FOOD & FUNCTION

Tomato powder impedes the development of azoxymethane-induced colorectal cancer in rats through suppression of COX-2 expression via NF-κB and regulating Nrf2/HO-1 pathway

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Received: February 28, 2012
Revised: April 28, 2012
Accepted: May 21, 2012

Cancer is one of the leading causes of death worldwide. Since dietary factors have been connected to a reduced risk of a diversity of human cancers, in this study we investigated the effects of tomato powder (TP) on the development of azoxymethane (AOM)-induced colorectal cancer in Wistar rats, and possible mechanism(s) by which TP shows its chemopreventive activity. Here we show that TP added to feed at 5% rate decreases the rate of aberrant crypt foci (ACF) and reduces the development of adenocarcinoma and growth of AOM-induced colorectal cancer in rats. In addition, we demonstrate that TP supplementation shows its chemopreventive activities through inhibition of cyclooxygenase-2 (COX-2) expression via NF-κB pathway and promotion of apoptosis, as well as regulating Nrf2/HO-1 signaling pathway in colorectal tissue of AOM-treated rats. Our findings identify an intimate connection between dietary supplementation of TP and the decreased risk of colorectal cancer in rats, and suggest that consumption of TP would be a natural candidate for the prevention of colorectal cancer in men.

Keywords:
Colorectal cancer / Cyclooxygenase-2 / NF-κB / Oxidative stress / Tomato powder

Cancer is still one of the leading causes of death all over the world. In 2008, about 13% of the deaths were caused by cancer estimated by the World Health Organization (WHO). Among many cancer types, colon cancer is one of the most common causes of cancer-related mortality in Western societies, and the death rate from colon cancer has also been stably increasing in Asia [1]. According to the American Cancer Society, more than 50 000 people die from colorectal cancer each year. Although many people are diagnosed with colorectal cancer every day, there is no reported preventative treatment for this deadly disease. However, it has been suggested that a diet loaded with natural antioxidant rich foods may reduce the risk of developing colorectal cancer [2].

Tomato and related tomato products are a good source of carotenoids including lycopene, neurosporene, gamma-carotene, phytoene, and phytofluene. Importantly, lycopene, a natural antioxidant compound, has been suggested to play important roles in cancer prevention via different mechanisms [3–6]. Despite its importance, humans are not able to produce lycopene similar to other carotenoids de novo, thus the requirement for lycopene in humans is met by the diet. It has been reported that more than 85% of our dietary lycopene comes from tomato and tomato-based products, and the rest coming from watermelon, pink grapefruit, guava, and papaya [4]. Due to its powerful antioxidant activity, tomato powder (TP) and lycopene have been extensively used in many

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Abbreviations: ACF, aberrant crypt foci; **AOM**, azoxymethane; **COX-2**, cyclooxygenase-2; **TP**, tomato powder

Table 1. The effects of tomato powder on the development of aberrant crypt foci (ACF), dysplasia, and adenocarcinoma in the indicated group of rats

	Groups				*P*	X^2
	Control	TP	AOM	AOM+TP		
ACF	0	0	47	20	–	–
Dysplasia	0^c	0^c	20^a	33^b	0.016	10.385
Adenocarcinoma	0^b	0^b	53^a	13^b	0.0001	14.196

Statistical analysis of the results was done with chi-square test using the PROC FREQ procedure (SAS). Differences among the groups were attained by Duncan's multiple comparison; *P* values of less than 0.05 were considered significant. Superscript letters a–c indicate differences among the groups.

studies [7–11]. In this study, we sought to investigate the effect of TP supplementation on the development and growth of azoxymethane (AOM)-induced colorectal cancer in rats and possible mechanism, if any, responsible for the chemoprevention effect.

