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Dietary tomato and lycopene impact androgen signaling- and carcinogenesis-related gene expression during early TRAMP prostate carcinogenesis

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Abstract

Consumption of tomato products containing the carotenoid lycopene is associated with a reduced risk of prostate cancer. To identify gene expression patterns associated with early testosterone-driven prostate carcinogenesis, which are impacted by dietary tomato and lycopene, wild type (WT) and transgenic adenocarcinoma of the mouse prostate (TRAMP) mice were fed control or tomato- or lycopene-containing diets from 4-10 wk-of-age. Eight-week-old mice underwent sham surgery, castration, or castration followed by testosterone-repletion (2.5 mg/kg/d initiated 1 wk after castration). Ten-wk-old intact TRAMP mice exhibit early multifocal prostatic intraepithelial neoplasia (PIN). Of the 200 prostate cancer-related genes measured by quantitative NanoString®, 189 are detectable, 164 significantly differ by genotype, 179 by testosterone status, and 30 by diet type ($P < 0.05$). In TRAMP, expression of *Birc5*, *Mki67*, *Aurkb*, *Ccnb2*, *Foxm1*, and *Ccne2* is greater compared to WT and is decreased by castration. In parallel, castration reduces Ki67-positive staining ($P < 0.0001$) compared to intact and testosterone-repleted TRAMP mice. Expression of genes involved in androgen metabolism/signaling pathways are reduced by lycopene feeding (*Srd5a1*) and by tomato-feeding (*Srd5a2*, *Pxn*, and *Srebfl1*). Additionally, tomato-feeding significantly reduced expression of genes associated with stem cell features, *Aldh1a* and *Ly6a*, while lycopene-feeding significantly reduced expression of neuroendocrine differentiation-related genes, *Ngfr* and *Syp*. Collectively, these studies demonstrate a profile of testosterone-regulated genes associated with early stages of prostate carcinogenesis that are potential mechanistic targets of dietary tomato components. Future studies on androgen signaling/

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metabolism, stem cell features, and neuroendocrine differentiation pathways may elucidate the mechanisms by which dietary tomato and lycopene impact prostate cancer risk.

Keywords

tomato; lycopene; testosterone; prostate carcinogenesis; TRAMP; murine

Introduction

Prostate carcinogenesis is an extended process that is likely impacted by a complex network of genetic, hormonal, and environmental factors throughout the life cycle, including the *in utero* development phase, the period of maximal growth during puberty, as well as the period of adulthood (1). Human prostate carcinogenesis clearly involves disruption of multiple molecular and biological processes, which contribute to its heterogeneity as a clinical entity (2). Normal prostate development, growth, and function, as well as the emergence of prostate cancer are androgen-dependent processes (3). Testosterone, the predominant androgen in males, is converted by 5 α -reductase (encoded by *Srd5a1* & *Srd5a2*) into dihydrotestosterone (DHT), the high-affinity ligand for the androgen receptor (AR) which, in conjunction with multiple cofactors, mediates transcriptional activity (3). While it is clear that androgens play a key role in the evolution of prostate cancer, the effects of dietary variables hypothesized to impact prostate cancer risk on androgen-driven processes are poorly understood. Transcriptomic analyses of human prostate cancer and non-cancerous tissue have revealed multiple dysregulated pathways during the development of human primary, metastatic, and hormone refractory prostate cancer (4). Yet it is more difficult to examine the molecular changes leading to the development of pre-malignancy in the human prostate, due to challenges of procuring such pre-malignant tissue samples. Rodent models of prostate carcinogenesis provide an opportunity to define androgen-driven gene expression signatures contributing to the emergence of premalignant histopathologic changes (5) and how dietary variables may modulate these signatures.

Epidemiologic and experimental studies of prostate cancer strongly suggest that disease risk is impacted by dietary patterns, specific nutrients, or phytochemicals (1). Notably, higher consumption of tomato products, estimated dietary lycopene intake, and circulating lycopene concentrations are inversely associated with prostate cancer risk in several human cohort studies (6). Laboratory studies provide evidence that tomato and lycopene may suppress oxidative damage, modulate intracellular signaling resulting in reduced proliferation, and enhance sensitivity to apoptosis, among other mechanisms (6). Some evidence suggests that tomato or lycopene intake may modulate testosterone production, serum concentrations, and metabolism, and may impact gene expression in human prostate cancer cells, normal rat prostate, and established prostate cancer xenografts (7-10). However, whether lycopene and/or other tomato phytochemicals inhibit prostate cancer by targeting androgen signaling and metabolism in early stages of prostate carcinogenesis has not been thoroughly investigated.

