

# $\beta$ -Carotene 9',10' Oxygenase Modulates the Anticancer Activity of Dietary Tomato or Lycopene on Prostate Carcinogenesis in the TRAMP Model

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## Abstract

The hypothesis that dietary tomato consumption or the intake of the carotenoid lycopene inhibits prostate cancer arose from epidemiologic studies and is supported by preclinical rodent experiments and *in vitro* mechanistic studies. We hypothesize that variation in activity of carotenoid cleavage enzymes, such as  $\beta$ -carotene 9',10'-oxygenase (BCO2), may alter the impact of dietary tomato and lycopene on prostate carcinogenesis and therefore examined this relationship in the TRAMP model. Starting at 3 weeks of age, TRAMP:*Bco2*<sup>+/+</sup> and TRAMP:*Bco2*<sup>-/-</sup> mice were fed either AIN-93G control, or semipurified diets containing 10% tomato powder or 0.25% lycopene beadlets until 18 weeks of age. Both tomato- and lycopene-fed TRAMP:*Bco2*<sup>-/-</sup> mice had significantly greater serum concentrations of total, 5-*cis*, other *cis*, and all-*trans* lycopene than TRAMP:*Bco2*<sup>+/+</sup> mice. Tomato- and lycopene-fed mice had a lower incidence of prostate cancer

compared with the control-fed mice. Although *Bco2* genotype alone did not significantly change prostate cancer outcome in the control AIN-93G-fed mice, the abilities of lycopene and tomato feeding to inhibit prostate carcinogenesis were significantly attenuated by the loss of *Bco2* ( $P_{\text{interaction}} = 0.0004$  and 0.0383, respectively). Overall, dietary tomato and lycopene inhibited the progression of prostate cancer in TRAMP in a *Bco2* genotype-specific manner, potentially implicating the anticancer activity of lycopene cleavage products. This study suggests that genetic variables impacting carotenoid metabolism and accumulation can impact anticancer activity and that future efforts devoted to understanding the interface between tomato carotenoid intake, host genetics, and metabolism will be necessary to clearly elucidate their interactive roles in human prostate carcinogenesis. *Cancer Prev Res*; 10(2): 161–9. ©2016 AACR.

## Introduction

The hypothesis that tomato products and their constituents, such as the carotenoid lycopene, may reduce prostate cancer risk, emerged from the Health Professional's Follow-up Study (HPFS), a large prospective cohort study of over 50,000 men (1–3). A negative association between circulating blood lycopene concentrations with prostate cancer, particularly more aggressive and lethal subtypes, has been observed over decades of continued

evaluation of the HPFS cohort (4) and supported by 2 recent meta-analyses relating circulating lycopene concentrations to disease risk (5, 6). Yet, consistency across the accumulated data from many other epidemiologic studies is lacking (7, 8). The reasons are likely multiple, including the concerns regarding studies of limited statistical power and differences in the precision of diet assessment tools to define relevant exposures over time. However, perhaps the greatest obstacle to diet and prostate cancer epidemiology is that the vast majority of studies cannot disentangle the impacts of heterogeneity in cancer screening across populations and the overdetection of cancers of limited clinical significance, which are followed by aggressive treatments and low cancer-specific mortality relative to incidence. Nevertheless, the variation in risk across populations globally continues to suggest an important role of environmental variables (8) including diet, nutrition, and lifestyle.

The ability of tomatoes and lycopene to impact prostate carcinogenesis has been examined in a variety of experimental models of prostate carcinogenesis (9–15) and tumorigenic progression (16–21). In carcinogenesis models, lycopene alone has shown anticancer activity when provided to the TRAMP model from weaning (12), when given at a higher concentration after DMAB carcinogen exposure in the F344 rat but not lower concentrations or during carcinogen exposure (9), when provided in combination with diet restriction (10), or with vitamin E and

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**Note:** Supplementary data for this article are available at Cancer Prevention Research Online (<http://cancerprevres.aacrjournals.org/>).

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**doi:** 10.1158/1940-6207.CAPR-15-0402

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selenium in the *Lady* transgenic model for longer durations (11, 15). In tumorigenesis models, lycopene was not effective in the Dunning MatLyLu orthotopic model (16) or the Dunning implantable tumor model either alone or in combination with selenium and vitamin E (19, 22), but did have anticancer activity in the BALB/c nude DU145 xenograft (17) and PC-3 xenograft (21), in the NMRI nu/nu PC-346C orthotopic model when given with vitamin E (18), and in the NCR-nu/nu DU145 xenograft model when provided with docetaxel (20). This suggests that certain combinations of agents may differently target the molecular processes of different cancer cell lines. In the Dunning implantable model of tumorigenesis, tomato feeding alone led to a nonsignificant decrease in tumor mass (22) but when fed with broccoli powder, led to a significant decrease in mass (22). In carcinogenesis models, tomato powder feeding increased survival with and without diet restriction in NMU-treated rats (10), and tomato powder alone or tomato paste with ketosamine (FruHis) increased survival whereas tomato paste alone did not (23). Tomato paste was also ineffective in another study of the TRAMP model (12), whereas tomato powder was effective in two other studies of TRAMP mice (13, 14), all of which suggest feeding tomato powder may be more effective than paste. Although the preclinical data could be viewed as "inconsistent," this variation can bear critical insights into scenarios in which lycopene and/or tomato are more or less effective, which most likely correlate with fundamental principles of cancer biology and pharmacology such as the stage and molecular features of the cancer, how much of and when the agent is administered, and which agent(s) is (are) coadministered. These preclinical studies are supported by a variety of anticarcinogenic tomato phytochemicals and metabolites in various *in vitro* and *in vivo* studies (24–27). Our recent study comparing the transcriptional signatures of tomato versus lycopene feeding in early TRAMP (27) carcinogenesis suggests there may be overlapping molecular targets of lycopene, its metabolites, and other tomato phytochemicals and metabolites.

