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LWT - Food Science and Technology xxx (2014) 1–8

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Lycopene induce apoptosis in human prostate cells and alters the expression of Bax and Bcl-2 genes

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A R T I C L E I N F O

Article history:
Received 22 January 2014
Received in revised form
11 April 2014
Accepted 13 April 2014
Available online xxx

Keywords:
Prostate cancer
Benign prostate hyperplasia
Lycopene
Chemoprevention

A B S T R A C T

Prostate cancer is the most common malignancy in men and the second leading cause of cancer-related mortality in men of the Western world. Lycopene has received attention due to its potential to prevent cancer. Alterations of the balance between proliferation and apoptosis are associated with cancer. In the present study, we evaluated the influence of lycopene on cell viability and apoptosis of human prostate cancer (PCa) cells and benign prostate hyperplasia (BPH) cells. We have studied prostate cells treated with lycopene for 96 h. Using MTT assay, we observed a decrease of viable PCa cells after treatment with lycopene. Flow cytometer analysis revealed that lycopene promoted up to two-fold increase of apoptotic cells in PCa cells when compared to the control group. Using real time PCR assay, we found that lycopene promoted an up-regulation of Bax gene and a downregulation of Bcl-2 gene in PCa cells. Less expressive effects were observed in BPH cells. Taken together, the present study supports the proposal that lycopene may have a protective effect on prostate cancer.

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1. Introduction

Prostate gland is a male accessory reproductive secretory organ, which expels its content in the urethra during ejaculation. In humans, the prostate is located immediately below the base of the bladder surrounding the neck region of the urethra. It is mainly associated with three types of disorders, namely, benign prostate hyperplasia (BPH), prostate cancer (PCa), and prostatitis. BPH and PCa are the most common pathophysiological conditions of prostate gland in elderly men (Nunzio et al., 2011). These diseases already represent significant challenges for health-care systems in most parts of the world. However, the cellular and molecular processes underlying the pathogenesis and development of BPH or PCa are poorly understood (Shen & Abate-Shen, 2010).

BPH is a slow progressive enlargement of the prostate gland leading to lower urinary tract symptoms (LUTS) in elderly men. It is characterized by hyperproliferation of epithelial and stromal cells in the transition zone of the prostate gland, which can be observed histopathologically (Schuster & Schuster, 1999). Despite of its obvious importance as a major health problem, little is known in terms of biological processes that contribute to the development of BPH. Changes in tissue consistency and cellular hyperplasia are accompanied by downregulation of apoptotic factors and increased level of antiapoptotic factors that decrease the rate of prostatic cell death, contributing to hyperproliferation of prostatic tissue (Kyprianou, Tu, & Jacobs, 1996). It has been reported that stromal to

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http://dx.doi.org/10.1016/j.lwt.2014.04.028
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epitelial ratio is altered in BPH, where the ratio increases from 2:1 in normal glands to 5:1 in BPH (Shapiro, Becich, Hartanto, & Lepor, 1992). Because stromal hyperproliferative activity is thought to promote the development of BPH, the existence of adult stem cells in the prostate stromal compartment is speculated to expand the stroma in response to stimuli during the pathogenesis of BPH (Lin et al., 2007).

Prostate cancer remains the second most common cancer-related death in men, and will result in more than 29.720 estimated deaths in 2013 (Siegel, Naishadham, & Jemal, 2012). With the characteristic slow progression for some tumors, and recurrence after treatment in many prostate cancer patients, the balance between over-aggressive and unneeded treatment coupled with the ability to identify cancers predisposed to aggressive phenotypes remains a challenge (Larkin & Kyprianou, 2013). Prostate cancer etiology, although not clear, is partly attributed to multigenic and epigenetic mechanisms and the heterogeneous nature of this disease (Maitland & Collins, 2005; Oldridge, Pellacani, Collins, & Maitland, 2011). Gleason and others described that when the transition of normal gland into adenocarcinoma of prostate takes place, its normal histological structure is disrupted and results in abnormal proliferation of the glandular structure, destruction of basement membrane, and progressive loss of basal cells ($<1\%$) (Gleason, 1966; Maitland, Frame, Polson, Lewis, & Collins, 2011).

Epidemiological studies have suggested that the inclusion of fruits, vegetables, and whole grains in dietary intake might prevent and even reverse the cellular changes associated with carcinogenesis at the initial stages, reducing tumor incidence (Ahmad & Mukhtar, 1999). These encouraging results have been documented in vitro and in vivo, as well as in clinical trials (Sporn & Liby, 2005). These beneficial data are attributed to bioactive compounds, including both essential nutrients (i.e., selenium, calcium, zinc, and vitamins C, D, and E) and nonessential componentes (carotenoids, flavonoids, allyl sulfide indole compounds, conjugated acids and $\omega-3$ fatty compounds). Bioactive compounds have been reported to modify specific carcinogenic processes, including cancer metabolism, hormonal balance, transcription factors, cell-cycle control, apoptosis, inflammation, angiogenesis, and metastasis (Ross & Davis, 2011).

