RESEARCH ARTICLE

Growth inhibitory efficacy of lycopene and β-carotene against androgen-independent prostate tumor cells xenografted in nude mice

Chih-Min Yang[1], Yeu-Torng Yen[1], Chin-Shiu Huang[2] and Miao-Lin Hu[1]

[1] Department of Food Science and Biotechnology, National Chung Hsing University, Taichung, Taiwan
[2] Department of Health and Nutrition Biotechnology, Asia University, Wufeng, Taichung, Taiwan

Received: July 7, 2010
Revised: September 23, 2010
Accepted: October 8, 2010

Scope: In this study, we evaluated the efficacy of lycopene against the growth of prostate cancer *in vivo*.

Methods and results: Athymic nude mice were implanted subcutaneously with human androgen-independent prostate carcinoma PC-3 cells. They were supplemented with a low or a high dose of lycopene (4 and 16 mg/kg) and a single dose of β-carotene (16 mg/kg) twice a week for 7 wk. At the end of the experiment, both lycopene and β-carotene strongly inhibited the tumor growth, as evidenced by the decrease in tumor volume and tumor weight. High-dosage lycopene and β-carotene significantly decreased the expression of proliferating cell nuclear antigen in tumor tissues and increased the levels of insulin-like growth factor-binding protein-3 in plasma. In addition, high-dosage lycopene supplementation significantly decreased the vascular endothelial growth factor (VEGF) levels in plasma. In contrast, β-carotene supplementation significantly increased the VEGF levels, as compared with tumor control group.

Conclusion: Lycopene and β-carotene supplementation suppressed the growth of prostate tumor cells, and the effects are likely associated with reduction of proliferation (attenuation of proliferating cell nuclear antigen expression) and with interference of the insulin-like growth factor 1 signaling (increased plasma insulin-like growth factor-binding protein-3 levels). Furthermore, the inhibition of VEGF by lycopene suggests that the antitumor mechanisms of lycopene also involve anti-angiogenesis.

Keywords:
β-carotene / Insulin-like growth factor-binding protein-3 / Lycopene / Prostate cancer / Vascular endothelial growth factor

1 Introduction

Prostate cancer, the most common diagnosed malignancy, is the second cause of cancer death in American men. The American Cancer Society estimated that there were 192 280 new cases and 27 360 deaths during 2009 [1]. Although the etiology of prostate cancer is still unclear, the well-recognized risk factors for prostate cancer are age, race, dietary habits and prostate androgen (dihydrotestosterone; DHT) levels [2, 3]. Numerous dietary components play an important role in modulating the development of prostate cancer [4]. Elevated intakes of dietary fats and total energy are positively associated with prostate cancer development, whereas higher intakes of vitamins, carotenoids and phytoestrogens are negatively associated [5, 6].

Lycopene, a tetraterpene hydrocarbon containing 40 carbon atoms and 56 hydrogen atoms, is one of more than 600 carotenoids synthesized by plants and photosynthetic

Correspondence: Professor Miao-Lin Hu, Department of Food Science and Biotechnology, National Chung Hsing University, 250 Kuo Kuang Road, Taichung, Taiwan 402 ROC, P. R. China
E-mail: mlhuhu@nchu.edu.tw
Fax: +886-4-2281-2363

Abbreviations: BW, body weight; **IGFBP-3,** insulin-like growth factor-binding protein-3; **IGF-1,** insulin-like growth factor 1; **PCNA,** proliferating cell nuclear antigen; **s.c.,** subcutaneously; **VEGF,** vascular endothelial growth factor

microorganisms [7]. Epidemiologic studies have suggested that elevated tomato or lycopene consumption is associated with a reduction of 30–40% risk of prostate cancer [8]. Lycopene is the major carotenoids in human serum and prostate tissues [9]. The higher levels of lycopene in serum are associated with decreased the risk of prostate cancer [10]. Previous *in vitro* studies have demonstrated that lycopene effectively inhibits proliferation of numerous cancer cells [11, 12]. However, very little is known about the anti-tumor effect of lycopene *in vivo*. We previously demonstrated that lycopene inhibits experimental metastasis of human hepatoma SK-Hep-1 cells in athymic nude mice [13]. Tang *et al.* [14] demonstrated that supplemental lycopene inhibits the growth of DU145, a human prostate tumor cell line, transplanted to BALB/c nude mice. In this study, we employed a xenograft tumor model in athymic nude mice injected (subcutaneously (s.c.)) once with PC-3 cells and supplemented orally with physiological doses of lycopene (4 and 16 mg/kg) in comparison with β-carotene (16 mg/kg) for 7 wk. We investigated the anti-tumor effects of lycopene and β-carotene and the possible anti-tumor mechanism by measuring the levels of insulin-like growth factor 1 (IGF-1), insulin-like growth factor-binding protein-3 (IGFBP-3) and the vascular endothelial growth factor (VEGF) in blood plasma as well as the proliferating cell nuclear antigen (PCNA) in primary tumor tissue of nude mice injected with PC-3 cells.

