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Cell Cycle Arrest and Induction of Apoptosis by Lycopene in LNCaP Human Prostate Cancer Cells

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ABSTRACT Lycopene is one of the major carotenoids and is found almost exclusively in tomatoes and tomato products. Since tomato consumption is associated with decreased risk of prostate cancer, characterizing the effects of lycopene on cell growth or survival, cell cycle progression, and apoptosis in LCNaP human prostate cancer cells might elucidate the mechanisms of actions of lycopene. To discover the possible anti-cancer mechanism of lycopene, water-soluble lycopene was used, and cell cycle arrest and apoptosis were measured. Placebo formulation at each lycopene dose at 0.1, 1, and 5 μM was used as a control. After 6, 24, and 48 hours of incubation, cells were harvested and measured for cell viability. Lycopene at 1 μM inhibited cell growth by 31%, compared with its placebo formulation after a 48-hour incubation. Lycopene at 5 μM increased the number of cells in the G_2/M phase of the cell cycle from 13% to 28% and decreased S-phase cells from 45% to 29%, while no shifts in cell cycle were detected in placebo-treated groups. Apoptosis was observed at the 5 μM lycopene formulation at the late stages during the 24- and 48-hour treatments. Lycopene, therefore, deserves further study as a potential chemopreventive/chemotherapeutic agent.

KEY WORDS: • ***apoptosis*** • ***cell cycle*** • ***lycopene*** • ***prostate cancer*** • ***tomato***

INTRODUCTION

LYCOPENE is the predominant carotenoid in tomatoes and tomato-based foods. It is one of the most potent antioxidants among dietary carotenoids.[1,2] Dietary intake of tomatoes and tomato products containing lycopene is associated with risk reduction of chronic diseases, such as cancer and cardiovascular disease.[3] Clinical intervention studies have found decreased carcinogenesis of advanced prostate cancer, reduced DNA damage, fewer tumors, increased apoptosis, and lower serum prostate-specific antigen concentrations with short courses of tomato or lycopene feeding in men with prostate cancer.[3–7] The general structure of lycopene is an aliphatic hydrocarbon with 13 conjugated carbon-carbon double bonds, making it soluble in fats and lipids and red in color. Being acyclic, lycopene possesses symmetrical planarity and has no vitamin A activity.[1] It is particularly susceptible to oxygen, light, extremes in pH, and high temperature.[8–10]

Prostate cancer is a leading cause of cancer death in (American) men, and evidence points to significant life style/diet components as risk factors for its development or prevention. A number of studies[3,4,11,12] have evaluated dietary components associated with both increased risk and reduced risk of prostate cancer, but there are no strong dietary predictors of prostate cancer risk or risk reduction. Interest in possible bioactive compounds in tomatoes for prostate cancer chemoprevention was generated with the publication of a large U.S. cohort study that found a protective association for greater tomato product consumption but not for any other major fruit or vegetable.[3] None of the tomato ingredients except lycopene contributed to the reduced risk of cancer.[3,4,11,12] (The study by Rock et al.[11] is not related to prostate, but concerns inhibition of breast cancer recurrence.)

Apoptosis is an area of growing interest in chemoprevention. It is a mode of cell death in which individual cells are deleted from tissue during normal tissue turnover[13] and is characterized by morphological changes including cell shrinkage, membrane blebbing, chromatin condensation, and DNA fragmentation.[13,14] In cancer, resistance to apoptosis affords tumor cells a survival advantage and the ability to be sustained and proliferate. In particular, resistance to apoptosis has been implicated in the development and progression of cancer.[15–17] Monitoring apoptosis as a biomarker in cancer prevention trials is gaining importance.[18,19] The purpose of this study was to determine effects of lycopene on androgen-sensitive LNCaP cell growth as well as cell cycle depression and apoptosis.

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MATERIALS AND METHODS

Cell culture

LNCaP cells originally were obtained from the American Type Culture Collection (Rockville, MD), and cell culture supplies were purchased from Sigma Chemical Co. (St. Louis, MO). LNCaP cells were maintained at 37°C with 5% CO_2 in RPMI 1640 (Invitrogen Co., Carlsbad, CA) media supplemented with 10% fetal bovine serum (FBS), 0.37% $NaHCO_3$, 4.5% glucose, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 m*M* sodium pyruvate, and 10 m*M* HEPES. The water-soluble lycopene was dissolved in dimethyl sulfoxide (DMSO) (Sigma) and added directly to cell culture medium at a final concentration of 0.1% DMSO.

