

A Dietary Tomato Supplement Prevents Prostate Cancer in TRAMP Mice

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Abstract

Transgenic adenocarcinoma of the mouse prostate (TRAMP) is a model for progressive prostate cancer that mirrors the stages of the human form. In this study, the effects of a diet enriched with processed whole tomatoes on survival, tumorigenesis, and progression of prostate cancer, and the antioxidant and inflammatory status of TRAMP mice were investigated. Tomato diet significantly increased overall survival ($P < 0.01$), delayed progression from prostatic intraepithelial neoplasia to adenocarcinoma, and decreased the incidence of poorly differentiated carcinoma. Biochemical data disclosed an increase in serum antioxidant activity and a reduction of serum inflammation/angiogenesis biomarkers of particular importance in prostate carcinogenesis. *Cancer Prev Res*; 3(10); 1284–91. ©2010 AACR.

Introduction

Prostate cancer is the most common noncutaneous malignant neoplasm in males in Western countries. It is responsible for 30,000 deaths per year in the United States (1), and its incidence is increasing rapidly in function of the growing number of men over 50 years old worldwide. It is an ideal candidate for chemoprevention. Typically diagnosed in elderly men, even a slight delay in its development could substantially reduce the occurrence of clinically detectable forms. Dietary constituents are regarded as promising tools for prostate cancer prevention (2, 3).

Epidemiologic studies were the first to indicate that tomatoes, especially processed tomato products, are associated with a 30% to 40% reduction in prostate cancer risk. A recent meta-analysis has revealed a reduction of the relative risk in the highest quartile of tomato intake of 0.89 and 0.81 for those consuming raw and cooked tomato products, respectively (4). An inverse relationship between serum lycopene, the primary tomato carotenoid, and prostate cancer has also been shown (5), whereas intervention studies have shown that biomarkers related to prostate carcinogenesis may be altered by dietary administration of tomato products (6). A systematic review by the Food

and Drug Administration, however, indicates that there is little robust evidence in favor of the supposed association between lycopene and reduction of prostate cancer risk (7). Most experimental carcinogenesis studies of fruit and vegetable compounds used a “reductionist” approach to examine pure chemical components, especially lycopene, whether alone or in combination. Lycopene is an O₂ quencher. It reduces oxidative DNA damage (8) and inhibits proliferation of various cancer cell lines (8, 9). In animal experiments, lycopene supplementation downregulates numerous inflammatory marker genes and the genes of insulin-like growth factor-1 and 5 α -reductase in prostate tissue, with subsequent downregulation of androgen target genes (10). However, in prostate cancer patients, tomato supplementation reduces serum prostate-specific antigen levels (11), whereas lycopene has no effect (12). In rats, tomato supplementation reduces carcinogenesis-induced mortality more efficiently than lycopene (13–15). Again, in the Dunning R3327-H prostate cancer model, the intake of whole foods, such as tomatoes and broccoli, slowed tumor growth more effectively than lycopene alone (16). Tomatoes contain other anticancer phytochemicals [see U.S. Department of Agriculture National Nutrient Database for Standard Reference: release 19 (2006), pdf available from http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/measure.pl?MSRE_NO=11548xyz1100xyzTomato%20powderxyzxyz], whereas constituents already present in small amounts or newly formed during processing also contribute to their final *in vivo* efficacy (14).

Transgenic adenocarcinoma of the mouse prostate (TRAMP) is a model in which the progression of prostate cancer mirrors the stages of the human form (17). Expression of the SV40 early genes driven by the prostate-specific promoter probasin leads to prostate cell transformation, and all TRAMP mice develop prostate cancer spontaneously. This model, therefore, is regularly used to assess chemoprevention of prostate cancer.

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This article compares the effects of a feed enriched with 10% whole tomato versus a tomato-free control feed in the TRAMP model. Its two primary outcomes were evaluation of (a) tumor incidence and (b) overall survival rate. The secondary outcome was evaluation of serum biomarkers of particular significance in prostate carcinogenesis.

Materials and Methods

Animals

Male and female TRAMP mice, heterozygous for the PB transgene, were maintained in a pure C57BL/6 background. Transgenic males were obtained by crossing C57BL/6 TRAMP females with C57BL/6 nontransgenic males, and bred in the Animal Care Facility (CeSI, G. d'Annunzio University Foundation, Chieti, Italy). Housing and care of the animals were in accordance with the guidelines established by the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes."