One of the key questions regarding the role of tomato products in prostate carcinogenesis is the relative contribution of lycopene to the anti-prostate cancer efficacy of tomato compared to other tomato phytochemicals. While tomato-feeding was more effective than lycopene in improving survival in rats with carcinogen- (NMU and androgens) induced prostate cancer (11), lycopene appears to be nearly as effective as tomato components in reducing cancer incidence and in increasing survival in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice (12). In addition, the timing of tomato or lycopene intervention during the life cycle may also be critical. When tomato feeding is initiated at 4 wk of age, it delays prostatic intraepithelial neoplasia (PIN) formation in 12-wk-old TRAMP mice (13). Similarly, tomato- and lycopene-feeding initiated at weaning and fed throughout life reduces prostatic lesion incidence and severity in TRAMP mice at 20 and 30 wk of age (12-14). Intriguingly, recent cohort findings suggest a stronger inverse association between tomato consumption reported at study enrollment and cumulative average lycopene intake over many years than with lycopene intakes recorded closer to the time of cancer diagnosis (15), possibly suggesting that longer term dietary tomato product or lycopene intake could be important in human prostate cancer prevention as well.

Together, the experimental and human data warrant further investigation of the impact of tomato and lycopene on the very early processes associated with prostate carcinogenesis. Prostate cancer risk in the TRAMP model is impacted by tomato, lycopene, and early castration (12-14, 16). In this model, the testosterone-driven rat probasin promoter induces prostate-specific expression of the SV40 antigen, initiating carcinogenic process, that over a predictable time frame, progresses from a hyperplastic epithelium, to PIN (~6-10 wk), to prostatic adenocarcinoma (~10-16 wk), to poorly differentiated carcinoma with metastasis (~18-24 wk) (17, 18), recapitulating features of human androgen-dependent cancer progression. The primary objective of the current study is to define the impacts of dietary tomato or lycopene on prostatic gene expression during early, androgen-driven carcinogenesis. To accomplish this objective, we first characterize the molecular pathways deregulated in the early, pre-malignant stages of TRAMP prostate carcinogenesis, and which pathways are subject to androgen regulation as determined by removal of testosterone by castration, and then the impacts of tomato- and lycopene-feeding on expression of genes associated with early androgen-driven prostate carcinogenesis are defined.

Methods and materials

Study design

All mouse experiments were performed in compliance with the Ohio State University Institutional Animal Care and Use Committee. Two, 3 x 3 factorial studies were utilized to investigate the effects of diet on androgen-driven prostate carcinogenesis in WT and TRAMP mice. Male TRAMP (+/-) C57BL/6xFVB/N hybrid and male C57BL/6 controls (Jackson Laboratory, Bar Harbor, ME) were used. Tomato and lycopene diets were formulated to provide blood and tissue concentrations of lycopene similar to humans (19). TRAMP mice weaned at 4 wk-of-age were randomized to one of three semi-purified AIN-93G-based diets (ResearchDiets, New Brunswick, NJ) with either 0.25% (w/w) placebo beadlets (DSM, Basel, Switzerland), 10% (w/w) tomato powder providing 384 mg

lycopene/kg diet (FutureCeuticals, Momence, IL) [with 0.25% (w/w) placebo beadlets], or 0.25% (w/w) lycopene beadlets (RediVivo 10% lycopene, DSM) providing 462 mg lycopene/kg diet, as previously described (19). Similar diets were fed to 4-wk-old WT but contained 0.04% (w/w) lycopene beadlets in the lycopene diet providing 20 mg lycopene/kg and 0.04% (w/w) control beadlets in control and tomato diets providing 40 mg lycopene/kg diet, respectively. Eight-wk-old mice within each diet group were randomized to undergo a sham (superficial incision only) surgery, castration, or castration followed by testosterone repletion (2.5 mg testosterone propionate/kg of body weight/d). Testosterone propionate (Sigma-Aldrich, St. Louis, MO) dissolved in sterile cyclodextrin (Sigma-Aldrich) was administered 1 wk after castration *via* mini osmotic pump implantation (Alzet, Durect, Cupertino, CA), per the manufacturer's instructions. All mice were sacrificed 5 d after pump implantation (12 d after surgery). Plasma was collected, prostate glands were procured by micro-dissection and were separated into constituent lobes [anterior (AP), ventral (VP), dorsal (DP), and lateral (LP)], and 5 prostates/experimental group designated for histological outcomes were fixed in 10% neutral buffered formalin overnight followed by processing and paraffin embedding. Three to five DP and LP designated for RNA isolation were preserved in RNAlater[®] solution (Life Technologies, Grand Island, NY).