Lycopene, a carotenoid found in high quantities in tomatoes and tomato-rich products, has been explored in association with prevention and treatment of various kind of diseases, including prevention of prostate cancer (Silberstein, Silberstein, & Saphier, 2013). In 2007, the World Cancer Research Fund reported that a high intake of fruit and vegetable may be a beneficial, reducing the risk of cancer, including lycopene uptake for prostate cancer (Kavanaugh, Trumbo, & Ellwood, 2007). Lycopene is found in the human prostate tissue, suggesting the biological possibility of a direct effect of this carotenoid in prostate function and carcinogenesis (Shami & Moreira, 2004).

Studies from around the world have demonstrated cancer chemopreventive properties of lycopene and other carotenoids specifically against PCa. Lycopene has been noted for its protective effects against PCa, acting as an antioxidant and inhibiting cell proliferation (Wei & Giovannucci, 2012). Although the exact mechanism by which lycopene reduces the growth of prostate cancer cells is still not well understood, it is frequently believed that this effect may be due to the protective action of lycopene against oxidative damage caused by reactive oxygen species (ROS) in cells. Lycopene has also been identified as an antioxidant compound with potential anti-cancer properties and no obvious side effects (Rackley, Clarck, & Hall, 2006; Silberstein et al., 2013).

PCa tissues, in particular, have high levels of oxidative damage. The risk of prostate damage has been reported to be lower in patients who regularly consume higher levels of lycopene. Patients with localized prostate adenocarcinoma who consumed tomato sauce-based dishes for three weeks (30 mg of lycopene per day) exhibited a statistically significant decrease in oxidative DNA damage in the prostate tissues (Chen et al., 2001).

Lycopene inhibits cell cycle progression and promotes apoptosis. It inhibits IGF-1 and androgen signaling and IL-6 expression in the prostate, while upregulating gap junction communication. These pathways were explored to determine the extent of the lycopene effect and the possible predictive value of the pathway for the development of prostate cancer and advanced prostate cancer (Bowen et al., 2002). A risk reduction of almost 35% was observed for a consumption frequency of 10 or more servings of tomato products per week, and the protective effects were even stronger for more advanced or aggressive prostate cancer (Giovannucci et al., 1995). Similarly serum and tissue levels of lycopene were inversely associated with prostate cancer risk in recent case-control and cohort studies (Gann et al., 1999; Rao & Agarwal, 1999).

Many investigators have reported growth inhibition with lycopene in numerous prostate cell lines. The effect appears to be through cell cycle arrest and apoptosis and has been shown at physiological concentrations. It has been demonstrated that treatment of LNCaP cells with physiologically attainable concentrations of lycopene (0.3–3.0 μM) significantly reduced mitochondrial transmembrane potential, induced the release of mitochondrial cytochrome *c*, and increased annexin V binding, confirming induction of apoptosis (Hantz, Young, & Martin, 2005). In PC-3 cell line, the growth and DNA synthesis were inhibited with the lycopene concentration increased, and lycopene also could change the cell cycle distribution, i. e. increasing the proportion of G0/G1 phase and descending the proportion of S and G2/M phase, and induce the apoptosis. Lycopene induced apoptosis of PC-3, change the cell cycle distribution and downregulate the expression of cyclin D1 and Bcl-2 gene and upregulate the expression of Bax gene and then restrain cell proliferation (Wang & Zhang, 2007).

The results of several studies reveal lycopene may have a role in prostate cancer prevention and treatment, however, further studies are warranted to specify the beneficial properties of lycopene and their role in disease prevention and treatment. In the present study, we evaluated the influence of lycopene on cell viability, cell cycle, and apoptosis in primary culture of human prostate cancer and benign prostate hyperplasia cells.

2. Material and methods

2.1. Reagents

All-trans lycopene was purchased from Sigma Chemical Company (St. Louis, MO). Water-soluble lycopene (96% pure) was provided by Roche (Rio de Janeiro, Brazil). Dulbecco's cell culture medium and bovine serum albumin were obtained from Sigma Chemical Company (St. Louis, MO), and fetal bovine serum (FBS) from Laborclin (Campinas, Brazil). Tissue culture flasks were obtained from Nunc (Roskilde, Denmark). All the chemicals were of analytical grade.

2.2. Isolation and characterization of primary cell cultures

Transurethral resection fragments of prostate tissues from two BPH surgeries were used to obtain the BPH cells. PCa cells were obtained from fragments of prostate tissues obtained from two cancer cases submitted to radical prostatectomy. Participants provided their written consent to participate in this study upon signature on the Consent Term established. The study was approved by the Ethics Committee of the Clementino Fraga Filho

University Hospital, Federal University of Rio de Janeiro, Protocol-CAAE0029.0.197.000-05.

Tissues were cut in fragments of 1–3 mm^3 that were grown in 24-well plates containing DMEM supplemented with 10% FBS and 1 μL/mL penicillin (Sigma). The medium was changed every two days. Cells were trypsinized and transferred to 25 mm^2 culture dishes. After six passages, a homogeneous cell population was obtained.