2 Materials and methods

2.1 Materials

Lycopene and β-carotene were purchased from Wako (Japan) and Sigma Aldrich (Germany). The purity of commercial lycopene and β-carotene was 97 and 97%, respectively, as claimed by the suppliers. PCNA and anti-mouse IgG-horseradish peroxidase polyclonal antibody were purchased from Santa Cruz Biotechnology. VEGF, IGF-1 and IGFBP-3 ELISA kit were purchased from R&D (R&D systems, USA). All chemicals used are of reagent or higher grade.

2.2 Prostate cancer cell for injection to nude mice

PC-3 prostate cancer cell line (BCRC Number: 60122) was grown in RPMI1640 containing 10% vol/vol fetal bovine serum, 0.37% wt/vol NaHCO3, penicillin (100 kU/L), streptomycin (100 kU/L) in a humidified incubator under 5% CO_2, and 95% air at 37°C. The survival rate of cells was always higher than 95% by trypan blue exclusion. Before injection into the mice, the cells were harvested by trypsinization and washed three times with cold serum-free medium and then injected in a total volume of 0.1 mL using a 1-mL latex-free syringe (BD) within 30 min of harvest.

2.3 Animals, diet and treatment

We have previously shown that athymic nude mice and gerbils are better accumulators of lycopene than F344 rats and BALB/c mice and are more useful for studying the *in vivo* effects of lycopene [15]. In this study, we employed athymic nude mice (6- to 8-wk-old, 17–22 g) from the Animal Center of the National Science Council. The study protocol was approved by the Animal Research Committee of National Chung Hsing University.

The mice were housed individually in hanging wire mesh cages with controlled temperature (25 ± 2°C), humidity (65 ± 5%) and alternating 12-h-light/-dark cycles. Upon arrival, the mice were acclimated for 1 wk, and then each mouse was implanted s.c. with 1×10^7 PC-3 cells (0.1 mL/mouse). On the following day (day 1), mice were randomly divided into four groups ($n = 6$ for each group) as follows: group 1, tumor control; group 2, tumor cell injection and oral supplementation with a low dose of lycopene (4 mg/kg); group 3, tumor cell injection and oral supplementation with a high dose of lycopene (16 mg/kg); group 4, tumor cell injection and oral supplementation with β-carotene (16 mg/kg). The mice were orally administered with lycopene or β-carotene in corn oil twice *per* week for 7 wk. During the entire experimental period (including acclimation), the mice consumed a standard rodent diet (Lab 5001, Purina Mills) and water *ad libitum*. The standard diet contained 59.8% carbohydrate, 23.4% protein, 4.5% crude fat and 4.5 mg β-carotene/kg but no detectable amounts of lycopene, as indicated by the supplier.

At the end of the experiment (7 wk after tumor injection), the mice were killed by CO_2 asphyxiation. Blood samples were collected from both the retro-orbital plexus and heart in a 10-mL vacutainer tube containing K_3EDTA and were centrifuged ($400 \times g$; 10 min) to obtain plasma.

2.4 Measurement of carotenoid levels in plasma

Carotenoids were extracted from plasma by a modification of a method described previously [16]. Briefly, aliquots (200 μL) of pooled plasma, kept on ice, were placed in 100×13 mm borosilicate glass tubes and extracted with 200 μL of 95% ethanol containing α-tocopherol acetate (200 μL/mL) and retinol acetate (570 ng/mL). After being vortexed for 45 s, they were extracted with hexane (1.0 mL, containing 0.01% BHT) and vortexed for 1 min. The phases were separated by centrifugation ($1400 \times g$, 10 min, 4°C). The upper organic phase was removed and evaporated in a glass test tube (as above) under a stream of nitrogen at room temperature. The residue was reconstituted in 15 μL of chloroform and vortexed for 40 s. Initial dissolution in chloroform was necessary for efficient recovery of carotenoids. A 35 μL volume of ACN–methanol (1:1) was added and vortexed for a further 40 s. A 20-μL aliquot of final extract was injected onto the HPLC system. Absorption

maxima of β-carotene and lycopene were 450 and 470 nm, respectively.