Feeds

Two feeds were used: (a) a commercial feed (Altromin MT diet; RIEPER) containing serum and milk proteins instead of soy and fish flour; this constituted the basic/control diet (CD); (b) a new feed in which the CD is supplemented with 10% tomato powder (hereinafter called the tomato diet or TD). This powder was spray dried from heat-processed paste from whole cherry tomatoes, including seeds and skins. The paste was concentrated by means of a standard Hot Break procedure up to 40° Brix and spray dried without additives to obtain a powder with less than 5% humidity. The compositions of the powder and the two diets are reported in Supplementary Tables S1 and S2, respectively. The caloric value of the two feeds was similar, with no significant difference in their macronutrient composition. They were stored at 4°C to 6°C in the dark.

Study design

Four-week-old male TRAMP mice were fed with CD for 1 week for adaptation. At 5 weeks of age, they were randomly distributed to the TD or CD group in accordance with a free software routine (GraphPad, <http://www.graphpad.com/quickcalcs/randomize1.cfm>). All the animals were matched for weight and general physical condition. Two protocols were designed in function of the outcomes: in protocol 1, mice (18 in the CD and 18 in the TD group) were allowed to live until natural death or killed at the end of the study period. In protocol 2, five CD and five TD mice surviving the lethal development of poorly differentiated (PD) tumors were killed at 12, 20, 25, 30, and 33 weeks. Ten mice were initially recruited for groups to be sacrificed at 12 and 20 weeks, 20 for the 25-week time point, and 30 for the last two time points. Blood (the first five samples obtained) and tissue samples were analyzed as described below to evaluate and correlate, in each animal and at each time point, carotenoid and flavo-

noid concentration, total antioxidant capacity, marker protein content, and pathologic status.

Blood and tissue collection and analyses

Animals were anesthetized by CO₂ inhalation. Blood samples collected by cardiac puncture in heparinized tubes were centrifuged in the dark at 250 × g for 10 minutes to separate plasma, which was stored at -80°C. After induction of death with a further CO₂ inhalation, prostate glands (dorsolateral, ventral, and anterior lobes) and seminal vesicles were removed and microdissected whenever possible. When a tumor obscured the boundaries of the lobes, it was taken as such.

Histology

Prostates and all major organs were examined for microscopic evidence of primary tumors/metastases. One-millimeter step sections were taken from formalin-fixed, paraffin-embedded tissue, and 5-mm slices were stained with H&E. Tumor volume was calculated from the formula $V = a^2 \times b/2$ (where *a* and *b* are the minimum and maximum in millimeters) and expressed as mean volume ± SD mm³ (irrespective of the finding). H&E and immunostained sections were examined with a Leica DMR microscope. Prostate lesions were classified as (a) low-grade prostatic intraepithelial neoplasia (PIN), (b) high-grade PIN, (c) well-differentiated adenocarcinoma, (d) moderately differentiated adenocarcinoma, and (e) PD tumors (18). The PD, androgen receptor (AR)-negative, synaptophysin-expressing tumor is currently the lethal phenotype in the TRAMP model. It is a separate form and not a progression from the PIN and the well-differentiated adenocarcinoma that arise from AR-mediated, SV40-Tag transgene expression in secretory epithelial cells (18–20). The histopathologic picture of TRAMP mice also includes a non-metastasizing, fibroepithelial (phyllodes) tumor in which proliferating mesenchymal cells lined by cuboidal to columnar epithelium form papillary/polypoid lesions in the seminal vesicles.

Immunohistochemistry

Immunohistochemistry was done on formalin-fixed, paraffin-embedded tissues. The primary antibodies were anti-SV40 large T antigen (BD Pharmingen), anti-mouse AR (Upstate), and synaptophysin (Biogenex). The biotinylated secondary antibodies were anti-rat IgG (1:200; DAKO) and goat anti-rabbit IgG (1:200; Santa Cruz Biotechnology).

Serum protein analysis

The concentrations of serum proteins (see the complete list in Supplementary Table S3) were measured at 12, 20, 25, 30, and 33 weeks of age (Rules-Based Medicine, Inc.) in a quantitative multiplexed immunoassay (Rodent Multi-Analyte Profile, <http://www.rulesbasedmedicine.com/products-services/rodentMAP-antigen.asp>), and expressed as means ± SEM pg/mL or ng/mL (*n* = 5). Because this technology requires only 70 μL of serum, sufficient aliquots

from the 20-, 25-, 30-, and 33-week samples were available for the simultaneous determination of carotenoid/flavonoid content and antioxidant capacity.

Serum carotenoids, flavonoids, and total antioxidant capacity

Serum carotenoid and flavonoid concentrations were determined at 20, 25, 30, and 33 weeks of age. Carotenoid determination, extraction and high-performance liquid chromatography analysis of sera, and serum flavonoid extraction and high-performance liquid chromatography/tandem mass spectrometry analysis of the extracts were done as previously described (21, 22). The ferric-reducing ability of plasma was assayed according to the original description (23). Data were reported as mean concentrations \pm SEM, expressed as nmol/L for carotenoids and flavonoids and μ mol TE/L for antioxidant capacity ($n = 5$).