One of the critical questions regarding lycopene and other tomato-derived carotenoids has emerged from the discovery of two carotenoid cleavage enzymes in mammals. The role of the  $\beta$ -carotene 15,15' monooxygenase (BCO1) enzyme is primarily for the central cleavage of carotenoids, which is essential in meeting vitamin A requirements after consumption of provitamin A carotenoids, such as  $\beta$ -carotene (28). A second enzyme,  $\beta$ -carotene 9',10' oxygenase (BCO2; aliases include CMO2 and BCDO2), appears to be primarily responsible for eccentric carotenoid cleavage (29) and is putatively involved in lycopene metabolism (30–32). Thus, the expression or activity of BCO2 may modulate the ability of lycopene to impact the host and is perhaps regulated by dietary, metabolic, or other environmental exposures as well as being impacted by genetic variability, such as SNVs (single nucleotide variants). In support of this hypothesis, SNVs in BCO1 have been shown to impact the relationship between lycopene intake and blood lycopene concentrations (33, 34), but to the authors' knowledge, no such data exist for BCO2. Our recent studies suggest that knockout of BCO2 is associated with higher blood concentrations of lycopene (30, 35). Another consideration raised regarding lycopene cleavage is the possibility that metabolites are biologically active (30), perhaps through interaction with multiple members of the steroid receptor superfamily (32, 36–40). Indeed, lycopene metabolites have been detected in plasma and tissues (30, 41–43).

We present a study designed to assess the impact of BCO2 on the ability of tomato or lycopene to reduce prostate carcinogenesis, an approach made possible by the characterization of *Bco2*<sup>-/-</sup> mice (29, 35, 44). We completed genetic crosses to produce mice with or without BCO2 activity in the "transgenic adenocarcinoma of the mouse prostate" (TRAMP) system. We chose TRAMP because its cancer progression over 18 weeks mimics many histopathologic features of human prostate carcinogenesis (45), and this system is associated with dysfunction p53 signaling, a critical pathway defective in human prostate cancers (46, 47). Whether the anticancer effects associated with tomato and lycopene feeding are BCO2-dependent or -independent remains critical to understanding the biological mechanisms of lycopene and tomato consumption. Our understanding of epidemiologic relationships will be greatly enhanced if specific diet and genotype interactions impacting carcinogenesis are appreciated.

## Materials and Methods

### Animal breeding strategies, diets, and experimental design

A 2 × 3 factorial design study was completed with two genotypes of mice (TRAMP<sup>+/-</sup>:*Bco2*<sup>+/+</sup> TRAMP<sup>+/-</sup>:*Bco2*<sup>-/-</sup>) and three previously described dietary interventions [a semipurified AIN-93G control diet, the control diet containing 10% tomato powder, or the control diet containing 0.25% lycopene beadlets (w/w); ref. 30]. Both TRAMP [C57BL/6-Tg(TRAMP)8247Ng/J; The Jackson Laboratory] and *Bco2*<sup>-/-</sup> (B6; 129S6-Bco2tm1Dnp; generated as described (44) and breeding pairs donated by Johannes von Lintig; Case Western Reserve University, OH) breeding colonies were established and maintained at The Ohio State University, and all murine protocols and procedures were reviewed and approved by The Ohio State University Institutional Animal Care and Use Committee. Briefly, TRAMP<sup>+/-</sup> mice were crossed with *Bco2*<sup>-/-</sup> mice. TRAMP<sup>+/-</sup>:*Bco2*<sup>-/+</sup> mice were crossed with TRAMP<sup>-/-</sup>:*Bco2*<sup>+/-</sup> mice to achieve TRAMP<sup>+/-</sup>:*Bco2*<sup>+/+</sup> and TRAMP<sup>+/-</sup>:*Bco2*<sup>-/-</sup> F2 progeny. At 3 weeks of age, TRAMP<sup>+/-</sup>:*Bco2*<sup>+/+</sup> or TRAMP<sup>+/-</sup>:*Bco2*<sup>-/-</sup> were randomized to one of the three experimental diets (*n* = 39–46 per genotype × diet groups). Both tomato- and lycopene-containing diets were formulated as previously described (30) to target similar concentrations of dietary lycopene (250 mg lycopene•kg<sup>-1</sup> diet). The final tomato- and lycopene-containing diets delivered 384 and 462 mg lycopene•kg diet<sup>-1</sup>, respectively, based upon our high-performance liquid chromatography (HPLC) analysis.

Diets were stored in the dark at -20°C, and fresh diet was provided every other day to minimize tomato phytochemical degradation. After 15 weeks of feeding, all mice (18 weeks of age) were euthanized by CO<sub>2</sub>, blood was collected by cardiac puncture for serum carotenoid analysis, and prostate lobes were microdissected and fixed in 10% neutral buffered formalin for histologic evaluation.

### Diet and serum carotenoid analysis

Tomato powder- and lycopene beadlet-containing diet carotenoids were extracted using a previously described extraction method (48) with the following minor modifications. Carotenoids were extracted from 0.025 g of tomato powder and 0.25 g lycopene beadlet diets (*n* = 5). Lycopene beadlet diet samples were suspended in 2 mL water to facilitate dispersion of the beadlets before hexane extraction. Extracts were stored under

argon gas at  $-20^{\circ}\text{C}$  for <48 hours before reconstitution in methyl *tert*-butyl ether (MtBE) for analysis. Serum samples were immediately analyzed by reconstitution in 35  $\mu\text{L}$  of mobile phase B [MeOH/MtBE/aqueous ammonium acetate (1.5%, w/v)], held at  $4^{\circ}\text{C}$  and 27  $\mu\text{L}$  were injected by autosampler. Serum carotenoid extraction was performed as previously reported with minor modifications (ref. 14;  $n = 5$  pooled serum samples/group,  $n = 6$  mice/pooled sample). Serum and diet samples were analyzed by HPLC using a photodiode array detector (HPLC-PDA; PDA 2996, Waters). The HPLC system and carotenoid standard preparation have been previously described (49). The analytic lab participates semiannually in the National Institute for Standards and Technology's Fat Soluble Vitamin and Carotenoid Round Robin activity to monitor protocol accuracy with respect to other analytical labs.