Source of lycopene

Water-soluble lycopene (10%) and placebo were obtained from Roche Vitamins, Inc. (Parsippany, NJ). Both contained sucrose, cornstarch, gelatin, corn oil, and small amounts of ascorbyl palmitate and DL-α-tocopherol. The placebo contained corn oil instead of lycopene. Tomato paste was purchased in a local food market.

Quantification of lycopene

The total lycopene concentration was measured by high-performance liquid chromatography (HPLC) as reported by Stacewicz-Sapuntzakis *et al.*[20] Lycopene was reconstituted with HPLC-grade ethyl ether and the mobile phase (1:3, vol:vol). The carotenoids were separated by isocratic HPLC (on a C_{18} Novapak column; 3.9 × 150 mm, 5 μm particle size; Waters Co., Milford, MA) with a mobile phase of methanol, acetonitrile, and tetrahydrofuran (THF) (50:45:5, by volume). The reconstituted samples were placed in autosampler vials, and a 10-μL volume was injected at a flow rate of 1 mL/min. The peaks were detected by a Waters model 490 Programmable Multiwavelength Detector. Our laboratory is a reference laboratory for the National Institute of Standards and Technology (Gaithersburg, MD) quality assurance program for carotenoids.[21]

Measurement of lycopene in cells

Cellular uptake of lycopene was measured as reported by Xu *et al.*[22] with some modification. Briefly, cells were plated at a density of 260,000 cells/well in 12-well plates. After preincubation overnight, the standard culture media were decanted and replaced with media containing lycopene as measured at final concentrations of 0.5, 1, and 5 μM. Treated cells were harvested after 6, 24, and 48 hours of incubation and centrifuged in a glass tube. Two hundred microliters of phosphate-buffered saline (PBS) was added to the cells. Cells were deproteinized with 100 μL of ethanol and vortex-mixed for 30 seconds followed by 100 μL of ethanol containing internal standard (0.5 μg/mL retinyl acetate in absolute ethanol). Then 2 mL of hexane (containing butylated hydroxytoluene [BHT], 100 mg/L) was added and centrifuged for 5 minutes. The upper hexane layer was removed to another glass tube. The aqueous layer was extracted with another 2 mL of hexane. The hexane fractions were combined in the glass tube and evaporated to dryness in a vacuum centrifuge. The residue was redissolved in 50 μL of ethyl ether and 150 μL of the HPLC mobile phase to a total volume of 200 μL. Extracts were kept at 0°C until HPLC analysis the same day.

Stability of lycopene in a culture medium

Stability of lycopene in a culture medium was measured as reported by Xu *et al.*[22] with some modification. The lycopene, dissolved in 0.1% DMSO, was added to the culture medium at a final concentration of 0.1, 1, or 5 μM. The mixture was incubated under the cell culture conditions. After incubation, a 200 μM aliquot of the mixture was mixed with 100 μL of ethanol and vortex-mixed for 30 seconds followed by additon of 100 μM ethanol containing internal standard (0.5 μg/mL retinyl acetate in absolute ethanol). Then 200 μL of hexane (containing BHT, 100 mg/L) was added and centrifuged at 20,000 *g* for 5 minutes. The rest of the extraction step was the same as described above in the measurement of lycopene in cells and medium.

Cell proliferation assay

Cell viability was determined by utilizing the ability of viable cells to exclude trypan blue.[23] Cells from triplicate wells were removed and pelleted by centrifugation at 800 rpm for 3 minutes. Cell pellets were then resuspended in 50 μL of 0.1% trypan blue (in PBS), and the percentage of viable cells was determined by microscopy, using a hemacytometer.

Protein analysis

Cellular protein content was measured using the Bio-Rad reagent (Sigma, St. Louis, MO) in triplicate.[24] Bovine serum albumin (Sigma) was used as the standard protein. A Micro Quant microplate reader (Bio-Tek Instruments, Inc., Winooski, VT) was used for the analysis

Flow cytometric measurement of cell cycle

Propidium iodide (PI) staining was used to analyze the DNA content.[25] LNCaP cells were plated in 10-cm culture dishes at concentrations determined to yield 50–60% confluence within 24 hours. Cells were than treated with lycopene or placebo at different concentrations. At 6, 24, and 48 hours, cells were harvested by centrifugation, washed in PBS, fixed with ice-cold 70% ethanol, and treated with 1 mg/mL RNase for 30 minutes. PI was added at a final concentration of 50 mg/mL. Data were collected and analyzed using a Coulter EPICS Elite ESP flow cytometer (Coulter Co., Miami, FL) equipped with a 15 mV air-cooled argon laser operating at 488 nm for excitation of the ethidium bromide (EB). From each sample, 20,000 cells were counted. Experiments were performed in triplicate.