2.5 ELISA for VEGF, IGF-1 and IGFBP-3

VEGF, IGF-1 and IGFBP-3 in plasma were quantified separately with the commercial kit by a solid-phase ELISA (R&D System) at a wavelength of 450 nm. Plasma samples were diluted with commercial dilute-solution (1:5) before assay. All experiments were conducted three times, with each experiment being conducted in duplicate.

2.6 PCNA protein expression

Protein expression of PCNA in tumor tissues was measured by Western blotting. Tumor tissues were homogenized in the RIPA buffer containing protease inhibitors and centrifuged (10 000 × *g*; 5 min). The supernatants were frozen at −80°C until use. An amount of protein (40 μg) from the supernatant was resolved by SDS-PAGE and transferred onto a nitrocellulose membrane. After blocking with TBS buffer (20 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.4) containing 5% nonfat milk, the membrane was incubated with PCNA monoclonal antibody followed by horseradish peroxidase-conjugated anti-mouse IgG, and then visualized using an ECL chemiluminescent detection kit (Amersham).

2.7 Statistical analysis

Values are expressed as means and standard deviations and analyzed by one-way ANOVA followed by LSD test for comparisons of group means. $p<0.05$ was considered statistically significant unless otherwise indicated.

3 Results

3.1 Lycopene and β-carotene inhibited tumor growth in PC-3 bearing nude mice

To determine the anti-tumor effects of lycopene and β-carotene *in vivo*, PC-3 bearing nude mice were given two doses of lycopene (4 and 16 mg/kg twice a week) and a single dose of β-carotene (16 mg/kg twice a week) for 7 wk. Solid tumors were observed on week 2 after injection with PC-3 cells. Tumor volumes of mice treated with high-dosage lycopene (16 mg/kg body weight (BW)) and β-carotene (16 mg/kg) were reduced by 67% ($p<0.001$) and 62% ($p<0.001$), as compared with that of tumor control group (Fig. 1). Furthermore, the tumor weights of mice treated with high-dosage lycopene or β-carotene were decreased by 65% ($p<0.001$) and 71% ($p<0.001$) (Fig. 2). Supplementation with either lycopene or β-carotene produced no overt toxicity in mice, as evidenced by a lack of difference in body weights among the four groups of nude mice during the 7-wk supplementation period (Fig. 3). There was a gradual decrease in body weights during the experiment (Fig. 3), and it is the usual trend in tumor-bearing mice, as such a decrease has been reported by others [17].

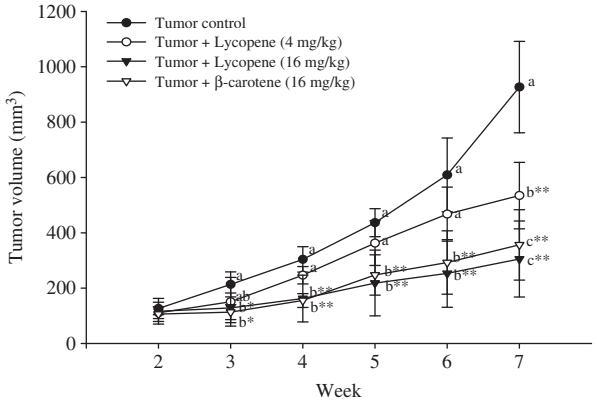

Figure 1. Effects of lycopene and β-carotene supplementation on tumor volume in PC-3 bearing-nude mice. After subcutaneous implantation of PC-3 cells, nude mice were treated with lycopene or β-carotene and then analyzed for the growth of primary tumor. Values are means ± SD, $n=6$; means without a common letter differ, $p<0.05$. $^{*}p<0.05$ at week 3; $^{**}p<0.001$ at other weeks compared with the tumor control.

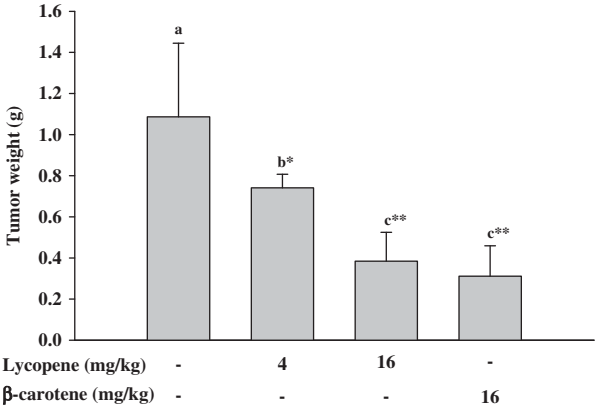

Figure 2. Effects of lycopene and β-carotene supplementation on tumor weight in PC-3 bearing-nude mice. After subcutaneous implantation of PC-3 cells, nude mice were treated with lycopene or β-carotene and then analyzed for the weight of primary tumor. Values are means ± SD, $n=6$; means without a common letter differ, $p<0.05$. $^{*}p<0.05$, $^{**}p<0.001$ compared with the tumor control.