Plasma lycopene measurement

Plasma carotenoids were extracted and analyzed as previous described (20).

Histopathology

Sections (5µm) of prostate tissues were stained with hematoxylin and eosin (H&E) and bright-field images were captured at 200X original magnification with a Nikon Eclipse E800 microscope (Nikon Instruments Inc, Melville, NY) equipped with a Nikon DS-Ri1 digital camera.

Immunohistochemistry and image analysis

Expression of Ki67, the protein product of the *Mki67* gene was validated using immunohistochemistry with antigen retrieval (1X Citra Plus Buffer; Biogenex, Fremont CA; 30 min in a steamer), monoclonal rat anti-mouse Ki67 (TEC3; 1:50; 60 min; Dako, Carpinteria, CA) followed by biotinylated rabbit anti-mouse IgG (1:200; 30 min; Dako). Staining was visualized with streptavidin/HRP (1:200; 30 min) and DAB (10 min; Dako) and counterstained with Mayer's hematoxylin (2 min; Sigma-Aldrich). Staining was quantified from scanned images (Aperio Scanscope XT scanner, Leica, Buffalo Grove, IL) using positive pixel counting [ImageScope 8.1 (Aperio[®]) digital image analysis package]. The Ki67 protein expression is expressed as a percentage of positive pixels relative to the total (positive + negative) pixels for a whole AP cross-section.

RNA isolation and mRNA expression

RNA was extracted from fresh-frozen pooled DP and LP preserved in RNAlater[®] at the time of dissection, using the RNeasy Mini kit (Qiagen, Valencia, CA). A custom-designed murine prostate carcinogenesis gene codeset of 200 genes, selected based on relevance to both human and murine prostate carcinogenesis as indicated by scientific literature

(Supplementary Table S1), was used to quantitate gene expression using nCounter technology (NanoString®, Seattle, WA) from 100ng total RNA, without enzymatic amplification. NanoString® nCounter technology was selected for quantitative gene expression analysis due to its documented precision, sensitivity comparable to RT-PCR, lack of reliance on enzymatic reactions and amplification, and multiplex capabilities with limited starting material (21). Expression was analyzed by subtraction of the negative controls from the sample data, followed by natural log transformation and normalization to *Ccng1* (NM_009831.2), which was identified as a relevant normalization factor based on low between-group variability ($P < 0.05$ by ANOVA), and high stability within groups [mean standard error = 0.0105].

Statistics

To define expression differences in response to the main and interaction effects of genotype, testosterone, and diet, normalized mRNA data on the log scale was analyzed by a 3-way ANOVA model assuming no interactions with diet. The latter assumption was examined and no evidence of important diet interactions was found [e.g. p -values closely followed the uniform distribution expected under the null hypothesis and none of the 189 genes were affected by diet $\times$ genotype, diet $\times$ testosterone, diet $\times$ genotype $\times$ testosterone interactions at the Bonferroni-adjusted significance level of 0.00026 (0.05/189)]. The model without diet interactions was then used to assess the prostatic gene expression changes by diet, testosterone, and genotype. Genes with a count number > 100 , and an expression fold-difference between genotypes (TRAMP *vs.* WT) or between testosterone statuses (intact *vs.* castrated) in the top 5% of 189 detected genes were defined as biologically significantly regulated. Plasma lycopene concentration, body weight, tissue weight, and Ki67 protein expression were evaluated by the same 3-way ANOVA model and then by 2-way ANOVA within genotypes. Pair-wise comparisons of groups were evaluated using the *post hoc* Sidak test. All statistics were calculated using the Stata12 statistical analysis package (StataCorp, College Station, TX).

Pathways analysis

The Ingenuity Pathway Analysis application (<http://www.ingenuity.com>) was used to detect prostatic molecular pathways differences between TRAMP *vs.* WT and intact *vs.* castrated mice. The gene expression linear ratios and pair-wise P -values for TRAMP *vs.* WT and castration *vs.* intact mouse were used as data inputs. Significance cutoff criteria was set to $P < 0.05$. Genes significantly impacted by genotype and testosterone status were then mapped onto the Global Ingenuity Knowledgebase Network and significance was calculated as previously described (19). The top 10 canonical pathways associated with early prostate carcinogenesis were selected based on P -value for each pathway.