Statistics

Overall survival was measured from the start of treatment to death and censored at the last follow-up (48 weeks for survivors). Its distribution was estimated using the Kaplan-Meier method, and the difference between groups was detected by means of the log-rank test. The effects of treatment and time on serum biomarker levels were evaluated by two-way ANOVA with repeated measures. Post hoc comparisons were then made with Bonferroni's *t* test for pairwise multiple comparisons, with 5% as the significance cutoff.

Results

Protocol 1

Survival. The Kaplan-Meier survival curves (Fig. 1A) revealed a significant increase in the overall survival rate of the TD mice (67% versus 11%; $P = 0.0018$). Histopathologic examination disclosed PD tumors in 10 CD mice (56%) and 3 TD mice (17%). All these animals died spontaneously within the 31st (CD mice) or 33rd week of age (TD mice). All the other mice developed phylloids tumors of the seminal vesicles that were significantly smaller (two-tailed Mann Whitney *U* test, $P < 0.01$) in the TD animals (volume 147 ± 141 mm³, $n = 15$, versus 428 ± 391 mm³, $n = 8$; Supplementary Fig. S1). This slow-growing tumor eventually gives rise to voluminous masses due to actively Tag-positive, fibroblast-like cells that obstruct seminal vesicles and urethers. Death is usually due to urinary blockage and consequent kidney failure.

Metastatic progression. Metastatic progression was confined to the mice with PD tumors. Six of 18 CD mice developed liver, lung, or periaortic lymph node metastases evident macroscopically in four animals and microscopically identifiable in two, whereas only one TD mouse displayed macroscopic liver and lung metastases.

Body weight. There were no significant differences in the mean body weights (Supplementary Fig. S2).

Protocol 2

Prostate tumor development and progression. To evaluate the effect of the treatment on progression from nonneoplastic tissue to PIN and then to adenocarcinoma, mice were studied at 12, 20, and 25 weeks of age (five CD and five TD mice for each time point). The mean percent areas of the dorsolateral prostate at these times were microscopically classified as nonneoplastic (normal), PIN (low- and high-grade PIN), and well- and moderately differentiated adenocarcinoma (Fig. 1B). Their histology and immunohistochemistry are illustrated in Fig. 2. TD had a significant effect on tumor areas. At 12 weeks of age, the

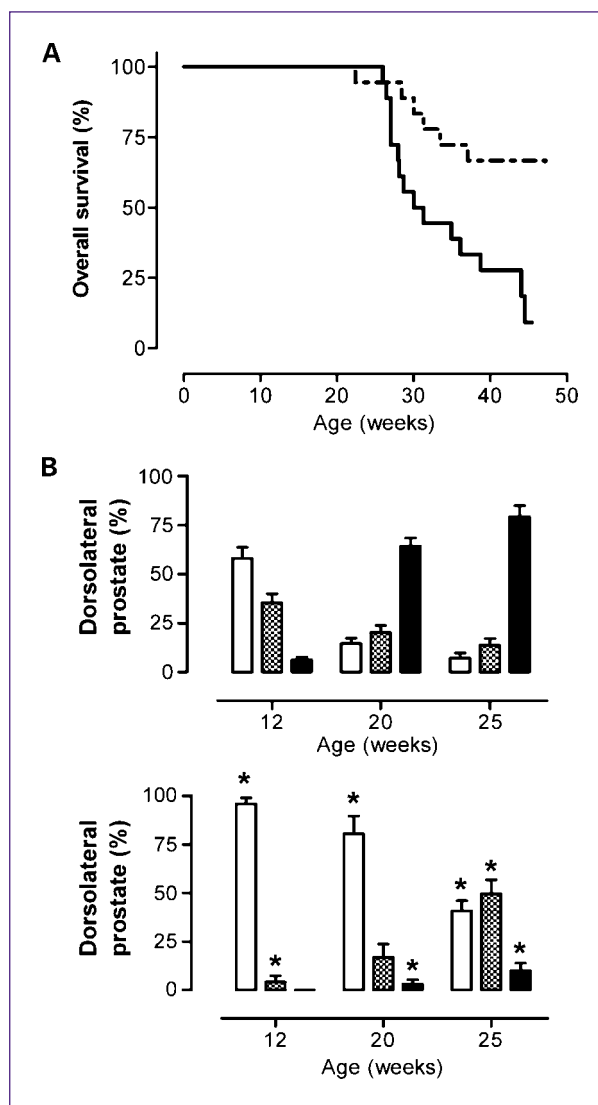


Fig. 1. A, Kaplan-Meier survival curves for TD mice (dashed line) and CD mice (continuous line). B, tumor progression in CD mice (top) and TD mice (bottom). Data are expressed as mean percent \pm SEM ($n = 5$ per each age point) area of prostate with normal histology (no neoplastic prostate, open columns), with PIN (dotted columns), and with adenocarcinoma (closed columns). *, $P < 0.01$ between TD group and CD group (two-tailed Mann-Whitney *U* test).