3.2 Effects of lycopene and β-carotene on plasma levels of VEGF, IGF-1 and IGFBP-3 at the end of carotenoid supplementation

Lycopene supplementation markedly decreased the plasma levels of VEGF, and this effect was dose-dependent, whereas

β-carotene significantly increased VEGF levels, as compared with tumor control group (Table 1). Both the high dose of lycopene and β-carotene supplementation markedly increased plasma levels of IGFBP-3, but did not affect the levels of IGF-1 in plasma (Table 1).

3.3 Inhibitory effects of lycopene and β-carotene on PCNA expression in tumor tissues

The Western blot was applied to analyze the effects of lycopene and β-carotene on PCNA protein expression in tumor tissues. Protein expression of PCNA in the tumor tissues was strongly elevated in the tumor control mice, and lycopene supplementation significantly reduced the level in a dose-dependent manner, with 83% inhibition ($p<0.001$) by the high-dosage lycopene (Fig. 4). Similarly, β-carotene supplementation significantly decreased the PCNA level (68%, $p<0.001$) in tumor tissues.

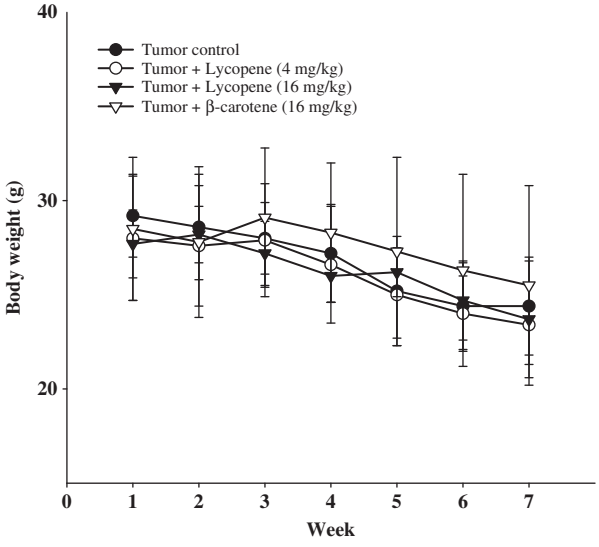

Figure 3. Effect of lycopene and β-carotene supplementation on body weight in PC-3 bearing-nude mice. Values are means ± SD, $n = 6$.

3.4 Effect of lycopene and β-carotene supplementation on total lycopene and β-carotene levels in plasma

No detectable amounts of lycopene or β-carotene were found in the plasma of the tumor control group, nor were in the plasma of mice supplemented with 4 mg lycopen/kg BW (Table 2). In mice supplemented with the high dosage lycopene (16 mg/kg) or β-carotene (16 mg/kg), the plasma lycopene and β-carotene concentrations were markedly increased. Notably, the plasma β-carotene concentration (556 ± 64 nmol/L) was 4.7-fold of plasma lycopene concentration (118 ± 56 nmol/L), suggesting that β-carotene is more bioavailable than lycopene in the nude mice.

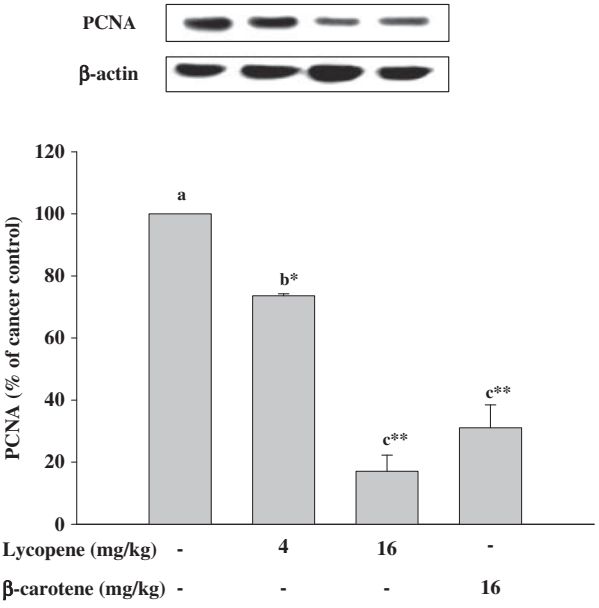

Figure 4. Protein expression of proliferating cell nuclear antigen (PCNA) determined by Western blotting in lung tissues of nude mice. One day after subcutaneous injection with PC-3 cells (2×10^7/100 μL), the mice were supplemented with 4 or 16 mg lycopene/kg BW and 16 mg β-carotene/kg BW twice a week for 7 wk. Values are means ± SD, $n = 6$; means without a common letter differ, $p<0.05$. $^*p<0.05$, $^{**}p<0.001$ compared with the tumor control.