Results

Mouse body and tissue weights

Mouse urogenital tract (UGT) weights were not changed by tomato- or lycopene-feeding, compared to control-feeding, regardless of genotype (Supplementary Fig. S1). Castration reduced UGT weight by 64% and 57% compared to those of intact and testosterone-repleted

mice ($P<0.001$ for both), respectively in WT, and by 69% and 60% compared to those of intact and testosterone-repleted TRAMP ($P<0.001$) mice, respectively. The body weights of WT mice were lower than those of TRAMP mice at the initiation of the study (14 ± 0.2 g vs. 20 ± 0.2 g, $P<0.0001$). Body weight gain from initiation of experimental diets to the day of surgery (4 wks) was not impacted by diet type. During the 12-d testosterone manipulation phase, castration caused weight loss while sham surgery did not in both WT (-0.05 ± 0.2 g and 0.88 ± 0.3 g, respectively, $P=0.0002$) and TRAMP (-0.23 ± 0.1 g and 0.87 ± 0.1 g, respectively, $P<0.0001$) mice. This effect was reversed by testosterone administration.

Plasma lycopene concentrations

Lycopene was not detected in the plasma of either control-fed WT or TRAMP mice (Supplementary Fig. S1). The achieved plasma lycopene concentrations were similar in tomato- and lycopene-fed WT and TRAMP mice. Compared to intact, castrated mice had lower plasma lycopene concentrations in tomato- and lycopene-fed WT and TRAMP by 35% ($P<0.05$) and 21% (N.S), respectively.

Histopathology of TRAMP prostate carcinogenesis

Testosterone and genotype impacted prostatic morphology in 10-wk-old mice (Fig. 1), with intact TRAMP having multifocal early PIN lesions in DP. Castration caused glandular atrophy in both WT and TRAMP. Testosterone-repletion stimulated glandular cell growth in both WT and TRAMP mice and was associated with early PIN lesions in the TRAMP. The current study focusing upon gene expression was not powered to define the more subtle dietary impact on histopathology.

Gene expression impacted by genotype, testosterone, and diet

Among the 200 genes in the codeset, expression of 189 genes was above the limits of detection, and expression of genes strongly clustered into genotype- and testosterone-responsive groups, with 164 being significantly changed by main effects of genotype, 179 by testosterone, and 30 by diet ($P<0.05$) (Fig. 2). The TRAMP mice had significantly up-regulated expression of 139 genes and down-regulated expression of 25 genes, compared to WT. Castration significantly increased expression of 139 and decreased expression of 35 genes compared to intact mice. Compared to sham surgery, castration significantly up-regulated 142 and 72 genes and down-regulated 14 and 55 genes in WT and TRAMP, respectively. Testosterone-repletion significantly reduced expression of 118 and increased expression of 43 genes compared to castration.

Expression of genes associated with early prostate carcinogenesis

The 5% of genes most significantly impacted by genotype fell primarily in the functional groups of cell growth and cell cycle regulation (Fig. 3 and Supplementary Table S2). The genes strongly up-regulated in TRAMP compared to WT included baculoviral IAP repeat-containing 5 (*Birc5*, encoding survivin), antigen identified by monoclonal antibody Ki67 (*Mki67*), aurora kinase B (*Aurkb*), cyclin B2 (*Ccnb2*), secreted phosphoprotein-1 (*Spp1*), forkhead box M1 (*Foxm1*), cyclin E2 (*Ccne2*), and E2F transcription factor 1 (*E2f1*). Genes

for which expression was biologically down-regulated in TRAMP relative to WT were epidermal growth factor (*Egf*) and probasin (*Pbn*).

Gene expression driven by testosterone

Castration significantly impacted expression of genes involved in cancer growth and cell migration in both WT and TRAMP. Although insulin-like growth factor binding protein 3 (*Igfbp3*), CD 44 antigen (*Cd44*), gap junction protein $\alpha 1$ (*Gja1*), and colony stimulating factor 1 (*Csf1*) were not differentially expressed between WT and TRAMP, they were significantly increased in castrated compared to intact mice (Fig. 4 and Supplementary Table S3). In contrast, castration significantly lowered expression of NK-3 transcription factor, locus 1 (*Nkx3.1*) and $\beta 2$ -microglobulin (*B2m*) expression, relative to intact mice.

Castration reversed expression of cell growth-related genes which were induced in TRAMP compared to WT mice (Fig. 3). Castration reduced expression of *Birc5*, *Mki67*, *Aurkb*, *Ccnb2*, *Foxm1*, and *Ccne2* and enhanced expression of *Spp1*, *Egf*, and *Pbn*, compared to that of the intact group.

