Lycopene exerts anti-inflammatory effect to inhibit prostate cancer progression

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Lycopene is a natural compound that alleviates oxidative stress and inflammation, exerting therapeutic effects in a number of cancers. The aim of this study is to investigate the efficacy of lycopene in inhibiting prostate cancer. Cell viability assays indicated the dose- and time-dependent toxicity of lycopene in prostate cancer cells. Annexin V/propidium iodide double-staining assays revealed the strong apoptotic effects of lycopene. The levels of inflammatory factors, including interleukin-1 (IL1), IL6, IL8, and tumor necrosis factor-α (TNF-α), in lycopene-treated cells were also reduced by lycopene treatment. With the increasing dose of lycopene, the survival of mice bearing prostate cancer xenografts was significantly improved (P < 0.01), and the tumor burden was significantly reduced (P < 0.01). Our results indicate that lycopene is a promising chemotherapy drug, which inhibits prostate cancer progression by suppressing the inflammatory response.

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INTRODUCTION
Prostate cancer is one of the leading causes of cancer-related death among men worldwide.¹ While the survival rate of patients with locoregional prostate cancer is close to 100%, the prognosis of patients with metastatic cancers is quite dismal. Hormone therapy, radiotherapy, and chemotherapy are used for the treatment of advanced prostate cancers.² However, chemoresistant tumors undergo recurrence, which results in the poor prognosis of prostate cancer patients.³ In addition, severe side effects resulting from these radical therapies greatly compromise the quality of life of prostate cancer patients.⁴ Therapies tailored for efficacious prostate cancer suppression, without significant side effects, are highly desired to improve the clinical management of prostate cancer.

Chronic inflammatory diseases and chronic infections are putative contributors to the development of epithelial malignancies. Recent study has indicated inflammation as an important precursor of prostate cancer.⁵ Because of this, a large array of new compounds targeting inflammation have been developed to impede cancer progression.⁶ Chronic prostatic inflammation, i.e., chronic prostatitis, is considered a potential risk factor for prostate cancer.⁷,⁸ Dietary supplements, such as natural antioxidants, are emerging as an important class of anti-cancer drugs by targeting inflammation.⁹,¹⁰ Lycopene is a natural compound derived from plants and microorganisms. It is a carotenoid with a highly unsaturated hydrocarbon chain commonly used as an antioxidant.¹¹ It provides protective effects against cardiovascular diseases and induces cell-cycle arrest and apoptosis in cancers.¹²,¹³ In prostate cancer, a significant reduction in lycopene levels was found to be correlated with the incidence of prostate cancer; in addition, the dietary intake of lycopene was associated with a reduced risk of prostate cancer.¹⁴,¹⁵ Consequently, the important role of lycopene has spurred interest in developing lycopene as a therapeutic agent for prostate cancer.¹⁶

Definitive evidence to support the anticancer effect of lycopene is still lacking. A comprehensive evaluation of the therapeutic efficacy of lycopene in prostate cancer tumor models would greatly facilitate the clinical translation of lycopene in prostate cancer therapy.

We aimed to evaluate the efficacy of lycopene in suppressing PC3, LNCaP, and DU145 prostate cancer xenografts in mice. We were also interested in assessing the correlation of lycopene in alleviating inflammation and prostate cancer progression. The data reported in this study could assist the further clinical investigation of the potential of lycopene in the clinical management of prostate cancer.

MATERIALS AND METHODS

Cell culture
Prostate cancer cells, LNCaP, PC3, and DU145 were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were maintained in Roswell Park Memorial Institute (RPMI)-1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific) in a humidified incubator (37°C and 5% CO₂).

Lycopene treatment
Lycopene was acquired from Wako (Osaka, Japan). Lycopene was dissolved in tetrahydrofuran/butylated hydroxytoluene (THF/BHT) solvent (Sigma-Aldrich, St. Louis, MO, USA) to a concentration of 10 mmol l⁻¹ as a stock solution, which was stored at −80°C until use. To prepare lycopene at treatment concentrations, the stock solution was diluted in THF/BHT solvent with FBS at a 1:9 ratio. Cells were seeded into 96-well plates (1000 cells per well); the lycopene solution
was then added to the medium, and the cells were incubated for various durations. In control groups, cells were incubated with medium supplemented with the same volumes of THF/BHT solvent.