TABLE 1. PERCENT REMAINING LYCOPENE IN THE MEDIUM WITH CELLS

Lycopene treatment	*Incubation time*			
	0 hour	*6 hours*	*24 hours*	*48 hours*
0.1 μM	100[a]	81 ± 6[b]	74 ± 4[c]	19 ± 2[f]
1 μM	100[a]	83 ± 5[b]	77 ± 6[c]	23 ± 2[f]
5 μM	100[a]	93 ± 7[a]	58 ± 3[c,d]	58 ± 6[c,d]

Data are mean ± SD percentages of the initial proportion of lycopene ($n = 3$).
The values with the same letter are not significantly different ($P < .05$).

Measurement of annexin V binding by flow cytometry

The percentage of cells actively undergoing apoptosis was detected with an annexin V-fluorescein isothiocyanate (FITC) kit (Oncogene Research, Cambridge, MA) according to the manufacturer's instructions. Briefly, cells were plated in 10-cm culture dishes at concentrations determined to yield 50–60% confluence within 24 hours. Cells were then treated with lycopene or placebo. After 6, 24, and 48 hours, cells were collected, washed with ice-cold PBS, and centrifuged at 1,000 g for 5 minutes. The cell pellet was resuspended in ice-cold binding buffer. After that, annexin V-FITC (1.25 μL/0.5 mL) and PI (10 μL/0.5 mL) solutions were added. The tube was incubated for 15 minutes in the dark and filtered through 41-μm (pore size) spectra/mesh nylon filters (Spectrum, Rancho Dominguez, CA). DNA content from 10,000 cells was analyzed using a Coulter EPICS Elite ESP flow cytometer equipped with a 15 mV air-cooled argon laser operating at 488 nm for excitation of the EB.

Statistical analysis

All experiments were replicated three times. Mean standard deviation, mean square errors, two-factor analysis of variance, correlation, and interaction of main effects were calculated using Sigmastat computer software version 1.0 (Jandel Corp., San Rafael, CA). Appropriate comparisons were made using the Student–Newman–Keuls Method for multiple comparisons. A $P < .05$ was considered statistically significant.

RESULTS

Measurement of lycopene in cells

LNCaP cells were cultured for up to 48 hours in medium containing lycopene or placebo at different concentrations. Cells were harvested at different time intervals and rinsed with PBS, and the cellular lycopene was extracted and measured using HPLC. The concentrations of lycopene at each time point were normalized to cellular protein levels, and the results are presented in Figure 1. Cellular lycopene levels increased in a dose-dependent manner from cell medium containing 0.1–5.0 μM lycopene.

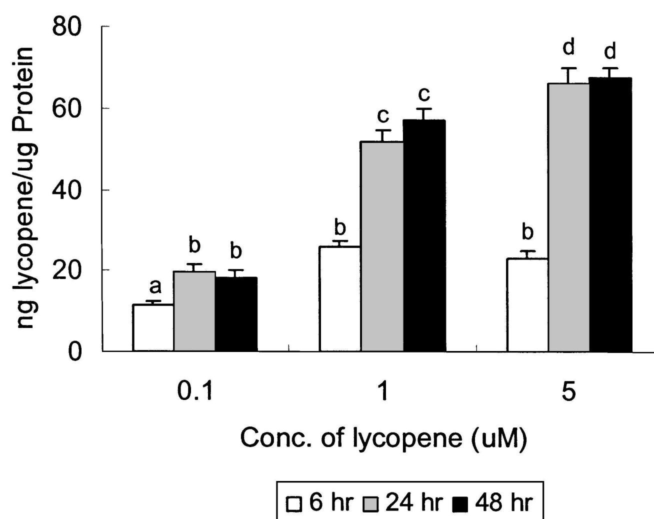

FIG. 1. Cellular uptake of lycopene. The values marked with the same letters are not significantly different from other treatments ($P < .05$).