A significant interaction between genotype and testosterone impacted expression of *Ccne2* and *E2f1* (Fig.4). *Ccne2* [fold change (FC)=6.13 $\pm$ 1.21, $P<0.0001$] and *E2f1* (FC=5.56 $\pm$ 1.21, $P<0.0001$) were significantly up-regulated in TRAMP compared to WT. They were down-regulated in castrated TRAMP ($P<0.0001$) but not in castrated WT, compared to intact TRAMP and WT, respectively.

Molecular pathways impacted by genotype and testosterone

Out of the 10 most significantly genotype-regulated canonical pathways, 9 were also impacted by castration compared to intact mice (Fig. 5 and Supplementary Table S4). While GADD45 signaling was within the top 10 pathways impacted by genotype, it was not in the top 10 pathways changed by castration, though it was still significantly impacted by castration ($P=4.71E-18$ or $-\text{Log}(P)=17.4$). The NF- κ B signaling pathway consequently fell into the top 10 canonical pathways altered by testosterone status.

Gene expression impacted by tomato and/or lycopene feeding

Tomato- and lycopene-feeding modulated expression of genes related to several biological targets. Remarkably, out of 189 detectable prostatic genes, tomato and lycopene similarly impacted expression of 26 genes and differentially impacted only 4 genes, those encoding nerve growth factor receptor (*Ngfr*), synaptophysin (*Syp*), *B2m*, and vitamin D receptor (*Vdr*) (Fig. 6). Among the 30 genes impacted by diet, 4 genes were increased by and 26 were decreased by tomato and lycopene. The genes can be functionally grouped into 1) androgen metabolism and signaling: 5 α -reductase 1 and 2 (*Srd5a1/2*), paxillin (*Pxn*), and sterol response element binding factor 1 (*Srebfl*); 2) MAPK signaling: transforming growth factor 1 and 2 (*Tgfb1/2*), fibroblast growth factor 2 (*Fgf2*), epidermal growth factor receptor (*Egfr*), *Kras* and *Nfkb*; 3) p53 signaling: insulin growth factor (*Igf*), caspases 2, 8 and 9 (Casp2/8/9); 4) cell adhesion molecules: P selection (*Selp*), cadherin 1 and 2 (*Cdh1/2*) and vimentin (*Vim*); 5) TR/RXR and VDR/RXR activation: solute carrier family 2 (facilitated glucose transporter), member 1 (*Slc2a1*), retinoid $\times$ receptor α (*Rxra*), and vitamin D

receptor (*Vdr*); 6) phase II detoxifying enzymes: glutathione peroxidase 3 (*Gpx3*) and superoxide dismutase 2 (*Sod2*); 7) complement and coagulation cascades: plasminogen activator, urokinase receptor (*Plaur*); 8) endocytosis: vascular epithelial growth factor receptor 1 (*Vegfr1*); 9) neuroendocrine differentiation: nerve growth factor receptor (*Ngfr*) and synaptophysin (*Syp*); and 10) stem cell features: aldehyde dehydrogenase 1A1 (*Aldh1a1*) and lymphocyte antigen 6a (*Ly6a*).

Tomato- and lycopene-feeding down-regulated androgen metabolism/signaling-related gene expression, relative to control-feeding (Fig. 6). Tomato- and lycopene-fed mice had lower expression of genes encoding the isoforms of 5 α -reductase, *Srd5a1* (lycopene vs. control FC=-1.18 $\pm$ 1.08, $P=0.03$) and *Srd5a2* (tomato vs. control FC=-1.25 $\pm$ 1.09, $P=0.04$). Two androgen receptor co-regulators were down-regulated in tomato-fed, compared to control-fed mice: *Pxn* (tomato vs. control FC=-1.19 $\pm$ 1.09, $P=0.04$) and *Srebfl* (tomato vs. control FC=-1.21 $\pm$ 1.11, $P=0.05$).

Neuroendocrine differentiation- and stem cell-related genes were down-regulated by tomato- and lycopene-feeding. Lycopene-fed mice had lower expression of prostatic *Ngfr* (FC=-1.61 $\pm$ 1.16, $P=0.02$) and *Syp* (FC=-1.72 $\pm$ 1.18, $P=0.02$) than control-fed mice. Tomato-fed mice had lower prostatic expression of *Aldh1a1* (FC=-1.14 $\pm$ 1.15, $P=0.04$) and *Ly6a* (FC=-1.34 $\pm$ 1.14, $P<0.001$) than control-fed mice.

Protein expression of Ki67

Ki67 protein expression was lower in castrated compared to intact mice in both WT and TRAMP (1.1% vs. 0.7% for WT and 3.6% vs. 0.2%, $P<0.0001$ for TRAMP), as predicted by mRNA patterns (Fig. 3). Dietary intervention did not result in a significant impact on Ki67 protein expression at this early time point.

