A 10% Tomato Diet Selectively Reduces Radiation-Induced Damage in TRAMP Mice

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ABSTRACT

Background: Tomatoes contain carotenoids that have the potential to alter the effects of external beam radiation therapy (EBRT).

Objectives: We hypothesized that dietary lyophilized tomato paste (TP) would reduce apoptosis within carotenoid-containing nonneoplastic tissues in EBRT-treated TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) mice.

Methods: Male TRAMP mice (n = 73) were provided an AIN-93G diet or a modified AIN-93G diet containing 10% TP (wt:wt) at 4 wk of age. Prostate tumor growth was monitored by ultrasound. The caudal half of the mouse was irradiated with 7.5 Gy (Rad) or 0 Gy (sham) at 24 wk of age or after the tumor volume exceeded 1000 mm³ with a Cobalt-60 source. Mice were euthanized 24 h postradiation. Carotenoids and α-tocopherol were measured by HPLC and compared by a t test. Tissues were assessed for radiation-induced changes (hematoxylin and eosin) and apoptosis [cleaved caspase-3 (CC3)] and compared by Kruskal–Wallis test or Freedman–Lane’s permutation test.

Results: Serum concentrations of lycopene (52% lower), phytoene (26% lower), and α-tocopherol (22% lower) were decreased in TP-fed irradiated mice (TP-Rad) compared with TP-fed sham mice (P < 0.05). CC3 scores increased within the prostate tumor with radiation treatments (P < 0.05), but were not affected by tomato consumption. In nonneoplastic tissues, TP-Rad had a lower percentage of CC3-positive cells within the cranial (67% lower) and caudal (75% lower) duodenum than irradiated mice on the control diet (Rad) (P < 0.005). Likewise, CC3 scores within the dorsolateral prostate of TP-Rad trended toward lower scores than for Rad (P = 0.07).

Conclusions: TP selectively reduces radiation-induced apoptosis in extratumoral tissues without decreasing radiation-induced apoptosis within the prostate tumor in TRAMP mice. Additional studies are needed to confirm and expand upon these findings. J Nutr 2021;00:1–10.

Keywords: tomato, carotenoids, prostate cancer, radiation, TRAMP

Introduction

Prostate cancer (PCa) is the second leading cause of cancer-related deaths among men in Western countries (1). External beam radiation therapy (EBRT) is one of the most common treatment approaches for PCa (2–4). Despite improvements to treatment, ~50%–60% of men who receive EBRT develop biochemical recurrence (5, 6). In clinical practice, the EBRT is limited by potential damage to the surrounding (nonneoplastic) tissues. Acute and chronic toxic effects such as bowel and sexual dysfunction (7) constrain the clinical tolerability of EBRT.

After PCa diagnosis, patients often seek information about food and supplements that may improve their response to therapies, quality of life, and survival. Tomatoes and lycopene are 2 of the most frequently mentioned foods or supplements by social media, lay press, and purveyors of alternative therapy as having a protective effect against PCa development and progression. Tomato and lycopene consumption has been associated with decreased risk of PCa in epidemiological studies (8, 9). Lycopene, in addition to other tomato bioactives, may decrease PCa tumor progression by modifying inflammatory status (10, 11), androgen and growth factor signaling (12), apoptosis (11,13,14), and cell cycle progression (11, 13, 14).

Lycopene and other tomato carotenoids are potent antioxidants that may improve natural defenses and scavenge free radicals generated during radiation therapy. Antioxidants may quench singlet molecular oxygen [reactive oxygen species (ROS)], suppress the highly proinflammatory environment after EBRT, and potentially alter apoptosis. Improving antioxidant status in normal tissues is radioprotective and may lead to fewer adverse events owing to decreased oxidative damage from
radiation therapy (15, 16). Although results suggest that tomato products may provide a benefit to patients who are diagnosed with PCa, the lack of preclinical and clinical data for men undergoing EBRT prevents more definitive trials from occurring and remains a gap in the literature.

The objective of this study was to determine the extent to which tomato feeding decreased apoptosis and cell death within tissues (neoplastic and nonneoplastic) that accumulate carotenoids. We hypothesized that carotenoids would reduce apoptosis within irradiated nonneoplastic tissues. To evaluate this hypothesis, we conducted 2 studies in the TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) model. The first study was a pilot study that established the timing of radiation that would be used in the dietary study. The second study evaluated whether lifelong tomato powder consumption decreased radiation-induced damage (apoptosis and inflammation) in prostate tumors and associated surrounding tissues. Tomato or lycopene consumption has not been previously evaluated as a method of reducing radiation-induced damage (inflammation or apoptosis) in prostatic tumors and surrounding organs, to our knowledge.

