Research Article

Lycopene Attenuated Hepatic Tumorigenesis via Differential Mechanisms Depending on Carotenoid Cleavage Enzyme in Mice

Blanche C. Ip, Chun Liu, Lynne M. Ausman, Johannes von Lintig, and Xiang-Dong Wang

Abstract

Obesity is associated with increased liver cancer risks and mortality. We recently showed that apo-10'-lycopenoic acid, a lycopene metabolite generated by beta-carotene-9',10'-oxygenase (BCO2), inhibited carcinogen-initiated, high-fat diet (HFD)-promoted liver inflammation, and hepatic tumorigenesis development. The present investigation examined the outstanding question of whether lycopene could suppress HFD-promoted hepatocellular carcinoma (HCC) progression, and if BCO2 expression is important using BCO2-knockout (BCO2-KO) and wild-type male mice. Results showed that lycopene supplementation (100 mg/kg diet) for 24 weeks resulted in comparable accumulation of hepatic lycopene (19.4 vs. 18.2 nmol/g) and had similar effects on suppressing HFD-promoted HCC incidence (19% vs. 20%) and multiplicity (58% vs. 62%) in wild-type and BCO2-KO mice, respectively. Intriguingly, lycopene chemopreventive effects in wild-type mice were associated with reduced hepatic proinflammatory signaling (phosphorylation of NF-kB p65 and STAT3; IL6 protein) and inflammatory foci. In contrast, the protective effects of lycopene in BCO2-KO but not in wild-type mice were associated with reduced hepatic endoplasmic reticulum stress–mediated unfolded protein response (ERUPR), through decreasing ERUPR-mediated protein kinase RNA-activated like kinase–eukaryotic initiation factor 2a activation, and inositol requiring 1a–X-box–binding protein 1 signaling. Lycopene supplementation in BCO2-KO mice suppressed oncogenic signals, including Met mRNA, β-catenin protein, and mTOR complex 1 activation, which was associated with increased hepatic microRNA (miR)-199a/b and miR214 levels. These results provided novel experimental evidence that dietary lycopene can prevent HFD-promoted HCC incidence and multiplicity in mice, and may elicit different mechanisms depending on BCO2 expression. Cancer Prev Res; 7(12); 1219–27. ©2014 AACR.

Introduction

Primary liver cancer is the third leading cause of cancer-related deaths worldwide (1, 2), and hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, accounting for 70% to 85% of cases (1, 2). Nonalcoholic fatty liver disease (NAFLD) is a pathology that is observed in 75% to 100% of overweight and obese adults and children (3), and its rising prevalence parallels closely with HCC’s escalating morbidity and mortality trends (3). The prevention of liver cancer progression through dietary means represents an important disease control strategy because HCC has a high mortality rate and a poor prognosis (4, 5).

Previous animal studies demonstrated that high-fat diet (HFD) and obesity promoted liver tumorigenesis by inducing chronic inflammation through the IL6/STAT3 pathway (6), with STAT3-activated tumors being more aggressive in humans (6–8). Metabolic surplus from excess calorie consumption can also elevate synthesis of hepatic enzymes, which creates excess demand on the endoplasmic reticulum (ER) for proper protein folding (9–11). This excess demand on the ER leads to the induction of ER stress–mediated unfolded protein response (ERUPR; refs. 9–11), which was associated with liver cancer development (11).

Observational studies have shown beneficial associations between lycopene-rich foods against various cancers (as reviewed in refs. 12–14), including those of gastrointestinal tract origin (14). Patients with NAFLD have
significantly reduced plasma lycopene (15), suggesting a potential interactions between low lycopene status and the development of liver diseases. In the rat model, dietary lycopene has been shown to reduce the liver-specific carcinogen diethylnitrosamine (DEN) initiation of hepatic neoplastic foci and macroscopic nodules (16–18), inhibit hepatic glutathione S-transferase placental-form–positive foci in rats that develop spontaneous liver tumors (19), and ameliorate DEN-initiated, HFD-promoted precancerous lesions (20). However, the primary outcomes for these rat studies were hepatic neoplastic lesions that may develop into tumors. There are currently no published in vivo studies to demonstrate whether lycopene can effectively reduce HCC development and progression. Our mechanistic understanding of how lycopene functions against hepatic tumorigenesis is also far from complete (21).