Cell viability assay
To assess cell viability and growth, after lycopene treatment, cell counting kit-8 (CCK-8, Dojindo Molecular Theranostics, Minato-ku, Japan) solution (10 μl) was added to the cells. After 4 h of incubation, the absorbance of the medium was measured at 450 nm in a microplate reader. The viability was calculated as follows: cell viability (%) = (OD [treated] – OD [0 h])/OD (0 h) × 100%, in which OD means optical density.

Annexin V/propidium iodide double-staining assay
To evaluate the apoptosis induced by lycopene, an Annexin V/propidium iodide double-staining kit (Sigma-Aldrich) was used. Cells were suspended in binding buffer at a density of 10^6 cells per ml. Subsequently, 5 μl of Annex V-fluorescein isothiocyanate and 5 μl of propidium iodide (Sigma-Aldrich) were added. After 15 min of incubation in the dark at room temperature, cell apoptosis was measured using a FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). The apoptosis level of untreated cells was used to normalize that of the treated cells.

Animal experiments
The Balb/c male nude mice were acquired from Jackson Laboratories (Bar Harbor, ME, USA). Tumor injection was carried out when mice were under anesthesia with 3% isoflurane. Cells (1 × 10^6) were suspended in 200 μl of phosphate-buffered saline (PBS) mixed with Matrigel (mixing ratio of 2:1; BD Biosciences, Bedford, MA, USA). A 25G needle (BD Biosciences, Franklin Lakes) was inserted subcutaneously into a site in the foreleg. Lycopene (0, 1, 5, or 10 mg kg^-1) was administered via gavage once per day for 7 days. Tumor size was then monitored with a caliper, and the survival data of mice were monitored for up to 180 days. This study was carried out in strict accordance with the recommendations in the guide for the care and use of laboratory animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Fourth Hospital of Hebei Medical University (Shijiazhuang, China).

Flow cytometry
Flow cytometry was used to quantify T-cell subsets, including Tc1, Th1, Tc17, and Th17 cells. The protein expression of CD3+CD8+IFN-γ+, CD3+CD4+IFN-γ+, CD3+CD8+IL17+, CD3+CD8−IL17+, and CD4+CD25+Foxp3+ was used to identify Tc1, Th1, Th17, Tc17, and Treg cells, respectively. Anti-CD3-PE-Cy5, anti-CD4-FITC, anti-CD8-FITC, anti-CD25-FITC, anti-IL10-PE-Cy7, anti-IL17-PE-Cy7, anti-IL17+PE-APC, and anti-Foxp3-PE were acquired from Thermo Fisher Scientific. Anti-CD56 (LS-C5374, LifeSpan BioSciences, Seattle, WA, USA), anti-CD15 (ORB181771, Biorbyt, San Francisco, CA, USA), anti-CD16 (ORB231476, Biorbyt), anti-F4/80 (126310, United States Biological, Salem, MA, USA) were also used for flow cytometry analysis of natural killer (NK), macrophage, and neutrophil immunity cells. The washing, fixation, and permeabilization of cells were performed according to the manufacturer’s recommendations. Flow cytometry was performed using a FACSCalibur instrument (BD Biosciences, Franklin Lakes).

Statistical analyses
Data are presented as the mean ± standard deviation (s.d.). At least, three independent experiments were performed for each result. One- or two-way ANOVA, followed by Tukey’s post hoc test, was used to assess the significance of differences, and P < 0.05 was considered statistically significant.