Methods

Diet

Tomato paste (TP) (Contadina®) was purchased from a local supermarket in September 2014, July 2015, and April 2016 and lyophilized in a VirTis Freezemobile 12SL/Unitop 600 SL freeze dryer (SP Scientific). Lyophilized TP was ground into a fine powder using a tabletop food processor, transferred to resealable gallon bags (air removed), and kept in the dark at −20 °C to preserve the carotenoids and other nutrients in the TP (17, 18).

Two experimental diets were used: a powdered, AIN-93G-based control diet and the same diet modified to contain 10% (wt:wt) lyophilized TP. Proximate analysis was performed on the 100% TP powder and diet formulas were balanced for total energy, carbohydrates, protein, fat, fiber, and moisture. AIN-93G vitamin and mineral mixes were provided to both diets in equal proportions. Supplemental Table 1 describes the composition of the control and tomato diets. Ingredients were mixed using a commercial mixer (Hobart). New diets were formulated every 2 mo. Carotenoid and α-tocopherol contents of each batch were analyzed by HPLC.

Mouse breeding, genotyping, and housing

The University of Illinois Laboratory Animal Care and Use Committee approved all experimental procedures (Protocol 18029). Male C57BL/6-Tg(TRAMP)8247Ng/J (C57BL/6 TRAMP +/+), female C57BL/6j, and female FVB/NJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Breeding and genotyping of these mice were conducted as previously described (18). Males carrying the probasin:SV40-Tag transgene (TRAMP mice) were weaned at 3 wk of age and enrolled into the study via rolling admission. Mice were housed under controlled conditions (12-h light/dark cycle, 22 °C, 55% humidity), weighed weekly, and diet was added 3 times/wk.

Pilot study

TRAMP mice were randomly assigned onto an AIN-93G control diet (n = 18) or 10% tomato powder diet (n = 20) at 4 wk of age. TP was not evaluated in the pilot study. Beginning at 10 wk of age, weekly in vivo ultrasound imaging was used for longitudinal tumor screening and tumor volume measurement. Ultrasound screening of TRAMP prostates with a Vevo 2100 preclinical ultrasonic imaging platform (VisualSonics, Inc.) has been previously described (18). Serial 2D image slices were used to generate prostate or tumor volume estimates as previously described (19).

Mice were irradiated with 7.5 Gy γ radiation by a Cobalt-60 source at a dose rate of 0.22 Gy/min (Theratron-780 Isocentric teletherapy, Theratronico®) (n = 12) or 0 Gy [sham treatment (Sham), n = 6] at 24 wk of age if a tumor mass was not detected or after the tumor grew to a specific size (500 mm3). The radiation was collimated to protect the cranial half of the mouse. Inhalation isoflurane was used for general anesthesia. Sham-treated mice underwent the same procedure as irradiated mice (including inhalation anesthesia) but received no radiation. A radiation dose of 7.5 Gy of radiation was delivered to PCa-bearing mice. The radiation dose was calculated using the linear-quadratic model to generate a radiation dose iso-effective to 25% of a human’s hypofractionated total dose used for the management of PCa (20–23).

Tomolarminosis dosimeters (University of Wisconsin Radiation Calibration Laboratory, Madison, WI) were placed under the caudal and cranial halves of the mouse and were used to confirm the delivered radiation dose (Supplemental Figure 1). Mice were euthanized 0 (sham mice), 24, or 72 h postradiation (n = 6/time point). Figure 1 shows study designs for the pilot and diet studies.

FIGURE 1 Study design. TRAMP mice were randomly assigned to dietary treatment groups after weaning (4 wk). Prostates were monitored weekly (pilot study; A) or biweekly (diet study; B) for tumor occurrence by ultrasound beginning at 10 wk of age. After tumor detection, mice were scanned weekly by ultrasound until tumors reached a volume of 500 mm3 (pilot study) or 1000 mm3 (diet study). At this volume, the caudal half of the animal was irradiated with 7.5 Gy by a Co-60 source. Mice were euthanized 24 or 72 h after radiation or after a sham treatment. TRAMP TRansgenic Adenocarcinoma of the Mouse Prostate.