Discussion

It is clear from studies in multiple laboratories that tomato components or lycopene reduce the progression of prostate carcinogenesis in TRAMP and other rodent models (9, 11-14). As in human prostate cancer, the progression in TRAMP is initially driven by testosterone and evolves into poorly differentiated castration-resistant disease (22). In this study we utilized a system of castration and testosterone-repletion in WT and TRAMP mice in order to examine how dietary tomato or lycopene impact androgen-driven gene expression early in carcinogenesis, prior to the development of significant histopathologic changes. As anticipated, prostate weight, histological features, and gene expression profiles were dramatically changed in response to testosterone status, with 95% of the detectable genes in our gene codeset being impacted by testosterone and 87% by genotype. With this foundation, these molecular signatures in testosterone-driven early prostate carcinogenesis can be used to address which processes are impacted by the dietary variables of tomato and lycopene. We observed that 16% (30 genes) of the measured genes were significantly impacted by the dietary interventions. Interestingly, 29 of these diet-sensitive genes are also subject to androgen-regulation (Fig. 1), and this marked overlap indicates that tomato- and lycopene-feeding modulate expression of androgen-regulated genes in early prostate carcinogenesis. In the context of similar plasma lycopene concentrations in the tomato and

lycopene fed mice, regardless of genotype, the effects of dietary tomato or lycopene are remarkably similar, both in the genes impacted and the magnitude of changes. These results suggest that lycopene accounts for much of the impact of tomato feeding; although a few very intriguing differences emerged. Interestingly, the dietary interventions down-regulated gene expression associated with androgen metabolism and signaling, neuroendocrine and stem cell features.

Molecular signature of early prostate carcinogenesis in the TRAMP model

Although minimal histopathologic changes are noted at 10 wks-of-age, expression of the SV40 construct in the TRAMP prostate yields a unique molecular signature compared to WT (Fig. 1 and 2). As predicted, based upon known inhibitory functions of T-antigen on expression of tumor suppressors p53 and Rb, expression of downstream genes controlling cell cycle regulation/proliferation and p53 signaling pathways are activated in early prostate carcinogenesis (Fig. 3 and 5). Many of these early changes observed in our study are known to persist in established cancer in 30 wk-old TRAMP mice, and indeed hyperproliferation is characteristic of the TRAMP system throughout its progression (23, 24). The up-regulation of *Birc5* (encoding survivin), *Mki67*, *Ccnb2*, *Foxm1*, *Ccne2*, and *E2f1* and down-regulation of *Egf* and *Pbn* (encoding probasin) are consistent with these changes. Ki67 expression is frequently used as a histopathologic biomarker of proliferative status and high expression is associated with decreased survival in prostate cancer patients (25). Survivin is an apoptosis inhibitor, and prostatic expression is correlated with cancer progression in humans (26). FOXM1 is a regulator of the cell cycle, which is up-regulated in metastatic prostate cancer tissue (4). These genes are included in the p53, cell cycle, and check-point regulation pathways (Supplementary Table S4), and these early changes are consistent with changes reported in more advanced TRAMP cancers (23).

Testosterone-driven gene expression in early prostate carcinogenesis

Modulation of testosterone activity remains a key therapeutic intervention extending the lives of prostate cancer patients (3). The use of weak anti-androgens targeting 5 α -reductase, which inhibits the conversion of testosterone to DHT, has shown significant activity in human chemoprevention studies with acceptable toxicity (27, 28). In parallel, early castration (4-wk-old) in TRAMP decreases prostate cancer formation and growth (16). One of the objectives of the current study is to define patterns of genes associated with early prostate carcinogenesis impacted by testosterone status, as these genes may provide insight into mechanisms and serve as targets for diet and chemopreventive agents. Castration suppressed cell cycle progression-related genes (*Mki67*, *Birc5*, *Aurkb*, *Ccnb2* and *Foxm1*) and the protein product of *Mki67*, Ki67 (Fig. 3). These findings support the concept that disruption of testosterone-driven signaling impacting cell cycle and proliferation should continue to be pursued for prostate cancer prevention.