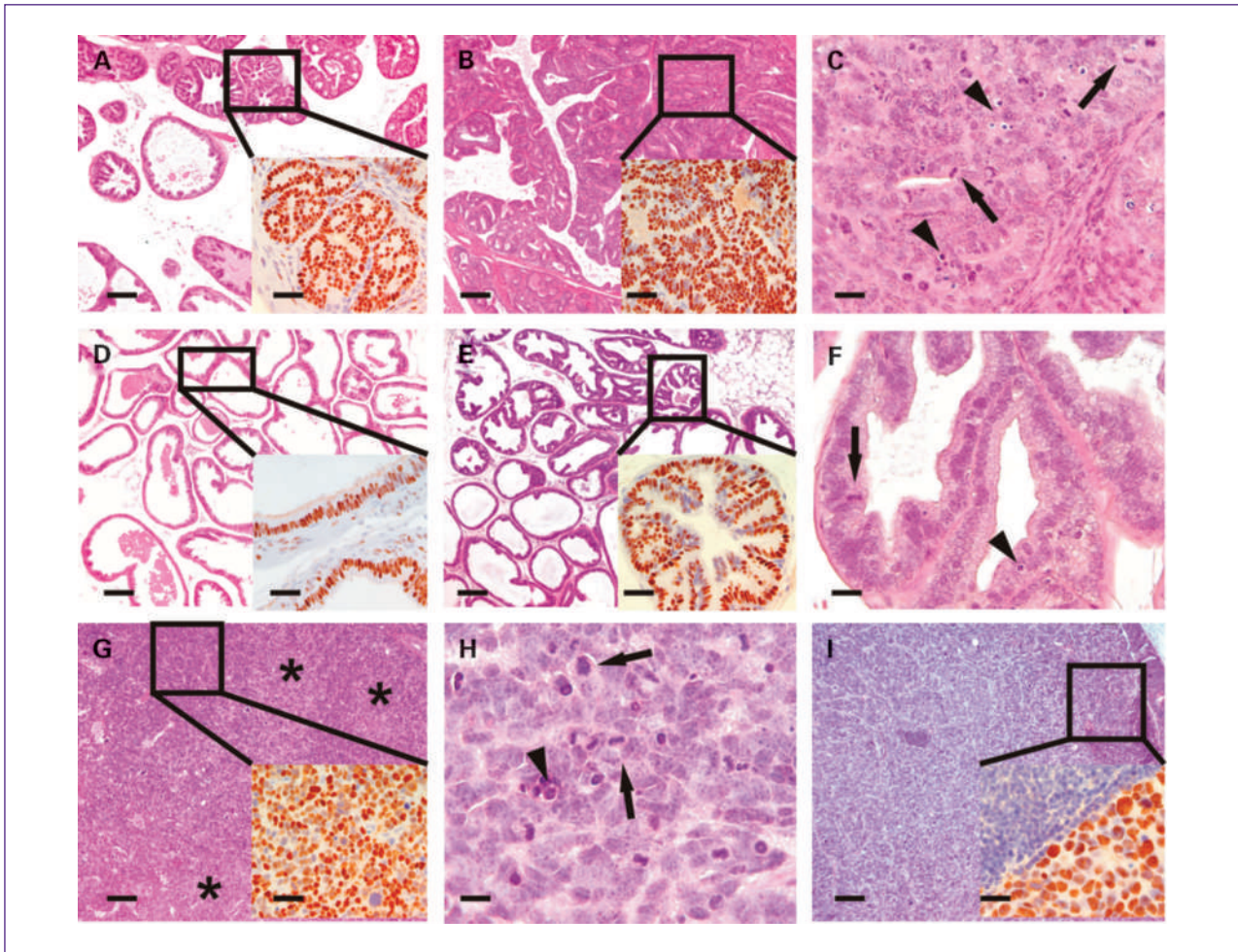


Fig. 2. H&E (A-I) and Tag-SV40 immunostaining (inserts) of prostate tissue from CD and TD mice at 12 weeks of age. Prostate of CD mice (A) shows large foci of PIN, which progress to diffuse adenocarcinoma at 25 weeks (B). Higher magnification of adenocarcinoma (C) showing loss of intraluminal spaces, crowded gland-like tumoral cords, numerous atypical mitoses (arrows), and apoptoses (arrowheads). In TD-treated mice at 12 weeks of age (D), there are few PIN foci in normal prostatic tissue; PIN is the most present lesion until 25 weeks (E), and adenocarcinoma lesions are minimal. F, higher magnification of PIN in tomato-treated mouse prostate: one or two layers of cells containing few mitoses (arrow) and apoptoses (arrowhead) line gland luminal spaces. G, representative image of PD tumor from control and tomato-treated groups showing diffuse sheets of cells with large necrotic areas (*), without remnants of glands. H, higher magnification of PD tumor: chaotic arrangement of anaplastic tumor cells, aberrant mitoses (arrows), and apoptoses (arrowhead). I, periaortic lymph node metastasis from PD prostatic carcinoma: tumor cells are clearly distinct from residual normal lymphoid tissue. Tag-SV40 transgene expression is constant throughout TRAMP mouse prostate cancer progression from PIN to adenocarcinoma (inserts in A, B, D, E). Tag-SV40 is also widely expressed in both primary and metastatic PD tumor (inserts in G and I) in both control and tomato-treated animals (A, B, D, E, G, I; bar, 140 μm ; C, F, H; bar, 50 μm ; inserts: bar, 90 μm).

area with normal histology was greater in the TD mice (Fig. 1B, bottom) than in the CD mice (Fig. 1B, top; $96.1 \pm 3.1\%$ versus $58.0 \pm 5.6\%$; $P < 0.01$ two-tailed Mann-Whitney U test). In addition, TD mice displayed a significantly smaller area covered by PIN lesions and no adenocarcinoma (Figs. 1B, bottom, and 2A and D). TD also had a striking effect on adenocarcinoma: adenocarcinoma involved $64.2 \pm 4.2\%$ (CD mice) versus $3.0 \pm 2.3\%$ (TD mice) of the dorsolateral prostate ($P < 0.01$) at 20 weeks, and $79.2 \pm 5.7\%$ versus $10.0 \pm 3.9\%$ at 25 weeks (Figs. 1B and 2B, C, E, and F; $P < 0.01$).