Administration of the colon-specific carcinogen AOM (for detailed information on methods, see Supporting Information) to rats induces the development of clusters of abnormal tube-like glands known as aberrant crypt foci (ACF) that may then lead to cancer [12]. Therefore, we first assessed the effect of TP supplementation on the development of precancerous ACF lesions in the colon, and found no ACF developed in the rats fed with standard diet and the rats fed with standard diet+TP (Table 1). However, administration of AOM induced the development of average 47 crypts in the animals fed with a standard diet (Table 1). Interestingly, TP supplementation significantly decreased the development of ACF to an average of 20 crypts in AOM-treated rats (Table 1). We then wanted to determine if TP supplementation has any effect on the development of adenocarcinoma and dysplasia. Not surprisingly, we found no adenocarcinoma and dysplasia development in the rats fed with standard diet and the rats fed with standard diet+TP (Table 1). Administration of AOM to the rats, however, induced adenocarcinoma development in 8/15 animals (53.3%) (Table 1). Interestingly, only 2/15 (13.3%) animals developed adenocarcinoma in the group of AOM-treated rats fed with a standard diet+TP supplementation indicating that TP significantly reduces the development of adenocarcinoma in rats (Table 1). Conversely, we found that TP supplementation to AOM-treated rats induced a serious dysplasia in two rats and a mild dysplasia in three rats, whereas AOM induced a mild dysplasia in three rats (Table 1).

We also microscopically examined the hematoxylin and eosin (H&E)-stained colon in tissue sections, and found a normal histology from the rats fed a standard diet and the rats fed with standard diet+TP (Fig. 1A and B). However, AOM administration induced the percentage of proliferating cells, therefore resulted in the destructions in the architecture of histology as shown in Fig. 1C. Importantly, supplementation of TP to the AOM-treated rats diminished the proliferation of the cells and the tumor growth, and reduced the most of changes caused by AOM (Fig. 1D). Collectively, our results showed that TP supplementation prevents the rate of the proliferating cells and tumor growth, and reduces the development of AOM-induced precancerous ACF, indicating its protective effects against AOM-induced colorectal cancer in the rats.

Cyclooxygenase-2 (COX-2) is an enzyme that is expressed at a very low level in normal tissues, but in pathological

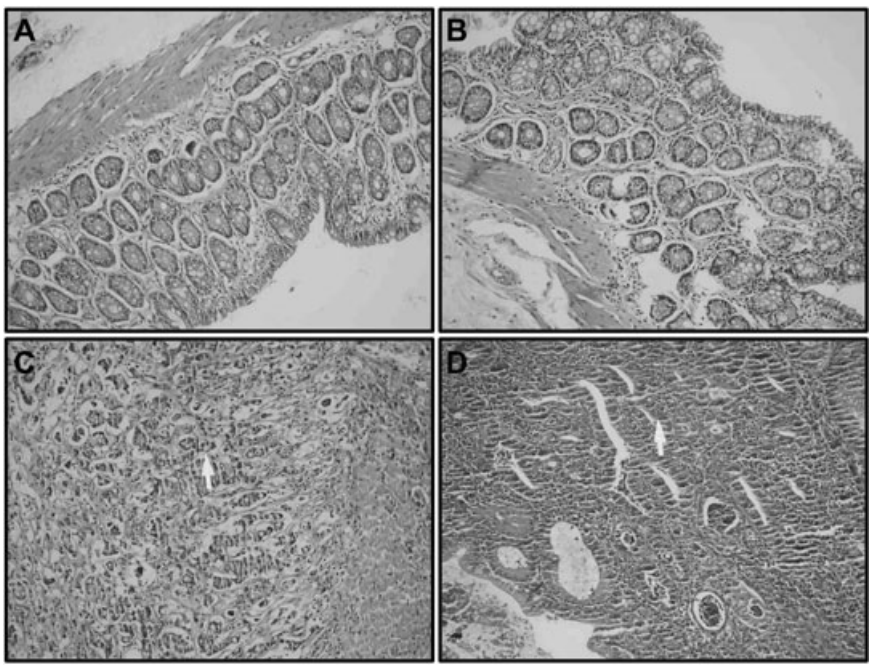

Figure 1. Representative histopathology of H&E stained colorectal tissue sections (A) control group fed with a standard diet (B) control group + TP fed with a standard diet + 5% TP (C) AOM-treated group fed with a standard diet (D) AOM + TP group fed with a standard diet + 5% TP (x 400 magnification).