Stability of lycopene in a culture medium

We determined the stability of lycopene in the medium with and without cells. Table 1 shows the percentage remaining of lycopene in the medium with cells. Lycopene loss was least during the 6–24-hour period, with 58–77% remaining in the cell medium at 24 hours. Losses increased dramatically at 48 hours, which could be explained by increases in cellar uptake of lycopene.

Effect of lycopene on cell number and viability

We examined the effect of lycopene on cell proliferation of the LNCaP cell line using the trypan blue exclusion assay (Fig. 2). When the harvested cells were counted, the number of control cells continued to increase, whereas the number of treated cells had decreased in a time- and dose-dependent manner. The maximum inhibitory effect on cell growth was registered at a concentration of 5 μM lycopene at all incubation times. Inhibitory effects were also seen at concentrations of 0.1 μM lycopene at 24 and 48 hours, but not at 6 hours.

Effect of lycopene on the cell cycle

To elucidate the mechanisms responsible for the reduction of cell number by lycopene, the population distribution throughout the cell cycle was examined via flow cytometry. Table 2 shows the mean percentages of cells in each phase from three independent experiments, after treatment with varying concentrations of lycopene and placebo at several time points. The effects of each treatment were time dependent. In the absence of lycopene, most cells (about 46%) were in S phase, because of the high proliferative state of this immortalized cancer cell line. There were no immediate effects of treatments at the 6-hour time point. At 24 hours there was a slight but not statistically significant build-up of

TABLE 2. EFFECT OF LYCOPENE AND PLACEBO ON CELL CYCLE PROGRESSION WITH RESPECT TO THE CONTROL

	6 hours			*24 hours*			*48 hours*		
Treatment	G_0/G_1	*S*	G_2/M	G_0/G_1	*S*	G_2/M	G_0/G_1	*S*	G_2/M
Control	40.9 ± 0.7	45.5 ± 1.2	13.7 ± 1.9	41.2 ± 1.8	42.9 ± 0.7	15.3 ± 2.5	43.2 ± 2.1	41.8 ± 0.1	15.1 ± 2.0
Lycopene									
0.5 μM	44.0 ± 1.2	43.1 ± 0.8	13.0 ± 0.4	49.9 ± 2.5	34.4 ± 0.1[a]	15.8 ± 2.5	45.8 ± 2.4	34.7 ± 0.8	19.6 ± 3.2
1 μM	42.2 ± 0.4	44.1 ± 0.4	14.8 ± 1.5	45.0 ± 1.9	34.7 ± 0.3[a]	20.4 ± 1.6	40.4 ± 1.6	36.5 ± 0.3[a]	26.7 ± 0.9[a]
5 μM	41.7 ± 0.1	44.9 ± 0.3	13.4 ± 0.1	40.3 ± 2.9	34.8 ± 0.4[a]	25.0 ± 2.5[a]	36.9 ± 0.7[a]	31.6 ± 0.3[a]	28.0 ± 1.8[a]
Placebo									
0.5 μM	42.2 ± 1.7	42.2 ± 1.1	15.7 ± 2.8	41.5 ± 2.8	40.0 ± 1.0	18.1 ± 1.8	42.8 ± 1.9	40.1 ± 0.1	17.2 ± 1.9
1 μM	40.9 ± 1.3	44.0 ± 2.0	15.1 ± 3.2	42.2 ± 3.5	40.2 ± 0.7	17.0 ± 2.8	40.3 ± 2.7	39.8 ± 0.6	20.0 ± 3.3
5 μM	41.0 ± 0.6	44.5 ± 1.1	14.6 ± 0.5	40.5 ± 2.1	39.2 ± 1.2	20.2 ± 3.3	40.1 ± 4.4	38.4 ± 0.6	20.6 ± 2.0

At the indicated time, distribution of the cells in G_0/G_1, S, and G_2/M phases was analyzed by flow cytometry as described in Materials and Methods. Results are expressed as the percentage of total cells. Data represent mean ± SD values of triplicate experiments.

[a]Different from control, $P < .05$.

cells in the G_0/G_1 phase for all lycopene-containing treatments except 5 μM lycopene, where there was a dramatic cell cycle arrest in the G_2/M phase that was sustained at the 48-hour time point. In the placebo-treated cells at 48 hours of incubation, G_1 phase slightly decreased from 41% to 40%, S phase also slightly decreased from 43% to 38%, and G_2/M phase increased a little, from 15% to 21%. Taken together, these results indicated that lycopene inhibited cellular proliferation of LNCaP cells via a G_1 and G_2/M phase arrest of the cell cycle.