At 25 weeks, neoplasia (i.e., areas with PIN and adenocarcinoma) occupied 92.8% of the CD mouse prostate

and only 59.4% of the TD mouse prostate. Also in TD mice, 50% of the prostatic tissue was replaced by PIN and 9.4% by adenocarcinoma, compared with 13.6% PIN and 79.2% adenocarcinoma in the CD mice. The histopathologic picture of the CD mice also included PD tumors in one of five mice (4.2 mm^3) and three of five mice (113 , 376 , and 402 mm^3 at 20 and 25 weeks of age, respectively). By contrast, a small PD tumor (4.2 mm^3) was observed at 25 weeks in only one TD mouse (Fig. 2G-I).

Serum biomarkers. The proteins quantitated with multiplexed immunoassay included several molecules widely recognized as significant prostate carcinogenesis biomarkers. The influence of TD on these serum biomarkers is

shown in Fig. 3. TD mice displayed lower serum vascular endothelial growth factor (VEGF) concentrations than CD mice ($P < 0.0001$, ANOVA). A significant effect of time (weeks of age; $P = 0.048$), as well as a significant interaction "weeks \times treatment" ($P = 0.036$), were also observed. Post hoc comparisons with Bonferroni's t test for pairwise multiple comparisons showed that TD significantly decreased serum VEGF at 12 and 25 weeks ($P < 0.05$ and $P < 0.001$, respectively), and also modulated the concentrations of several angiogenic/inflammatory chemokines. TD mice sera had significantly lower concentrations of the angiogenic KC/GRO- α , MIP-2/GRO- β , and MIP-1 β (the angiogenic CCL3 chemokine) than CD mice. No differences were found for interleukin-8 (IL-8), MIP-1 α , MIP-1 γ , and MIP-3 β serum concentrations. The peak level of KC/GRO- α , observed at 12 weeks in CD mice, was completely abolished by TD. Similarly, peak levels of MIP-1 β and MIP-2 recorded at 20 weeks in CD mice were not present in TD mice. Although less impressive in terms of magnitude, a significantly lower concentration of the angiostatic IP-10 chemokine was observed in TD mice at 25 weeks (Supplementary Fig. S3A).