### Histologic analysis of prostate lobes

Histologic evaluation was performed on 5- $\mu\text{m}$ -thick formalin-fixed, paraffin-embedded tissue sections of the individual prostate lobes stained with hematoxylin and eosin. The lesions in each prostate lobe were evaluated according to the refined grading scheme for TRAMP mice [grade 0: normal, grade 1: low-grade prostatic intraepithelial neoplasia (PIN); grade 2: moderate-grade PIN; grade 3: high-grade PIN; grade 4: Phyllodes-like tumor; grade 5: well-differentiated adenocarcinoma; grade 6: moderately differentiated adenocarcinoma; grade 7: poorly differentiated carcinoma (includes both neuroendocrine and adenocarcinoma types); ref. 45]. The most severe and most common lesions within a lobe were initially defined, and then the distribution of each lesion in each lobe was determined to be either focal (fewer than three foci within the lobe), multifocal (three or more foci within the lobe, but with less than 50% of the lobe), or diffuse (greater than 50% of the lobe). Pathology was examined in each of the four lobes for the highest score, a combination of grade and distribution, and the overall highest score for the entire prostate. The overall highest scores indicative of the most advanced lesions were examined among all mice and dichotomized as cancer or noncancer for analysis of cancer incidence.

### Statistical analysis

The study design was a two-factor factorial experiment with two genotypes and three diets. Of particular interest was the hypothesis that the effect of diet on cancer incidence would vary by genotype, and this was tested in a statistical model as an interaction term (50). Models for these data included main

effects for genotype and diet as well as interaction terms between genotype and diet. Hypotheses were tested by using the corresponding linear contrast from the full model. Animal weights and organ weights were analyzed using linear regression, adjusting the organ outcomes for animal weights. Carotenoid concentrations were analyzed using linear regression after being log-transformed to improve normality and homoscedasticity. Logistic regression was used for the dichotomous outcome of cancer incidence and generalized estimating equations (GEE) were used for the lobe analyses to adjust for multiple measures within the same animal (51). Fisher's exact test was used to compare the distribution of lesion categories between groups with adjustment for multiplicity by the step-down Bonferroni (Holm) method (52). All analyses were conducted in SAS v9.3 (SAS Institute).

## Results

### Body weight and organ weights

There was a very modest impact of genotype on body weight ( $Bco2^{-/-} > Bco2^{+/+}$  by 1.1 g,  $P = 0.02$ ). The tomato- and lycopene-fed mice were slightly heavier than control-fed mice (2.7 g,  $P < 0.001$  and 3.7 g,  $P < 0.001$ , respectively). Body weight and organ weights (liver, kidney, spleen, and testes) by diet and genotype are presented in Supplementary Table S1.

### The status of BCO2 enzyme impacts serum lycopene concentrations

Lycopene was not detected in control-fed mice, and serum lycopene concentrations in both tomato- and lycopene-fed mice were approximately 0.25  $\mu\text{mol/L}$  in the TRAMP: $Bco2^{+/+}$  mice (Table 1 and Supplementary Fig. S1). The absence of the BCO2 enzyme was associated with 55% greater concentrations of total lycopene in serum ( $P = 0.0004$ ). The increase in serum lycopene concentrations upon loss of BCO2 was similar across lycopene geometric isomers: *trans*-lycopene (51% increase;  $P = 0.0006$ ), 5-*cis*-lycopene (52% increase;  $P = 0.0006$ ), other-*cis*-lycopene (64% increase;  $P = 0.0003$ ). There were no significant differences in total, *trans*-, 5-*cis*-, or other *cis*-lycopene between tomato- and lycopene-fed mice. The proportion of total lycopene found as the 5-*cis* isomer modestly differed between tomato-fed BCO2 genotypes (fold-difference WT vs. KO = 0.90; 95% confidence interval (CI), 0.82–0.99;  $P = 0.0315$ ). For this study, the proportion of lycopene found as 5-*cis* in lycopene-fed as well as other *cis* and all-*trans* lycopene in both tomato- and lycopene-fed mice did not approach statistical significance.

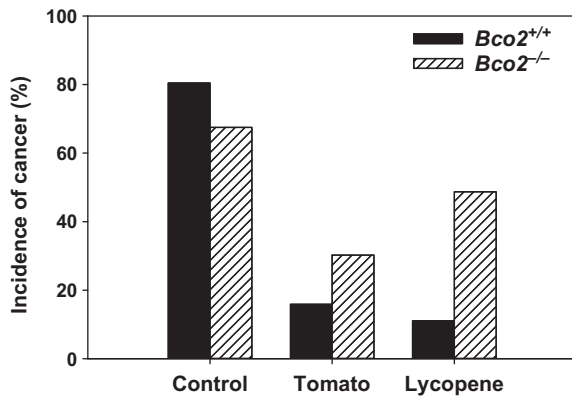
**Table 1.** Serum carotenoid concentrations by genotype and diet<sup>a</sup>