The cells were immunocytochemically characterized as follows. Cells were washed twice with PBS and fixed with 4% paraformaldehyde-PBS (Sigma) for 10 min. After fixation, they were washed with PBS and incubated in a 50 nM NH_4Cl for 30 min. The nonspecific antibody binding was blocked with PBS/BSA 5%, and the primary antibodies were incubated overnight. We used antibodies against vimentin (Sigma) and α-smooth muscle actin (Sigma) for BPH. We used antibodies against cytokeratin 5 (Cell Marque) and alpha-methylacyl CoA racemase (P504S-Cell Marque) for primary PCa cells. After incubation with primary antibodies, cells were washed with PBS and incubated for two additional hours with either goat antimouse Alexa 488 or goat antirabbit Alexa 488/546 secondary antibodies (Invitrogen). Cell nuclei were stained with DAPI (Santa Cruz Biotechnology). Finally, cells were washed in distilled water and mounted on histological slides with N-propylgallate (Sigma). Images were captured using a confocal microscopy (Olympus IX81) and a Hamamatsu OrcaR2 digital camera.

2.3. Cell culture and treatment protocol

All cells were plated in 25 cm^2 tissue culture flasks, (5.0×10^6 cells/flask) and maintained routinely in the Dulbecco's medium (DMEM) supplemented with 10% FBS and 2 g/L HEPES buffer, pH 7.4, under 5% CO_2 atmosphere. Stock flasks were grown to 70% confluence and subcultured routinely. Medium renewal was done 2–3 times weekly. For each experiment, all the cells were seeded at 10^4 cells/cm^2 density in 6- and 96-well plates for cell cycle and cell proliferation analyses, respectively. After 24 h, medium was removed and cells were treated with increasing concentrations of lycopene (0.5; 1; 2.5; 5; 10 and 20 μM) dissolved in DMEM at 50 °C. The controls were included on each plate. The cells were then incubated for 96 h with daily medium replacement.

2.4. Cell viability assay

Cell viability was monitored by MTT assay (Amresco, Solon, OH). MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) is a pale yellow substrate that is reduced by living cells to yield a dark blue formazan product. This requires active mitochondria, and even recently dead cells do not reduce significant amounts of MTT. Exponentially growing cells were adjusted to $1.0 \times 10^4/cm^2$ with DMEM, plated in 96-well plates (Corning, Tewksbury, MA) at 200 μL/well and incubated for 12 h according to the routine procedure. The cells were then incubated with lycopene (0.5–20 μM) for 24, 48, 72 and 96 h (6 wells for each sample). Each well was also incubated with MTT (20 μL/well; 5 g/L) for 4 h. The liquid was removed and 100 μL/well sodium dodecyl sulfate was added to dissolve the solid residue. Finally, the absorbance was measured using a microplate reader (BIO-RAD 2550) at 570 nm. The cell proliferation inhibition rate (CPIR) was calculated using the following formula: CPIR = (1 − average A value of experimental group/average A value of control group) × 100%.

2.5. Cell cycle assays

Cell cycle analysis was done using the propidium iodide assay. Briefly, cells were resuspended in 500 μL propidium iodide solution (PBS, 0.1% Triton X-100, 0.1% RNAse and 50 μg/mL propidium iodide; Sigma). Cell cycle analysis was assessed by flow cytometry (FACScalibur, BD Bioscience, San Jose, CA), and after acquisition of 20.000 events the data were analyzed in Cell Quest software.

2.6. Apoptosis assays

In apoptotic cells, phosphatidylserine translocates from the inner to the outer leaflet of the cell membrane, where annexin V conjugated to fluorescein isothiocyanate (FITC) can bind. Coupled with propidium iodide staining, disruption or increased permeability of the plasma membrane can also be determined. Cells were resuspended in 400 μL binding buffer containing 5 μL of annexin V FITC and 5 μL propidium iodide (Apoptosis Detection Kit II, BD Biosciences) for 15 min at room temperature. Annexin V binding was evaluated by flow cytometry (FACScalibur, BD Biosciences), and after acquisition of 20.000 events the data were analyzed in Cell Quest software.

2.7. Gene expression analysis

Total RNA was extracted from the studied cells using RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. RNA yield and quality were determined by a spectrophotometer NanoDrop ND-1000 V3.2 (Nanodrop Technologies, Wilmington, DE). Equal amounts (1 μg) of RNA from cells were reverse transcribed with cDNA Syntesis kit "Superscript II First-Strand Synthesis System for RT-PCR" (Invitrogen) and Oligo (dT) primer (Invitrogen). The cDNA was used as a template for subsequent real-time polymerase chain reaction (RT-PCR).

Quantitative RT-PCR was done in a CFX96 Real Time System (BIORAD) C1000 Thermal Cycler using SYBR Green and TaqMan assays (Applied Biosystems, Grand Island, NY) following the manufacturer's instructions. The expression level of Bax, Bcl-2, PPARγ, RXR, PTOV1 and Tp53 mRNA were all normalized with β-actin and ubiquitin expression level. To evaluate the quality of the RT-PCR products, melt curve analyses were performed after each assay. Relative expression was determined using the ΔΔCT method with β-actin and ubiquitin rRNA as the reference genes.