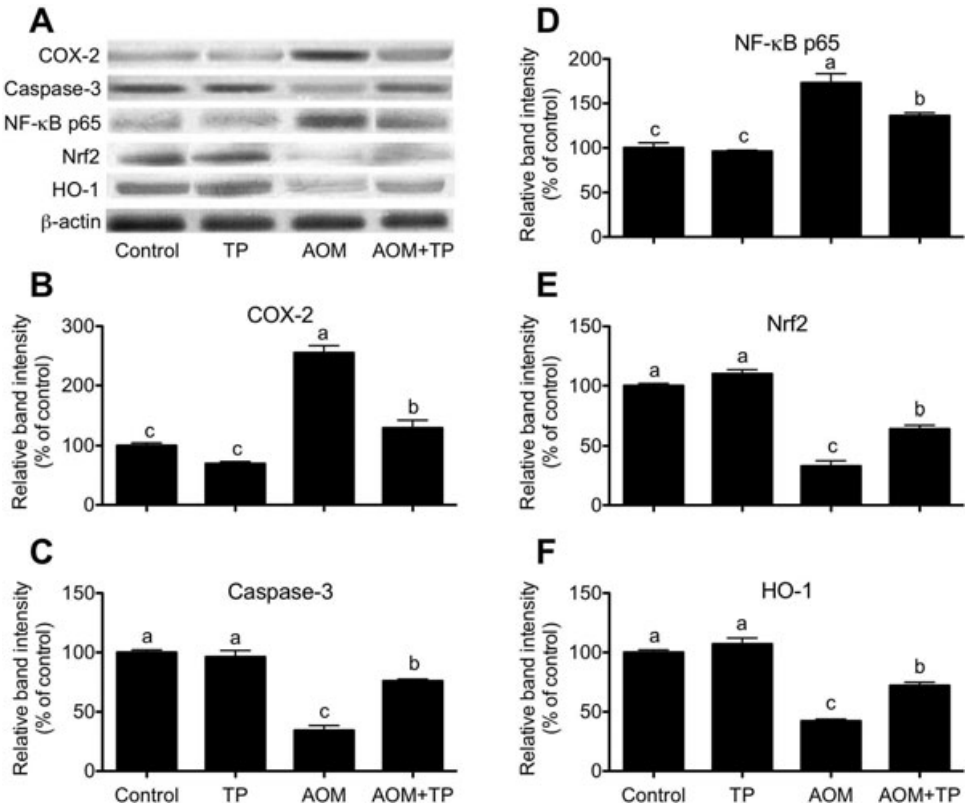

Figure 2. (A) Representative western blot analysis of COX-2, Caspase-3, NF-κB, Nrf2, and HO-1 expressions in the colon of rats from the indicated groups. Blots were repeated at least four times. β-actin was included to ensure equal protein loading. (B-F) Bar graphs show the densitometric quantification of normalized protein expression levels to β-actin. Data are shown as percentage of control and represented as ±SD. Small letters on top of each bar indicate significant difference.

circumstances and oncogenesis, the expression of COX-2 is upregulated [13, 14]. COX-2 is responsible for the biosynthesis of prostaglandins through the conversion of arachidonic acid [14]. There is now a compelling evidence that abnormal arachidonic acid metabolism via COX pathway can support carcinogenesis. To find out the possible mechanism involved in the prevention of AOM-induced colorectal cancer by TP supplementation in rats, we first tested the expression of COX-2 by Western blotting. The protein expression of COX-2 was lower in the rats fed with standard diet and the rats fed with standard diet+TP, but it was drastically increased in the AOM-treated rats due to an augment in cancerous cells by the carcinogen (Fig. 2A and B). Nevertheless, TP supplementation to the AOM-treated animals significantly reduced the protein expression of COX-2 (Fig. 2A and B). Given that the COX-2 is associated with the development of carcinogenesis, tumor promotion and inhibition of apoptosis [13, 15], it is proposed that the targeted inhibition of COX-2 may have beneficial effects on diminishing the development and growth of the colorectal cancer. Although the use of selective COX-2 inhibitors offers some advantages in the anticancer therapy, diet-based natural products have not yet been clearly shown to play any protective role in the inhibition of COX-2. However, we here showed that TP is capable of inhibiting AOM-induced activation of COX-2 in rats, thus the progression and proliferation of colorectal cancer cells which prompts us to suggest TP as a natural inhibitor of COX-2 enzyme. Although the mechanism underlying inhibition of COX-2 expression by TP supplementation needs further examination to be fully elucidated, as discussed further below the findings suggest a nuclear factor-kappa B (NF-κB)-dependent inhibition of COX-2.