	Serum concentration ( $\mu\text{mol/L}$ ) $\pm$ SE				P values for comparisons	
	$Bco2^{+/+}$		$Bco2^{-/-}$		Across LYC and TP $Bco2^{+/+}$ vs. $Bco2^{-/-}$	Within TP $Bco2^{+/+}$ vs. $Bco2^{-/-}$
	LYC	TP	LYC	TP		
Lycopene	0.245 $\pm$ 0.013	0.252 $\pm$ 0.009	0.387 $\pm$ 0.051	0.404 $\pm$ 0.051	0.0004	—
All- <i>trans</i> <sup>b</sup>	0.064 $\pm$ 0.003 (26%)	0.072 $\pm$ 0.003 (29%)	0.104 $\pm$ 0.015 (27%)	0.106 $\pm$ 0.012 (26%)	0.0006	—
5- <i>cis</i> <sup>b</sup>	0.106 $\pm$ 0.006 (43%)	0.111 $\pm$ 0.005 (44%)	0.164 $\pm$ 0.022 (42%)	0.175 $\pm$ 0.023 (43%)	0.0006	—
Total <i>cis</i> <sup>b</sup>	0.075 $\pm$ 0.005 (31%)	0.069 $\pm$ 0.005 (27%)	0.119 $\pm$ 0.015 (31%)	0.123 $\pm$ 0.017 (30%)	0.0003	—
Phytoene	—	0.013 $\pm$ 0.002	—	0.019 $\pm$ 0.004	—	n.s.
Phytofluene	—	0.025 $\pm$ 0.003	—	0.042 $\pm$ 0.008	—	0.0342
$\zeta$ -Carotene	—	0.019 $\pm$ 0.002	—	0.030 $\pm$ 0.004	—	0.0408

Abbreviations: LYC, lycopene diet; TP, tomato powder diet.

<sup>a</sup> $n = 6$  mice/pooled sample.

<sup>b</sup>Concentrations are followed by % of total lycopene, rounded to the nearest 1%.



**Figure 1.**

Prostate cancer incidence in mice by diet and *Bco2* genotype. The impact of tomato and lycopene feeding on TRAMP prostate carcinogenesis at 18 weeks of age is dependent upon the *Bco2* genotype. The effect of lycopene or tomato on cancer reduction significantly differed by *Bco2* genotype (interaction effect of lycopene versus control by genotype  $P = 0.0004$  and interaction effect of tomato versus control by *Bco2* genotype,  $P = 0.0383$ ).  $n = 39$ – $46$  mice per group.  $P$  values for differences: Within the TRAMP: *Bco2*<sup>+/+</sup> mice: lycopene versus control,  $P < 0.0001$ ; tomato versus control,  $P < 0.0001$ . Within TRAMP: *Bco2*<sup>-/-</sup> mice: lycopene versus control,  $P = 0.0928$ , n.s.; tomato versus control,  $P = 0.0009$ . Within the lycopene-fed mice: TRAMP: *Bco2*<sup>-/-</sup> mice versus TRAMP: *Bco2*<sup>+/+</sup>,  $P = 0.0004$ . Between lycopene and tomato and within genotypes: tomato versus lycopene in *Bco2*<sup>+/+</sup>,  $P = 0.5096$ ; *Bco2*<sup>-/-</sup>,  $P = 0.0889$ ;  $P_{\text{interaction}} = 0.1239$ .

***Bco2* genotype impacts serum lycopene precursor concentrations**

The carotenoids phytoene, phytofluene, and ζ-carotene are biosynthetic precursors of lycopene and are present in tomatoes. As expected, all precursors were only detected in tomato-fed mice (Supplementary Fig. S2). The lack of BCO2 resulted in an increase in phytofluene concentrations (63%;  $P = 0.0342$ ) and ζ-carotene concentrations (55%;  $P = 0.0408$ ). A similar trend was observed for phytoene (41%;  $P = 0.2742$ , n.s.).

**Dietary tomato and lycopene modulate TRAMP carcinogenesis**

Histopathologic images of the most common grades of prostate carcinogenesis in 18-week-old TRAMP mice are presented in Supplementary Fig. S3. The impact of tomato and lycopene feeding on the incidence of prostate cancer (presence of cancer in at least one lobe) was dependent upon the *Bco2*

genotype (Fig. 1). There was a significant difference between the genotypes in the benefit received from the lycopene diet versus control ( $P_{\text{interaction}} = 0.0004$ ). Among the TRAMP: *Bco2*<sup>+/+</sup> mice, the cancer rate dropped from 80.4% with control feeding to 11.1% with lycopene feeding ( $P < 0.0001$ ). For the TRAMP: *Bco2*<sup>-/-</sup> mice, lycopene feeding led to a smaller reduction in incidence: from 67.5% in the control-fed mice to only 48.7% in the lycopene-fed mice ( $P = 0.0928$ , n.s.). The effect of tomato feeding versus control also significantly differed by *Bco2* genotype ( $P_{\text{interaction}} = 0.0383$ ). The cancer incidence in tomato-fed TRAMP: *Bco2*<sup>+/+</sup> was 15.9% (compared with 80.4% in the respective control-fed mice;  $P < 0.0001$ ), whereas the incidence in tomato-fed TRAMP: *Bco2*<sup>-/-</sup> mice was 30.2% (compared with a rate of 67.5% in respective control-fed mice;  $P = 0.0009$ ). Within the lycopene-fed animals, the incidence of cancer is greater in the TRAMP: *Bco2*<sup>-/-</sup> mice compared with TRAMP: *Bco2*<sup>+/+</sup> mice ( $P = 0.0004$ ). However, in spite of the large number of mice used in this study, this study was not sufficiently powered to precisely determine if the relative impacts of *Bco2* genotype on the cancer incidence differed between lycopene-fed versus tomato-fed mice are significantly different (tomato vs. lycopene in *Bco2*<sup>+/+</sup>,  $P = 0.5096$ ; *Bco2*<sup>-/-</sup>,  $P = 0.0889$ ;  $P_{\text{interaction}} = 0.1239$ ).