The authors reported no funding received for this study. Author disclosures: the authors report no conflicts of interest.
Diet study
The diet study was designed as a 2 x 2 factorial design with diet and radiation treatment as the main variables. Male TRAMP mice were randomly assigned to consume control diet (n = 35) or 10% TP (n = 38). Power analyses (power = 0.80 and α = 0.05) indicated that 15 animals/group would be sufficient to detect a 40% change in oxidative damage in nontumor tissues (24).

Histopathology and immunohistochemistry
Tissues selected for evaluation included prostate tumors, adjacent prostate lobes (dorsolateral, ventral, and anterior lobes), visibly enlarged abdominal lymph nodes (e.g., those containing metastases), urethra, bladder, kidney, liver, lung, and small intestine. Tissues were dehydrated and embedded in paraffin. Tissues were sectioned at 4-µm thickness for staining with hematoxylin and eosin (H&E). A board-certified veterinary pathologist (MAW) evaluated the tissues in a blinded fashion to identify morphological changes including cell death (apoptosis and necrosis), edema, and inflammation (or infiltration by scavenger macrophages). The pathologist also evaluated the occurrence and severity of neoplasia in prostate and tumor sections in a blinded fashion as previously described (26). Metastases were confirmed by H&E and SV-40 staining. Cell death scores (apoptosis and necrosis combined) were evaluated within the PCa tumor and selected adjacent tissues (urethra, bladder, small intestine, and liver) in blinded fashion, using a semiquantitative scoring scale (Supplemental Appendix 1). Apoptosis was evaluated by cleaved caspase-3 (CC3) immunohistochemical evaluation (Cell Signaling Technology) stained slides. Diffuse pale brown to dark brown cytoplasmic staining was considered positive. Within the prostate and tumor, CC3 was evaluated using a semiquantitative scale (Supplemental Appendix 2). The entire small intestine was evaluated for a global estimation of apoptosis at a low magnification in 100-crypt segments as previously validated (27). The number of CC3-positive cells in each segment was counted and averaged for each section of the small intestine.

Necropsy
TRAMP mice were exsanguinated by cardiac puncture under deep anesthesia followed by cervical dislocation. When possible, the prostate was dissected into individual lobes (anterior, dorsal, lateral, and ventral). Suspected malignant prostate masses (tumors) were dissected from the remaining prostate. Sections of individual prostate lobes, malignant prostate tumors, seminal vesicles, liver, lungs, and epididymal adipose tissue were weighed, snap frozen in liquid N2, and stored at −80°C for future analysis. Sections of the prostate tumors, adjacent prostatic lobes (dorsolateral, ventral, and anterior lobes), visibly enlarged abdominal lymph nodes (e.g., those containing metastases), urethra, bladder, kidney, liver, lung, and small intestine were fixed in 10% neutral buffered formalin for no more than 48 h and stored at −80°C in paraffin. Tissues were sectioned (μm thickness for staining with hematoxylin and eosin (H&E).

Antioxidant analysis
Diet and tissue concentrations of carotenoids and α-tocopherol were extracted and analyzed by HPLC as previously described (28, 29). Approximately 25 mg diet, 300 mg tumor tissue, 200 μL serum, and 100 mg liver tissue were used for analysis. Anterior prostates from 10 mice/treatment condition (1 lobe/mouse) were pooled into 2 individual replicates (n = 5 replicate).

Cytokine analysis
Serum samples were stored at −80°C until the day of analysis. Serum concentrations of IL-1β, IFN-γ, TNF-α, IL-6, IL-10, and IL-17a were measured using a Bio-Plex multiplex assay (Bio-Rad) as previously described (30). Serum concentrations of C-reactive protein (CRP) were assessed by a commercial ELISA kit following the manufacturer’s instructions (Abcam). Portions of the liver, epididymal adipose tissue, anterior prostate, and prostate tumor were homogenized in PBS and frozen at −20°C overnight. Tissue samples were thawed and centrifuged at 4°C to 2000 x g for 15 min. TNF-α was quantified in the resultant tissue-derived supernatant by an ELISA following the manufacturer’s instructions (Thermo Fisher Scientific).