Lycopene can be preferentially metabolized by the enzyme beta-carotene 9,10-oxygenase (BCO2), and generate apo-10'-lycopenoids, including apo-10'-lycopenal, -lycopenol and -lycopenoic acid (APO10LA; refs. 22, 23). It is important to understand whether lycopene effects on various cellular functions and signaling pathways are the results of intact lycopene or apo-10'-lycopenoids (13). We have recently shown that APO10LA supplementation significantly reduced hepatic inflammation (decreased inflammatory foci, TNFα, IL6, NF-κB p65 protein expression, and STAT3 activation) and tumorigenesis in HFD-fed mice (24). Therefore, lycopene metabolites such as APO10LA, may exhibit protective effects against obesity-associated hepatic inflammation and tumorigenesis. The outstanding question is whether BCO2 expression is critical for the potential biologic effects of lycopene against HFD-promoted liver tumorigenesis. This information is critically needed because 19 SNPs of BCO2 have been found in humans (25). These BCO2 SNPs in humans are associated with increased circulatory proinflammatory IL18 expression (25), and with reduced circulatory high-density lipoprotein (25), suggesting a gene–diet interaction between the BCO2 enzyme and dietary lycopene on human health outcomes. We hypothesize that lycopene is effective in inhibiting HFD-promoted liver tumorigenesis, and lycopene biologic actions could be different in the absence of BCO2 expression.

Using the BCO2-knockout (BCO2-KO) and wild-type mice in the present study, we investigated the potential inhibitory effects of lycopene against HFD-promoted hepatic tumorigenesis, and elucidated the underlying mechanisms by which lycopene exhibited these chemopreventive effects.

Materials and Methods

Study design

The in vivo experimental protocol was adapted from previous publications that studied hepatic tumorigenesis (6, 24, 26). All study protocols were approved by the Institutional Animal Care and Use Committee at the Jean Mayer-USDA Human Nutrition Research Center on Aging at Tufts University. The generation of BCO2-KO mice with BCO2 ablation at the protein level was described in the previous study (27). The respective wild-type control mice with a 129Svl/SvEvTac F1 generation–mixed genetic background were established by conventional cross-breeding in our animal facility. The rationale for the selected wild-type background was based upon the embryonic stem (ES) cell mouse strain used to generate the BCO2-KO mice (27). Therefore, using mice that share the same genetic background as these ES cells would sufficiently represent the biologic effects of BCO2 enzyme expression. The schematic for the study design is shown in Fig. 1A. Study mice were fed the standard laboratory chow (Harlan Laboratories), maintained on a 12-hour light/dark cycle in a controlled temperature and humidity room, and given water ad libitum. Two-week-old male wild-type and BCO2-KO mice were injected i.p. with a liver-specific carcinogen, DEN (Sigma-Aldrich) at a dosage of 25 mg/kg body weight as previously described (6, 24). At 6 weeks of age, wild-type and BCO2-KO mice were randomized to either an obesogenic HFD.
Liver tumors quantification and liver tissue processing

Whole livers were removed from study mice after euthanization and processed as previously described (24). Briefly, two investigators unaware of treatment groups counted the surface liver tumors (tumor multiplicity). Livers were weighed and washed with saline for further processing. Surface liver tumors were removed, snap-frozen in liquid nitrogen and stored at −80°C. The left lobe of mouse liver was fixed in 10% buffered formalin solution (Thermo Fisher Scientific), processed and embeded in paraffin for serial sectioning as described in the previous study (24). The remaining sections of liver were divided into smaller portions, snap-frozen in liquid nitrogen and stored at −80°C.

Histopathologic evaluation of liver tissue

Sections of (5 μm) formalin-fixed, paraffin-embedded liver tissue were stained with hematoxylin and eosin (H&E) for histopathologic examination. H&E-stained liver slides were examined by two independent investigators blinded to treatment groups under light microscopy (Zeiss). Liver histopathology of non-tumor areas was graded in 20 random fields at ×100 magnification, according to the degree of liver inflammation severity as described previously (24, 34). Briefly, inflammatory foci were evaluated by the number of inflammatory cell clusters, which mainly constitute the infiltration of mononuclear inflammatory cells. Mean foci per field were calculated and reported as inflammatory cell clusters per cm². The liver tumor was confirmed as HCC by two independent investigators according to the following criteria: (i) the presence of trabecular pattern with 3+ cell-thick hepatocellular plates/cords; (ii) mitotic figure; (iii) enlarged convoluted nuclei or high nuclei/cytoplasmic ratio; (iv) the presence of tumor giant cells with compact growth pattern; and (v) the presence of endothelial cells lining of sinusoids that surround enlarged hepatocellular plates/cords.