The distribution of the highest histopathologic grade (low-grade PIN, moderate-grade PIN, high-grade PIN, well-differentiated adenocarcinoma, moderately-differentiated adenocarcinoma, and poorly-differentiated adenocarcinoma) across treatment groups is presented in Table 2 (Supplementary Fig. S4). In the TRAMP: *Bco2*<sup>+/+</sup> mice fed control diet, the predominant grade was poorly-differentiated adenocarcinoma (54%), followed by well-differentiated adenocarcinoma (20%) and high-grade PIN (20%). In the TRAMP: *Bco2*<sup>-/-</sup> mice fed control diet, poorly-differentiated adenocarcinoma (33%) and well-differentiated adenocarcinoma (35%) were the predominant grades, followed by high-grade PIN (28%). Tomato and lycopene feeding reduced the lesion severity in TRAMP: *Bco2*<sup>+/+</sup> mice, with high-grade PIN being the most common lesion, followed by moderate-grade PIN in mice fed tomato powder (64% and 25%, respectively) and lycopene (62% and 24%, respectively), and cancer was evident in only 11% of mice in each diet group. However, tomato and lycopene feeding had less of an impact in TRAMP: *Bco2*<sup>-/-</sup> mice, where, in mice fed tomato powder, 42% presented with high-grade PIN, 35% with moderate grade PIN, 14% with well-differentiated cancer, and 9% with poorly differentiated cancer. Similarly, TRAMP: *Bco2*<sup>-/-</sup> mice fed lycopene presented with 36%

**Table 2.** Frequency and distribution of highest pathologic grades in all mice, by *Bco2* genotype and dietary treatment

Grade	Frequency (percentage)						Adjusted $P$ -values for differences in pathologic distributions <sup>a</sup>				
	<i>Bco2</i> <sup>+/+</sup>			<i>Bco2</i> <sup>-/-</sup>			Within genotype		Between genotypes		
	CON	TP	LYC	CON	TP	LYC	<i>Bco2</i> <sup>+/+</sup>	<i>Bco2</i> <sup>-/-</sup>	<i>Bco2</i> <sup>+/+</sup> vs. <i>Bco2</i> <sup>-/-</sup>		
Low-grade PIN	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	CON vs. TP	<0.0001	0.0002	CON	0.5121
Moderate-grade PIN	0 (0%)	11 (25%)	11 (24%)	1 (3%)	15 (35%)	8 (21%)	CON vs. LYC	<0.0001	0.0513	TP	0.5121
High-grade PIN	9 (20%)	28 (64%)	28 (62%)	11 (28%)	18 (42%)	14 (36%)	TP vs. LYC	0.6521	0.5121	LYC	0.0513
Well-differentiated adenocarcinoma	9 (20%)	3 (7%)	5 (11%)	14 (35%)	6 (14%)	12 (31%)					
Moderate-differentiated adenocarcinoma	3 (7%)	0 (0%)	0 (0%)	1 (3%)	0 (0%)	2 (5%)					
Poorly differentiated adenocarcinoma	25 (54%)	2 (5%)	0 (0%)	13 (33%)	4 (9%)	3 (8%)					

NOTE: See Supplementary Fig. S4 for a stacked bar graph depicting the data. Abbreviations: CON, control diet; TP, tomato powder diet; LYC, lycopene diet.

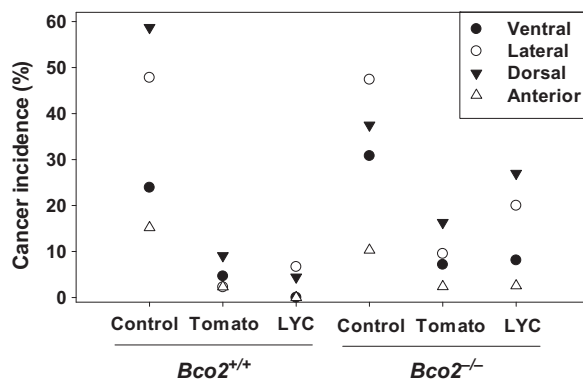
<sup>a</sup>Significant differences in pathologic distributions were evaluated by Fisher's exact test using the Bonferroni step-down adjustment method for repeated hypothesis testing.

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high-grade PIN, 31% well-differentiated adenocarcinoma, 21% moderately differentiated adenocarcinoma, and 8% poorly differentiated adenocarcinoma. These results are indicative of a shift to a more advanced cancer phenotype in mice lacking BCO2 compared with those with intact BCO2 when fed the carotenoid-containing diets. Pairwise analysis of the distributions of pathologic grades between groups was conducted. *Bco2* genotype was not associated with a significant difference in the distribution of pathologic grades for mice on the control diet (TRAMP: *Bco2*<sup>+/+</sup> vs. TRAMP: *Bco2*<sup>-/-</sup>, *P* = 0.5121). Consistent with the overall cancer grading data, tomato- and lycopene-fed TRAMP: *Bco2*<sup>+/+</sup> mice had significantly different pathologic distributions (both *P* < 0.0001) than TRAMP: *Bco2*<sup>+/+</sup> mice compared with control. In parallel, the distributions were significantly different in TRAMP: *Bco2*<sup>-/-</sup> mice fed tomato versus control (*P* = 0.0002), but a nonsignificant trend when fed lycopene versus control (*P* = 0.0513). There was also a nonsignificant trend in the distribution between the TRAMP: *Bco2*<sup>+/+</sup> and TRAMP: *Bco2*<sup>-/-</sup> animals fed the lycopene-containing diets (*P* = 0.0513), but no trend with tomato-containing diets (*P* = 0.5121). There were no significant differences in the distribution of pathologic grades between mice fed tomato-containing and those fed lycopene-containing diets for either of the genetic backgrounds.