NF-κB is a ubiquitous transcription factor involved in the inflammatory and innate immune responses as well as cell survival, proliferation, apoptosis, and cell migration. Constitutively active NF-κB has been shown to be involved in many types of cancer cells including colon cancer cell lines and human tumor cells [16, 17]. Given that COX-2 is a NF-κB target gene [18] and that COX-2 expression was suppressed by TP supplementation, we next tested the effect of TP supplementation on the expression of NF-κB. Although NF-κB is expressed at a constant level in colorectal tissue of the rats fed with standard diet and the rats fed with standard diet+TP, administration of AOM to the rats resulted in the activation of NF-κB (Fig. 2A and D). This concurs with the notion that NF-κB is constitutively active in many different types of tumors [16, 17]. However, TP supplementation significantly reduced the expression of NF-κB (Fig. 2A and D), possibly showing its chemopreventive activity by inhibiting NF-κB and therefore, stopping the proliferation of cancerous cells. This finding is very interesting because it has been thought that NF-κB inhibitors might have a protective role in colorectal cancer through the suppression of COX-2 expression as COX-2 is a NF-κB target gene [18]. In lights of these findings, we visualize a model in which TP administration shows its chemopreventive activity by inhibiting COX-2 expression via NF-κB activity.

We also assessed the expression of Caspase-3, an important protein in apoptosis, and found a higher expression in the animals fed with standard diet and the rats fed with standard diet+TP, but it was decreased by AOM treatment most likely due to the inhibition of apoptosis in cancerous cells (Fig. 2A and C). Interestingly, TP supplementation led to an increase in the protein expression of Caspase-3 and therefore initiates apoptosis in cancerous cells induced by AOM (Fig. 2A and C). This indicates that TP may also show its chemopreventive function through induction of Caspase-3-dependent apoptosis in colon cells.

Numerous cellular responses to oxidative stress have been found to involve signaling proteins that act through the antioxidant response element (ARE) and the transcription factor erythroid 2 related factor 2 (Nrf2) [19]. Transcription factor Nrf2 plays a central role in the regulation of phase II genes. Under basal conditions, Nrf2 is bound to Keap1 in the cytoplasm due to an interaction between a single Nrf2 protein and a Keap1 [20]. Exposure to a number of stressors and inducing agents leads to dissociation of Nrf2 from Keap1 thereby rescuing Nrf2 from proteasomal degradation and allowing for entry into the nucleus. Thus, activation of Nrf2 is considered to be an important molecular target of cytoprotective agents [20–22]. To test if TP supplementation plays any role in Nrf2/HO-1 signaling, we analyzed the protein expression of Nrf2 and HO-1 and found that the higher basal expression of Nrf2 and HO-1 in colorectal tissue of the rats fed with standard diet and the rats fed with standard diet+TP was drastically reduced by the AOM treatment (Fig. 2A, E, and F). TP supplementation, however, significantly increased the protein expression of Nrf2 and HO-1 in colorectal tissue of the rats injected with the AOM (Fig. 2A, E, and F). Although the exact mechanisms behind the activating the Nrf2-dependent HO-1 induction by TP remain unknown, TP may contribute to the activation of Nrf2, which is controlled through the multiple regulatory mechanisms, including Keap1-mediated ubiquitination and degradation, subcellular distribution, and phosphorylation [23]. Taken together, the modulation of the Nrf2/HO-1 signaling pathway may also be an important molecular mechanism involved in the chemoprevention by TP.

In conclusion, here we have shown that dietary supplementation of TP prevents the growth and incidence of AOM-induced colorectal cancer in the Wistar rats by inhibiting COX-2 expression via NF-κB pathway. Moreover, TP supplementation was also able to induce apoptosis through a Caspase-3-dependent mechanism, thus stop proliferation of cancerous cells. Finally, we have demonstrated that TP supplementation plays an important role in the oxidative stress by controlling Nrf2 and HO-1 expressions. All together, our findings suggest that consumption of TP could be a potential candidate for the prevention of colorectal cancer in humans. However, it should be noted that although lycopene, the primary red carotenoid in tomatoes, might be the principal photochemical responsible for the chemopreventive activity, there are other carotenoids, noncarotenoid compounds, flavonoids, alkaloids, and other cytotoxic compounds as well as a large number of unknown phytochemicals in tomatoes that may potentially have relevant activity. Since this study was conducted to test the chemoprevention effect of whole TP on the AOM-induced colorectal cancer, further studies are needed to test the contribution of different tomato constituents to the colorectal cancer prevention.

The authors acknowledge Firat University (FUBAP-1798) for supporting this project. Dr. Omer Kucuk is a Georgia Cancer Coalition Distinguished Cancer Scholar.

The authors have declared no conflict of interest.

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