Research Article

Cancer Prevention Research

The Interactions of Dietary Tomato Powder and Soy Germ on Prostate Carcinogenesis in the TRAMP Model

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Abstract

The interactions between bioactive-rich food components within a complex human diet for the inhibition of prostate carcinogenesis are largely unknown and difficult to quantify in humans. Tomato and soy products have each shown anti-prostate cancer (PCa) activity in laboratory studies. The objective of this study was to determine the efficacy of dietary tomato and soy germ, alone and in combination, for the inhibition of PCa in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. At 4 weeks of age, male C57BL/6 × FVB TRAMP mice ($n = 119$) were randomized to consume: AIN-93G control, 10% whole tomato powder (TP), 2% soy germ powder (SG), or 10% tomato powder with 2% soy germ powder (TP+SG) for 14 weeks. One hundred percent of mice fed the control diet had PCa, whereas PCa incidence was significantly lower in mice consuming TP (61%, $P < 0.001$), SG (66%, $P < 0.001$), and TP+SG (45%, $P < 0.001$). Although the protection offered by the combination of TP and SG was not synergistic, it was the most effective intervention. TP, SG, and TP+SG increased apoptotic index (AI) and modestly reduced the proliferative index (PI) in the prostate epithelium of TRAMP mice exhibiting primarily prostatic intraepithelial neoplasia. The dramatic reduction in the PI/AI ratio by the dietary interventions suggests that the control mice experience a stronger stimulus for malignant progression in the prostate microenvironment. Maximally effective and safe strategies for PCa prevention may result from optimizing combinations of nutrients and bioactives through an orchestration of dietary patterns. *Cancer Prev Res; 6(6); 548–57. ©2013 AACR.*

Introduction

The combination of pharmacologic agents based upon selection of different mechanisms of action and nonoverlapping toxicity has led to dramatic success in the chemotherapy for certain cancers (1, 2). Yet, these principles have not been effectively applied to translational human cancer prevention studies. Numerous epidemiologic studies suggest that consumption of soy foods or tomato products is associated with a lower risk of prostate cancer (PCa; ref. 3, 4) and this manuscript explores the potential benefit of combining them for PCa prevention. Soy products are a rich source of bioactive components, such as isoflavones, saponins, and lignans. Tomatoes also provide various polyphenols, but are best known as a source of carotenoids such as lycopene and precursors, phytoene and phytofluene (5). Anti-PCa activity of soy or tomato components tested in cell culture or rodent models include protection against oxidative stress, inhibition of proliferation, enhanced sensitivity to apoptotic death signals, inhibition of inflammation and angiogenesis, and disruption of hormonal and growth factor signaling, among others (5–8). Experimental models of prostate tumorigenesis and carcinogenesis suggest that both soy and tomato products and several of their components have significant anticancer bioactivity (8–18).

Our research team is particularly interested in examining the hypothesis that whole foods or novel food products containing a diverse array of bioactive phytochemicals, each individually at modest concentrations, may have greater activity for cancer prevention than any individual component developed as a pharmaceutical chemopreventive agent. For example, we found that tomato powder is more effective than lycopene in reducing testosterone and PCa in rodents (9, 19). Recent *in vitro* evidence suggests that whole soy extract is more effective than individual isoflavones and sera from men consuming tomato paste was more effective than sera from men consuming lycopene alone in inducing cell-cycle arrest and apoptosis in PCa cells (20, 21). People consume complex diets, yet there is limited research on the potential additive or interactive anticancer effects from consumption of multiple foods in combination. The combination of tomato and broccoli was more effective at reducing PCa progression in rats than when these vegetables were consumed individually (9), and the combination of dietary soy and tea, but not soy or tea alone, was effective in reducing inflammation and the development of prostate neoplasms in rats (10). The reductionist approach of testing

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single bioactive components derived from foods is useful in identifying potential anticarcinogenic drugs or to provide insight into mechanisms of action for a food; however, it is increasingly important to investigate whole foods and combinations of foods, which may be more efficacious due to the presence of multiple bioactive compounds with complementary or synergistic activity.

Epidemiologic and laboratory studies support the hypotheses that diets rich in soy or tomato products may reduce the risk of PCa, and the limited, but positive research on the added benefits of whole foods and mixtures of foods suggest that a combination of tomato and soy may be more protective. High compliance for a dietary intervention with the combination of tomato and soy products in a short phase II study in men with active PCa has previously been completed (22), supporting feasibility and justifying future investigations regarding the efficacy of the combination of these foods for biologic activity in the prostate. *In vivo* models provide an opportunity to examine the efficacy of interventions for future translation into clinical prevention studies. The TRAMP (transgenic adenocarcinoma of the mouse prostate) model, which develops a prostate epithelium-specific cancer, is well-characterized and has been used extensively for investigation of dietary and pharmacologic chemoprevention strategies (23). The objective of this study was to test the hypotheses that dietary interventions with tomato or soy germ, alone and in combination, would reduce the incidence of PCa in TRAMP mice.