Effect of lycopene on cell apoptosis

Table 3 presents our apoptosis results. Most cells were in late apoptosis so late and early apoptotic cells were combined and reported as percent apoptotic cells. Apoptosis was observed at the highest dose (5 μM) of lycopene at every time point, and most were at late stages of apoptosis during the treatment (at 24 and 48 hours). The increasing proportion of lycopene with the annexin V-FITC conjugate indicates that target cells start to die as late as 24 hours after treatment. The percentage of treatment-induced apoptosis in lycopene was significantly higher ($P < .01$) than apoptosis in the placebo group. After 48 hours of incubation of LNCaP cells, 13% of 5 μM lycopene-treated cells were stained with annexin V-FITC. After 48 hours of treatment, the percentage of stained cells with annexin V-FITC rose to 21% in the lycopene-treated cells, while the increase was only 5.4% in placebo-treated cells. More importantly, there was a modest but statistically significant increase in apoptosis at 24 and 48 hours at the physiologically relevant 1 μM lycopene concentration.

DISCUSSION

Lycopene is highly hydrophobic, which is problematic in aqueous cell culture media. Vehicles such as THF, DMSO,

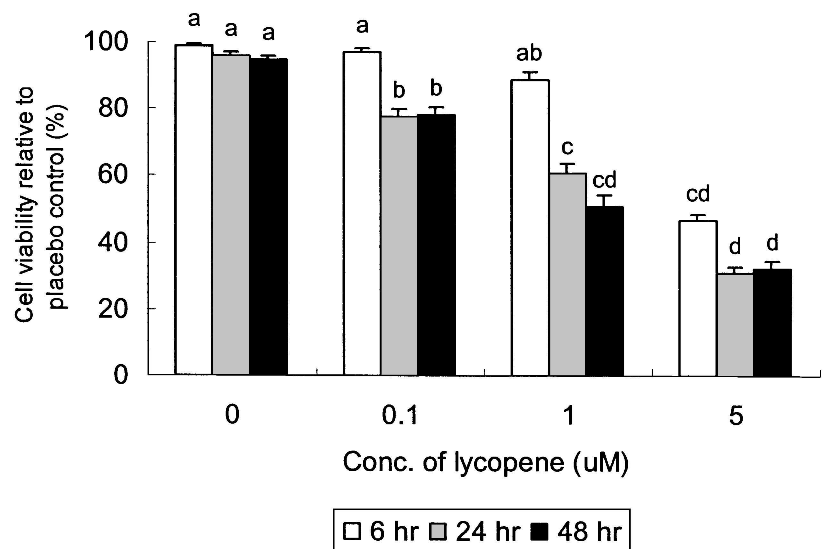

FIG. 2. Effect of lycopene on LNCaP cell growth. The values marked with the same letters are not significantly different from other treatments ($P < .05$).

TABLE 3. INCIDENCE OF APOPTOSIS (PERCENT) AS DETECTED BY ANNEXIN V IN LNCaP HUMAN PROSTATE CANCER CELLS

	Incubation time		
Treatment	*6 hours*	*24 hours*	*48 hours*
Control	1.1 ± 0.13[a]	1.7 ± 0.61[a]	2.6 ± 0.32[a]
Lycopene			
0.5 μM	1.8 ± 0.32[a]	2.3 ± 0.15[a]	3.7 ± 0.12[b]
1 μM	2.7 ± 1.21[a]	3.0 ± 0.21[b]	5.9 ± 1.36[c]
5 μM	4.8 ± 1.21[b]	13.2 ± 4.24[c,d]	20.6 ± 5.39[d]
Placebo			
0.5 μM	1.3 ± 0.17[a]	1.6 ± 0.09[a]	2.7 ± 0.95[a]
1 μM	1.2 ± 0.09[a]	1.7 ± 0.11[a]	2.6 ± 0.74[a]
5 μM	1.9 ± 0.15[a]	4.7 ± 1.76[b]	5.4 ± 1.46[b]

Percent apoptosis was defined as the sum of early and late apoptotic cells.