Statistical analysis
Statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc.), GraphPad Prism 8.4 for Windows (GraphPad Software), and RStudio Version 1.4.1103 (RStudio). Tissue weights, serum cytokines, and tissue cytokines were compared using 1- or 2-factor ANOVA with multiple-comparison adjustments by Tukey’s method. Data were log transformed when assumptions of normality were not met. Histological scores for prostate tumor, nonprostatic prostate lobes, lungs, liver, kidney, urethra, and regional lymph nodes were assessed using a Kruksal-Wallis test with multiple comparisons by Dunn’s test. Histology scores for the small intestine were evaluated using a randomized permutation test for interaction effects using Freedman and Lane’s approach with the package Permuno (31). Tissue accumulation of carotenoids was evaluated using a t-2ailed t test. Unless otherwise stated, a P value < 0.05 was considered statistically significant. All values are reported as mean ± SEM.

Results
Whole-animal and tissue alterations—pilot study
There were no differences between the body weights at euthanasia across the time points that were evaluated (Supplemental Figure 2A, B). In addition, there were no differences in the epididymal adipose tissue, lungs, heart, and total prostate weights (Supplemental Table 2). After radiation treatment (24 or 72 h after radiation), the mean weight of the spleen decreased by 50% (P < 0.001). Mean testicular weight also decreased by 10% after 72 h (P < 0.01). Mean PCa tumor weight was numerically 68% lower than sham-treated mice 72 h postirradiation; however, statistical comparisons were not possible (Supplemental Table 2).

By 24 wk of age, 100% of mice developed histologically confirmed prostate adenocarcinoma (Supplemental Table 3). Malignant masses that extended out of the prostatic envelope were confirmed tumors. Histological evaluation of tumors suggested that radiation increased the median cell death scores within the tumor from 1 (0%–1% cell death) in sham-treated mice to 3 (25%–50% cell death) 24 and 72 h postirradiation (Supplemental Table 4). Statistical evaluation of these scores was not appropriate because of the low number of mice with tumors in the 72-h group (n = 2). No significant lesions or morphological changes were noted in the small intestine, bladder, urethra, prostate, or liver with radiation exposure (data not shown). Serum CRP concentrations were 50% lower than in...
the other treatment conditions 72 h postradiation \((P = 0.005)\) (Supplemental Figure 3A).

**Whole-animal and tissue alterations—diet study**

Unlike the pilot study where 100% of mice developed histologically confirmed prostate adenocarcinoma, 59% of all mice (58% of control-fed and 59% of TP-fed mice) developed PCa by 24 wk (Table 1). There were no differences in mean body weight at euthanasia regardless of diet or treatment condition (Supplemental Figure 2C, D). There were no differences in liver, epididymal adipose tissue, lungs, heart, testes, prostate tumor, individual prostatic lobes, and total prostate weights (Supplemental Table 5). For the spleen, there was a main effect of radiation such that irradiated mice had lower mean spleen weights than sham-treated mice \((P < 0.0001)\).

**Carotenoid and \(\alpha\)-tocopherol content of diet and accumulation in tissues**

Supplemental Table 1 describes the ingredients for each diet. Figure 2 shows the carotenoid composition of the tomato diet, whereas Table 2 shows tissue carotenoid accumulation. Comparisons for carotenoids were only carried out between TP-Sham and TP-Rad owing to the absence of carotenoids in the control diet. Lycopene was the predominant carotenoid \((39 \text{ nmol/g, 21 mg/kg, 49% of total carotenoids})\) in the TP diet. The AIN-93G base for both diets also provided \(~120 \text{ nmol/g (75 IU) \(\alpha\)-tocopherol}. The profile of carotenoids in the liver and serum largely reflected the composition of the tomato diet for sham-treated mice. Radiation decreased serum concentrations of lycopene \((P < 0.0001)\), phytoene \((P < 0.01)\), and \(\alpha\)-tocopherol \((P < 0.01)\) compared with sham-treated mice by 48%, 26%, and 22%, respectively (Table 2). Although serum lycopene and other antioxidants were lower after radiation, tissue concentrations in the liver and prostate were not modified by radiation. Antioxidants in the prostate were not statistically compared; however, mean \(\alpha\)-tocopherol concentrations within the prostate were 42% lower in irradiated mice than in sham-treated mice.

