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ORIGINAL ARTICLE

Lycopene differentially induces quiescence and apoptosis in androgen-responsive and -independent prostate cancer cell lines ☆

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Summary
Background & aims: Lycopene has been credited with a number of health benefits including a decrease in prostate cancer risk. Our study investigates the molecular mechanism underlying anti-cancer activity of lycopene-based products in androgen-responsive (LNCaP) and androgen-independent (PC3) cells.
Methods: The effect of lycopene-based agents on prostate cancer growth and survival were examined using proliferation assays, bromodeoxyuridine incorporation and flow cytometric analysis of cellular DNA content. Biochemical effects of lycopene treatment were investigated by immunoblotting for changes in the absolute levels and phosphorylation states of cell cycle regulatory and signalling proteins.
Results: LNCaP and PC3 cells treated with the lycopene-based agents undergo mitotic arrest, accumulating in G0/G1 phase. Immunoblot screening indicated that lycopene's antiproliferative effects are likely achieved through a block in G1/S transition mediated by decreased levels of cyclins D1 and E and cyclin dependent kinase 4 and suppressed

Abbreviations: IGF, insulin-like growth factor; IGF-BP IGF, binding protein; IGF-IR IGF, type 1 receptor; CDK, cyclin dependent kinase; HPLC, high performance liquid chromatography; SDS, sodium-dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis; BrdU, bromodeoxyuridine; BSA, bovine serum albumin; Rb, retinoblastoma.

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Retinoblastoma phosphorylation. These responses correlated with decreased insulin-like growth factor-I receptor expression and activation, increased insulin-like growth factor binding protein 2 expression and decreased AKT activation. Exposure to lycopene at doses as low as 10 nM for 48 h induced a profound apoptotic response in LNCaP cells. In contrast PC3 cells were resistant to apoptosis at doses up to 1 μM.
Conclusions: Lycopene exposure can suppress phosphatidylinositol 3-kinase-dependent proliferative and survival signalling in androgen-responsive LNCaP and androgen-independent PC3 cells suggesting that the molecular mechanisms for the cytostatic and cytotoxic actions of lycopene involve induction of G0/G1 cell cycle arrest. This study supports further examination of lycopene as a potential agent for both the prevention and treatment of prostate cancer.

Introduction

Therapeutic options for patients with advanced prostate cancer (PCa) are limited, consequently there is a need to explore new therapies and pursue the biochemical mechanisms underlying positive pre-clinical and clinical data. Lack of curative therapies and their potential for adverse effects are factors that have led many men to turn to a potpourri of herbal preparations and dietary supplements in the hope that these will help them prevent or overcome PCa.[1] These preparations and supplements cover a range of compound classes including vitamins, (e.g., vitamin E), minerals, (e.g., selenium), herbal preparations, (e.g., Saw Palmetto) and phytochemicals, (e.g., lycopene).

Lycopene (ψ, ψ-carotene) is an unsaturated carotenoid with a total of 13 double bonds, 11 of which are conjugated, thus contributing to the compound's potent antioxidant properties. Studies *in vitro* have shown lycopene to be twice as potent as β-carotene and ten times more potent than α-tocopherol in terms of its singlet oxygen quenching ability.[2] Serum lycopene levels were shown to increase significantly into the μM range after the consumption of tomato products and supplements, with a concomitant decrease in the biomarkers of oxidation including the oxidation of serum lipids, low density lipoprotein cholesterol, serum proteins and DNA.[3]

The beneficial properties of tomato products were largely attributed to lycopene by one of the earliest epidemiological studies showing an inverse relationship between the consumption of tomatoes and/or tomato products and PCa risk.[4] Over the last decade numerous epidemiological, experimental and tissue culture studies and reviews have been published which describe an association between lycopene supplementation or a tomato rich diet and decreased positive markers for PCa.[5–7] In addition three phase II clinical studies have been reported recently, two conducted in India which report favorable outcomes regarding PSA decline in patients with either metastatic disease (10 mg Lycored softules daily for 3 months) or who were undergoing concurrent orchiectomy (2 mg twice daily for 3 months); and one dose escalation study (15–120 mg/kg daily for 1 yr) conducted in the US in patients with recurrent disease which concluded no significant impact of Lyc-O-Mato™ (6% lycopene) on PSA.[8–10] Further clinical study is required to establish whether the timing of lycopene intervention and/or combination with hormone withdrawal are important factors regarding outcome, as well as dose and formulation of lycopene. Much of the literature makes use of uncharacterized lycopene containing tomato extracts rather than pure lycopene, therefore, perhaps wrongly assuming consistent lycopene stability while also honing in on lycopene as the active ingredient.[11] Due to its highly unsaturated anti-oxidant properties lycopene readily undergoes trans- to cis-isomerization when heated and when exposed to oxygen, especially if present in solid or powdered form.[12] In order to overcome these stability issues, we have used two lycopene-based products, both of which have now been characterized through independent quality control testing and contain 3% and 38% lycopene by weight.

Beyond its antioxidant properties, the biological actions of lycopene are not fully delineated, although several cell culture studies suggest effects on cell cycle progression. Lycopene has been indicated to have antiproliferative effects on prostate[13–16] and breast cancer cell lines.[17,18] In breast cancer models, reduced expression of cell cycle regulatory proteins, such as cyclins D1 and E and the cyclin dependent kinases 2 and 4, as well as suppression of insulin-like growth factor (IGF-I) action have been correlated with lycopene's effects on proliferation.[17–20]

Both of the cell lines used in this study lack the tumor suppressor protein, phosphate and tensin homologue (PTEN). PTEN is among the most commonly mutated genes in epithelial cancers[21] and its function is lost in the majority of lethal PCas.[22] PTEN is a phospholipid phosphatase that dephosphorylates the 3′ position of phosphatidylinositol 3,4,5-trisphosphate (PIP3), thus diminishing a major substrate for plextrin homology domain-containing kinases including AKT/PKB, PDK1 and PDK2/ILK, all of which serve as positive effectors of phosphatidylinositol 3-kinase (PI3 K) signalling.[23] Pro-tumorigenic activity results from the dysregulation of these downstream signalling events which promote tumor cell growth and elicit protection from cytotoxic therapies.

It is therefore important to identify agents that can overcome the therapeutic resistant properties of PTEN deficient tumor cells. With this study we investigate the molecular basis for lycopene's antiproliferative action in two PTEN-null PCa cell lines through use of two characterized lycopene-based products. We show that lycopene-induced