HPLC analysis

Lycopene (all-trans and 5-, 9-, and 13-cis-isomers) and lycopene metabolites, including apo-10'-lycopenal, apo-10'-lycoenoic acid concentrations in liver tissue and diets, were measured by gradient reverse phase HPLC consisted of a Waters 2695 separations module and a Waters 2996 photodiodearray detector (Waters) as previously described (23, 29). Lycopene and metabolites were quantified relative to the internal standard by determining peak areas calibrated against known amounts of standard.

RNA and microRNA extraction and quantitative real-time PCR

Total RNA was extracted from frozen liver sections with TRIzol reagent (Invitrogen), as previously described (24). cDNA was prepared from the RNA samples using M-MLV (for mRNA; Invitrogen) or M-MuLV [for microRNA (miR); BioLabs] reverse transcriptases and an automated thermal cycler PTC-200 (MJ Research). Quantitative real-time PCR (qRT-PCR) was performed using FastStart Universal SYBR Green Master (ROX; Roche). Relative gene expression was determined using the 2−ΔΔCt method. Primer sequences are listed in Supplementary Table S1.

Protein isolation and Western blotting

Both tissue protein preparation and Western blotting analysis were as described previously (24). The following antibodies were used for Western blotting: mTOR, NF-xB p65, eukaryotic initiation factor (eIF) 2α, phosphorylated-eIF2α (Ser51), phosphorylated NF-xB p65 (Ser536), phosphorylated-STAT3 (Tyr705), phosphorylated S6 (Ser235/236), S6, STAT3 (Cell Signaling Technology), IL6 (R&D Systems), CCAAT/enhancer–binding protein homology protein (CHOP), and cyclin D1 (Santa Cruz Biotechnology). Proteins were detected by a horseradish peroxidase–conjugated secondary antibody (Bio-Rad). The specific bands were visualized by the SuperSignal West Pico Chemiluminescent Substrate Kit (Pierce) according to the manufacturer’s instructions. Dilution series and calibration curve were performed for each of the antibodies used to quantify protein. Antiactin antibody (Sigma-Aldrich) was used to detect β-actin for loading normalization of some proteins. Intensities of protein bands were quantified using GS-710 Calibrated Imaging Densitometer (Bio-Rad).
**Statistical analysis**

SAS 9.3 software was used to perform the statistical analysis. Two-way ANOVA analysis with Tukey adjustments for multiple comparisons was used to evaluate the effects of BCO2 protein expression, lycopene supplementation, and the potential interactions between these two factors. The \( \chi^2 \) test was used to examine the effects of mouse strains or dietary lycopene on liver tumor incidence. Student \( t \) test or Wilcoxon signed-rank test was used to test for the differences between the following comparisons: (i) WT and WT+Ly; (ii) KO and KO+Ly. Statistical significance was \( P < 0.05 \).

**Results**

**Lycopene supplementation inhibited HCC development in both wild-type and BCO2-KO mice without altering body/liver weights**

Food intake by weight was similar among the four groups of mice (Table 1). Lycopene had no significant effect on body or liver weight in either mouse strain (Table 1), although BCO2-KO mice exhibited significantly lower final body or liver weight in either mouse strain (Table 1), irrespective of mouse strain. Dietary lycopene supplementation (Table 1), BCO2-KO mice accumulated a great proportion of hepatic all-trans lycopene (65%), as compared with WT (51% as all-trans lycopene, Table 1). Using our HPLC analysis, we did not detect measurable amounts of lycopene metabolites in hepatic tissue from all groups of mice (data not shown).

