafny-Yalin et al., 2010; Tzulker et al., 2007). The different accessions were harvested from the end of August up to the end of October in the year 2013, as previously described (Tzulker et al., 2007).

The arils were separated, aril juice was prepared by squeezing the arils through a nylon sieve, and the juice and the seeds were collected. From each of the whole peels and seeds, 100 g dry weight was homogenised (for 3 min) using a cold blender with 200 ml of distilled water to prepare the homogenate (Orgil et al., 2014; Tzulker et al., 2007). The homogenates and aril juice were then centrifuged (8000 *g* for 15 min), and supernatants were collected and stored frozen in aliquots at –80 °C for further analysis (that was performed over the next 10 months).

2.2. *Cancer cell lines*

Human breast cancer cells, MCF-7 and MDA-MB-453, and human prostate cancer cells, LNCaP and PC-3, were purchased from ATCC (Rockville, MD, USA). The cells were maintained in RPMI 1640 medium with L-glutamine, supplemented with 10% foetal calf serum (FCS) and 1% PenStrep (penicillin + streptomycin) and 1 mg/ml G418 antibiotic (Sigma-Aldrich, St Louis, MO, USA) (Petrova et al., 2009). Cells were grown in a humidified incubator at 37 °C with 5% CO_2 in air and fed twice a week with fresh medium. Construction of MCF7-N1: the NF-κB TA Luci reporter plasmid was transfected into MCF7 cells according to manufacturer's instructions (Clontech, USA). Resistant clones were selected, and the new cell line (MCF7-N1) was validated by monitoring levels of luciferase in response to exposure of known modulators of the NF-κB transcription factor.

2.3. *Cell proliferation assay (MTT assay)*

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed, as previously described (Orgil et al., 2014; Petrova et al., 2009). The MTT assay is based on the ability of mitochondrial dehydrogenase of living cells to reduce the MTT (yellow) to a purple formazan product (Fotakis & Timbrell, 2006), which can be related directly to the number of viable (living) cells (Fotakis & Timbrell, 2006). The cancer cell lines were placed into 96-well plates and maintained in the appropriate medium for 24 h. The pomegranate