Materials and Methods

Animals and experimental design

The University of Illinois Laboratory Animal Care Advisory Committee approved all animal procedures. Male C57BL/6 × TRAMP mice, heterozygous for the probasin-Tag transgene, female C57BL/6 and male and female FVB/NJ mice were purchased from The Jackson Laboratory. Female and male heterozygous TRAMP (C57BL/6) mice from our colony were bred with FVB/NJ mice to obtain [TRAMPxFVB/NJ] F_1 offspring used for the study. Mouse tail DNA from male offspring was isolated with an Extract-N-Amp™ Tissue PCR kit (Sigma Aldrich), and mice were genotyped via PCR-based DNA screening. Offspring were weaned at 3 weeks of age, individually housed in shoe-box cages under controlled conditions (12 hours light-dark cycle, 22°C, 60% humidity) and acclimated to a powdered, modified AIN-93G diet for 1 week. At 4 weeks of age, mice were randomized to consume experimental diets: AIN-93G control ($n = 29$), 10% whole tomato powder ($n = 31$; FutureCeuticals), 2% soy germ powder ($n = 32$; Frutarom), and 10% tomato powder with 2% soy germ powder ($n = 27$). Diets were balanced for protein, fat, energy, and fiber (Table 1), and stored at 4°C in the dark. Nontransgenic littermates ($n = 5$ per a dietary group) were included in the study to confirm the effects of the transgene. Mice were weighed weekly, and individual feed intake was measured 3 times a week when fresh diet was provided.

At 18 weeks of age, mice were asphyxiated by CO_2 and blood was collected by cardiac puncture. When possible,

Table 1. Composition of experimental diets

	Grams/100 g Total diet			
	Control	**10% Tomato powder[a]**	**2% Soy germ[b]**	**10% Tomato powder + 2% soy germ[a,b]**
Cornstarch	39.75	36.5	39.75	36.5
Casein	20.0	18.7	19.12	17.82
Maltodextrin	13.2	9.95	13.2	9.95
Sucrose	10.0	10.0	10.0	10.0
Fiber[c]	5.0	3.3	4.36	2.66
Mineral mix[d]	3.5	3.5	3.5	3.5
Vitamin mix[e]	1.0	1.0	1.0	1.0
L-Cystine	0.3	0.3	0.3	0.3
Choline bitrate	0.25	0.25	0.25	0.25
Cottonseed oil	7.0	6.5	6.52	6.02
Tomato powder[f]	0.0	10.0	0.0	10.0
Soy germ[g]	0.0	0.0	2.0	2.0

[a]Contains 268 μg total lycopene per gram of diet.
[b]Contains 200 μg total daidzein, 156 μg total glycitein, and 45 μg total genistein (aglycone equivalents) per gram of diet. For all 3 isoflavones, glucosides accounted for 58%, acetyl glucosides 32% to 39%, and aglycones 4% to 10% of total isoflavones.
[c]Non-nutritive cellulose.
[d]AIN93-MX formulation.
[e]AIN93-VX formulation.
[f] FutureCeuticals Tomato Powder 20 N8
[g]Frutarom SoyLife Complex Micro.

the prostate was microdissected into individual lobes (anterior, dorsal, lateral, and ventral). Liver, testes, and half of each prostate lobe were flash frozen in liquid nitrogen and stored at −80°C. All animals were thoroughly examined for gross metastases. Lungs, liver sections, enlarged periaortic lymph nodes, and half of each prostate lobe were fixed in 10% phosphate-buffered formalin overnight and transferred to 70% ethanol.

Histopathology

Tissues were paraffin embedded and 4 μm sections were stained with hematoxylin/eosin for pathologic grading. A blinded examiner (S.K. Clinton), with extensive experience with transgenic mouse models of PCa, evaluated all prostate lobes for incidence and severity of pathology according to a grading scheme which has been previously described (24).