RESULTS

Prostate cancer cells were inhibited by lycopene in vitro
We first examined whether the anti-cancer effect of lycopene was dose-dependent. The viability of LNCaP, PC3, and DU145 cells was monitored for 72 h following treatment at 0–5 μmol l^-1 (Figure 1a). In all three cell lines, lycopene treatment led to a decrease in cell viability, as opposed to the increase in cell viability without treatment (THF/BHT solvent only). The inhibition of cell viability by lycopene occurred in a dose- and time-dependent manner. At 1 μmol l^-1 and 5 μmol l^-1, the differences between the treated and nontreated groups at 24 h, 48 h, and 72 h were significant (all P < 0.01). LNCaP demonstrated the most marked inhibition of viability (~50%) in the presence of lycopene. In line with the decrease in viability, we also found that cells treated with 1 μmol l^-1 and 5 μmol l^-1 exhibited the most dramatic increase in apoptotic rates (Figure 1b).

We also evaluated the toxicity of lycopene in a normal prostate cell line, PrEC, and demonstrated that no observable toxicity was documented at 0–5 μmol l^-1 lycopene (Supplementary Figure 1). Our results supported our hypothesis that lycopene inhibited the viability of prostate cancer cells by inducing apoptosis, as evidenced by the increasing caspase-3 levels measured by PCR (Supplementary Methods) under treatment with higher lycopene concentrations (Supplementary Figure 2). Taken together, these data corroborated the anti-cancer effect of lycopene in prostate cancer cells and spurred our interest in understanding the mechanism of this anti-cancer effect and evaluating the suppressive effects of lycopene in prostate cancer in vivo.

Lycopene lowered the expression of inflammatory factors in prostate cancer cells
Given the close relationship between inflammation and prostate cancer progression, we investigated how lycopene affects the level of inflammatory factors in prostate cancer cells. To this end, normal prostate epithelial cells, as well as LNCaP, PC3 and DU145 cells, were treated with lycopene, followed by evaluation of the expression of interleukin-1 (IL1), IL6, IL8, and tumor necrosis factor-α (TNF-α). Our data showed that while prostate cancer cells demonstrated significant upregulation (P < 0.01) of all these inflammatory factors, lycopene treatment attenuated this upregulation (P < 0.01; Figure 2). In addition, we also characterized IL10 and transforming growth factor-beta (TGF-β) expression by prostate cancer cells after lycopene treatment. IL10 and TGF-β secreted by tumor cells are known to enable immune evasion. In prostate cancer cells, IL10 and TGF-β levels were markedly reduced by lycopene at 0.5, 1, and 5 μmol l^-1 (P < 0.01), and this reduction occurred in a dose-dependent manner (Supplementary Figure 3). In normal prostate cells, PrEC, IL10, and TGF-β expression did not show observable changes. Therefore, lycopene decreased IL10 and TGF-β in tumor cells, and this impaired production of anti-inflammatory cytokines by tumor cells may have a positive impact on tumor immunity.

Lycopene improved the survival of mice bearing prostate cancer xenografts
We proceeded to investigate the correlation of lycopene treatment and the survival of prostate cancer-bearing mice. The Kaplan–Meier plot indicated that a marked improvement of survival was induced...
by lycopene treatment and that higher lycopene doses induced better survival in all three prostate tumor models (Figure 3).

**Figure 1:** Lycopene dose/time-dependent suppressed the viability and increased the apoptosis capacity of prostatic carcinoma cancer cell lines (LNCaP, PC3, and DU145 cells). (a) Prostatic carcinoma cell lines were placed on 96-well plates (1000 cells per well) and incubated with fresh medium containing 0, 0.1, 0.5, 1, or 5 µmol l⁻¹ lycopene for 0, 24, 48 and 72 h. Viability of individual treated cells and non-treated cell lines were detected by CCK-8 kit. Absorbance was measured at 450 nm and the cell viability was represented as: cell viability (%) = (OD [treated] − OD [0 h])/OD (0 h) × 100%. (b) Lycopene induces prostatic carcinoma cancer cell apoptosis. Annexin V/propidium iodide double-staining assay was performed to detect the apoptosis levels of lycopene-treated or not prostatic carcinoma cell lines at the indicated concentrations for 72 h. Relative expression values represent mean and standard deviation from three independent experiments. The apoptosis levels of untreated cells were used to normalize those of the treated cells and the data were represented in percentages. *P < 0.01, groups treated with lycopene versus control (0 µmol l⁻¹). OD: optical density; CCK-8: cell counting kit-8.