<table>
<thead>
<tr>
<th>Study group</th>
<th>WT</th>
<th>WT+Ly</th>
<th>KO</th>
<th>KO+Ly</th>
<th>P values for two-way ANOVA or Wilcoxon signed-rank test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food consumption, g/d</td>
<td>2.6 ± 0.1</td>
<td>3.1 ± 0.5</td>
<td>3.0 ± 0.1</td>
<td>2.9 ± 0.1</td>
<td>&lt;0.01 &lt;0.06 0.54 0.11</td>
</tr>
<tr>
<td>Final body weights, g</td>
<td>52.2 ± 1.1</td>
<td>49.1 ± 1.3</td>
<td>43.6 ± 1.9</td>
<td>41.6 ± 1.7</td>
<td>&lt;0.01 &lt;0.01 0.71</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>2.4 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>&lt;0.01 &lt;0.14 &lt;0.01 0.98</td>
</tr>
<tr>
<td>Liver/body weight, %</td>
<td>4.6 ± 0.2</td>
<td>4.6 ± 0.2</td>
<td>3.4 ± 0.1</td>
<td>3.2 ± 0.1</td>
<td>&lt;0.01 &lt;0.64 &lt;0.01 0.55</td>
</tr>
<tr>
<td>Liver tumor outcomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence, %</td>
<td>88</td>
<td>71</td>
<td>100</td>
<td>80 (( P = 0.10 ))</td>
<td>NA 0.03 0.48 NA</td>
</tr>
<tr>
<td>Multiplicity, n</td>
<td>17.8 ± 4.5</td>
<td>7.4 ± 1.8(^a)</td>
<td>10.3 ± 2.2</td>
<td>4.0 ± 0.7(^b)</td>
<td>0.05 0.01 0.52 0.98</td>
</tr>
<tr>
<td>Hepatic lycopene, nmol/g tissue</td>
<td>ND</td>
<td>19.4 ± 4.1(^b)</td>
<td>ND</td>
<td>18.2 ± 3.2(^b)</td>
<td>0.01 &lt;0.01 0.82 0.90</td>
</tr>
<tr>
<td>All-trans/cis-isomers, %</td>
<td>ND</td>
<td>51.49</td>
<td>ND</td>
<td>65:35</td>
<td>NA NA &lt;0.01 NA</td>
</tr>
<tr>
<td>Inflammatory Foci, n/cm(^2)</td>
<td>0.95 ± 0.6</td>
<td>0.55 ± 0.1 (( P = 0.06 ))</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.1</td>
<td>NA 0.29 0.04 NA</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not applicable; ND, not detected.

\(^a\)Values are means ± SEMs or n (%). Two-way ANOVA was used to examine the overall, diet, strain, and diet-strain effects. Student \( t \) test, \( \chi^2 \) test, or Wilcoxon signed-rank test was used to compare between WT and WT+Ly or KO and KO+Ly.

\(^b\)Different from WT or KO, \( P < 0.05 \).
Lycopene supplementation was associated with decreased mTOR activation and protooncogene Met expression in BCO2-KO but not wild-type mice

Chronic mTORC1 activation promoted HCC development in mice, through inducing mTOR protein expression and the activation of S6 ribosomal protein by phosphorylation (36). Elevation in protooncogenes Met and β-catenin is both positively associated with increased hepatocarcinogenesis, partially through promoting cell proliferation (37, 38). Lycopene chemopreventive effects in BCO2-KO mice were associated with reduced Mtor mRNA (27%; Fig. 2F), mTOR protein (43%; \( P = 0.06 \); Fig. 3A), Met mRNA (17%; Fig. 2F), cell proliferation marker cyclin D1 protein (44%; Fig. 3B), β-catenin protein (33%; Fig. 3C), but not β-catenin mRNA (Ctnnb1; Fig. 2F). In wild-type mice, dietary lycopene also lessened Mtor mRNA (20%; Fig. 2F), mTOR protein (42%; Fig. 3A), cyclin D1 protein (44%; Fig. 3B), and β-catenin protein (37%; Fig. 3C) expression. However, lycopene reduced mTOR signaling as measured by S6 ribosomal protein phosphorylation was only observed in BCO2-KO mice (61%, Fig. 3D), but not in wild-type mice. miR199a/b directly targeted Mtor and Met mRNA, leading to the subsequent downregulation of their protein products (37, 39). miR214 induction was shown to reduce β-catenin protein without altering its mRNA (Ctnnb1) expression (38, 40). Decrement in miR199a/b and miR214 has been associated with HCC development (37, 38, 40–42), and linked to ER\textsuperscript{UPR} (41). We observed that the lycopene-mediated reduced ER\textsuperscript{UPR} in BCO2-KO mice coincided with the significant elevation in hepatic miR199a/b (25%; Fig. 3E) and miR214 expression (23%; Fig. 3E), but not in wild-type mice.