**Morphologic changes in PCa, prostate, and nonprostatic tissues**

There were no significant lesions or morphologic changes, including cell death within the urethra, bladder, small intestine, lungs, or liver, as a result of diet or radiation treatments (data not shown). However, there were substantial foci of necrosis and apoptosis within many prostate tumors that were highly variable in size and distribution across all of the diet and treatment conditions. Often, these foci were in dense clusters with obviously necrotic foci. Within these foci, inflammation contained neutrophils with more subtle infiltration by scavenger macrophages. Both processes were present, with most of the focus being composed of nonidentifiable cell debris, in most foci. Hence, both were combined and scored as “cell death.” Cell death scores within the prostate were between 1.5 and 2.5 for all treatment conditions, indicating that, on average, 10%–25% of most tumors had substantial cell death (Table 3). Neither diet nor treatment altered the overall proportion of cell death within the tumor \((P = 0.33)\).

CC3 expression was used to identify cells in the process of apoptosis. CC3 scores are shown in Tables 4 (prostate tumors and associated nonneoplastic prostate) and 5 (small intestine). CC3 expression was negligible to absent in all other tissues (urethra, bladder, lung, kidney, and liver) regardless of treatment (data not shown). Within the tumor, the median CC3 scores substantially increased in irradiated mice from 1 in Sham mice to 4 in Rad mice (Table 4) \((P < 0.001)\), with >50%
of cells in Rad and TP-Rad tumors apoptotic based on CC3 staining. In nonneoplastic prostate, apoptosis was not modified by treatment or diet within the anterior (P = 0.85) or ventral prostates (P = 0.35). However, the median score for CC3 was altered in the dorsolateral prostate. TP-Sham had lower CC3 scores than Sham and Rad (Table 4) (P < 0.05). Likewise, TP-Rad had a trend toward lower CC3 scores than Rad (P = 0.07) for nonneoplastic dorsolateral prostate. The lateral lobe of the prostate has been reported to have the highest concentration of carotenoids in the rodent prostate (32).

CC3-positive cells within the small intestine (Table 5) were confined almost exclusively to cryptal enterocytes in all segments. In general, sham-treated mice had very low scores for CC3 expression across all segments of the small intestine regardless of diet. Radiation treatment increased the scores in all 3 segments of the small intestine compared with sham-treated animals (P < 0.01). There was a significant effect of diet such that mean scores for CC3-positive cells were lower in TP-fed mice for the cranial duodenum (P = 0.001), the caudal duodenum (P = 0.004), and the jejenum (P = 0.04). There was also a trend for diet to reduce CC3 expression within the ileum (P = 0.061). Likewise, there was a significant interaction between diet and radiation treatment within the cranial duodenum (P = 0.02) and caudal duodenum (P = 0.01). These are the regions of the intestine that absorb carotenoids and other antioxidants (33, 34). Within the duodenums of radiation-treated mice, TP-fed mice had 66.5% fewer CC3-positive cells within the cranial duodenum and 74.7% fewer CC3-positive cells within the caudal duodenum than their radiation-only counterparts. There was no interaction between treatment and diet for the jejunum (P = 0.23) or ileum (P = 0.32). However, concentrations of carotenoids and antioxidants in these segments are generally expected to be lower than in the duodenum (33, 34).

### Inflammatory markers

Four proinflammatory (TNF-α, IFN-γ, IL-17a, and IL-6) cytokines and 1 anti-inflammatory (IL-10) cytokine were measured in the serum. There were no main effects of diet on the measured cytokines. Radiation decreased circulating TNF-α (P < 0.0001), IFN-γ (P < 0.001), IL-6 (P < 0.01), IL-17a (P < 0.01), and IL-10 (P = 0.01) concentrations by 50%, 35%, 35%, 35%, and 22%, respectively (Figure 3A). TNF-α concentrations within the liver (P = 0.30), adipose tissue (P = 0.22), prostate (P = 0.27), and tumor (P = 0.63) were not significantly modified by diet or radiation (Figure 3B). Serum CRP concentrations were not modified by diet (P = 0.24) (Supplemental Figure 3B).