2.8. Statistical analysis

The presented data are mean values ± standard error of three independent experiments done in duplicate ($n = 6$). Statistical comparisons were carried out by analysis of variance and post hoc Tukey's test using Graph Pad Prism 5.0 and Statistical 6.0 program. The differences were considered significant when $P < 0.05$.

3. Results

3.1. Characterization of cells

Homogeneous primary cell populations were obtained from BPH and PCa surgery resections. All cells had normal growth characteristics expected under standard in vitro culture conditions. Previous studies have shown that after six passages the primary prostate cell cultures were characterized by an increase in expression of cytoskeleton proteins, such as vimentin and α-smooth muscle actin, as described in Soares et al. (2013). PCa cells were also positive for alpha-methylacyl CoA racemase (Fig. 1A), which is considered to be a useful marker for neoplastic transformation in the prostate (Jiang et al., 2002; Molinie et al., 2004). Iwasa, Mizokami, Miwa, Koshida, and Namiki (2007)

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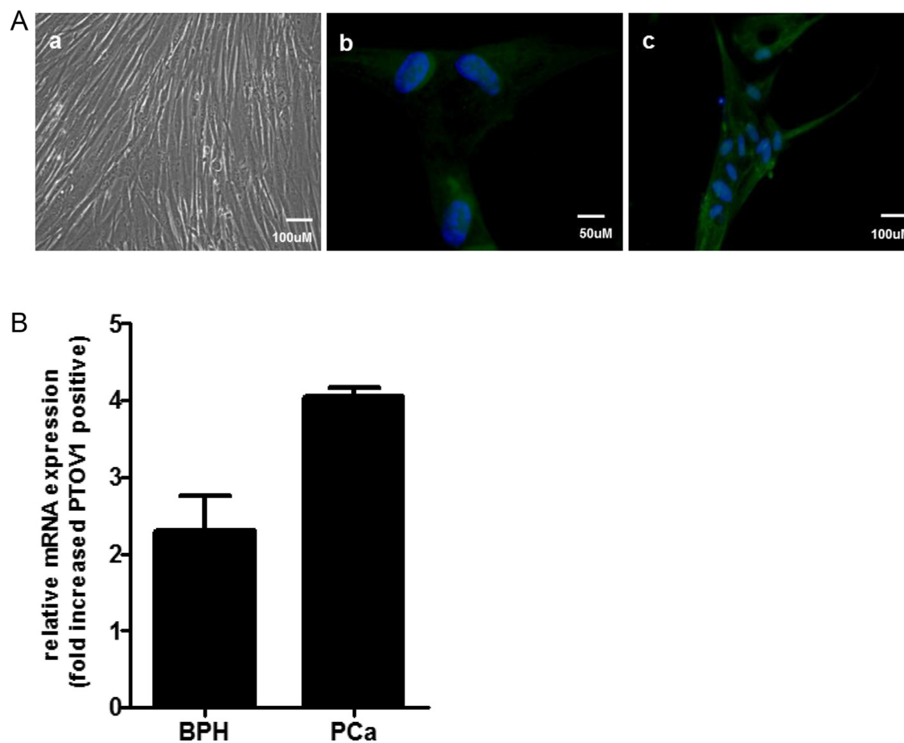

Fig. 1. Characterization of prostate cancer cells. 1A. Phase-contrast micrograph of primary prostate cancer cells (a). Immunocytochemistry α-smooth muscle actin (b) and racemase (c) in PCa cells. 1B. PTOV1 gene expression in BPH and PCa cells.

demonstrated presence of this racemase in about 94% prostate adenocarcinomas.

We evaluated the relative PTOV1 expression (Fig. 1B). The prostate tumor cells overexpressed-1 (PTOV1) protein that was not detected in normal prostate cells. PTOV1 expression is associated to increased cancer proliferation in vivo and in vitro (Fernandéz et al., 2011). In PCa cells, PTOV1 expression was two fold higher as compared to BPH cells.

3.2. Effect of lycopene on cell viability

Previous studies reported that lycopene formulated as 10% water soluble granules was not toxic. Hereafter, all the results refer to lycopene with this vehicle, already used in previous studies in vitro (Teodoro, Perrone, Martucci, & Borojevic, 2009). The plating of cells was followed by 24 h recovery, and cells were subsequently incubated with 0.5, 1, 2.5, 5, 10 or 20 μM lycopene during 24, 48, 72, and 96 h (Figs. 2 and 3). All data showed no effect of lycopene after 24 h incubation. We used the MTT assay to monitor the cell viability.