Discussion

To the best of our knowledge, the present study provides the first experimental evidence that lycopene supplementation is effective in inhibiting DEN-initiated HCC incidence and multiplicity in two different strains of mice, the BCO2-KO strain and its respective wild-type. The final body weight difference between wild-type and BCO2-KO mice in the present study did not impede the beneficial effects of dietary lycopene against HCC development. This result underscores the potential chemopreventive effects of dietary lycopene against HFD-promoted tumorigenesis in mice, regardless of the amount of body weight gain. Moreover, the hepatic lycopene concentrations in lycopene-supplemented mice (18.2–19.4 nmol/g tissue) were within ranges for humans (0.1–20.7 nmol/g tissue; ref. 43). Therefore, we believe that the lycopene-supplemented dosage used in the present study was physiologically relevant to lycopene biologic effects in human conditions. It should be

Type mice. Lycopene supplementation in wild-type mice significantly reduced hepatic proinflammatory biomarkers, including IL6 (58%; Fig. 2A) protein expression, phosphorylation of NF-kB p65 (Ser536; 42%; Fig. 2B), and STAT3 (Tyr705; 43%; Fig. 2C), as compared with nonsupplemented mice. None of these lycopene-mediated modulations were observed in BCO2-KO mice. Reduced HCC development by dietary lycopene was associated with attenuated expression of hepatic ER stress markers in BCO2-KO but not wild-type mice

Dietary lycopene significantly suppressed markers of ER\textsuperscript{UPR}-mediated protein kinase RNA-activated like kinase (PERK)-eIF2α signaling in BCO2-KO mice, but not wild-type mice, including the activation of eIF2α by phosphorylation (65%; Fig. 2D), and the expression of CHOP protein (39%; Fig. 2E). Similarly, lycopene supplementation also significantly reduced ER\textsuperscript{UPR}-mediated activation of inositol requiring (IRE) 1α-X-box–binding protein 1 (XBP1) system in BCO2-KO but not wild-type mice, as measured by the splicing of XBP1 mRNA (Xbp-1s; 16%; Fig. 2F).

Type mice. Similarly, lycopene supplementation also

Published OnlineFirst October 7, 2014; DOI: 10.1158/1940-6207.CAPR-14-0154

www.aacrjournals.org Cancer Prev Res; 7(12) December 2014 1223

Downloaded from cancerpreventionresearch.aacrjournals.org on September 14, 2017. © 2014 American Association for Cancer Research.
pointed out that the in vivo study design for the present study was selected to investigate how lycopene can inhibit HFD-promoted hepatic tumorigenesis after the carcinogen initiation (e.g., i.p. injection of DEN to the animals at 2 weeks of age). It was not our intention to evaluate lycopene effects on the initiation stage of hepatocarcinogenesis in this study.

The present study suggests that the molecular mechanisms for lycopene chemopreventive effects may be mouse-strain specific. The lycopene-mediated chemoprevention in wild-type mice was associated with reduced hepatic inflammatory foci, lowered hepatic IL6 protein, as well as with decreased activation of NF-κB p65 (by phosphorylation), and the oncogenic transcription factor STAT3. These lycopene-mediated mechanistic modulations were similar to the chemopreventive effects of the lycopene metabolite APO10LA in C57Bl/6J wild-type mice (24). Intriguingly, our study also revealed that dietary lycopene exhibits chemopreventive effects in the absence of BCO2 expression. In contrast with wild-type mice, lycopene-mediated chemopreventive effects in BCO2-KO mice were associated with reduced ER<sub>UPR</sub> (IRE1α–XBP1 and PERK–eIF2α) and mTORC1 activation, as well as with suppressed oncogenic Met gene and β-catenin protein expression.