TD markedly affected serum concentrations of many IL-6-type cytokines, namely IL-6 itself, oncostatin M, and IL-11 (Fig. 3), but not leukemia inhibitory factor. In CD mice, IL-6 concentration peaked at 25 weeks (as in the case of VEGF), and IL-11 and oncostatin M at 20 weeks. In TD mice these cytokines were undetectable or barely detectable at 12 weeks, and their concentration curves were flattened at significantly lower levels. TD was also significantly effective in lowering fibroblast growth factor-9 (FGF-9) and IL-7 (Fig. 3) from the 12th to the 25th week. As shown in the supplementary material, TD lowered the serum concentrations of IL-10 (20-30 weeks), TNF- α (Supplementary Fig. S3A), SCF (20-25 weeks), MMP-9 (20 weeks), and MCP-1 (25 weeks; Supplementary Fig. S3B). Effects on IL-1 α and IL-1 β were only observed at the latest times (from the 25th to the 33rd weeks; Supplementary Fig. S3C).

TD had no significant effect on the concentrations of epidermal growth factor receptor, endothelin-1, FGF-basic, glutathione S-transferase, interleukins (from IL-2 to IL-5, IL-12, and IL-17), RANTES, tissue inhibitor of

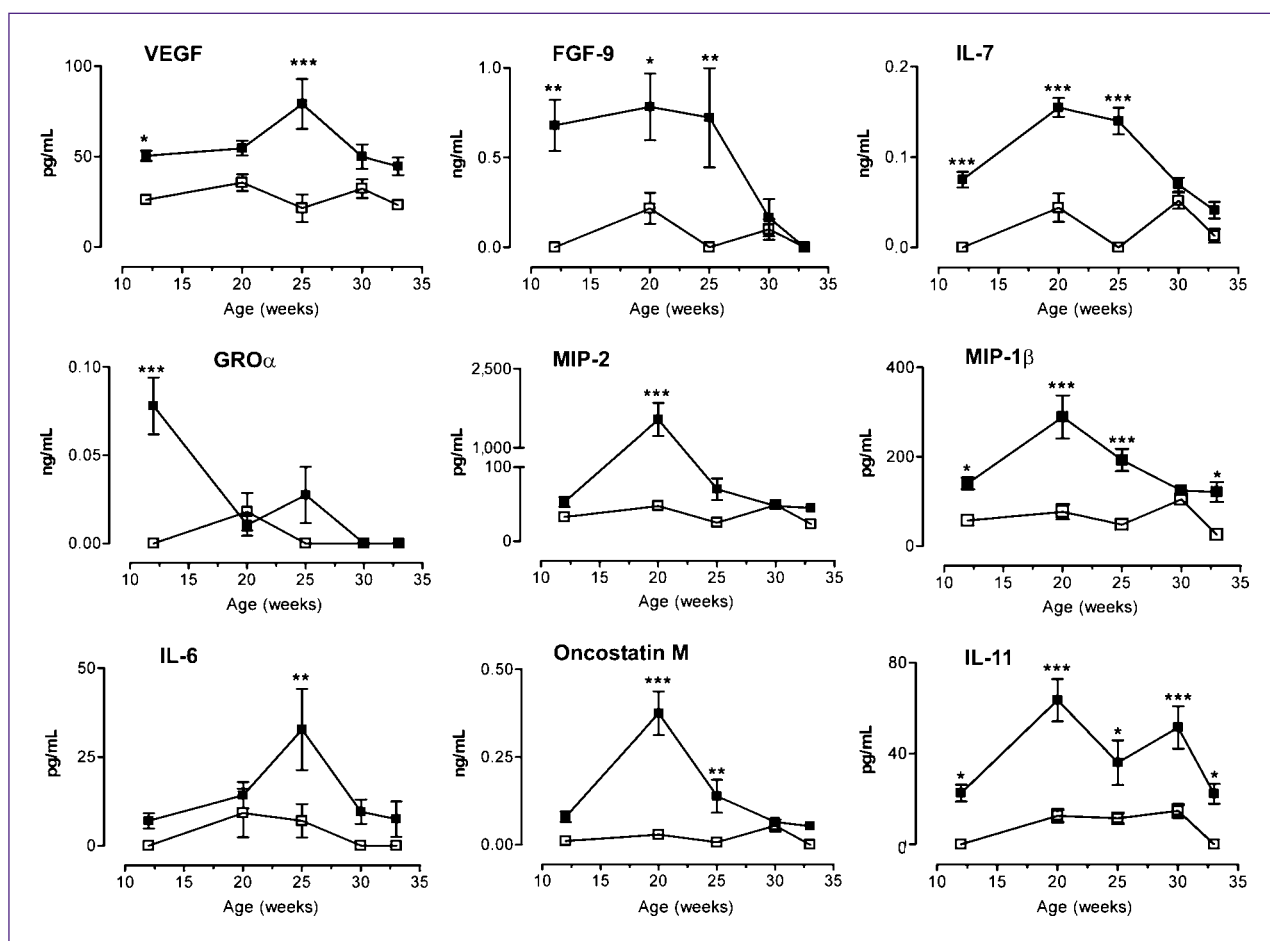
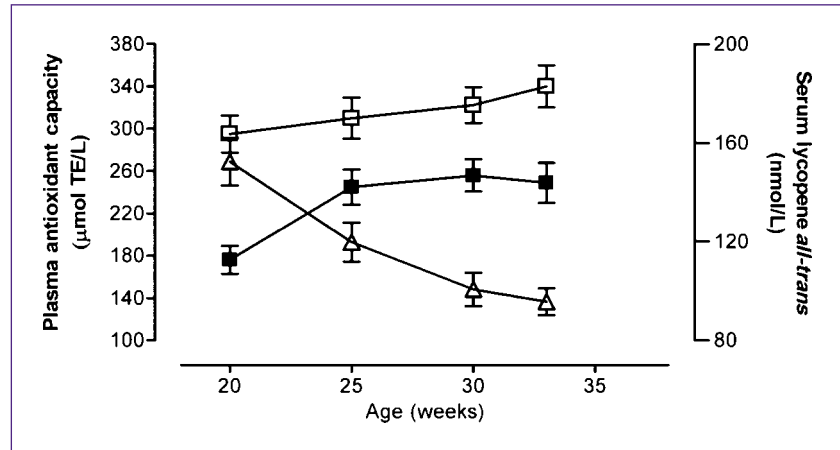