#### The murine prostate lobe-specific distribution of cancer incidence

We present the lobe-specific distribution of pathology in Fig. 2. It is important to place these results in context with the genetics of our model. The incidence and severity of cancer in the dorsal, ventral, lateral, and anterior lobes is greater in the more commonly used F1 generation of C57BL/6-Tg(TRAMP)8247Ng/J x FVB/NJ (45) compared with the currently used model C57BL/6-Tg(TRAMP)8247Ng/J, which was not crossed to the FVB/NJ background (53). In our study, it was not feasible to integrate the TRAMP<sup>+/+</sup>:FVB F1 cross with the *Bco2*<sup>-/-</sup> genotype due to the breeding scheme necessary to generate



**Figure 2.** Prostate lobe-specific incidence of cancer by diet and *Bco2* genotype. Overall, the incidence of cancer was dorsal > lateral > ventral > anterior. The control diet-fed TRAMP: *Bco2*<sup>+/+</sup> mice at 18 weeks of age exhibited carcinoma in 58.7% of dorsal lobes, 47.8% of lateral, 23.9% of ventral, and 15.2% of anterior lobes. Statistical modeling of the cancer incidence using generalized estimating equations, showed that there was a significant impact of lobe (*P* < 0.0001) and an interaction of genotype\*diet (*P* = 0.0014). *n* = 39–46 mice per group. LYC, lycopene.

the TRAMP: *Bco2*<sup>-/-</sup> mice. Thus, our study was conducted in mice without the FVB/NJ cross. The currently utilized mixed background composed of C57BL/6-Tg(TRAMP)8247Ng/J and B6 bears strong similarity to TRAMP mice on a pure B6 background, which are known to have less aggressive cancer and relatively low incidence of neuroendocrine carcinoma or neuroendocrine markers (54). In these 18-week-old TRAMP: *Bco2*<sup>+/+</sup> mice fed control diet, the incidence of cancer was 58.7% in dorsal lobes, 47.8% in lateral, 23.9% in ventral, and 15.2% in anterior lobes. Within the control diet, the BCO2 genotype did not significantly impact the incidence of cancer in any of the four lobes (anterior, *P* = 0.4997; dorsal, *P* = 0.0517; lateral, *P* = 0.9667; ventral, *P* = 0.4792). Modeling the cancer incidence using a logistic GEE approach demonstrated that the cancer incidence was significantly different by lobe (*P* < 0.0001; Fig. 2). Compared with the odds of cancer in the anterior lobe, the odds of cancer in the dorsal lobe was 7.37 times higher (95% CI, 4.03–13.48; *P* < 0.0001), 5.87 times higher in the lateral lobe (95% CI, 3.15–10.92; *P* < 0.0001), and 2.55 times higher in the ventral lobe (95% CI, 1.29–5.07; *P* = 0.0074). In this strain of mice, the ventral lobe was less likely to have cancer compared with the lateral lobe (OR 0.44; 95% CI, 0.28–0.68; *P* = 0.0003) or the dorsal lobe (OR 0.35; 95% CI, 0.22–0.55; *P* < 0.0001). There was no difference between the lateral and dorsal lobes (OR 0.80; 95% CI, 0.52–1.21; *P* = 0.29). As in the model for overall cancer incidence, there was a significant genotype-diet interaction (*P* = 0.0014) impacting cancer incidence within each lobe. However, in this study, the effect of diet, genotype, or diet-genotype interaction did not differ by lobe, as indicated by the lack of a significant 3-way interaction between genotype-diet-lobe (*P* = 0.80) or 2-way interaction between diet-lobe (*P* = 0.3702) or genotype-lobe (*P* = 0.4377).

Frequently, the weight of the urogenital tract or individual lobe weights are used as surrogates for disease index. However, at this early age where we are capturing the transition from the preneoplastic lesion to the histopathologic cancer, few mice experience a large tumor mass, and the urogenital tract or lobe weights were not strong surrogates for early progression of prostate carcinogenesis (Supplementary Table S2).

## Discussion

This study illustrates that a specific genetic variation impacting carotenoid metabolism alters the ability of a dietary carotenoid (lycopene) to impact prostate cancer risk in parallel with changes in serum carotenoids. Thus, this highly controlled preclinical study has important implications for human clinical trials and epidemiologic studies addressing the impact of tomatoes or lycopene on prostate cancer risk. We hypothesized that tomato or lycopene feeding would reduce early carcinogenesis at the transition from PIN to adenocarcinoma in the TRAMP model, and the results support that both interventions had a similar impact in this system. However, most interestingly, the effect of lycopene feeding was strongly *Bco2*-dependent, with *Bco2*<sup>-/-</sup> mice having greater cancer incidence and, in general, more severe lesion scores than lycopene-fed *Bco2*<sup>+/+</sup> mice. The effect of *Bco2* genotype was still significant but less pronounced in tomato-fed mice, where some of the anticancer impact may be maintained by other phytochemicals present in tomato powder. Together, these data indicate for the first time that part of the antiprostata cancer bioactivity of lycopene is BCO2 dependent. The mechanistic

implications are even more provocative when placed in context of serum carotenoids. The loss of *Bco2* in TRAMP mice causes an increase in serum lycopene and several other tomato carotenoids, yet the anticancer activities of tomato and lycopene are reduced. This finding implicates cleavage products of tomato carotenoids as potentially mediating some of the anticancer activity in the TRAMP model.