Lycopene decreased the number of viable PCa cells within 48 h, from the 2.5 μM lycopene concentration on. At 72 h incubation, the cell growth inhibition remained similar to 48 h ($P > 0.05$) but the inhibitory effect could be observed also for lower lycopene doses (0.5 μM). A potent inhibitory effect on PCa cell viability at 96 h of treatment was observed, reaching 40% (Fig. 2). No statistical difference was observed between the used doses indicating that the saturation of the lycopene activity was possibly reached in doses lower than 0.5 μM. No change on cell viability was induced in BPH cells treated with lycopene at any time interval examined or carotenoid concentration used (Fig. 3). Taken as a whole, these data indicate that lycopene's effect was cell-specific and time-dependent, and that lycopene can be a potent inhibitor of human PCa cells growth.

3.3. Effect of lycopene on cell cycle progression

To monitor the influence of lycopene on cell cycle, we treated cells with lycopene (5–10 μM) for 96 h and quantified the cells percentage in different cell cycle phases (Table 1 and Fig. 4). In PCa cells, an increase of cells in G0/G1 phase and a decrease in G2/M phase were observed for treatment with lycopene. In BPH cells, lycopene promoted a decrease of cells in G0/G1 phase and an increase in G2/M phase for both doses of treatment.

3.4. Apoptosis

Quantification of apoptosis can be a useful measure of cancer cell kinetics (Table 2and Fig. 5). Alterations of the balance between proliferation and apoptosis are associated with cancer. Lycopene promoted apoptosis in PCa cells with a maximum 2.19-fold increase, at the highest lycopene concentration (10 μM). In BPH cells no statistically significant difference was observed after lycopene treatment.

3.5. Gene expression profile

To study putative molecular mechanisms by which lycopene interferes in prostate tumor progression, we investigated expression profile of several related genes (Fig. 6). In PCa cell, lycopene treatment promoted an upregulation of PPARγ, RXR, Tp53 and Bax genes and a downregulation of Bcl-2 gene. Conversely, in BPH cells, the Bcl-2 gene was upregulated similar to the other genes studied.

4. Discussion

The present study has shown that lycopene has a specific effect on prostate cells in the used in vitro experimental model. The cells showed a consistent time and dose-dependent response to lycopene, in terms of modifications of cell cycle, gene expression and

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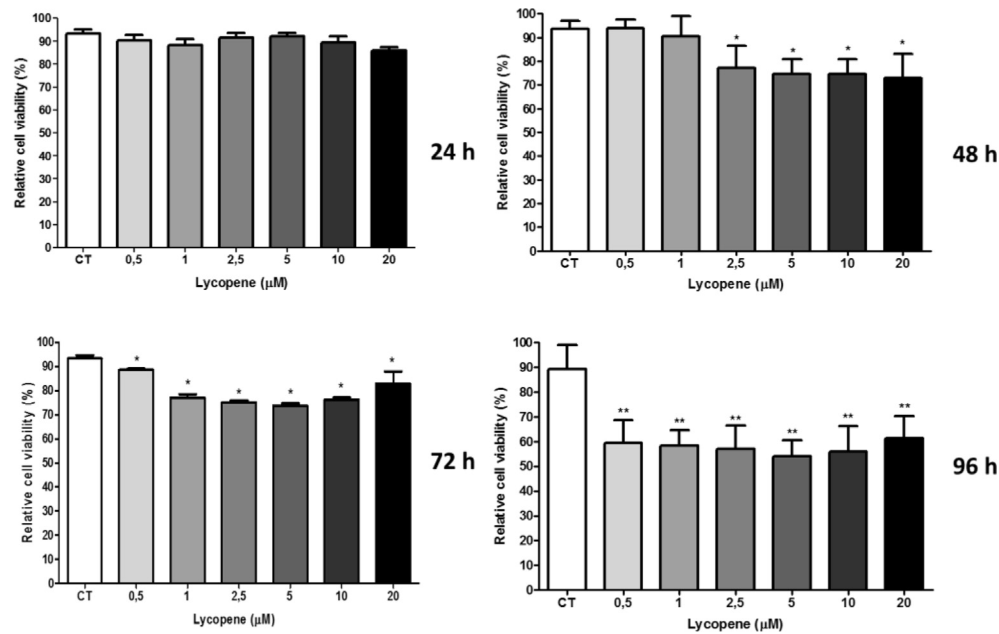

Fig. 2. Effect of lycopene on viability of PCa cells at different time intervals after exposure using MTT assays. Legend: The experiment is expressed as mean ± standard error and differences significant between treated cells with lycopene were compared using the Tukey test (*$p < 0.05$, **$p < 0.01$).

apoptosis. We may affirm that lycopene activity is cell type dependent. The same results confirm that the lycopene, in concentrations used throughout the study, is not toxic and does not interfere with the basic metabolic activities of the cells.

Prostate cancer is a common form of cancer with high incidence worldwide, ant it is a common cause of death in developed countries. The interest on lycopene-rich diets and supplements for the prevention or therapy of prostate cancer has much increased during the last years (Holzapfel et al., 2013). Epidemiological studies support the possibility that lycopene can reduce cancer risk. An increase in dietary consumption of lycopene was associated with decreased prostate cancer development (Nambiar & Singh, 2013).