Immunohistochemical analysis

Paraffin-embedded tissue sections (4 μm) were prepared, deparaffinized, and rehydrated. Slides were placed in a decloaking chamber and treated in a citrate buffer (pH 6.0) for 30 seconds at 125°C and 10 seconds at 90°C for antigen retrieval. The subsequent steps were completed in a BioGenex i6000 Automated Staining System (BioGenex). Endogenous peroxidase was quenched with a 3% H_2O_2 solution for 15 minutes. The slides were blocked with Power Block™ (BioGenex) for 10 minutes, avidin blocked for 15 minutes, and biotin blocked for 15 minutes. The slides were incubated with rabbit antiproliferating cell nuclear antigen (PCNA) antibody (Abcam) or rabbit anticleaved caspase-3 (Cell Signaling) for 30 minutes and visualized using a SuperSensitive™ Link-Label IHC Detection System (Biogenex). The slides were stained with DAB and counterstained with hematoxylin. Tissue from mouse small intestine was used as a positive control for PCNA, and mouse thymus was used as a positive control for cleaved caspase-3. Negative control slides were obtained by omitting the primary antibody. Stained slides were scanned with a NanoZoomer 2.0-HT digital slide scanner (Hamamatsu) with Olympus Uplansapo 20× objective at 40× digital zoom, giving 0.23 μm resolution. Images were captured with NDP view software (Hamamatsu). A representative image of the dorsal lobe and the lateral lobe of the prostate was captured for each mouse. Proliferation index (PI) and apoptotic index (AI) were calculated as previously described in our laboratory (9, 25). AI from cleaved-caspase-3–stained prostate sections was calculated as: AI = (cleaved caspase-3 positive cell count/total epithelial cells counted) × 100. PI from PCNA-stained prostate sections was calculated as: PI = (PCNA positive epithelial cell count/total epithelial cells counted) × 100. These indices were established by counting at least 1,000 cells from each image.

Carotenoid analysis

Carotenoid extraction and analysis of diet, serum, and tissues were conducted as previously described (26). Serum and testes were pooled within groups to facilitate HPLC detection of carotenoids, and liver was extracted in duplicate.

Isoflavone analysis

Serum, prostate, and diet samples were analyzed for genistein, glycitein, and daidzein and their conjugates and metabolites at The Ohio State University Comprehensive Cancer Center Nutrient and Phytochemical Analytic Shared Resource. Briefly, 100 to 200 μL of serum was extracted with 2 volumes of acetonitrile. Extracts were resuspended in 2:1 acetonitrile/water, probe sonicated, centrifuged, and the supernatant was collected. This was repeated, and the supernatants were pooled, dried under nitrogen, and resuspended in 0.5 mL 2 mol/L acetate buffer (pH 5.5). Isoflavones were deconjugated with β-glucuronidase and sulfatase in 2 mol/L acetate buffer (pH 5.5) for 1 hour at 37 °C. Digests were extracted twice with 3 volumes of ether and dried under nitrogen. Extracts were redissolved in 150 μL methanol with bath sonication and filtered through 0.2 μm nylon before injection. Approximately 40 mg of prostate tissue was transferred to a 1.5 mL microcentrifuge tube, suspended in 400 μL of water and probe sonicated with a Fisher dismembrator at 35% amplitude setting for 5 seconds. Eight hundred microliters of acetonitrile was added to induce protein precipitation and extract isoflavone metabolites. Samples were centrifuged for 5 minutes at 21,500 rcf to pellet solids, and supernatants were transferred to a 4 mL glass vial. Pellets were resuspended in 1.2 mL of 2:1 acetonitrile/water, probe sonicated, and centrifuged. Supernatants were pooled with first supernatants in a speedvac (45°C, 0.1 vacuum) until dry (1.5 hours). Residues were resuspended in 1 mL of 2 mol/L acetate (pH 5.5) and isoflavones were deconjugated with β-glucuronidase and sulfatase in 0.2% NaCl for 3 hours at 37°C. Extracts were digested twice with 3 volumes of ether (3 mL). Ether extracts were pooled, dried under nitrogen, redissolved in 150 μL methanol with bath sonication, and filtered through 0.2 μm nylon before injection. Samples were analyzed by UPLC/MS with a Phenomenex Synergi Fusion RP Column (2 × 50 mm, 2.5 μm) and quadrupole mass spectrometer (Quattro Ultima, Micromass) via an electrospray probe operated at negative polarity. Authentic standards of daidzein, dihydrodaidzein, *o*-desmethylangolensin, equol, glycitein, genistein, and dihydrogenistein were used as external calibrants.

Serum VEGF

Serum VEGF was analyzed by ELISA according to the manufacturer's instructions (R&D Systems).

Statistical analysis

SAS (version 9.3; SAS Institute) was used for all statistical analysis, and a $P < 0.05$ was considered significant. Serum lycopene and isoflavones were analyzed by Student *t* test. Weight gain, feed intake, gain:feed ratio, serum VEGF, proliferation index, and apoptotic index were compared by 2-tailed analysis of variance using the mixed model procedure in SAS with a Dunnett's post-hoc test. Fisher's exact test was used to compare incidence of pathology between an experimental group and the controls.