It is clear that any single rodent model of prostate cancer cannot capture the full spectrum of human prostate cancer biology and clinical behavior, which is notoriously heterogeneous. Each unique model may provide insight into specific cancer pathways involved in subtypes of human prostate cancer. The TRAMP model was one of the first genetically manipulated systems available for prostate cancer investigators but over time has often been criticized for its progression to poorly differentiated cancers with neuroendocrine features at very late stages of growth (55). These features are characteristic of advanced castrate- and chemotherapy-resistant prostate cancers associated with the lethal phases of human prostate cancer progression. However, over the first 18 weeks of life, the TRAMP model clearly exhibits a progression from normal epithelia to prostatic intraepithelial neoplasia to localized adenocarcinoma, the rate of which is dependent upon genetic background (strain) of mice and shows a histopathologic process similar to humans (53, 55). Perhaps more critically, the emerging molecular genomics of human prostate cancer demonstrates marked heterogeneity, with several genes and networks showing high frequencies of genetic damage, including those of the androgen receptor (63%), *TP53* (53%), *PTEN* (41%), and *ETS* fusions (57%) (46). The SV40-driven TRAMP system disrupts p53 signaling (56), contributing to dysregulation of the cell cycle, enhanced cell proliferation, increased threshold for apoptotic cascade activation, and promotion of genomic instability, and is thus mimicking key genomic and pathologic signatures of human prostate cancer (46). One potential mechanism with the TRAMP model may be that interventions which may impact androgen signaling, such as tomato and lycopene (27), may disrupt androgen-driven expression of the SV40 transgene; however, inspection of prostatic SV40 staining in 10-week-old mice after consuming control, 10% tomato powder- and 0.25% lycopene beadlet-containing diets from weaning does not support any diet effect on SV40 expression (Supplementary Fig. S5 and Supplementary Methods). Thus, the recent elucidation of the molecular defects in human prostate cancer progression and histopathologic evolution suggest that the TRAMP system is a relevant experimental system, particularly for preclinical evaluation of dietary and chemopreventive strategies during early carcinogenesis (57).

It is useful to compare this study with the accumulating literature of tomato products and lycopene in various preclinical models. To date, we are aware of nine publications in models of *de novo* prostate carcinogenesis (9–15, 23, 58). In the NMU/androgen-induced model of rat prostate carcinogenesis, feeding of tomato powder emerged as more effective in the inhibition of cancer risk than lycopene, which was modestly effective (10). The concept of greater bioactivity of tomato products compared with lycopene is generally supported by studies in transplantable models using cell lines (22). There are three publications (13, 14, 58) in TRAMP evaluating lycopene at various dosages and durations, with significant inhibition of prostate cancer reported among those studies providing higher lycopene concentrations. One previous study showed that tomato powder reduced

TRAMP carcinogenesis, which was potentiated by additional soy intake (48). This study represents the first comparison of lycopene and tomato powder in the TRAMP model showing significant and very similar anticancer bioactivity and serum concentrations of lycopene. It is our impression that the TRAMP system is particularly sensitive to lycopene, often producing quantitative reductions in the carcinogenesis cascade that are more substantial than observed in other models. This specific sensitivity suggests that the anticancer activity of lycopene directly targets mechanisms of carcinogenesis impacted by SV40, such as the p53 pathway. Our recent study, evaluating nearly 200 prostate cancer-related genes, supports the concept that lycopene and tomato products produce similar impacts upon gene expression patterns in the TRAMP system, suggesting that much of the impact of tomato products in the TRAMP model may be due to lycopene content (27). Detailed analysis of the pathologic grading from this study (Table 2; Supplementary Fig. S3) indicates that dietary tomato and lycopene reduced lesion severity and overall incidence in TRAMP mice, which aligns with recent findings from a 15 study meta-analysis indicating that greater blood lycopene concentrations, resulting from dietary intake of lycopene-containing foods, are inversely correlated with severity and lethality (5). Overall, studies of cancer prevention in rodent models (9–15, 23, 58) provide substantial support for the epidemiologic associations between tomato components and reduced risk of prostate cancer; nonetheless, one should remain cognizant of the potential for publication bias.

It is appreciated that diet and genetics are likely to impact human cancer risk, yet few of these interactions have been fully documented and characterized, which reduces the sensitivity and specificity of nutritional epidemiologic studies. Murine models allow remarkable precision for the evaluation of such interactions, due to the control of dietary intake combined with the ability to manipulate the genome of mice. This study is the first to demonstrate that *BCO2* can impact carcinogenesis, although this effect is diet dependent. In the TRAMP mice fed a control AIN diet, we do not detect an impact of *Bco2*<sup>-/-</sup> compared with *Bco2*<sup>+/+</sup> genotype. There is new evidence that exogenous *Bco2* expression leads to inhibition of prostate cancer cell growth *in vitro*, in a lycopene- and enzyme activity-independent manner, (59) highlighting a potential *BCO2* function in prostatic tissue, that was not detectable in the TRAMP system designed to test prevention. When fed tomato or lycopene, we see an attenuation of the anticancer effect in the *Bco2*<sup>-/-</sup> compared with *Bco2*<sup>+/+</sup> mice. Although our study was not powered to detect a difference in the tomato- versus lycopene-fed group, it appears that the impact of genotype may be stronger in the lycopene-fed mice compared to those fed tomato. We can speculate that perhaps other components, such as noncarotenoid phytochemicals with anticancer activity found in the tomato powder (24) may overcome the loss of lycopene's impact in the *Bco2*<sup>-/-</sup> mice.