Table 1. Plasma concentrations of VEGF, IGF-1 and IGFBP-3 in four groups after 7 weeks treatment[a),b)]

Group	VEGF (pg/mL)	IGF-1 (ng/mL)	IGFBP-3 (ng/mL)
Tumor control	158 ± 60^A	0.52 ± 0.17	0.52 ± 0.03^A
Tumor+lycopene (4 mg/kg)	118 ± 18^A	0.67 ± 0.28	0.55 ± 0.09^A
Tumor+lycopene (16 mg/kg)	38 ± 11^B	0.65 ± 0.20	4.39 ± 0.06^B
Tumor+β-carotene (16 mg/kg)	298 ± 17^C	0.67 ± 0.06	4.23 ± 0.05^C

a) Values are means ± SD, $n = 6$. Means in a column with superscripts without a common upper case letter differ significantly, $p<0.05$.
b) Nude mice were injected s.c. with 100 μL (1×10^7 cells) of PC-3 cells. After 24-h injection, lycopene (4 and 16 mg/kg) and β-carotene (16 mg/kg) were administered twice a week for 7 consecutive weeks.

Table 2. Plasma concentrations of carotenoid in four groups after 7 weeks treatment[a),b)]

Group	Lycopene (nmol/L)	β-carotene (nmol/L)
Tumor control	bd[c)]	bd[c)]
Tumor+lycopene (4 mg/kg)	bd[c)]	bd[c)]
Tumor+lycopene (16 mg/kg)	118±56	bd[c)]
Tumor+β-carotene (16 mg/kg)	bd[c)]	556±64

a) Nude mice were injected s.c. with 100 μL (1 × 107 cells) of PC-3 cells. After 24 h, lycopene (4 and 16 mg/kg) and β-carotene (16 mg/kg) were administered twice a week for 7 consecutive weeks.
b) Values are expressed as mean±SD, $n = 6$.
c) bd: Below the detection limit: 0.03–0.07 μmol/L for lycopene; 0.04 μmol/L for β-carotene.

4 Discussion

In the present study, we employed PC-3-bearing nude mice to investigate the effects of lycopene and β-carotene supplementation on primary tumor growth *in vivo*. We showed that lycopene markedly inhibited tumor growth in a dose-dependent manner. β-carotene supplementation (16 mg/kg) also markedly decreased tumor growth, and its effect was similar to that of the high-dosage lycopene supplementation. Our results support the finding of Tang *et al.* [14], which indicated that lycopene (100 and 300 mg/kg; 5 d/wk) inhibits the growth of human prostate cancer cells (DU145) injected (s.c.) into BALB/c nude mice. However, the supplemental doses of lycopene used in the present study are relatively low, as compared to those used by Tang *et al.* [14]. The high-lycopene dose used in this study (16 mg/kg body weight, twice a week) is equivalent to 0.114 mg/mouse/d (base on the average body weight of a mouse, 25 g), which translate into 19 mg/d for a 70-kg person, as calculated by the difference in daily food intake, *i.e.* 3 g (dry weight) for a mouse and 500 g (dry weight) for a person with an energy intake of 2000 kcal/day [18]. In patients with localized prostate adenocarcinoma, it has been shown that lycopene (30 mg/d) not only increases the lycopene concentration in serum and prostate but also decreases the serum prostate-specific antigen and prostate tissue oxidative DNA damage [19]. Lycopene is regarded as a safe supplement, as long-term use of lycopene at doses up to 75 mg/d has been shown to produce no apparent toxicities [20].

Several possible anti-cancer mechanisms of lycopene have been suggested, which include the following: (i) the arrest of the cell cycle at G0/G1 phase in leukemic cells [21]; (ii) interference with IGF-1 signaling in mammary cancer cells [22]; (iii) induction of connexin 43 expressions and gap junction intercellular communication [22, 23]; and (iv) inhibition of cancer invasion and migration [13, 24, 25]. In the present study, we further showed that the promotion of IGFBP3 level in plasma by lycopene may also be involved in the anti-tumor actions of lycopene. High IGF-I blood levels have been shown to promote cancer cell growth through IGF-1R in an autocrine/paracrine manner of many tumors including prostate cancer [26]. IGFBP3 inhibits IGF-1 binding to IGF-1R, leading to the inhibition of IGF-induced cell proliferation [27]. In addition, IGFBP-3 itself has been shown to induce the caspase-dependent apoptosis *via* the death receptor-mediated pathway in MCF-7 human breast cancer cells [28]. Some natural compounds, such as graph seed extract, possesses anticancer efficacy against prostate cancer *in vivo*, and that this effect is associated with its antiproliferative, proapoptotic and antiangiogenic activities together with upregulation of IGFBP-3 [29]. Intriguingly, we found that supplemental lycopene or β-carotene increased the IGFBP-3 levels, but did not affect the IGF-1 levels in plasma. The results suggest that the underlying action of lycopene and β-carotene may involve the increase in IGFBP-3, which inhibits IGF-1 binding to IGF-1R.