In parallel, castration also activates expression of genes associated with suppression of cellular growth, such as, *Igfbp3* and *Gjal* (Fig. 4). IGFBP3 inhibits the growth-stimulating functions of IGF by sequestering IGF-1, and may impact autocrine, paracrine, and endocrine actions of IGF (29). The inverse association between IGFBP3 and prostate cancer risk remains somewhat speculative, yet this finding illustrates the importance of the intimate

orchestration that exists between steroid and protein hormone/growth factor action in prostate carcinogenesis (29). In the current study, castration increased expression of *Gjal* (encoding a gap junction communication protein, connexin 43), which is consistent with the effect of castration on the wild type rat prostate (30). The putative function of connexin 43 in prostate cancer development is stage-specific: in primary prostate cancer cells, loss of connexin promotes metastatic potential, and in a human prostate cancer cell line, connexin 43 inhibits proliferation, independent of gap junction function (31). Overall, castration reduced/reversed expression of genes associated with the canonical pathways induced in early prostate carcinogenesis (Fig. 5), with a marked reduction of proliferation and cell cycle regulation pathways.

Tomato and lycopene similarly impact gene expression

The plasma lycopene concentrations were comparable in both WT and TRAMP mice (0.34 and 0.36 $\mu\text{mol/L}$ in WT and TRAMP, respectively), were within the achievable range of plasma concentrations found in humans (32), and tomato and lycopene similarly altered expression of 26 genes, with no effect on 159 genes. Only 4 genes showed divergent expression, yet these are also very interesting: *Ngfr*, *Syp*, *Vdr* and *B2m* (Fig. 6). Thus it seems that lycopene largely contributed to the impact of tomato feeding on gene expression in early prostatic carcinogenesis. Genes encoding proteins related to androgen metabolism/signaling, MAPK signaling, p53 signaling and phase 2 detoxification enzymes largely constituted the genes similarly impacted by lycopene-and tomato-feeding.

Interestingly, the neuroendocrine-related genes, *Syp* and *Ngfr*, are expressed in the 10 wk-old mouse prostate, and expression is inhibited by dietary lycopene. Neuroendocrine cells (identified by synaptophysin) have also been detected in PIN lesions of 12-wk-old TRAMP and in advanced, poorly differentiated TRAMP tumors (33, 34), and it is important to recognize that neuroendocrine cells are present in normal human prostates and benign prostatic hyperplasia (35). High grade and late stage prostate tumors, especially castration refractory cancers, often exhibit a neuroendocrine phenotype, as identified by SYP staining (36). Higher intake of lycopene is associated with lower risk of aggressive and lethal prostate cancer (15). To our knowledge, this is the first report to show lycopene significantly down-regulates *Syp* and *Ngfr* in prostate carcinogenesis, suggesting lycopene may attenuate prostate carcinogenesis by reducing neuroendocrine features associated with poorly differentiated and more aggressive cancer.

Impacts of tomato- and lycopene-feeding on androgen metabolism-related gene expression

Regulation of androgen metabolism and signaling are among the proposed mechanisms by which tomato and lycopene exert inhibitory effects in prostate cancer. We observed that tomato and lycopene feeding decreased *Srd5a1* and *Srd5a2* (encoding 5 α -reductase 1 and 2) expression (Fig. 6). Other studies have also suggested similar effects in various, yet quite different, types of scenarios (8-10, 37). Pharmacologic inhibition of SRD5A enzyme activity by finasteride and dutasteride reduced overall prostate cancer incidence by 23-25% compared to placebo in brief chemoprevention trials (27, 28, 38), and thus it may be possible that lycopene or tomato exhibit anticancer activity by reducing overall enzyme

content and subsequent activity. Thus, the effect of dietary tomato and lycopene on functional changes and enzymatic activity of 5 α -reductase warrant future mechanistic investigation.

Expression of AR coregulators were also impacted by tomato and lycopene feeding in early prostate carcinogenesis, suggesting another mechanism by which tomato and lycopene may reduce androgen signaling. Previously, tomato and lycopene were reported to inhibit prostatic steroid binding protein mRNA expression, AR coregulator (Protein DJ1), and an androgen receptor-stabilizing chaperone protein (HSP90) in the prostate of healthy rats, the Dunning rat model, and human primary prostate epithelial cells (9, 10, 39). That *Pxn* is impacted by tomato and lycopene is a novel finding. SREBP (encoded by *Srebf1*) and paxillin (encoded by *Pxn*), are AR co-activators which bind to the AR promoter region in human prostate cancer cells (40, 41). SREBP overexpression enhances proliferation and prostate cancer progression and is associated with progression of human prostate cancer (42). Therefore the inhibitory effect of tomato and lycopene *Srd5a1/2*, *Srebf1* and/or *Pxn* might contribute to the inhibition of prostate carcinogenesis by impacting key co-regulators of androgen signaling.