Table 2. Serum and tissue lycopene and isoflavones

	Control	10% Tomato powder	2% Soy germ	10% Tomato powder + 2% soy germ
Serum (μmol/L) and tissue (nmol/g) lycopene				
Serum	ND[a]	0.67 ± 0.07	ND[a]	0.44 ± 0.04[b]
Liver	ND[a]	4.56 ± 0.91	ND[a]	3.14 ± 0.56
Prostate Tumor	ND[a]	0.25 ± 0.05	ND[a]	0.18 ± 0.04
Testes	ND[a]	1.42 ± 0.23	ND[a]	0.47 ± 0.10[b]
Serum isoflavones (μmol/L)				
Total isoflavones	NA[c]	NA[c]	3.93 ± 0.60	4.02 ± 0.26
Total parent	NA[c]	NA[c]	0.61 ± 0.22	0.74 ± 0.14
Daidzein	NA[c]	NA[c]	0.29 ± 0.85	0.48 ± 0.09
Glycitein	NA[c]	NA[c]	0.14 ± 0.39	0.24 ± 0.05
Genistein[d]	NA[c]	NA[c]	0.18 ± 0.10	0.01 ± 0.01
Total metabolites	NA[c]	NA[c]	3.32 ± 0.49	3.28 ± 0.17
Dihydrodaidzein	NA[c]	NA[c]	0.02 ± 0.01	0.08 ± 0.03
Dihydrogenistein	NA[c]	NA[c]	0.01 ± 0.01	0.03 ± 0.01
O-desmethylangolensin	NA[c]	NA[c]	0.03 ± 0.01	0.07 ± 0.03
Equol	NA[c]	NA[c]	3.26 ± 0.49	3.10 ± 0.18
Prostatic isoflavones (nmol/g)				
Total isoflavones	NA[c]	NA[c]	0.201 ± 0.083	0.224 ± 0.068
Total parent	NA[c]	NA[c]	0.048 ± 0.022	0.041 ± 0.005
Daidzein	NA[c]	NA[c]	0.035 ± 0.017	0.029 ± 0.003
Glycitein	NA[c]	NA[c]	0.010 ± 0.004	0.008 ± 0.002
Genistein	NA[c]	NA[c]	0.003 ± 0.001	0.003 ± 0.001
Total metabolites	NA[c]	NA[c]	0.152 ± 0.062	0.183 ± 0.063
Dihydrodaidzein	NA[c]	NA[c]	0.032 ± 0.014	0.034 ± 0.011
Dihydrogenistein	NA[c]	NA[c]	0.003 ± 0.001	0.003 ± 0.001
O-desmethylangolensin	NA[c]	NA[c]	0.001 ± 0.001	0.002 ± 0.001
Equol	NA[c]	NA[c]	0.117 ± 0.047	0.142 ± 0.050

NOTE: Values are means ± SEM. Liver $n = 7$, serum and testes lycopene $n = 5$, prostate tumor lycopene $n = 5$–8, serum isoflavones $n = 7$ to 8, prostate isoflavones $n = 4$.

[a]Not detectable.

[b]Significant difference between 10% tomato powder and 10% tomato powder + 2% soy germ ($P < 0.05$).

[c]Isoflavones were not detected in the analysis of AIN-93G or 10% tomato powder diets; therefore serum from mice consuming these diets was not analyzed for isoflavones.

[d]Genistein was not detected in 3 mice in the 2% soy germ group and 6 mice in the 10% tomato + 2% soy germ group.

Results

All experimental diets readily consumed by TRAMP mice

There were no significant differences in weight gain or gain:feed ratio between a dietary intervention group and the control. Feed intake in the TP and SG groups was not significantly different from the controls. The average daily feed intake in the TP + SG group (5.57 ± 0.03 g) was significantly lower than the control group (5.71 ± 0.03 g, $P = 0.003$); however, this minimal difference in feed intake is likely not biologically significant.

Tomato powder feeding increases serum and tissue lycopene accumulation

Lycopene, the primary carotenoid in tomatoes, was detected in serum and tissues of mice consuming TP or TP + SG (Table 2). Mean serum lycopene levels were significantly higher in TP fed mice than mice fed TP + SG ($P = 0.03$). Testes lycopene was nearly 3 times higher in mice that consumed TP compared with TP + SG ($P = 0.006$). Although not statistically significant, lycopene accumulation in the prostate tumors ($P = 0.2$) and liver ($P = 0.3$) was also lower in TP + SG fed mice.

Isoflavones and isoflavone metabolites are detected in serum and prostate of TRAMP mice consuming soy germ

Parent isoflavones and isoflavone metabolites were detected in the serum and prostate of SG and TP + SG fed mice (Table 2). Individual and total isoflavone concentrations in the serum and prostate were not significantly different between SG or TP + SG fed mice. Equol, a metabolite of daidzein, was the primary isoflavone detected in the serum and prostate, and equol concentrations were higher