### Discussion

Males who undergo treatment of PCAs often seek information about supplements containing lycopene or tomato components with the goal of improving their therapeutic outcomes. However, to our knowledge no epidemiological or preclinical experiments have evaluated the potential for tomato consumption to modify the PCAs and surrounding adjacent tissue response to therapy. The current study addresses a gap in the literature by evaluating the hypothesis that tomato consumption decreases radiation-induced inflammation and apoptosis after radiation within the prostate tumor and other surrounding organs. To our knowledge, no previous studies have evaluated the interactions between tomato consumption...
and radiation using a preclinical model. We hypothesized that a diet containing lycopene would reduce apoptosis within carotenoid-containing nonneoplastic tissues without decreasing apoptosis within prostate tumors. Data from this study support our hypothesis that tomato consumption during radiation therapy reduces apoptosis in tissues that contain carotenoids without decreasing apoptosis within the prostate tumor.

About two-thirds of radiation-induced damage is caused indirectly through action of ROS (35). The use of antioxidants during radiation therapy is controversial because some have hypothesized that antioxidants might protect the targeted tissue from the effects of radiation (36–39). Tomato consumption increases carotenoid concentrations in the blood and several tissues (including the prostate), which may result in increased antioxidant protection and decreased inflammation (40, 41). It is possible that lycopene and other tomato carotenoids may decrease the efficacy of EBRT by quenching ROS in the tumor before substantial DNA damage occurs (35, 42). However, TP consumption did not decrease apoptosis and cell death scores in irradiated TRAMP prostate tumors, suggesting that TP did not protect prostate tumors in our study.

We found that some nontumor tissues were less susceptible to the harmful effects of EBRT in mice fed TP. This was most notable in the small intestine within the current study. The composition of tumor interstitial fluid is highly heterogeneous (43). Within the prostate tumor, carotenoid concentrations were low (∼50-fold lower than in the liver). This suggests that the prostate tumor is less protected from the effects of radiation partially due to a relatively low concentration of carotenoids that are not evenly distributed through the tumor. Lycopene and other carotenoids have been shown to also reduce angiogenesis within tumors by altering expression of vascular endothelial growth factor (VEGF) and inflammatory markers (44, 45). This would further decrease the ability of the prostate tumor to acquire carotenoids and other antioxidants because these compounds are rapidly quenched during radiolysis.

In the diet study, circulating concentrations of antioxidants (such as lycopene and α-tocopherol) were decreased by 35%–50% 24 h after radiation in TP-Rad compared with TP-Sham. Without an adequate supply of antioxidants from the diet, such as lycopene and α-tocopherol, serum and tissue concentrations would rapidly become depleted, and nonneoplastic tissues could be more heavily damaged through indirect activity of ROS. As injuries in these tissues accumulate, clinical acute and late-stage toxicities may occur leading to digestive, urinary, bowel, or sexual dysfunction (7). Tomato carotenoids are absorbed and accumulate within several tissues (46). TP consumption during radiation (TP-Rad) resulted in lower rates of CC3-positive cells within the cranial (67% lower) and caudal (75% lower) duodenum than for control-fed (Rad) mice (P < 0.05). This decrease suggests that TP consumption can reduce radiation-induced damage within the small intestine, which is a common site for acute and late-stage toxicities (7). Although intestinal carotenoids were not directly measured in the current study owing to a lack of tissue, other studies by our group and others have observed carotenoid accumulation within the small intestine (33, 34). Future studies are needed to evaluate the changes in carotenoid content within the small intestine after EBRT.

Dietary preclinical and clinical studies are critical for men who are being treated with EBRT because many men who are diagnosed with PCa choose to improve their diet after diagnosis. Although consumption of fruits and vegetables generally increases after PCa diagnosis, tomato consumption remains relatively constant (<15% increase) after diagnosis (47). Tomatoes are the second most commonly consumed vegetable in the United States, representing 19% of all vegetable consumption (48). To our knowledge, there is currently only 1 small study in humans (n = 17) that evaluated the interactions between tomato consumption and EBRT (49). Men were supplemented with 0 mL, 118 mL, 237 mL, or 355 mL tomato juice during radiation therapy (49). The goal of that study was to evaluate the tolerance and adverse events associated with tomato supplementation. No adverse events were noted with tomato consumption, indicating its safety. Although the sample size was small, an important finding was that higher serum lycopene concentrations were associated with less cachexia and improved therapeutic outcomes among patients treated with EBRT (49). Larger clinical and preclinical interventional studies are necessary to determine the role that tomato consumption has during EBRT.