The values with the same letter are not significantly different ($P < .05$).

or lipid micelles have been used to aid lycopene stability in cell culture media. Most researchers use THF as a co-solvent, which can be toxic to some cell lines (including prostate cell lines), limiting the concentrations of lycopene delivered, and does not stabilize lycopene in the cell medium. We used a new 10% water-soluble lycopene, and this was relatively stable during the experimental period. Most studies report the concentration of lycopene in the cell medium and do not measure the cellular concentration of lycopene, which could be highly variable from one cell line to the next and between treatment approaches. We showed significant uptake of lycopene by this prostate cell line with cellular concentrations reaching levels comparable to, if not higher than, concentrations found in human prostate tissue.[6]

Lycopene has been previously shown to have growth inhibitory activity in several human cancer cell lines, including HL-60 human leukemia cells,[26] KB-1 human oral tumor cells,[27] and MCF-7 human breast carcinoma cells.[28,29] It has been found that lycopene treatment decreases mammary (MCF-7), lung (NCI-H226), and endometrial (Ishikawa) human cancer cell viability with half-maximal inhibitory concentrations of 1–2 μM.[28] In KB human oral tumor cells, 3 μM lycopene inhibited cell proliferation by 47% after 3 days of incubation, and 7 μM lycopene led to 89% inhibition after the same time.[27]

Our study demonstrated that the antitumor properties of lycopene on LNCaP cell growth and its antiproliferative effect are due to its ability to induce G_2/M-phase arrest and apoptotic cell death especially, in unsynchronized cells. Although these are modest effects, small arrests in the cell cycle have profound effects on cell growth and viability. Contrary to our results, Karas *et al*.[29] showed no changes in the cell cycle phase distribution in unsynchronized cells, but when they treated cells with serum starvation (0.5% fetal calf serum) they found that lycopene slowed cell cycle progression in MCF-7 human breast cancer cells. Serum starvation of lycopene-treated and untreated cells resulted in an accumulation of cells in the G_0/G_1 phase, with the cell numbers increasing from 52% in unstarved cells to 70% in starved cells. A concomitant decrease from 33% to 20% and from 15% to 10% was noted in the S and G_2/M phases, respectively. These results strongly indicate that the lycopene is capable of slowing the cell cycle.

In conclusion, lycopene decreased prostate cancer cell viability at physiological lycopene concentrations, likely through cell cycle arrest and apoptosis. These data suggest that lycopene is the strongest candidate for the apparent protective effect of tomato consumption on the incidence of prostate cancer.

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REFERENCES

1. Ong ASH, Tee ES: Natural source of carotenoids from plants and oils. *Methods Enzymol* 1992;213:142–167.
2. Khachik F, Goli MB, Beecher GR, Holden JM, Lusby WR, Tenorio MD, Barrera MR: Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *J Agric Food Chem* 1992;40:390–398.
3. Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC: Intake of carotenoids and retinol in relation to risk of prostate cancer. *J Natl Cancer Inst* 1995;87:1767–1776.
4. Giovannucci E, Rimm EB, Liu Y, Stampfer MJ, Willett WC: A prospective study of tomato products, lycopene, and prostate cancer risk. *J Natl Cancer Inst* 2002;94:391–398.
5. Schuurman AG, Goldbohm RA, Dorant E, van den Brandt PA: Vegetable and fruit consumption and prostate cancer risk: a cohort study in the Netherlands. *Cancer Epidemiol Biomarker Prev* 1998;7:673–680.
6. Chen L, Sapuntzakis M, Duncan C, Sharifi R, Ghosh L, van Breemen R, Ashton D, Bowen PE: Serum and oxidative DNA damage in prostate cancer patients consuming tomato sauce. *J Natl Cancer Inst* 2001;93:1872–1879.
7. Kucuk O, Sarkar FH, Sakr W, Djuric Z, Pollak MN, Khachik F, Li YW, Banerjee M, Grignon D, Bertram JS, Crissman JD, Pontes EJ, Wood DP: Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. *Cancer Epidemiol Biomarkers Prev* 2001;10:861–868.
8. Henry LK, Catignani GL, Schwartz SJ: Oxidative degradation kinetics of lycopene, lutein, 9-cis and all-trans β-carotene. *J Am Oil Chem Soc* 1998;75:823–829.
9. Moss GP, Weedon BCL: Chemistry of the carotenoids. In: *Chemistry and Biochemistry of Plant Pigments, Vol. 1,* 2nd ed. (Goodwin TW, ed), Academic Press, New York, 1976, pp. 149–224.
10. Scita G: Stability of β-carotene under different laboratory conditions. *Methods Enzymol* 1992;213:175–185.
11. Rock CL, Flatt SW, Wright FA: Responsiveness of carotenoids to high vegetable diet intervention designed to prevent breast cancer recurrence. *Cancer Epidemiol Biomarkers Prev* 1997;6:617–623.