**Figure 2:** The changes of inflammatory cells with the treatment of lycopene. Expressions of (a) IL1, (b) IL6, (c) IL8, and (d) TNF-α proteins from media culturing three prostatic carcinoma cancer cells (LNCaP, PC3, and DU145 cells), treated with or without lycopene by ELISA, were compared with normal prostatic epithelial cells PrEC in the absence of lycopene. *P < 0.01. ELISA: enzyme-linked immunosorbent assay; IL1: interleukin-1; TNF-α: tumor necrosis factor-α.

**Figure 3:** Lycopene potently prolonged the survival time of prostatic carcinoma mice model in vivo. There prostatic carcinoma cell lines (a) LNCaP, (b) PC3, and (c) DU145 cells) were injected with 1 × 10⁸ cells into the left foreleg in mice. Then the model mice were treated with different concentration (0, 1, 5, or 10 mg kg⁻¹) of lycopene by gavage once per day for 7 days and the survival was monitored to up to 180 days. To further clarify the immunoregulatory role of lycopene in prostate cancer, Ki67, a proliferation marker, was measured in tumor tissue harvested from both the treatment and control groups. To our surprise, no significant difference was observed in Ki67 levels in tumors with or without treatment (*P = 0.462; Supplementary Figure 4). Furthermore, lycopene had no significant effect on the level of necrosis of prostatic carcinoma in vivo, as revealed by Annexin V/FITC assays of the tumors (*P = 0.271; Supplementary Figure 5).

To elucidate the involvement of the immune regulatory role of lycopene in tumor suppression, lycopene treatment was also performed in immune-competent mice bearing TRAMPC2 tumors, followed by an analysis of immune cell accumulation in tumors. Here, we showed that tumor inflammatory cells, including Tc1, Th1, Tc17 and Th17 cells, were increased while tumor Tregs were reduced by lycopene (*P < 0.01; Figure 6). Further evaluation of CD56+CD16+ (NK immunity cell marker), F4/80+ (macrophage immunity cell marker) and CD15+CD16+ (neutrophil immunity cell marker) cells demonstrated that lycopene promoted immune cell accumulation in tumors, thus activating tumor immune attack (*P < 0.01; Supplementary Figure 6). Taken together, these data indicate that lycopene can effectively reduce tumor growth by exerting an immune regulatory role to prolong the survival of tumor-bearing mice.
Lycopene potently inhibited LNCaP prostatic carcinoma growth in vivo. Three prostatic carcinoma cell lines (LNCaP, PC3 and DU145 cells) were injected with $1 \times 10^6$ cells into the left foreleg in mice. Then the model mice were treated with different concentration (0, 1, 5, or 10 mg kg$^{-1}$) of lycopene by gavage per day for 1 week. Expression of protein of (a) IL1, (b) IL6, (c) IL8 and (d) TNF-α from serum of mice implanted with three prostatic carcinoma cancer cells treated with lycopene by ELISA compared with normal non-treated mice. $^*P < 0.05$, $^{**}P < 0.01$. ELISA: enzyme-linked immunosorbent assay; IL1: interleukin-1; TNF-α: tumor necrosis factor-α.