The prostatic glandular epithelium includes a subpopulation of cells with self-renewal potential (43). In normal and cancerous human and murine prostate, stem cell antigen-1 (Sca1, encoded by *Ly6a* gene), has been used as a biomarker of prostate stem/progenitor cells (44). Expression of ALDH1A1 is also increased in progenitor cells in both human prostate cancer and rodent models (45). In human clinical samples, ALDH1A1 is positively correlated with Gleason score and pathological stage and is inversely associated with patient survival (45, 46). The inhibitory effect of tomato and lycopene on *Ly6a* and *Aldh1a* expression (Fig. 6) indicates that inhibition of stem cell features and therefore promotion of terminal differentiation, might be a novel mechanism by which tomato consumption reduces prostate cancer risk, warranting investigation beyond the expression of these two relevant genes.

Conclusion

In conclusion, gene expression profiles are clearly deregulated as early as 10 wk-of-age in the TRAMP model, a time representing the emergence of morphological changes in the mouse prostate. In addition, testosterone regulation of carcinogenic gene expression patterns likely mimics early stages of human carcinogenesis. While the impacts of tomato- and lycopene-feeding on gene expression were of a lesser magnitude than those observed between genotypes or androgen statuses, this was to be expected of the physiologically-relevant dietary intervention, as opposed to the much larger impacts of the TRAMP genotype and castration. Nonetheless, the differences are illustrative of the broad impacts of tomato and lycopene feeding on androgen-driven early carcinogenic gene expression. Our evidence suggests that the cancer preventive effects of tomato and lycopene are partially mediated by their inhibition of androgen activity and co-regulators of androgen receptor activation. Novel mechanisms of tomato and/or lycopene on neuroendocrine markers and cancer stem cell biology deserve further investigation. Remarkably, our gene expression signatures in early carcinogenesis indicate that lycopene and tomato have nearly equivalent

impact on androgen-driven gene expression in the TRAMP model, suggesting that lycopene is the major bioactive tomato component impacting early TRAMP carcinogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Representative histological images (magnification = 200x) of H&E stained 10-wk-old mouse anterior prostate lobes from WT or TRAMP, after either sham surgery (Intact), castration surgery (Castration), or castration surgery followed by testosterone repletion (Repletion). Arrow indicates an early PIN lesion (**A-F**).

Figure 2.

Hierarchical clustering of 189 detectable prostatic genes in response to genotype and testosterone status. I, intact; C, castration; R, castration followed by testosterone repletion (A). Venn diagram depicting the numbers of prostatic genes for which expression was significantly ($P < 0.05$) impacted by either the main effects of genotype, testosterone, or diet (non-overlapping portions) or a combination of main effects (overlapping portions) (B).

Figure 3.

Gene expression was strongly up-regulated (A-D) or down-regulated (E&F) in TRAMP compared to WT mice with a significant main effect of genotype ($P < 0.05$), in intact, castration, or castration followed by testosterone repletion (repletion) groups, and was significantly decreased in castration compared to intact and testosterone repletion group ($P < 0.0001$; not denoted on figures). Fold-change of expression is relative to the intact, WT group. Data are expressed as mean $\pm$ SE ($n = 3-5$ /group). *** Denotes a significant between-genotype, within surgical treatment difference ($P < 0.0001$). Castration significantly decreased Ki67 protein expression of AP in WT (G) and TRAMP (H). *** Denotes a significant differences in castration compared to intact and repletion in both WT and TRAMP, regardless of diet type.

Figure 4.

Prostatic gene expression significantly impacted by androgen status (A-F) and a significant genotype $\times$ testosterone status interaction (G&H). *** Indicates significant differences between androgen status groups, within genotype ($P < 0.0001$ compared to intact and repletion groups). Fold change of expression is relative to intact, WT. Data are expressed as mean $\pm$ SE (n=3-5/group).

Figure 5.

The top 10 canonical pathways significantly impacted by genotype (**A**) and castration (**B**), as calculated from gene expression data by IPA.

Figure 6.

Prostatic gene expression significantly impacted by an overall main effect of diet ($P<0.05$). Bars represent gene expression in tomato- or lycopene-fed mice, relative to control-fed mice, in which equal expression is represented as “1” ($n=21-22/\text{group}$). ^ Indicates significant differences between tomato- vs. control-fed and * indicates significant differences between lycopene- vs. control-fed mice ($P<0.05$). (A). Gene expression of testosterone metabolism and signaling-related genes down-regulated with tomato or lycopene-feeding compared to control-feeding. Fold change is calculated as a ratio of gene expression to control group and expressed as mean $\pm$ SE ($n=21-22/\text{group}$) (B-E). * Denotes a significant between-diet group difference, relative to control-feeding, ($P<0.05$).