Fig. 3. Dietary treatments influenced serum levels of several proteins related to prostate carcinogenesis. Values represent mean \pm SD ($n = 5$). P values (ANOVA) for each marker are reported. P values for post hoc comparisons (Bonferroni's t test for pairwise multiple comparisons) are also shown: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. ■, CD; □, TD.

Fig. 4. Plasma antioxidant capacity in CD and TD mice. Serum lycopene *all-trans* concentration is only shown for the TD group because it is undetectable in CD mice. Data are expressed as mean \pm SEM ($n = 5$).



metalloproteinase-1, and the other proteins listed in Supplementary Table S3.

Carotenoids, flavonoids, and antioxidant activity in blood samples. *All-trans* lycopene was the only serum carotenoid. Its time course concentration is only illustrated (Fig. 4) for TD mice because it was not detectable in CD mice. Flavonoids were only detected in low amounts. Rutin traces below the quantification threshold were present at 20 and 25 weeks, whereas a concentration of 4.10 nmol/L was found at 30 and 33 weeks. Traces of isohorramnetin 3-glucuronide were only present at 30 and 33 weeks. The antioxidant activity of these serum samples is illustrated in Fig. 4.

Post hoc comparison with Bonferroni's *t* test for pairwise multiple comparison showed that TD significantly increased the antioxidant activity of blood ($P < 0.0001$, $P < 0.05$, $P < 0.05$, and $P < 0.01$ at 20, 25, 30, and 33 weeks, respectively).

SV40 Tag antigen expression in the dorsolateral prostate

A major concern was that the preventive effect of TD might be due to direct suppression of the probasin promoter, resulting in reduced expression of the Tag transgene. As shown by immunohistochemistry (Fig. 2, inserts in A, B, D, E, G, and I), the Tag oncoprotein was expressed in the prostates of both CD and TD mice. Tag was also present in primary and metastatic PD. These observations suggested that the mechanism of tomato action against prostate cancer is not related to Tag expression but to direct suppression of carcinogenesis.

Discussion

TRAMP mice on the TD survived longer than the CD controls and displayed a lower incidence of PD carcinoma. This is the first indication that whole-tomato supplementation improves TRAMP mouse survival.

This improvement (Fig. 1A) was probably due to the ability of whole tomato to modulate both the tumor bur-

den, leading to urethral compression, uroastasis, and renal failure, and systemic metastasis. TD, in fact, reduced adenocarcinoma and phyllode tumor growth, as well as the incidence of metastasis from the AR-negative PD tumor. As already mentioned, this is the main "lethal phenotype" in TRAMP mice due to both its rapid growth and consequent acute renal damage by compression, and as a source of distant metastases and systemic cachexia (18–20).

Our TD was specifically elaborated to have a high content and bioavailability of all bioactive tomato compounds. Prolonged heating of whole tomatoes, including their seeds and skins, maximized the concentration of flavonoids and carotenoids (21) and promoted the formation of ketosamine (14).