12. Gann PH, Ma J, Giovannucci E, Willett W, Sacks FM, Hennekens CH, Stampfer MJ: Low prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. *Cancer Res* 1999;59:1225–1230.
13. Kerr JFK, Wyllie AH, Currie AH: Apoptosis, a basic biological phenomenon with wider implications in tissue kinetics. *Br J Cancer* 1972;26:239–245.
14. Wyllie AH, Kerr JFK, Currie AR: Cell death: the significance of apoptosis. *Int Rev Cytol* 1980;68:251–306.
15. Raffo AJ, Perlman H, Chen M-W, Day ML, Streitman JS, Buttyan R: Overexpression of Bcl-2 protects prostate cancer cells from apoptosis *in vitro* and confers resistance to androgen depletion *in vivo*. *Cancer Res* 1995;55:4438–4445.
16. Cardillo M, Berchem G, Tarkington MA, Krajewski S, Krajewski M, Reed JC, Tehan T, Ortega L, Lage J, Gelmann EP: Resistance to apoptosis and upregulation of Bcl-2 in benign prostatic hyperplasia after androgen deprivation. *J Urol* 1997;158: 212–216.
17. Tang DC, Li L, Chopra DP, Porter AT: Extended survivability of prostate cancer cells in the absence of trophic factors. Increased proliferation, evasion of apoptosis, and the role of apoptosis proteins. *Cancer Res* 1998;58:3466–3479.
18. Trock BJ: Validation of surrogate endpoint biomarkers in prostate cancer chemoprevention trials. *Urology* 2001;57:241–247.
19. Riss TL: Apoptosis as a biomarker in chemoprevention trials. *Urology* 2001;57:141–142.
20. Stacewicz-Sapuntzakis M, Bowen PE, Kikendall JW, Burgess M: Simultaneous determination of serum retinol and various carotenoids: their distribution in middle-aged men and women. *J Micro Anal* 1987;3:27–45.
21. Duewer DL, Kline MC, Sharpless KE, Thomas JB, Stacewicz-Sapuntzakis M, Sowell AL: NIST micronutrients measurement quality assurance program: characterizing individual participant measurement performance over time. *Anal Chem* 2000;72: 3611–3619.
22. Xu X, Wang Y, Constantinou AI, Stacewicz-Sapuntzakis M, Bowen PE, van Breemen RB: Solubilization and stabilization of carotenoids using micelles: delivery of lycopene to cells in culture. *Lipids* 1999;34:1031–1036.
23. Beausoleil HE, Labrie V, Dubreuil JD: Trypan blue uptake by Chinese hamster ovary cultured epithelial cells: a cellular model to study *Escherichia coli* STb enterotoxin. *Toxicon* 2002;40: 185–191.
24. Hwang E-S, Jeffery EH: Effect of different processing methods on induction of quinine reductase by dietary broccoli in rats. *J Med Food* 2004;7:95–99.
25. Tyagi A, Bhatia N, Condon MS, Bosland MC, Agarwal C, Agarwal R: Antiproliferative and apoptotic effects of silibinin in rat prostate cancer cells. *Prostate* 2002;53:211–217.
26. Nara E, Hayashi H, Kotake M, Miyashita K, Nagao A: Acyclic carotenoids and their oxidation mixtures inhibit the growth of HL-60 human promyelocytic leukemia cells. *Nutr Cancer* 2001; 39:273–283.
27. Livny O, Kaplan I, Reifen R, Polak-Charcon S, Madar Z, Schwartz B: Lycopene inhibits proliferation and enhances gap-junction communication of KB-1 human oral tumor cells. *J Nutr* 2002;132:3754–3759.
28. Levy J, Bosin E, Feldman B, Giat Y, Miinster A: Lycopene is a more potent inhibitor of human cancer cell proliferation than either α-carotene or β-carotene. *Nutr Cancer* 1995;24:257–266.
29. Karas M, Amir H, Fishman D, Danilenko M, Segal S, Nahum A, Koifmann A, Giat Y, Levy J, Sharoni Y: Lycopene interferes with cell cycle progression and insulin-like growth factor I signaling in mammary cancer cells. *Nutr Cancer* 2000;36:101–111.