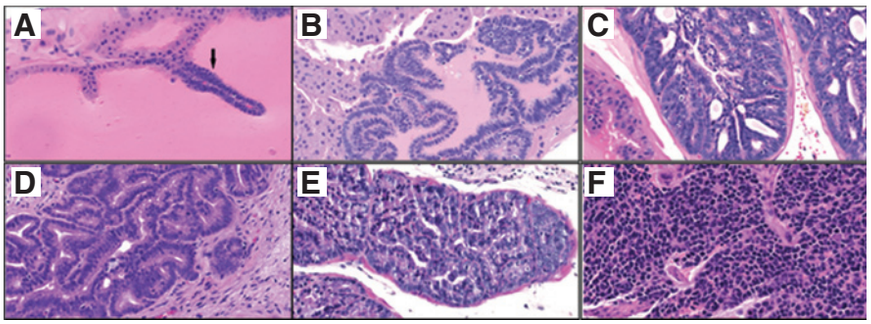

Figure 1. Prostate pathology in 18-week-old TRAMP mice. Low-grade PIN (A), moderate-grade PIN (B), high-grade PIN (C), well-differentiated adenocarcinoma (D), moderately-differentiated adenocarcinoma (E), poorly-differentiated carcinoma (F). Images were captured from Nanozoomer-scanned slides with NDP view software at ×40 digital zoom.

than all other metabolites and parent isoflavones combined. Serum genistein was below detection limits in 3 of 7 SG fed mice and 6 of 8 TP + SG mice (limit of detection = 5×10^{-9} mol/L).

Range of pathology identified in TRAMP mice

Nontransgenic mice in all of the dietary groups had histologically normal prostates. At the time of necropsy (age 18 weeks), all TRAMP mice had evidence of prostatic intraepithelial neoplasia (PIN) or PCa. Representative images of the range of pathologic progression in mice observed are shown in Fig. 1. Cancerous lesions categorized as well-differentiated (WD), moderately-differentiated (MD) or poorly-differentiated (PD) carcinoma were identified in 68% of the TRAMP mice (Table 3). As previously described in the TRAMP model, cancer lesions were more frequent in the dorsal, lateral, and ventral lobes (27), and the most common pathology was PD carcinoma.

Consumption of tomato powder, soy germ, and the combination significantly reduced prostate cancer incidence

Cancer incidence was defined as the presence of an adenocarcinoma (WD, MD, PD) lesion in at least one prostatic lobe. One hundred percent of mice fed control diets showed evidence of PCa in at least one lobe, and compared with the controls, overall incidence was significantly lower in TRAMP mice consuming TP (61%, $P < 0.001$), SG (66%, $P < 0.001$), and TP + SG (45%, $P < 0.001$; Table 4). In parallel to the low cancer incidence, TRAMP mice in the dietary intervention groups had significantly higher incidence of noncancerous PIN lesions compared with the controls.

We also employed a histopathologic scoring system to assign a numerical score to each lobe based on the severity and overall extent of pathology within the prostate (24). Reassuringly, the quantitative numerical scoring system also identified a significant impact of diet on PCa. The most severe lesion score in the prostate was significantly lower in the TP (14.7 ± 1.1, $P < 0.01$), SG (15.6 ± 1.1, $P = 0.03$), and TP + SG (13.5 ± 1.2, $P < 0.001$) groups compared with the control (19.3 ± 0.4).

Table 3. Distribution of pathology (most severe lesion) by prostatic lobe in 18-week-old TRAMP mice

	PIN			Adenocarcinoma		
	LG	MG	HG	WD	MD	PD
Anterior	3%	39%	53%	0%	3%	2%
Ventral	5%	24%	17%	3%	3%	48%
Dorsal	2%	21%	27%	4%	2%	44%
Lateral	1%	21%	18%	8%	3%	49%

Abbreviations: PIN, prostatic intraepithelial neoplasia; LG, low grade; MG, moderate grade; HG, high grade; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated.

Dietary interventions impacted the severity of carcinogenesis in prostate lobes

Compared with the controls, PCa incidence in the SG group was significantly lower in the lateral and anterior lobes, and TP and TP + SG groups had significantly lower PCa incidence in the dorsal, lateral, and ventral lobes (Table 5). The incidence of MG PIN in the anterior lobe was significantly higher in TP, SG, and TP + SG groups compared with the controls due to the higher incidence of more severe pathology in the controls. Although not significantly different from TP or SG, cancer incidence in the dorsal, lateral, and ventral lobes was lowest in the TP + SG group.