Elevated ER<sub>UPR</sub> is associated with liver cancer development (11). ER<sub>UPR</sub> consists of three distinct pathways regulated by ER membrane-bound proteins: IRE1α–XBP1 system, PERK–eIF2α signaling, and activating transcription factor (ATF) 6α (9–11). Elevated IRE1α and ATF6α signaling activation in HCC tissue was correlated with increased severity of HCC histologic grading (44), and can induce PERK–eIF2α signaling (44). Induced protooncogene Met expression, β-catenin protein and chronic mTORC1 activation through S6 phosphorylation promoted HCC development in mice (36–38). These oncogenic signals can be stimulated by ER<sub>UPR</sub> through suppressing miR199a/b and miR214 expression (45–48). The miRNA profile or the “miRNome” identified in human liver tumors found that miR199a/b and miR214 are decreased in human HCC (37, 39, 41, 42, 43). Interestingly, we observed in BCO2-KO mice that lycopene-mediated ER<sub>UPR</sub> inhibition coincided with increased miR199a/b and miR214 expressions. miR199a/b upregulation inhibited proliferation and invasiveness of HCC cell lines (42). miR199a/b can directly degrade protooncogene Met and Mtor mRNA and reduced their encoded protein products (37, 39, whereas transfection of miR199a/b into HCC-derived cell lines inhibited phosphorylation of S6 (37). Therefore, the present findings suggested that lycopene chemopreventive effects in BCO2-KO mice were associated with reduced mTORC1 activation, potentially through ameliorating ER<sub>UPR</sub>.

Lycopene supplementation in wild-type mice also reduced mTOR mRNA and protein, but had no effects on S6 phosphorylation. The mTORC1 pathway integrates inputs from at least five cellular and extracellular signaling, and mTORC1 kinase activity can be modulated by modifying mTOR-associated proteins within the mTORC1 complex (49). It is plausible that lycopene supplementation in wild-type mice induced upstream signaling(s) that increased mTORC1 activity and counteracted the suppressive effect on S6 phosphorylation from mTOR protein reduction.

Downregulation of hepatic miR214 was associated with cell growth, cell invasion, stem-like traits, and early recurrence of HCC (38, 40). miR214 overexpression inhibited proliferation of HCC cells in vitro (41), reduced HCC tumorigenicity and β-catenin protein in vivo (38). We observed in BCO2-KO mice that lycopene-induced hepatic miR214 expression was associated with decreased cell proliferation marker cyclin D1 protein, β-catenin protein but not mRNA. These results from the present study were consistent with previous findings, in which miR214

![Figure 3. Effects of lycopene supplementation on hepatic tumorigenic biomarkers and miRNA expression. The study design is described in Fig. 1. Protein or miRNA expressions in liver lysates (WT, WT+Ly, KO, KO+Ly n = 16–20) were analyzed by Western blotting and β-actin was used as loading control unless specified otherwise. Graphical representation of fold changes in: A, mTOR, B, cyclin D1, C, β-catenin, D, S6 (Ser235/236) phosphorylation (S6 as loading control), E, miR199a/b and miR214 (5S as loading control). Representative Western blots with one sample per group are shown. Fold changes normalized to WT or KO; values are means ± SEMs; , different from WT; and #, different from KO; P = 0.05 KO, knockout on HFD; Ly, lycopene; p-, phosphorylated; T-, total; WT, wild-type on HFD.](Image 88x354 to 313x735)
15,15 is responsible for the central cleavage of carotenoids at the accumulated marginally higher percentage of colleagues (50), in which they found that BCO2KO mice in our laboratory to examine whether lycopene metabolites with BCO1/BCO2 double KO mice are currently ongoing in tumors in patients with HCC (57). Further investigations acyclic retinoids was shown to inhibit secondary primary genesis. It may not deliver comparable potent effects as other pharmacologic drugs against tumor progression, as shown by its modest effects on miR199a/b and miR214 induction. During our article preparation, Tan and colleagues (50) showed that lycopene-mediated hepatic gene regulation in mice could be dependent or independent of BCO2 status. The beta-carotene-15,15′-oxigenase (BCO1) is responsible for the central cleavage of carotenoids at the 15,15′ double bond (51–54). It remains controversial to whether lycopene is a potential substrate for BCO1, as numerous studies found no detectable activity of BCO1 towards lycopene (21, 52, 54). Nevertheless, the central cleavage product apo-15-lycopenal (acyclo-retinal) was recently detected when lycopene was incubated in purified recombinant human BCO1 (55), and previously in recombinant murine BCO1 (56). Because we did not see a difference on hepatic lycopene levels between WT and BCO2 KO mice, it is possible that lycopene can be cleaved by both BCO1 and BCO2. Apo-15-lycopenoic acid (acyclo-retinoic acid), an oxidative products of apo-15-lycopenal, is structurally similar to acyclic retinoic acid (21). Treatment with acyclic retinoids was shown to inhibit secondary primary tumors in patients with HCC (57). Further investigations with BCO1/BCO2 double KO mice are currently ongoing in our laboratory to examine whether lycopene metabolites generated by BCO1-mediated cleavage inhibit hepatic tumorigenesis.