Benign prostatic hyperplasia (BPH) is the most common benign alteration in aging men and a frequently occurring chronic condition in the male population (Manchanda, Kibler, Zhang, Ravi, & Bid, 2012). It is a complex and multifactorial disease from the etiological and pathophysiological point of view. BPH is defined histologically by hyperproliferation of stromal and epithelial cells of the prostate, caused by complex cellular alterations including changes in proliferation, differentiation, apoptosis and senescence (Manchanda et al., 2012).

It has been reported that stromal to epithelial ratio is altered in BPH, where the ratio increases from 2:1 in normal glands to 5:1 in BPH (Shapiro et al., 1992). Because stromal hyperproliferative activity is thought to promote the development of BPH, the existence

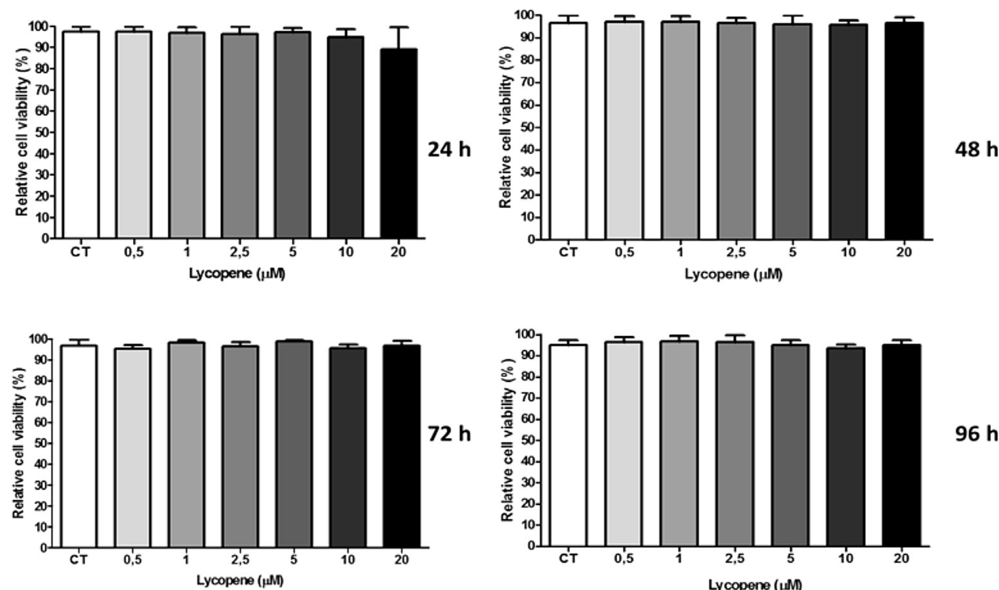

Fig. 3. Effect of lycopene on viability of BPH cells at different time intervals after exposure using MTT assays. Legend: The experiment is expressed as mean ± standard error and differences significant between treated cells with lycopene were compared using the Tukey test (*$p < 0.05$, **$p < 0.01$).

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Table 1
Effect of lycopene on cell cycle progression in BPH and PCa cells after 96 h exposure.

	Cell cycle phase	% Cells		
		Control	5 μM	10 μM
BPH	G0/G1	34.2 ± 0.01[a]	31.30 ± 0.01[b]	29.23 ± 0.04[b]
	S	16.01 ± 0.01[a]	16.10 ± 0.01[a]	14.03 ± 0.04[b]
	G2/M	25.03 ± 0.04[a]	26.24 ± 0.05[a]	27.01 ± 0.01[b]
PCa	G0/G1	62.2 ± 0.01[a]	70.23 ± 1.51[b]	78.12 ± 1.83[c]
	S	3.64 ± 0.94[a]	3.69 ± 0.55[a]	4.78 ± 1.59[b]
	G2/M	23.75 ± 1.54[a]	24.31 ± 0.58[a]	17.75 ± 1.08[b]

Legend: The cell cycle phases and quantitative results are illustrated in accordance with the exposure time and carotenoid concentration. The experiment is expressed as mean ± error standard. Small different letters mean there are significant differences ($P < 0.05$) within same line among different concentrations versus control group.

Table 2
Effect of lycopene on programmed cell death after 96 h exposure.

Relative apoptotic cells expression compared to control			
	Control	5 μM	10 μM
BPH	1.00[a]	1.12 ± 0.01[a]	1.11 ± 0.01[a]
PCa	1.00[a]	1.45 ± 0.01[b]	2.19 ± 0.01[c]

Legend: The flow cytometric analyzes are shown according to carotenoid concentration. The quantitative results of lycopene on cell lines are shown. The experiment is expressed as mean ± error standard. Small different letters mean there are significant differences ($P < 0.05$) within same line among different concentrations versus control group.

of adult stem cells in the prostate stromal compartment is speculated to expand the stroma in response to stimuli during the pathogenesis of BPH (Lin et al., 2007). The cells obtained in the primary cultures of BPH were the connective tissue stroma cells. Stromal cells are important for proliferation of prostate epithelial cells, because they provide the required supplementary growth factors and adhesive ligands (Li, Wu, Febbo, & Olumi, 2007).