The amount of lycopene within the TP diet of the diet study is achievable through the diet for humans. TP consumption in this study resulted in blood lycopene concentrations that are relevant to humans (12, 50–52). The TP diets in this study contained ~3 mg lycopene/kg body weight. This concentration is necessary in a mouse to achieve blood lycopene

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**TABLE 4** CC3 evaluation in TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) tissues (diet study)†

<table>
<thead>
<tr>
<th>Tumor</th>
<th>n</th>
<th>Mean ± SEM</th>
<th>Median ± SE</th>
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<tr>
<td>Sham</td>
<td>9</td>
<td>1.1 ± 0.4</td>
<td>1 ± 0.5 50</td>
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<tr>
<td>TP-Sham</td>
<td>7</td>
<td>2.8 ± 0.5</td>
<td>3 ± 0.5 4</td>
<td></td>
</tr>
<tr>
<td>Rad</td>
<td>5</td>
<td>4.2 ± 0.5</td>
<td>4 ± 0.5 9</td>
<td></td>
</tr>
<tr>
<td>TP-Rad</td>
<td>7</td>
<td>4.0 ± 0.4</td>
<td>5 ± 0.4 4</td>
<td></td>
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<tr>
<td>Dorsolateral prostate</td>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>Sham</td>
<td>5</td>
<td>1.6 ± 0.2</td>
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<tr>
<td>TP-Sham</td>
<td>10</td>
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<td>1 ± 0.2 4</td>
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</tr>
<tr>
<td>Rad</td>
<td>8</td>
<td>1.6 ± 0.2</td>
<td>2 ± 0.2 4</td>
<td></td>
</tr>
<tr>
<td>TP-Rad</td>
<td>9</td>
<td>1.0 ± 0.0</td>
<td>1 ± 0.0 4</td>
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</tr>
<tr>
<td>Ventral prostate</td>
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</tr>
<tr>
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<td>0.5 ± 0.4</td>
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<td>0 ± 0.3 1</td>
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<td>13</td>
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<td>1 ± 0.3 1</td>
<td></td>
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</table>

1Values are means ± SEMs and medians ± SEs of the median. Medians without a common letter differ (Kruskal–Wallis test followed by Dunn’s multiple-comparisons test; P < 0.05). †TP-Rad compared with Rad in the dorsolateral prostate trended toward significance (P = 0.07). A CC3 score of 0 represents no increase over background (0–1 positive cell/lobular profile); a score of 1 represents a focal increase above background (>25% lobular profiles with 2–5 positive cells/profile); a score of 2 represents “widespread” increases above background (>25% lobular profiles with >5–25 positive cells/profile); a score of 3 represents a “massive” increase above background (>25% lobular profiles with >6 positive cells/profile); a score of 4 represents 50% of lobules affected, with >50% having 6 to >10 positive cells, generally associated with foci of cell death and occasional aggregates of positive cells; and a score of 5 represents 50% of lobules affected, with >50% having >10 cells/lobule centered around the edges of foci of cell death plus scattered aggregates of positive cells. CC3, cleaved caspase-3; Rad, mice that received 75 Gy of radiation; Sham, mice that received 0 Gy of radiation; TP-Rad, mice that were fed 10% lycophized tomato powder and received 75 Gy of radiation; TP-Sham, mice that were fed 10% lycopene tomato powder and received 0 Gy of radiation.
concentrations similar to those of humans owing to the poor absorption of carotenoids in rodents (53). The ranges of blood lycopene concentrations found in this and similar studies correlate well with blood concentrations in American men over the ranges that are associated with a significant reduction in the risk of lethal PCa in the Health Professionals Follow-Up Study (HPFS) prospective cohort trial and other studies (45, 54, 55). This could be achieved with a half-serving of tomato sauce (1/4 cup, 60 g) per day. Lycopene blood concentrations found in this and similar studies correlate well with blood concentrations in American men over the ranges that are associated with a significant reduction in the risk of lethal PCa in the Health Professionals Follow-Up Study (HPFS) prospective cohort trial and other studies (45, 54, 55). 