DISCUSSION

Natural compounds with anticancer capacities have increasingly become attractive therapeutics to improve the efficacy of cancer suppression and the general health of affected patients. Because of its high prevalence, lifetime risk, and relatively slow rate of progression, prostate cancer is an ideal candidate disease for chemoprevention. Pharmacologic or nutritional interventions, which could potentially result in a substantial reduction in the incidence of clinically detectable disease, can considerably increase the survival of prostate cancer patients. Lycopene is the predominant carotenoid in tomatoes, watermelons, and grapefruits. A reduced level of plasma lycopene is a characteristic of prostate cancer. Indeed, lycopene has shown a plethora of potential beneficial effects on human health, including anti-inflammation, anti-oxidation, and immunomodulation effects. Lycopene serves as a dietary antioxidant that offers protection against free radicals. This property of lycopene confers preventative and protective effects in cardiovascular tissues. Here, we performed a comprehensive evaluation of the therapeutic effect of lycopene on PC3, LNCaP, and DU145 tumor models, showing that lycopene potently inhibited tumor progression and improved mouse survival. LNCaP tumors demonstrated the most striking difference in tumor volume after lycopene treatment. Consistent with what is observed in breast cancers and LNCaP tumors, lycopene dose-dependently induced cell apoptosis in all prostate cancer cells investigated in this study. This result is in accordance with previous findings that lycopene is a potent inhibitor of human cancer cell proliferation. Notably, the highest therapeutic efficacy was found in androgen-dependent LNCaP tumors. This result is unsurprisingly considering that LNCaP possesses lower proliferation and metastatic abilities. Despite this, PC3 and DU145 tumors, which are androgen-independent tumors, are also subject to significant suppression by lycopene. In vivo, the decreases in tumor volume in PC3 and DU145 tumor models were relatively smaller. However, the dramatic improvement in the survival of PC3 and DU145 tumor-bearing mice could be due to induced necrosis in these two tumors. Taken together, lycopene itself has the potential to suppress prostate cancer progression and could be used to supplement existing therapeutic regimens without severe side effects.

Further, we demonstrate that the anticancer effect of lycopene is linked to its role in suppressing inflammatory responses. We analyzed the changes in IL1, IL6, IL8, and TNF-α levels in response to lycopene treatment. It has been shown that prostate cancer coincides with elevated inflammation, while lycopene normalizes the levels of these inflammation levels. This observation is consistent with previous evidence that lycopene alleviates inflammation to induce ameliorating effects in a large array of diseases. This is evidenced by the fact that pro-inflammatory cytokines are crucial for the activation of the innate and adaptive immune responses to control tumor progression. The inflammation-induced response of Treg cells, including Th2 cells, was associated with tumor immune evasion in mice studies. Here, SCID mice were used, as they have no T-cell-mediated immunity. However, the expression levels of CD56+CD16+ (NK immunity cell marker), F4/80+ (macrophage immunity cell marker), and CD15+CD16+ (neutrophil immunity cell marker) were analyzed from tumor tissues with or without lycopene treatment in vivo and in vitro. These markers were upregulated after lycopene treatment. For further validation, we performed lycopene treatment in an immune competent mouse model (C57BL/6) inoculated with TRAMPC2 cells.

Immune cells, including Tc1, Th1, TC17, and Th17 cells, were upregulated ($P < 0.01$), and the accumulation of Tregs in the tumor microenvironment decreased ($P < 0.01$). This evidence further validates the immunoregulatory role of lycopene. Hence, lycopene treatment represents one of the anti-inflammatory strategies for cancer therapy, which has been increasingly investigated in recent years. Radiotherapy is associated with inflammation that compromises anticancer efficacy. The anti-inflammatory effects of lycopene may also explain recent findings on the radioprotective property of lycopene.
in cancer.\textsuperscript{31} Here, we did not explore the precise molecular mechanism of its action. It is possible that this anticancer effect stems from the regulation of the nuclear factor-κappa B (NF-κB) pathway by lycopene, as implicated in the previous study.\textsuperscript{34} Lycopene may also regulate the level of cyclin D, which is a key protein in maintaining a normal cell cycle, to induce the cell cycle arrest of prostate cancer cells.\textsuperscript{35} Further investigation on the link between the anti-inflammatory effect of lycopene and cancer suppression is warranted to better elucidate the mechanism of lycopene in cancer.

In sum, we evaluated the therapeutic potential of lycopene in three prostate cancer models, LNCaP, PC3, and DU145, and demonstrated that lycopene efficiently suppressed all these tumors in a dose- and time-dependent manner. Lycopene downregulated IL1, IL6, IL8, and TNF-α levels, and its modulation of inflammation contributed to tumor suppression. We believe that lycopene has potential as a new dietary agent for the treatment of prostate cancer. It may induce more potent anti-inflammatory effects than other carotenoids, such as phytoene and β-carotene, because of its superior antioxidative activity.\textsuperscript{36,37} When used alone or in combination with conventional treatment for prostate cancer, lycopene could improve the clinical management of the disease.