Dietary impacts on regional lymph node metastases

Due to our desire to examine early phases of carcinogenesis and the young age (18 weeks) at which mice were necropsied, the overall metastasis rate was low and the study lacked statistical power to fully assess local/regional or distant metastatic rates. However, the descriptive data reinforces the conclusions regarding dietary impact on prostate disease. The incidence of gross lymph node metastasis was lower in TP (9.4%), SG (12.9%), and TP + SG fed mice (11.1%) compared with the controls (17.2%). In mice with advanced, diffuse, PD carcinoma ($n = 25$), we observed that 6 mice had distant micrometastases, defined as the presence of microscopic T-antigen positive PCa cells in the liver and/

Table 4. Histopathological analysis (most severe lesion) of the entire prostate in TRAMP mice fed tomato powder or soy germ, alone or in combination

		PIN			Adenocarcinoma			
Treatment	***n***	**LG**	**MG**	**HG**	**WD**	**MD**	**PD**	**Prostate Cancer (WD – PD)**
Control	29	0%	0%	0%	10%	7%	83%	100%
10% Tomato	31	0%	7%	32%***	6%	0%	55%*	61%***
2% Soy Germ	32	0%	6%	28%**	6%	7%	53%*	66%***
10% Tomato + 2% Soy Germ	27	0%	7%	48%***	0%	4%	41%**	45%***

NOTE: LG, low grade; MG, moderate grade; HG, high grade; PIN, prostatic intraepithelial neoplasia; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated. Results are the incidence of each stage of pathology and overall prostate cancer incidence (sum of WD-PD) within dietary groups. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with control group by Fisher's exact test.

or lungs (data not shown), and half of those animals ($n = 3$) were control fed mice.

Dietary interventions with TP, SG, and TP + SG altered apoptosis

We chose a histologic subset of prostate samples to examine PI and AI. Sections from the dorsal and lateral prostate lobes in which the predominant pathology was PIN were stained and quantitated. The AI in the prostate of control fed mice ($0.7 \pm 0.2\%$) was nearly half that of mice consuming TP ($1.2 \pm 0.2\%$, $P = 0.007$), SG ($1.4 \pm 0.2\%$, $P = 0.002$), and TP + SG ($1.3 \pm 0.2\%$, $P = 0.004$). Proliferation in TP ($77 \pm 4\%$), SG ($74 \pm 3\%$), and TP + SG ($76 \pm 4\%$) groups was quantitatively lower but not significantly different than the controls ($81 \pm 3\%$). The ratio of the PI to the AI in control fed mice (154 ± 17) was over 2 times higher than mice consuming TP (71 ± 18, $P = 0.007$), SG (63 ± 17, $P = 0.002$), and TP + SG (70 ± 18, $P = 0.006$).

Table 5. Histopathological analysis (most severe lesion) of individual prostate lobes in TRAMP mice fed tomato powder or soy germ, alone or in combination

		PIN			Adenocarcinoma			
	n	**LG**	**MG**	**HG**	**WD**	**MD**	**PD**	**Prostate Cancer (WD – PD)**
Dorsal Prostate								
Control	29	0%	4%	17%	10%	7%	62%	79%
10% Tomato	31	0%	26%*	35%	7%	0%	32%*	39%**
2% Soy Germ	32	6%	16%	28%	0%	0%	50%	50%
10% Tomato + 2% Soy Germ	27	3%	41%***	26%	0%	0%	30%*	30%***
Lateral Prostate								
Control	29	0%	0%	14%	7%	10%	69%	86%
10% Tomato	31	0%	26%**	22%	10%	0%	42%*	52%**
2% Soy Germ	31	3%	32%***	13%	0%	0%	52%	52%**
10% Tomato + 2% Soy Germ	27	0%	41%***	18%	8%	0%	33%*	41%***
Ventral Prostate								
Control	29	0%	14%	7%	7%	7%	65%	79%
10% Tomato	31	7%	32%	22%	3%	0%	36%*	39%**
2% Soy Germ	32	6%	28%	7%	3%	3%	53%	59%
10% Tomato + 2% Soy Germ	27	8%	22%	33%*	0%	0%	37%	37%**
Anterior Prostate								
Control	27	0%	11%	74%	0%	8%	7%	15%
10% Tomato	28	4%	57%***	39%*	0%	0%	0%	0%
2% Soy Germ	30	3%	43%**	54%	0%	0%	0%	0%*
10% Tomato + 2% Soy Germ	22	5%	45%**	45%	0%	5%	0%	5%

LG, low grade; MG, moderate grade; HG, high grade; PIN, prostatic intraepithelial neoplasia; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated. Results are the incidence for each histopathologic grade and overall prostate cancer incidence (sum of WD-PD) within dietary groups. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with control group by Fisher's exact test.

Serum VEGF is increased in mice with poorly differentiated and metastatic adenocarcinoma

Serum VEGF was not significantly different between groups (Control = 97 ± 11 pg/mL, TP = 97 ± 11 pg/mL, SG = 97 ± 11 pg/mL, TP + SG = 86 ± 11 pg/mL, diet main effect $P = 0.88$). In this study of early carcinogenesis, we observed no major overall elevation in serum VEGF in TRAMP compared with normal, wild-type mice, or an overall impact of diet on baseline VEGF concentrations. However, serum VEGF levels were significantly elevated in TRAMP mice that had PD carcinoma with metastasis (Wild-Type = 68 ± 19 pg/mL; TRAMP without metastasis = 89 ± 5 pg/mL, TRAMP mice with metastasis = 139 ± 15 pg/mL, $P = 0.01$). Since too few mice had metastatic disease, the study is underpowered to assess an impact of diet on cancer associated elevations in serum VEGF.