Among the possible animal models available, we selected the TRAMP model. This model is well established and exhibits a predictable histological progression from low-grade hyperplasia to poorly differentiated adenocarcinoma and local as well as distant metastasis, resembling development of the human PCa (29, 52, 62). Neuroendocrine tumors arise in a high proportion of the prostate, and prostate lycopene concentrations are positively correlated with toxicities occur weeks after the onset of the treatment (63, 64). A single radiation dose of 7.5 Gy was used. Many acute toxicities occurred after 72 h. Cell death scores remained similar at 24 and 72 h postradiation. CC3 signaling cascades begin within hours after radiation exposure (27), and secondary necrotic/phagocytic programs are initiated soon after. By 72 h, we hypothesized that most acute apoptosis was beginning to resolve. Therefore, endpoints were evaluated 24 h postradiation to evaluate the acute effects of TP consumption. In the diet study, TP provided a bioavailable vehicle for multiple bioactive compounds; TP is readily available, convenient, and inexpensive for consumers. Preliminary data in humans also indicate that TP supplementation during EBRT is well tolerated with no notable side effects (49).

These studies were informed by the outcomes of a pilot study. Within the pilot study, dosimetry confirmed the dose of radiation received, and radiation-sensitive tissues (e.g., spleen and testes) rapidly decreased in weight postradiation. Serum CRP concentrations and tumor weights appeared to also decrease after 72 h. Cell death scores remained similar at 24 and 72 h postradiation. CC3 signaling cascades begin within hours after radiation exposure (27), and secondary necrotic/phagocytic programs are initiated soon after. By 72 h, we hypothesized that most acute apoptosis was beginning to resolve. Therefore, endpoints were evaluated 24 h postradiation to evaluate the acute effects of TP consumption. In the diet study, TP provided a bioavailable vehicle for multiple bioactive compounds; TP is thermally processed and contains dietary lipids that can enhance the absorption of carotenoids. TP is readily available, convenient, and inexpensive for consumers. Preliminary data in humans also indicate that TP supplementation during EBRT is well tolerated with no notable side effects (49).

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within the diet study than in the pilot study. Although we have observed similar rates of cancer incidence in previous studies (51, 52), the median values for cell death scores were very similar. We do not believe the lower incidence in the diet study influenced any of the results. In addition, this study only tested a single concentration of dietary TP throughout the life span. These results are more generalizable to a man who consumes TP through his lifespan rather than one that changes his dietary pattern after PCA diagnosis. Although multiple doses were not evaluated, men accumulate lycopene in the prostate with moderate doses and short periods of tomato consumption (62). As previously mentioned, 1 small study found that higher serum lycopene concentrations were associated with less cachexia and improved therapeutic outcomes among patients treated with EBRT (49). Future studies should evaluate the effectiveness of consuming TP at the time of diagnosis (tumor detection) to determine if TP is effective as a dietary therapeutic intervention.

To our knowledge, no other preclinical studies have evaluated the potential for tomato consumption to alter the response to EBRT within the tumor and within the surrounding tissues. This work provides preliminary data that tomato consumption with radiation therapy does not diminish the damage occurring within prostate tumors. Importantly, these data suggest that TP modifies the detrimental effects of radiation in other tissues (especially the small intestine). Reduced damage in nonneoplastic tissues reduces the number of adverse effects that may be experienced by the patient. Future preclinical studies should evaluate the potential for dietary tomatoes and lycopene to alter apoptosis with multiple doses of radiation. Future clinical studies should also evaluate the concentration of lycopene in the blood to determine whether increased circulating lycopene correlates positively or negatively with treatment-related outcomes.

Acknowledgments
We thank Molly Black and Patrick McQueen for overseeing animal breeding and colony maintenance; vivarium staff for providing high-quality animal care; Renee Walker for histocchemistry and immunohistochemistry work; Stoyan Toshkov for assistance acquiring lead shielding; Jesus Sarol and Amandeep Kaur from the Illinois Biostatistics, Epidemiology, and Research Design (BERD) core for assistance with the statistical evaluation of the data; and Elizabeth Jeffery for providing access to freeze-drying equipment. The authors’ responsibilities were as follows—JLR, KAS, TMF, RJM, WDO, and JWE: designed the research; JLR, MAW, and KAS: conducted the research; JLR: analyzed the data, performed the statistical analysis, and wrote the paper; JLR and JWE: had primary responsibility for the final content; and all authors: read and approved the final manuscript.

References


43. Simone CB, 2nd, Simone NL, Simone V, Simone CB. Antioxidants and other nutrients do not interfere with chemotherapy or radiation therapy and can increase kill and increase survival, part 1. Altern Ther Health Med 2007;13:22–8.