We observed greater hepatic all-trans lycopene (65%) accumulation in BCO2-KO than WT mice (51%). This observation is different from results published by Tan and colleagues (50), in which they found that BCO2KO mice accumulated marginally higher percentage of cis lycopene (68%) than WT mice (63%). The disparities between our results and those published by Tan and colleagues could be due to the difference in lycopene dosage (100 vs. 250 mg/kg diet), length of lycopene supplementation (24 vs. 3 weeks), or the strains of mice selected as the WT (129Svl/SvEvTac F1 vs. C57BL/6 × 129/Svl F1). Interestingly, certain members of the carotenoid cleavage enzyme family have intrinsic isomerase activity concurrently with carotenoid cleavage (58–60). For example, BCO1-mediated conversion of 9-cis-β-carotene to 9-cis-retinal occurred with a suboptimal output, indicating that this enzyme can catalyze cis to trans isomerization (59). It is plausible that the BCO1 isomerization capacity in our BCO2-KO study mice in conjunction with a long-term lycopene supplementation yielded the observed hepatic lycopene isomers distribution. Future investigation is also required to determine whether BCO2 can function as an isomerase.

In summary, our results demonstrated that lycopene elicited differential mechanism of chemopreventive effects against hepatic tumorigenesis in mice depending on the present or absence of BCO2. The lycopene-mediated chemopreventive effects were associated with reduced hepatic inflammatory responses in wild-type mice, but were associated with inhibition of ERα response in BCO2-KO mice. Together with our previous report on APO10LA's efficacy against liver cancer, these findings suggest that both lycopene and lycopene metabolites could be effective dietary agents for preventing liver cancer or reducing cancer risk for patients with NAFLD.

Disclosure of Potential Conflict of Interest
No potential conflicts of interest were disclosed.

Disclaimer
Any opinions, findings, conclusions, and recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the sponsors.

Authors’ Contributions
Conception and design: B.C. Ip, C. Liu, L.M. Ausman, X.-D. Wang Development of methodology: B.C. Ip, C. Liu, L.M. Ausman, X.-D. Wang Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B.C. Ip, C. Liu, L.M. Ausman, X.-D. Wang Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B.C. Ip, C. Liu, L.M. Ausman, X.-D. Wang Writing, review, and/or revision of the manuscript: B.C. Ip, C. Liu, L.M. Ausman, J. vonLintig, X.-D. Wang Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B.C. Ip, C. Liu, L.M. Ausman, J. vonLintig, X.-D. Wang Study supervision: X.-D. Wang

Acknowledgments
The authors thank Drs. Alice H. Lichtenstein and Martin S. Obin for their valuable comments. The authors also thank Ms. Kang-Quan Hu and other members of the Nutrition and Cancer Biology Laboratory, Dr. Donald E. Smith of the Comparative Biology Unit, John N. Lomartire, as well as Shahin Sarkarati Smith and Stephanie Thea Leon Valliere of the Nutrition Evaluation Laboratory (HNRCa at Tufts University) for their assistance and support.

Grant Support
This work was supported by the NIH grants CA104932 (to X.D. Wang), CA176256 (to X.D. Wang), and U54/AR5 grant 1950.51000-0745 (to X.D. Wang). B.C. Ip was supported by the NHLBI/NIH training grants 5T32HL069772-10 and 2T32HL069772-11A1.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 13, 2014; revised September 23, 2014; accepted September 26, 2014; published OnlineFirst October 7, 2014.

References


Published OnlineFirst October 7, 2014; DOI: 10.1158/1940-6207.CAPR-14-0154

Downloaded from cancerpreventionresearch.aacrjournals.org on September 14, 2017. © 2014 American Association for Cancer Research.


Lycopene Attenuated Hepatic Tumorigenesis via Differential Mechanisms Depending on Carotenoid Cleavage Enzyme in Mice

Blanche C. Ip, Chun Liu, Lynne M. Ausman, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1940-6207.CAPR-14-0154

Supplementary Material
Access the most recent supplemental material at:
http://cancerpreventionresearch.aacrjournals.org/content/suppl/2014/10/08/1940-6207.CAPR-14-0154.DC1

Cited articles
This article cites 60 articles, 22 of which you can access for free at:
http://cancerpreventionresearch.aacrjournals.org/content/7/12/1219.full#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.