Factors predisposing to the development of BPH or PCa include hormonal imbalance, oxidative stress, environmental pollutants, inflammation, hereditary, aging, and, more particularly, stromal to epithelial cells crosstalk (Barclay, Woodruff, Hall, & Cramer, 2005). So far, variety of growth factors and hormonal factors, including androgens and estrogens, has been described in the hyperplastic development of the prostate gland (Marcelli & Cunningham, 1999; Mimeault & Batra, 2006).

PTOV1 is one of the genes most discriminant between the normal and carcinomatous prostate, differentially expressed in prostate cancer and regulated by androgens (Santamaría et al., 2003). Morote et al. (2008) demonstrated that the high expression of PTOV1 correlated with proliferative status, as assessed by Ki67 immunoreactivity, and was associated with nuclear localization of the protein, suggesting a functional relationship between PTOV1 overexpression, proliferative status, and nuclear localization. The pattern of PTOV1 experssion in our model validated the difference between tye studied BPH and PCa cells.

The time and dose-response of all the studied cells to lycopene are somewhat surprising and indicate the required further studies of lycopene interactions with cells. In sensitive cells such as PCa the initial effect was observed from 48 h on. These are primary cancer cells with typical epithelial characteristic, corresponding to the basal proliferative cells of the prostate epithelium. They showed relatively early and high proliferation inhibition in presence of low quantity of lycopene (2.5 μM), which is in the range of lycopene concentration in blood of healthy persons with diet rich in fruits and vegetables that are sources of carotenoids, reported to be in the range of 3.8 μM (Yang, Zhang, Penniston, Binkley, & Tanumihardjo, 2008). The inhibitory effect was already saturated at 96 h with this low lycopene content and it did not increase with much higher extracellular lycopene, indicating again that lower but sustained concentration may reach the maximal inhibition level.

The loss of the ability to regulate the cell-cycle is characteristic for cancer cells and results in uncontrollable proliferation. The cell progression through the first gap (G1) phase of the cell cycle is a step which is frequently disordered in cancer (Diehl, 2002). The cell cycle modification in PCa cells by lycopene involved a marginal increase in G0/G1 and the corresponding decrease in G2/M. The cell arrest in G0/G1 can be long; it is reversible and it is of lesser importance for a cell population in situ as compared to the arrest in G2/M, which leads to apoptosis when cells cannot recover and proceed to cell division. However, PCa cells showed a significant increase in apoptosis, suggesting that another mechanism may be involved. Indeed, the equilibrium of Bcl-2/Bax expression was highly modified in PCa cells exposed to lycopene and there was also an upregulation of Tp53 gene. This cue may be relevant for understanding of lycopene effect of on PCa cells.

In contrast to PCa cells, in BPH no effect was observed on cell viability when comparing the treated to the control group. However, lycopene promoted changes on cell cycle of hyperplastic prostate cells. We demonstrated a decrease in G0/G1 percentage cells and an increase in G2/M cells. Whilst arrest in G0/G1 can be reverted, and cells can proceed with proliferation after interruption of the treatment, G2/M arrest leads potentially to apoptosis. However, there was a proportional increase in Bax and Bcl-2 gene expression profile, which could justify a balance in percentage of apoptotic and non-apoptotic cells.

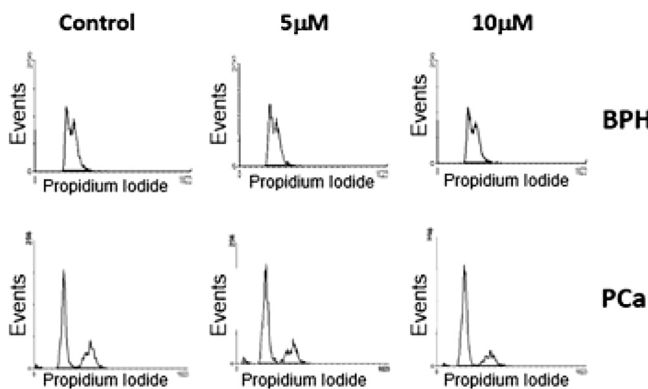

Fig. 4. Effect of lycopene on cell cycle progression in BPH and PCa cells after 96 h exposure. Legend: The flow cytometric analyzes are shown according to carotenoid concentration.

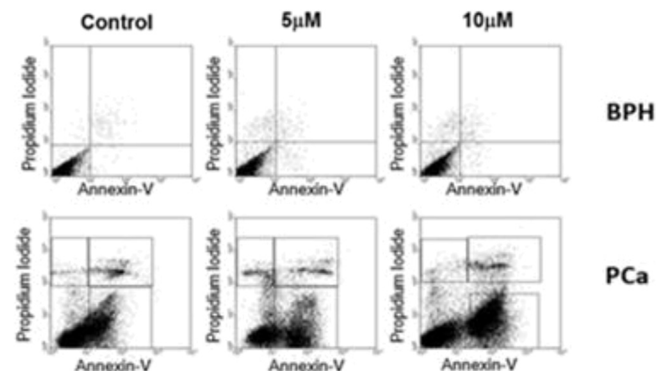

Fig. 5. Effect of lycopene on programmed cell death after 96 h exposure. Legend: The flow cytometric analyzes are shown according to carotenoid concentration.