AUTHOR CONTRIBUTIONS
LNJ and YBL carried out the experiments. LNJ participated in the statistical analysis and drafted the manuscript. BHL conceived of the study, and designed and drafted the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS
All authors declare no competing interests.

Supplementary Information is linked to the online version of the paper on the Asian Journal of Andrology website.

REFERENCES
1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7–30.
2. Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, et al. EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and local treatment with curative intent-update 2013. Eur Urol 2014; 65: 124–37.
3. Edwards BK, Noone AM, Mariotto AB, Simard EP, Boscoe FP, et al. Annual Report to the Nation on the status of cancer, 1975–2010, featuring prevalence of comorbidity and impact on survival among persons with lung, colorectal, breast, or prostate cancer. Cancer 2014; 120: 1290–314.
4. Fizzoli K, Faivre L, Lesaunier F, Delva R, Gravis G, et al. Androgen deprivation therapy plus docetaxel and estramustine versus androgen deprivation therapy alone for high-risk localised prostate cancer (GETUG 12): a phase 3 randomised controlled trial. Lancet Oncol 2015; 16: 787–94.
5. Gurel B, Lucia MS, Thompson IM, Goodman PJ, Tangen CM, et al. Chronic inflammation in benign prostate tissue is associated with high-grade prostate cancer in the placebo arm of the prostate cancer prevention trial. Cancer Epidemiol Biomarkers Prev 2014; 23: 847–56.
6. Hofseth LJ, Wargovich MJ. Inflammation, cancer, and targets of ginseng. J Nutr 2007; 137: 1836–4s.
7. Stanos KS, De Marzo AM. Prostate cancer and inflammation: the evidence. Histopathology 2012; 60: 199–215.
8. Vaara MH, Mehik A, Ohtonen P, Hellstrom PA. Prostate cancer incidence in men with self-reported prostatitis after 15 years of follow-up. Oncol Lett 2016; 12: 1149–53.
9. Duergger A, Heidegger I, Ofer P, Perktold B, Ramorer R, et al. The use of dietary supplements to alleviate androgen deprivation therapy side effects during prostate cancer treatment. Nutrients 2014; 6: 4491–519.
10. Aravindiram K, Yang NS. Anti-inflammatory plant natural products for cancer therapy. Planta Med 2010; 76: 1103–17.
11. Xu K, Mucci L, Rosner BA, Clinton SK, Loda M, et al. Dietary lycopene, angiogenesis, and prostate cancer: a prospective study of the prostate-specific antigen era. J Natl Cancer Inst 2014; 106: djl430.
12. Gloria NF, Soares N, Brand C, Oliveira FL, Borovejc R, et al. Lycopene and beta-carotene induce cell-cycle arrest and apoptosis in human breast cancer cell lines. Anticancer Res 2014; 34: 1377–86.
13. Aizawa K, Liu C, Tang SY, Veeramachaneni S, Hu KQ, et al. Tobacco carcinoend induces both lung cancer and non-alcoholic steatohepatitis and hepatocellular carcinomas in ferrets which can be attenuated by lycopene supplementation. Int J Cancer 2016; 139: 1171–81.
14. Gann PH, Ma J, Giovannucci E, Willett W, Sacks FM, et al. Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. Cancer Res 1999; 59: 1225–30.