Discussion

There are a number of *in vivo* and *in vitro* studies that have investigated the anticarcinogenic properties of pure phytochemicals derived from foods using the principles of pharmacognosy and pharmacology in hopes of developing novel drugs for cancer chemoprevention. However, there are far fewer studies focusing upon interventions with whole foods or novel-processed food products. In this study, we examine the combination of soy and tomato products for their anticancer activity in the TRAMP model to guide future development of food products for human PCa prevention strategies.

Preclinical rodent studies of PCa with tomato products alone have been promising. The current study shows that consumption of 10% tomato powder reduced the overall incidence of early PCa in the TRAMP model by nearly 40% ($P < 0.001$). Tomato powder at 10% of the diet has also been shown to increase survival and reduce the incidence of advanced, poorly differentiated PCa in the TRAMP model (11), and interventions in other rodent models also support the protective effects of tomato products on PCa (9, 12, 19). Lycopene concentrations in the serum (0.36–0.84 μmol/L) and prostate (0.11–0.48 nmol/g tissue) of TRAMP mice in this study are comparable with levels observed in men consuming 25 to 30 mg lycopene/day from tomato products (22, 28, 29), which can be achieved by intake of approximately 1 cup of tomato sauce, $^1/_2$ cup tomato paste, or 6 cups of raw tomatoes. Therefore, the tissue and serum concentrations of lycopene achieved in this study can be obtained by intake of whole foods, along with an array of bioactives, in humans without intake of pure lycopene supplements.

The majority of interventions with soy protein isolate or soy phytochemical extracts in rodent models of PCa have shown anticancer activity, suggesting that soy consumption may inhibit carcinogenesis, reduce tumorigenesis, and reduce expression of biomarkers related to proliferation or angiogenesis (13, 30). In this study, consumption of soy germ was selected as it is a phytochemical-rich fraction of the soybean that can be utilized by food scientists to produce novel functional foods for future cancer prevention studies or commercial products (31, 32). To our knowledge, this is the first study to evaluate the potential anticarcinogenic properties of soy germ. Soy germ, the hypocotelydon of the soybean, is separated during milling of whole soybeans and is a concentrated source of bioactives including isoflavones, saponins, and phytosterols. In contrast to soy protein isolates, concentrations of daidzein and glycitein in soy germ are considerably higher than genistein (as quantified by LC/MS). The anticarcinogenic properties of genistein have been extensively studied and may be one contributor to the anticancer activity associated with consumption of soy products (33). However, in our study, diets containing soy germ provided a modest 45 mg genistein/kg diet, which is substantially lower than doses previously suggested to be protective with *in vivo* studies (16, 17, 34–36). In fact, serum genistein was below detection limits from several of the mice that consumed diets containing soy germ and was only 4% to 5% of total isoflavones in the prostate. Rather, equol, produced from the metabolism of daidzein by intestinal microflora, was the predominant isoflavone in the serum and prostate. Equol has a longer half-life and is a more potent antioxidant than parent isoflavones (37). Equol has a greater affinity to estrogen receptors than daidzein and can concentrate in the prostate and the prostatic fluid at higher concentrations than genistein (37, 38). However, it is estimated that only 30% to 40% of the Western population can metabolize daidzein to equol, and it is of increasing interest to determine if health benefits from soy consumption are related to equol synthesis or other metabolites produced by the host or the intestinal microflora. Thus, our findings suggest that other soy phytochemicals, in addition to genistein may contribute to the anticancer activity of soy germ. Prostatic isoflavone concentrations in this study are comparable with what have been identified in men consuming soy foods or isoflavone supplements (39–41). Asian populations, which historically have shown a substantially lower incidence of PCa compared with Western countries, consume an average of 25 to 50 mg isoflavones/day (42), which can be achieved in the diet by consumption of 1 to 2 servings of soy foods (One serving = 4oz tofu, 1oz soy nuts or 8oz soymilk), including a recently developed tomato–soy juice (31).

Soy isoflavones and tomato carotenoids have distinctly different mechanisms of absorption, yet, long-term combined consumption of TP+SG resulted in reduced serum and testes lycopene than when TP was consumed alone. Results from this study support our previous findings where SG consumption significantly reduced carotenoid accumulation in the liver, testes, seminal vesicles, and prostate of male rats (26). In our previous study, reduced carotenoid bioaccumulation was not explained by mRNA expression of scavenger receptor class B type I (a protein involved in carotenoid absorption) or carotenoid metabolizing enzymes in the prostate, liver, or duodenal mucosa (26). Future studies should measure parent lycopene and their metabolites (cleavage products that may be more

bioactive) to identify potential interactions between SG and TP that may impact biodistribution and activity (43).