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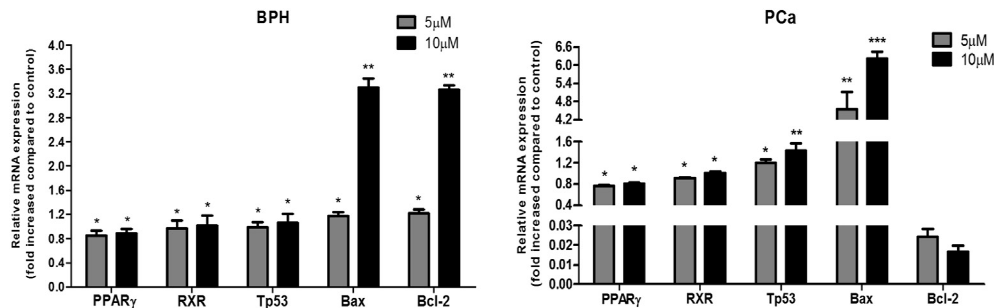

Fig. 6. Profile of gene expression in BPH and PCa cells. Legend: Quantitative analysis of real-time PCR in different genes associated with cancer progression, after 96 h incubation with lycopene. The experiment is expressed as mean ± error standard and differences significant between treated cells with lycopene (5–10 μM) were compared using Tukey test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

The tumor suppressor p53 protein plays an important role in DNA repair, cell cycle arrest, and apoptosis (Tomkova, Tomka, & Zajac, 2008). Induction of apoptosis is considered to be central to the tumor suppressive function of p53 (Schmitt et al., 2002). Through transcription-dependent pathways, p53 functions as a transactivator to up-regulate downstream proapoptotic genes, such as Bax, and/or functions as a repressor to down-regulate anti-apoptotic genes, such as Bcl-2, promoting apoptosis. (Green & Kroemer, 2009; Kolluri et al., 2008).

The Bax protein an important effect in the regulation of cell apoptosis. High Bax expression levels and formation of homo- or heterodimers with Bcl-2 may lead to cell death (Liu, Wang, Wang, Song, & Zhou, 2013). Our results have demonstrated that the expression of Bax was distinctly increased at 5 and 10 μM lycopene in PCa cells, which was consistent with cell cycle and apoptosis results. This indicated that the expression of Bax altered the balance between the genes promoting and those inhibiting apoptosis.

Bcl-2 is the key cell apoptosis inhibitory protein. Its regulatory effect depends on its expression, although the Bcl-2/Bax ratio is also considered a key factor (Basu & Haldar, 1998). When the Bcl-2/Bax ratio increases, cell apoptosis is inhibited and when the ratio is decreased, apoptosis is promoted. The expression levels of Bcl-2 were low at 5 and 10 μM lycopene in PCa cells. The low levels of Bcl-2 expression and the decrease of the Bcl-2/Bax ratio may be the reason that resulted in cell death.

Overexpression of Bcl-2 protein decreases the pro-apoptotic response to such cellular insults as irradiation, chemotherapy, and androgen withdrawal, leading to resistance to the treatments (Karnak & Xu, 2010). The increase of Bcl-2 expression has been described in patients with locally spread or metastatic forms of prostate cancer treated using hormone ablation therapy (Tsuji, Murakami, Kanayama, Sano, & Kagawa, 1998). For this reason Bcl-2 expression is a negative prognostic indicator in these patients (Bruckheimer & Kyprianou, 2000).

Krajewska et al. described Bcl-2, Bax, Bcl-XL and Mcl-1 expression in primary and metastatic prostate carcinomas. High grade carcinomas and metastasis expressed antiapoptotic proteins more frequently and with higher intensity than lower grade tumors. In contrast, proapoptotic protein Bax was expressed in each tested sample regardless of tumor grade (Krajewska et al., 1996).

Our study showed that lycopene positively regulates PPARγ and RXR expression leading potentially to a lower proliferation rate of the primary tumor. This may suggest a possible relationship between PPARγ activation and decreased proliferative activity, which is apparently promoted by lycopene. Therefore, these pathways may play an important role in cancer treatment and prevention. Furthermore, modulating PPAR signaling pathways would represent a potential novel strategy for inhibiting carcinogenesis and progression.

5. Conclusions

Lycopene inhibits cell proliferation, arrests cell cycle in different phases, and increases apoptosis in human PCa cells and BPH cells, in a time and dose-dependent manner. Taken together the present study support the proposal that lycopene may have a protective effect on prostate cancer.

Acknowledgments

This research received financial support from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) (161712/2012-6), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) (161712/2012-6) and FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro) (E-26/110.662/2011).

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