Another possible anti-cancer mechanism of lycopene suggested by the present study is the inhibition of angiogenesis, as evidence by the reduced plasma VEGF level by lycopene supplementation. Angiogenesis is a critical process for the solid tumor growth and progression [30]. VEGF plays an important role in tumor angiogenesis, as demonstrated by the findings that neutralizing VEGF antibodies inhibit both angiogenesis and tumor growth [31]. The higher levels of VEGF are present in prostate cancer than that in normal prostate cells [32]. In addition, VEGF has been shown to enhance the growth of PC-3 human prostate adenocarcinoma xenotransplanted into nude mice [33]. The present results confirm our previous findings that lycopene supplementation (20 mg/kg; twice a week for 12 wk) decreased VEGF levels in the plasma of nude mice transplanted with human hepatoma SK-Hep-1 cells [13]. In the present study, we demonstrate that lycopene inhibited the tumor growth in nude mice bearing PC-3 cells and that this action is probably related to the inhibition of angiogenesis. In contrast, the VEGF level in the β-carotene-supplemental group was significantly higher than that of tumor control group (Table 1). To date, the literature data on the role of β-carotene in angiogenesis is inconsistent. For example, β-carotene was shown to have a pro-angiogenic activity in a matrigel plug model in mice [34] and to stimulate angiogenesis at an early step by activation of cell migration and matrix reorganization in HUVECs [35]. In contrast, β-carotene was found to suppress tumor-specific angiogenesis in B16F10 melanoma-bearing C57BL/6 mice [36]. Our finding that the increased VEGF in β-carotene-treated mice did not affect the anticancer effect of β-carotene suggests that the angiogenic or anti-angiogenic effects of the carotenoids may not be important in their inhibition of the growth of transplanted primary prostate tumor cells.

Still another possible anti-tumor mechanism of lycopene suggested by the present study is the reduction of cell proliferation that leads to reduced tumor size, as we showed

that supplemental lycopene or β-carotene significantly decreased the tumor cell proliferation, as evidenced by the reduced protein expression of PCNA in tumor tissues. The expression of PCNA, a proliferation-associated marker used in different tumors, is positive in many tumor cells [37, 38].

The plasma levels of lycopene and β-carotene in nude mice fed 16 mg lycopene or β-carotene *per* kg BW were 0.118 and 0.556 μM, which are comparable to the plasma levels of carotenoids (0.39 ± 0.22 μM for lycopene and 0.63 ± 0.52 μM for β-carotene) in healthy humans [39]. Surprisingly, the plasma lycopene was undetectable in mice fed 4 mg lycopene/kg body weight. We suspect that the undetectable plasma lycopene concentration in the 4 mg lycopene/kg group was possibly due to the lower sensitivity of instrumentation. Our detection limit for plasma lycopene was 0.03–0.07 μmol/L, whereas the plasma lycopene in the 4 mg/kg group is roughly predicted to be about 0.029 μmol/L, which is calculated by dividing the plasma lycopene level of 118 nmol/L (or 0.118 μmol/L) for the 16 mg/kg lycopene group by 4, *i.e.* $0.118 \div (16\ \text{mg}/4\ \text{mg}) = 0.029$ μmol/L.

In conclusion, the present study demonstrates that lycopene supplemented at physiological doses effectively decrease the tumor growth in mice injected with PC-3 prostate cancer cells and that the effect of β-carotene is similar, but not identical, to that of lycopene. The anticancer effects of lycopene and β-carotene are likely associated with reduction of proliferation (attenuation of PCNA expression and IGFBP3 levels) and interfered with the IGF-1 signaling (reduction of IGFBP3 levels) in PC-3 bearing nude mice. However, the inhibition of VEGF by lycopene suggests that the antitumor mechanisms of lycopene also involve anti-angiogenesis. These results warrant further studies of lycopene as a potential chemopreventive or chemotherapeutic agent.

The authors have declared no conflict of interest.

5 References

[1] American Cancer Society, Cancer facts and figures 2009. Atlanta, Georgia 2009.