15. Graff RE, Pettersson A, Lis RT, Ahearn TU, Markt SC, et al. Dietary lycopene intake and risk of prostate cancer defined by ERG protein expression. Am J Clin Nutr 2016; 103: 851–60.
16. Holzapfel NP, Holzapfel BM, Champ S, Feldthuesen J, Clements J, et al. The potential role of lycopene for the prevention and therapy of prostate cancer: from molecular mechanisms to clinical evidence. Int J Mol Sci 2013; 14: 14620–46.
17. Principe DR, DeCant B, Mascarinas E, Wayne EA, Diaz AM, et al. TGFbeta signaling in the pancreatic tumor microenvironment promotes fibrosis and immune evasion to facilitate tumorigenesis. Cancer Res 2016; 76: 2525–39.
18. Che FY, Heng XF, Zhang HY, Su QP, Zhang BX, et al. Novel B7-H4-mediated crosstalk between human non-Hodgkin lymphoma cells and tumor-associated macrophages leads to immune evasion via secretion of IL-6 and IL-10. Cancer Immunol Immunother 2017; 66: 717–29.
19. Rowles JL 3rd, Ranard KM, Smith JW, An R, Erdman JW Jr. Increased dietary and circulating lycopene are associated with reduced prostate cancer risk: a systematic review and meta-analysis. Prostate Cancer Prostatic Dis 2017; 20: 361–77.
20. Cheng HM, Koutsidis G, Lodge JK, Ashor AW, Siervo M, et al. Lycopene and Tomato and risk of cardiovascular diseases: a systematic review and meta-analysis of epidemiological evidence. Crit Rev Food Sci Nutr 2017 Doi: 10.1080/10408398.2017.1362630. (Epub ahead of print).
21. Hantz HL, Young LF, Martin KR. Physiologically attainable concentrations of lycopene induce mitochondrial apoptosis in LNCaP human prostate cancer cells. Exp Biol Med (Maywood) 2005; 230: 171–9.
22. Levy J, Bosin E, Feldman B, Giat Y, Minister A, et al. Lycopene is a more potent inhibitor of human cancer cell proliferation than either α-carotene or β-carotene. Nutr Cancer 1995; 24: 257–66.
23. Rick FG, Fahrenholz CD, Garcia MI, Block NL, Schally AV, et al. Preclinical efficacy of growth hormone-releasing hormone antagonist MIA-602 for androgen-dependent and castration-resistant human prostate cancer. J Clin Oncol 2014; 32: 221.
24. Okamoto M, Lee C, Oyasu R. Interleukin-6 as a paracrine and autocrine growth factor in human prostatic carcinoma cells in vitro. Cancer Res 1997; 57: 141–6.
25. Pina-Zentella RM, Rosado JL, Gallegos-Corona MA, Madrigal-Perez LA, Garcia OP, et al. Dietary lycopene improves diet-mediated recuperation in rat model of nonalcoholic fatty liver disease. J Med Food 2016; 19: 607–14.
26. Giri AK, Rawat JK, Singh M, Gautam S, Kaithwas G. Effect of lycopene against gastroesophageal reflux disease in experimental animals. BMC Complement Altern Med 2015; 15: 110.
27. Prema A, Janakiramani U, Manivashagam T, Thennmozhi AJ. Neuroprotective effect of lycopene against MPTP induced experimental Parkinson’s disease in mice. Neurosci Lett 2015; 595: 12–9.
28 Jhou BY, Song TY, Lee I, Hu ML, Yang NC. Lycopene inhibits metastasis of human liver adenocarcinoma SK-Hep-1 cells by downregulation of NADPH oxidase 4 protein expression. *J Agric Food Chem* 2017; 65: 6893–903.