The tomato or lycopene diet × *Bco2* genotype interaction observed on prostate carcinogenesis provides the opportunity to develop mechanistic hypotheses. We also observe in this long-term study that *Bco2*<sup>-/-</sup> mice attain greater serum lycopene concentrations than *Bco2*<sup>+/+</sup> mice, as suggested by our prior research (30, 35). Thus, this pattern suggests that lycopene cleavage metabolites produced by *BCO2* may have anti-prostate cancer bioactivity and that their production is diminished in the *Bco2*<sup>-/-</sup> mice. Previous studies on the effect of the lycopene metabolite, apo-10'-lycopenoic acid, have shown it to reduce hepatic



inflammation, steatosis, and tumorigenesis in mice (60, 61), and other studies of apo-8'-lycopenal in models of liver cancer have been indicative of bioactivity (38, 39), increasing the plausibility of lycopene metabolite activity in the prostate. We also demonstrate that tomato-fed *Bco2*<sup>-/-</sup> accumulate lycopene precursors, phytofluene, ζ-carotene, and a suggestion for phytoene, opening the door for hypotheses regarding the potential for BCO2 to cleave additional carotenoids. The continued development of HPLC-MS technology is beginning to elucidate the *in vivo* cleavage products of lycopene (30, 41). However, we should be very open to alternative hypotheses, as we are just beginning to understand the role(s) of BCO2, and we anticipate that future studies will provide greater precision in understanding the findings we report. For example, a recent report shows that *Bco2* expression increases in response to oxidative stress and that loss of *Bco2* leads to excessive accumulation of carotenoids in tissues, which may then function as pro-oxidants (62), potentially leading to procarcinogenic activity. However, increases in plasma lycopene in the *Bco2*<sup>-/-</sup> mice observed in this study are not remarkably greater than those in the wild-type mice and are within typical plasma ranges found in humans. Therefore, this alternative hypothesis can be considered as one potential possibility among many regarding why, in the current study, *Bco2*<sup>-/-</sup> mice fed carotenoids had greater cancer incidence than similarly fed wild-type mice.

Our studies also suggest that additional investigation of human BCO2 enzymatic activity—how it is regulated and its impact on serum and prostate lycopene and metabolites—are warranted. One direction with the potential for impact will be the evaluation of BCO2 SNVs and the relationship of the genotype to plasma and tissue concentrations of lycopene and metabolites. This information, when evaluated in human epidemiologic cohort studies where an integrated assessment of genotype, dietary intake, and serum lycopene and/or metabolites is possible, should greatly improve our ability to define more accurately and quantitatively the potential for tomato products to alter risk. Although large-scale prostate cancer prevention studies with tomato products or lycopene are unlikely to be undertaken in the near future due to budget limitations by funding agencies, short-term clinical studies focusing upon biomarkers of carcinogenesis are achievable, and SNVs for BCO2 and other genes impacting lycopene metabolism (63) may be critical for interpretation of results. Another key element of elucidating the diet × genotype interaction will focus upon the tissue-specific expression of *Bco2*. At present, it appears that *Bco2* is very modestly expressed in prostate tissue of mice (unpublished observations), suggesting that the major sites of lycopene metabolism by *Bco2* are liver and other tissues (30, 31, 35). Thus, the impact of lycopene metabolites will require an understanding of their distribution to the prostate for direct impact or, conversely, an examination of how metabolites indirectly impact prostate carcinogenesis through systemic effects on anticancer immunity, endocrine pathways, or other host mechanisms. Finally, the presence of *Bco2* has anticancer benefit in the presence of lycopene, and thus we can propose that the loss of *Bco2* by an evolving

prostate cancer is potentially one additional mutation which ensures resistance and promotes progression.

In conclusion, we demonstrate that dietary tomato and lycopene, producing serum concentrations of lycopene in mice that are similar to those found in humans, significantly reduces prostate carcinogenesis in the TRAMP model. Interestingly, the deletion of *Bco2* gene, an eccentric carotenoid cleavage enzyme, may reduce the efficacy of lycopene as a chemopreventive agent. These studies support the hypothesis that lycopene cleavage metabolites may have anticancer activity. In addition, the interactions between diet and genotype on prostate carcinogenesis demonstrated in the rodent model suggest that additional research to elucidate the potential roles for BCO2 polymorphisms in humans consuming tomato products may be useful in the interpretation of cohort and clinical studies. With additional preclinical and clinical studies, we will continue to refine the molecular mechanisms, the timing, and the genetic interactions by which tomato consumption may impact prostate cancer.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Authors' Contributions

**Conception and design:** H.-L. Tan, J.W. Erdman Jr, S.K. Clinton

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**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** H.-L. Tan, J.M. Thomas-Ahner, N.E. Moran, J.W. Erdman Jr, G.S. Young, S.K. Clinton

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### Acknowledgments

R.C. Rengel assisted in the establishment of the breeding colonies necessary for the generation of the experimental genotypes. We are grateful to Johannes von Lintig and Adrian Wyss for providing the initial breeding stock (*Bco2*<sup>-/-</sup> mice) to establish our colony and complete the described studies.

### Grant Support

This work was supported by the NIH [NCI-R01125384; principal investigator (PI): J.W. Erdman, Jr.] and the American Institute for Cancer Research (AICR; PI: S.K. Clinton) with additional resources provided by the Ohio State University Molecular Carcinogenesis and Chemoprevention Program supported by The Ohio State University Comprehensive Cancer Center (NIH/NCI P30 016058), The Center for Advanced Functional Food Research and Entrepreneurship, The Food Innovation Center, and The James Development Prostate Cancer Prevention Fund (302024). N. Moran was funded by a Pelotonia Postdoctoral Fellowship.

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Received December 1, 2015; revised October 7, 2016; accepted October 23, 2016; published OnlineFirst November 2, 2016.

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