[2] Crawford, E. D., Understanding the epidemiology, natural history, and key pathways involved in prostate cancer. *Urology* 2009, *73*, S4–S10.

[3] Roddam, A. W., Allen, N. E., Appleby, P., Key, T. J., Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. *J. Natl. Cancer Inst.* 2008, *100*, 170–183.

[4] Kelloff, G. J., Crowell, J. A., Steele, V. E., Lubet, R. A. *et al.*, Progress in cancer chemoprevention: development of diet-derived chemopreventive agents. *J. Nutr.* 2000, *130*, 467S–471S.

[5] Giovannucci, E., Ascherio, A., Rimm, E. B., Stampfer, M. J. *et al.*, Intake of carotenoids and retinol in relation to risk of prostate cancer. *J. Natl. Cancer Inst.* 1995, *87*, 1767–1776.

[6] Clinton, S. K., Lycopene: chemistry, biology, and implications for human health and disease. *Nutr. Rev.* 1998, *56*, 35–51.

[7] Olson, J. A., Krinsky, N. I., Introduction: the colorful, fascinating world of the carotenoids: important physiologic modulators. *FASEB J.* 1995, *9*, 1547–1550.

[8] Giovannucci, E., A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. *Exp. Biol. Med. (Maywood)* 2002, *227*, 852–859.

[9] Clinton, S. K., Emenhiser, C., Schwartz, S. J., Bostwick, D. G. *et al.*, cis-trans lycopene isomers, carotenoids, and retinol in the human prostate. *Cancer Epidemiol. Biomarkers Prev.* 1996, *5*, 823–833.

[10] Gann, P. H., Ma, J., Giovannucci, E., Willett, W. *et al.*, Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. *Cancer Res.* 1999, *59*, 1225–1230.

[11] Levy, J., Bosin, E., Feldman, B., Giat, Y. *et al.*, Lycopene is a more potent inhibitor of human cancer cell proliferation than either alpha-carotene or beta-carotene. *Nutr. Cancer* 1995, *24*, 257–266.

[12] Livny, O., Kaplan, I., Reifen, R., Polak-Charcon, S. *et al.*, Lycopene inhibits proliferation and enhances gap-junction communication of KB-1 human oral tumor cells. *J. Nutr.* 2002, *132*, 3754–3759.

[13] Huang, C. S., Liao, J. W., Hu, M. L., Lycopene inhibits experimental metastasis of human hepatoma SK-Hep-1 cells in athymic nude mice. *J. Nutr.* 2008, *138*, 538–543.

[14] Tang, L., Jin, T., Zeng, X., Wang, J. S., Lycopene inhibits the growth of human androgen-independent prostate cancer cells *in vitro* and in BALB/c nude mice. *J. Nutr.* 2005, *135*, 287–290.

[15] Huang, C. S., Chuang, C. H., Hu, M. L., Effects of lycopene supplementation on plasma and tissue lycopene levels in various rodent strains. *Int. J. Vitam. Nutr. Res.* 2006, *76*, 377–384.

[16] Su, Q., Rowley, K. G., O'Dea, K., Stability of individual carotenoids, retinol and tocopherols in human plasma during exposure to light and after extraction. *J. Chromatogr. B Biomed. Sci. Appl.* 1999, *729*, 191–198.

[17] Jia, L., Shang, Y. Y., Li, Y. Y., Effect of antidepressants on body weight, ethology and tumor growth of human pancreatic carcinoma xenografts in nude mice. *World J. Gastroenterol.* 2008, *14*, 4377–4382.

[18] Liu, J. F., Lo, A., Yang, F. L., Wang, T. Y. *et al.*, Analysis of vitamin E, selenium, and other nutrients in planed balanced diets in Taiwan. *Nutr. Sci. J.* 2002, *27*, 221–231.

[19] Chen, L., Stacewicz-Sapuntzakis, M., Duncan, C., Sharifi, R. *et al.*, Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. *J. Natl. Cancer Inst.* 2001, *93*, 1872–1879.

[20] Shao, A., Hathcock, J. N., Risk assessment for the carotenoids lutein and lycopene. *Regul. Toxicol. Pharmacol.* 2006, *45*, 289–298.

[21] Amir, H., Karas, M., Giat, J., Danilenko, M. *et al.*, Lycopene and 1,25-dihydroxyvitamin D3 cooperate in the

inhibition of cell cycle progression and induction of differentiation in HL-60 leukemic cells. *Nutr. Cancer* 1999, *33*, 105–112.

[22] Karas, M., Amir, H., Fishman, D., Danilenko, M. *et al.*, Lycopene interferes with cell cycle progression and insulin-like growth factor I signaling in mammary cancer cells. *Nutr. Cancer* 2000, *36*, 101–111.