The serum lycopene concentration in the 20-week-old TD mice (150 nmol/L) was similar to that in rats on a 25% enriched TD (176 nmol/L; reviewed in ref. 24). Its decrease to 90 nmol/L at the 33rd week (Fig. 4) may have been due to disease-related factors, such as androgen status, oxidative stress, and aging (15).

Reduced feed intake was suggested as a cause of diminished serum lycopene levels in Dunning prostate cancer rats on a diet supplemented with lycopene beadlets (25). This seems unlikely in our TRAMP model because the body weights of the TD and CD mice were similar. As already mentioned, lower lycopene concentrations may have been due to the oxidative stress accompanying cancer progression, as recently shown in the TRAMP model (26). Suppression of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase, heme oxygenase-1, and phase II detoxifying enzymes) may provoke the *in vivo* "consumption" of lycopene, and hence the formation of potentially bioactive oxidation and/or unselectively cleaved products, namely "lycopenoids" (27, 28). These compounds, together with the flavonoids (rutin and isohorramnetin 3-glucuronide) found in sera of TD-fed mice, may activate the antioxidant responsive elements and thus both ameliorate the oxidative status of TD mice and contribute to the antitumoral activity of TD (28, 29). This suggestion

is in line with the higher antioxidant capacity of TD mouse plasma.

Chronic inflammation/angiogenesis is also of importance in both mouse and human prostate cancer development and progression (30, 31). This article is the first report of a significant reduction of the serum levels of several proinflammatory/proangiogenic molecules of significance in prostate carcinogenesis following tomato treatment. According to a recent hypothesis for inflammation-induced prostate carcinogenesis, activated prostate epithelial stem cells acquire a survival advantage by expressing one or more of the same cytokines, such as IL-6. Establishment of one or more autocrine signaling loops expands these cells in the absence of inflammation as a potential first tumor development stage (32). The highly bioavailable cocktail of tomato phytochemicals may thus act pleiotropically at different levels. TD chemoprevention, in fact, was accompanied by a significant systemic reduction of proinflammatory/proangiogenic factors. Several factors were reduced at 12 weeks, before tumor formation, and hence were probably involved in the chemoprevention mechanisms of TD (33).

VEGF is highly correlated with angiogenesis and metastasization in both men and TRAMP mice (34–36). It was inhibited by TD, which delayed the angiogenetic switches needed for the development of PIN and PD tumors (35). In addition, TD selectively reduced the concentrations of other proangiogenic/proinflammatory chemokines such as KC/GRO- α , MIP-2/GRO- β , and MIP-1 β , but not those of MIB-1 β , MIB-1 γ , and MIB-3 β . Increased production of angiogenic chemokines by prostate tumor cell lines and in prostate cancer patients has been documented. Furthermore, prostate cancer xenograft growth is inhibited by antibody neutralization of KC/GRO- α (CXCL1; ref. 37).

Prostate tumors from TRAMP mice also had higher mRNA for MIP-2 than noncancerous prostate tissues. This alteration was associated with increased activation of the NF- κ B transcription factor, which regulates the expression of angiogenic CXC chemokines (38). MIP-1 induced vigorous migratory responses in DU-145 prostate cancer cells (39) and inhibits apoptosis (40).

The TD-dependent reduction of IL-7 concentrations may also result in an environment less favorable for cancer development: IL-7 induces VEGF-independent proliferation of microvascular endothelial cells (41). TD markedly re-

duced serum concentrations of some IL-6-type cytokines, such as IL-6, oncostatin M, and IL-11. These cytokines and their receptors are expressed in the prostate and regulate its growth in an autocrine and paracrine manner (see ref. 42 for review). Because serum IL-6 and IL-11 concentrations increase in patients with metastatic and hormone-refractory prostate cancer (43), measurement of IL-6 concentrations leads to more accurate prediction of disease progression and survival. In addition, IL-6 plays an important role in the transition from an androgen-dependent to an androgen-independent state, promotes neuroendocrine differentiation, and contributes to cachexia in prostate cancer patients (44, 45). Targeting IL-6 has thus been proposed as a potential collateral treatment for prostate cancer.

Downregulation of serum FGF-9 concentration by TD at 12 weeks of age may also contribute to the antineoplastic activity of tomatoes. Prolonged FGF signaling leads to prostate neoplastic transformation and tumor progression through autocrine and paracrine loops (46).

Conclusions

Daily consumption of a tomato-rich diet was highly effective in preventing prostate cancer in TRAMP mice.

In addition to its direct effects on tumor cells, tomato, a functional food containing a mixture of pleiotropic compounds (47), can be regarded as a biological response modifier whose establishment of an anti-inflammatory and antiangiogenic environment prevents tumor onset and progression.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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