It is a challenge to identify specific mechanisms of action when investigating whole food products containing an array of bioactives with diverse activities. Indeed, many pathways may be modulated. We assessed the dynamic changes in the epithelial cell proliferation/apoptotic relationship. To accurately examine this relationship, we chose a similar area of the prostate (dorsal and lateral lobes) exhibiting the same histopathologic grade of cancer progression (PIN) from mice on each diet. We clearly observed that TP, SG, and TP+SG increased AI and modestly reduced the PI, resulting in a dramatic reduction in the PI/AI ratio, a finding that would favor the accumulation of malignant epithelial cells in the control group. These findings from an *in vivo* and very aggressive model support the *in vitro* findings suggesting that many polyphenols in soy and tomato can inhibit growth factor signaling transduction pathways and enhance sensitivity to activation of apoptotic cascades (15, 44, 45). Tomato sauce consumption has previously been reported to increase apoptosis in both PCa and benign prostatic hyperplasia (46). *In vitro*, isoflavone and isoflavone metabolites induced apoptosis in benign prostatic epithelial cells at concentrations within ranges identified in the prostatic fluid of men consuming a soy product (47). Isoflavones and lycopene have both been suggested to reduce proliferation by modulating IGF-1 signaling (20, 35, 44). *In vitro* evidence is useful in identifying potential bioactive compounds and mechanisms of action and provides preliminary evidence and support for preclinical trials investigating the efficacy of a whole food containing multiple bioactive compounds. Our findings of reduced cancer incidence by TP, SG, and TP + SG are supported by a large body of *in vitro* evidence, including hundreds of published studies on soy or tomato polyphenols, that bioactive compounds in these foods have anticarcinogenic properties.

We have previously observed a decline in serum VEGF in men with metastatic PCa fed tomato and soy components (22). We did examine serum VEGF in this study, but the early age of termination at 18 weeks, when most mice have microscopic cancer or PIN, was too early for the clear assessment of diet on serum VEGF or other markers of angiogenesis. Indeed, when evaluating our data, only mice with advanced, poorly differentiated cancers with regional or distant metastases showed elevations in serum VEGF. Thus, our study, terminated at an early point in the carcinogenesis process, prevented an assessment of the impact of diet on serum VEGF as seen in our human study.

Interestingly, we observed a reduction of PCa by TP and SG at a level of protection similar to what has been calculated from meta-analyses of epidemiologic studies measuring soy or tomato product consumption (3, 4). This is the first study to investigate the efficacy of a combination of tomato and soy for inhibition of PCa in an animal model. Over half of the TRAMP mice that consumed the combination of TP + SG were without a single cancerous lesion in the prostate compared to 100% cancer incidence in the control group. The combination of TP and SG was quantitatively the most effective intervention and suggests a positive interaction between tomato and soy foods for prevention of PCa. Interestingly, in patients with PCa, lycopene supplementation was more effective at reducing serum prostate-specific antigen (PSA) progression than the combined supplementation of lycopene and soy isoflavones (48), suggesting a potential negative interaction between soy isoflavones and lycopene. The small clinical trial had no control group and did not measure the efficacy of soy isoflavones alone; therefore, it is difficult to know if the soy isoflavones had any impact on cancer outcomes and if there was truly a negative interaction between these bioactive components.

This study reinforces the public health recommendations of many organizations such as the American Institute for Cancer Research and the United States government through the 2010 Dietary Guidelines for Americans which recommends that a variety of fruits and vegetables should serve as the foundation of a healthy diet (49, 50). In addition to supporting national public health goals, this work suggests that continued efforts to develop novel functional foods containing an array of bioactives are a reasonable strategy for PCa prevention or as an adjunct to therapy. The shown benefit from the combination of dietary components that have been selected on the basis of evidence derived from laboratory and epidemiologic studies provides a stimulus for food scientists to develop novel tomato–soy food products with defined and consistent composition (22, 31) for future clinical trials. Maximally effective and safe strategies for PCa prevention may result from optimizing combinations of nutrients and bioactives through an orchestration of dietary patterns or the development of novel food products that can be tested in prospective trials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: K. Zuniga, S.K. Clinton, J.W. Erdman, Jr.
Development of methodology: K. Zuniga, S.K. Clinton, J.W. Erdman, Jr.
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Zuniga, S.K. Clinton
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. Zuniga, S.K. Clinton, J.W. Erdman, Jr.
Writing, review, and/or revision of the manuscript: K. Zuniga, S.K. Clinton, J.W. Erdman, Jr.
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Zuniga, S.K. Clinton
Study supervision: S.K. Clinton, J.W. Erdman, Jr.

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