[23] Fornelli, F., Leone, A., Verdesca, I., Minervini, F., Zacheo, G., The influence of lycopene on the proliferation of human breast cell line (MCF-7). *Toxicol. In Vitro* 2007, *21*, 217–223.

[24] Huang, C. S., Fan, Y. E., Lin, C. Y., Hu, M. L., Lycopene inhibits matrix metalloproteinase-9 expression and down-regulates the binding activity of nuclear factor-kappa B and stimulatory protein-1. *J. Nutr. Biochem.* 2007, *18*, 449–456.

[25] Huang, C. S., Shih, M. K., Chuang, C. H., Hu, M. L., Lycopene inhibits cell migration and invasion and upregulates Nm23-H1 in a highly invasive hepatocarcinoma, SK-Hep-1 cells. *J. Nutr.* 2005, *135*, 2119–2123.

[26] Kimura, G., Kasuya, J., Giannini, S., Honda, Y. *et al.*, Insulin-like growth factor (IGF) system components in human prostatic cancer cell-lines: LNCaP, DU145, and PC-3 cells. *Int. J. Urol.* 1996, *3*, 39–46.

[27] Mohseni-Zadeh, S., Binoux, M., Insulin-like growth factor (IGF) binding protein-3 interacts with the type 1 IGF receptor, reducing the affinity of the receptor for its ligand: an alternative mechanism in the regulation of IGF action. *Endocrinology* 1997, *138*, 5645–5648.

[28] Kim, H. S., Ingermann, A. R., Tsubaki, J., Twigg, S. M. *et al.*, Insulin-like growth factor-binding protein 3 induces caspase-dependent apoptosis through a death receptor-mediated pathway in MCF-7 human breast cancer cells. *Cancer Res.* 2004, *64*, 2229–2237.

[29] Singh, R. P., Tyagi, A. K., Dhanalakshmi, S., Agarwal, R., Agarwal, C., Grape seed extract inhibits advanced human prostate tumor growth and angiogenesis and upregulates insulin-like growth factor binding protein-3. *Int. J. Cancer* 2004, *108*, 733–740.

[30] Folkman, J., Role of angiogenesis in tumor growth and metastasis. *Semin. Oncol.* 2002, *29*, 15–18.

[31] Kim, K. J., Li, B., Winer, J., Armanini, M. *et al.*, Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth *in vivo*. *Nature* 1993, *362*, 841–844.

[32] Ferrer, F. A., Miller, L. J., Andrawis, R. I., Kurtzman, S. H. *et al.*, Vascular endothelial growth factor (VEGF) expression in human prostate cancer: in situ and *in vitro* expression of VEGF by human prostate cancer cells. *J. Urol.* 1997, *157*, 2329–2333.

[33] Gridley, D. S., Andres, M. L., Slater, J. M., Enhancement of prostate cancer xenograft growth with whole-body radiation and vascular endothelial growth factor. *Anticancer Res.* 1997, *17*, 923–928.

[34] Razny, U., Polus, A., Kiec-Wilk,B., Wator, L. *et al.*, Angiogenesis in Balb/c mice under beta-carotene supplementation in diet. *Genes Nutr.* 2010, *5*, 9–16.

[35] Dembinska-Kiec, A., Polus, A., Kiec-Wilk, B., Grzybowska, J. *et al.*, Proangiogenic activity of beta-carotene is coupled with the activation of endothelial cell chemotaxis. *Biochim. Biophys. Acta* 2005, *1740*, 222–239.

[36] Guruvayoorappan, C., Kuttan, G., Beta-carotene inhibits tumor-specific angiogenesis by altering the cytokine profile and inhibits the nuclear translocation of transcription factors in B16F-10 melanoma cells. *Integr. Cancer Ther.* 2007, *6*, 258–270.

[37] Robbins, B. A., de la Vega, D., Ogata, K., Tan, E. M., Nakamura, R. M., Immunohistochemical detection of proliferating cell nuclear antigen in solid human malignancies. *Arch. Pathol. Lab. Med.* 1987, *111*, 841–845.

[38] Weber, K. L., Doucet, M., Price, J. E., Baker, C. *et al.*, Blockade of epidermal growth factor receptor signaling leads to inhibition of renal cell carcinoma growth in the bone of nude mice. *Cancer Res.* 2003, *63*, 2940–2947.

[39] Borel, P., Moussa, M., Reboul, E., Lyan, B. *et al.*, Human plasma levels of vitamin E and carotenoids are associated with genetic polymorphisms in genes involved in lipid metabolism. *J. Nutr.* 2007, *137*, 2653–2659.