29 Chang WJ, Du Y, Zhao X, Ma LV, Cao GW. Inflammation-related factors predicting prognosis of gastric cancer. *World J Gastroenterol* 2014; 20: 4586–96.

30 Osawa E, Nakajima A, Fujisawa T, Kawamura YI, Toyama-Sorimachi N, et al. Predominant T helper type 2-inflammatory responses promote murine colon cancers. *Int J Cancer* 2006; 118: 2232–6.

31 Xanthinaki A, Nicolatou-Galitis O, Athanassiadou P, Gonidi M, Kouloulias V, et al. Apoptotic and inflammation markers in oral mucositis in head and neck cancer patients receiving radiotherapy: preliminary report. *Support Care Cancer* 2008; 16: 1025–33.

32 Barker HE, Paget JT, Khan AA, Harrington KJ. The tumour microenvironment after radiotherapy: mechanisms of resistance and recurrence. *Nat Rev Cancer* 2015; 15: 602–25.

33 Gajowik A, Dobrzynska MM. Lycopene – antioxidant with radioprotective and anticancer properties. A review. *Rocz Panstw Zakl Hig* 2014; 65: 263–71.

34 Hadad N, Levy R. The synergistic anti-inflammatory effects of lycopene, lutein, β-carotene, and carnosic acid combinations via redox-based inhibition of NF-κB signaling. *Free Radic Biol Med* 2012; 53: 1381–91.

35 Nahum A, Hirsch K, Danilenko M, Watts CK, Prall GW, et al. Lycopene inhibition of cell cycle progression in breast and endometrial cancer cells is associated with reduction in cyclin D levels and retention of p27(Kip1) in the cyclin E-CDK2 complexes. *Oncogene* 2001; 20: 3428–36.

36 Rao AV, Agarwal S. Role of antioxidant lycopene in cancer and heart disease. *J Am Coll Nutr* 2000; 19: 563–9.

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**SUPPLEMENTARY METHODS**

*Apoptosis determination by quantitative reverse transcriptase polymerase chain reaction*

To verify the increases in apoptosis in prostate cancer cells induced by lycopene treatment, we performed qRT-PCR analysis of caspase-3, a putative apoptosis marker, in the three prostate cancer cell lines treated with 0-5 µM lycopene.

**Supplementary Figure 1:** The toxicity test of lycopene to PrEC cell lines. 0.5 × 10⁴ PrEC cells were placed in 96-well plates and incubated with fresh medium containing 0, 0.1, 0.5, 1, or 5 µmol l⁻¹ lycopene for 48 h. Growth curves of individual lycopene-treated and nontreated prostatic carcinoma cells were detected at least three independent experiments in triplicate by CCK-8 kit. CCK-8: cell counting kit-8.

**Supplementary Figure 2:** The changes of Caspase-3 with the treatment of lycopene in vivo. Expression of mRNA of Caspase-3 from three prostatic carcinoma cancer cells treated with or without lycopene was tested by PCR (**P < 0.01). PCR: polymerase chain reaction.
Supplementary Figure 3: The changes of IL-10 and TGF beta levels with the treatment of lycopene in vitro. Expression of protein of IL-10 and TGF beta from media culturing three prostatic carcinoma cancer cells and normal prostatic epithelial cells PrEC treated with Lycopene by ELISA compared with non-treated cells (*P < 0.01). ELISA: enzyme-linked immunosorbent assay; IL1: interleukin-1; TNF: tumor necrosis factor.
Supplementary Figure 4: The changes of Ki67 expression with the treatment of lycopene in vivo. Three prostatic carcinoma cell lines were injected with $1 \times 10^5$ cells into the left foreleg in mice. Then the model mice were treated with different concentration (0, 1, 5, or 10 mg kg$^{-1}$) of lycopene by gavage per day for 1 week. Expression of protein of Ki67 from mice treated with lycopene by real-time PCR compared with non-treatment mice ($P = 0.462$). PCR: polymerase chain reaction.

Supplementary Figure 5: Lycopene had no significant effect on necrosis level of prostatic carcinoma cancer in vivo. Three prostatic carcinoma cell lines were injected with $1 \times 10^5$ cells into the left foreleg in mice. Then the model mice were treated with different concentration (0, 1, 5, or 10 mg kg$^{-1}$) of lycopene by gavage per day for 1 week. The tissues were dissociated into single cells. Necrosis levels of mice treated with lycopene by Annexin V/propidium iodide double-staining assay compared with non-treatment mice. Graphs represent mean ± standard deviation from three independent experiments ($P = 0.271$).
Supplementary Figure 6: Lycopene induced the activity of immunity system of prostatic carcinoma in vivo and vitro. Three prostatic carcinoma cell lines were injected with $1 \times 10^5$ cells into the left foreleg in mice. Then the model mice were treated with different concentration (0, 1, 5, 10 mg kg$^{-1}$) of Lycopene by gavage once per day for 1 week. Expressions of proteins of CD56+/CD16+ (NK immunity cell marker), F4/80+ (macrophage immunity cell marker) and CD15+/CD16+ (neutrophil immunity cell marker) in tumor from mice with or without Lycopene treatment were tested by flow cytometry ($^*P < 0.01$).