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Lycopene in the prevention of renal cell cancer in the TSC2 mutant Eker rat model

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Abstract

Renal cell carcinoma (RCC) is the most frequent upper urinary tract cancer in humans and accounts for 80–85% of malignant renal tumors. Eker rat represents a unique animal model to study RCC since these rats develop spontaneous renal tumors and leiomyoma, which may be due to tuberous sclerosis 2 (TSC2) mutation resulting in the activation of the mammalian target of rapamycin (mTOR) pathway. This study examines the role of a lycopene-rich diet in the development of RCC in the TSC2 mutant Eker rat model. Ten-week old female Eker rats ($n = 90$) were assigned in equal numbers to receive 0, 100 or 200 mg/kg of lycopene as part of their daily diet. After 18 months the rats were sacrificed and the kidneys were removed.

Immunohistochemical staining with antibodies against mTOR, phospho-S6 and EGFR were performed, as well as hematoxylin–eosin staining for histologic examination of the tumors. Tumors were counted and measured in individual kidneys. Presence of tumor decreased from 94% in control animals to 65% in the experimental group, but the difference was not statistically significant ($P < 0.12$). However, mean numbers of renal carcinomas were statistically significantly decreased in the lycopene-treated rats ($P < 0.008$) when compared to untreated controls. In the lycopene group, tumor numbers decreased ($P < 0.002$) and the numbers tended to decrease linearly ($P < 0.003$) as supplemental lycopene increased from 0 to 200. Control rats fed only basal diet had a greater length of tumors (23.98 mm) than rats fed lycopene supplement groups (12.90 mm and 11.07 mm) ($P < 0.05$). Moreover tumor length decreased ($P < 0.02$) and tumor length tended to decrease linearly ($P < 0.03$) as supplemental lycopene increased from 0 to 200 mg/kg. All tumors showed strong staining with antibodies against mTOR, phospho-S6 and EGFR. In conclusion, dietary supplementation with lycopene attenuates the development of renal cell cancers in the predisposed TSC2 mutant Eker rat model. These results suggest that lycopene may play a role in the prevention of RCC.

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Keywords

Keywords: Renal cell cancer; Lycopene; Eker rat

Introduction

Renal cell carcinoma (RCC),¹ the most common type of kidney cancer in adults, has been increasing worldwide, at least until the mid 1990's. RCC accounted for approximately 3.8% of adult malignancies and 90–95% of neoplasms arising from the kidney in 2010 [16]. There were 58,240 new cases of and 13,040 deaths from renal cancer in 2010 in the United States, accounting for 2.3% of all cancer deaths in the United States [16].

Several studies have demonstrated that the gene responsible for renal carcinogenesis in the Eker rat is the rat homologue of the human tuberous sclerosis 2 gene (*TSC2*) gene [14]. The tumor suppressor tuberin, the *TSC2* gene product, negatively regulates the mammalian target of rapamycin (mTOR) pathway, which is a key regulator of cell growth and proliferation and increasing evidence suggests that its deregulation is associated with human chronic diseases, including cancer [14]. Studies also revealed that tuberin can inhibit the phosphatidylinositol 3-kinase (PI3K)-dependent activation of p70 S6 kinase activity (p70S6K) via the inhibition of mTOR [15,30]. Phosphorylated S6 protein, a substrate of p70S6K, was expressed in the early lesions in Eker rats, and this expression was suppressed by the treatment of rapamycin, an inhibitor of mTOR [14].

Dietary factors have been linked to RCC [8]. For example, several studies indicate an increased risk of RCC with increased consumption of meat [21], fried meats [20], dairy products [22], margarine and oils [27], reduced risks of RCC have been observed with increased intake of vegetables and fruit [11,21,31]. On the other hand, micronutrients with anticancer and antioxidant capacity such as carotenoids were associated with a reduced risk of renal cancer [20]. Lycopene, a major carotenoid present in tomatoes, is one of more than 600 carotenoids synthesized by plants and photo-synthetic microorganisms [23]. Several studies reported that lycopene treatment inhibits some cancer species in animal models and humans. It was shown that elevated tomato and/or its products consumption are associated with a reduction of 30–40% risk of prostate cancer [10]. A previous study has demonstrated that lycopene acts as a chemopreventive agent against the growth and progression of colorectal cancer in a mouse xenograft model. However, the *in vivo* anti-cancer effects of lycopene on the growth of renal cancer have not been demonstrated yet. Eker rat model, originally reported by R. Eker in 1954 [7], is one of the best characterized models of tuberous sclerosis complex (TSC) and has been used extensively for study of the function of the *TSC2* tumor suppressor gene. This makes it a good animal model for screening potential agents for testing in the prevention and treatment of human renal cancer. We therefore investigated the anti-tumor effects of lycopene and the possible anti-tumor mechanism by staining with antibodies against mTOR, phospho-S6 and EGFR.

¹Abbreviations used: RCC, renal cell carcinoma; TSC2, tuberous sclerosis 2; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; p70S6K, p70 S6 kinase activity; TBS, Tris-buffered saline; ABC, avidin–biotin complex.

Materials and methods

Animals and experimental design

Ninety-nine female Eker rats that carry the Eker *TSC2* mutation obtained from Dr. Cheryl Walker (MD Anderson Cancer Center) were used in the study. The rats were 10-week old at the initiation of the study and weighed 150-200 g. The experiment was conducted in accordance with animal welfare at Wayne State University (WSU), Detroit, MI, USA. The use of the animals in these studies followed the guidelines provided in the *Guide for the Care and Use of Laboratory Animals* (and the protocols were approved by the Institutional Animal Care and Use Committees of WSU). The rats were assigned randomly according to their body weights, to three treatment groups as 33 rats per group. The rats were fed either a basal diet or the basal diet supplemented with either 100 or 200 mg lycopene per kilogram of diet. The dose of lycopene was chosen based on our previous studies in the Japanese quail model [25]. Lycopene supplement was provided by DSM Nutritional Products Ltd, Switzerland, a 10% pure lycopene product. Small amounts of the basal diet were first mixed with the respective amounts of lycopene as a small batch, and then mixed with a larger amount of the basal diet until the total amounts of the respective diets were homogeneously mixed. Diets were stored in black plastic containers at 4 °C to protect against oxidation. Food cups containing the diet were also protected from light. Water and diets were offered ad libitum throughout the experiment. Rats were housed in pairs, in polycarbonate cages or in stainless steel cages with hardwood bedding, under environmental conditions of controlled temperature and humidity. A 12-h light/12-h dark photo cycle was maintained. The study was conducted over 18 month's period of time.

Animals were killed humanely under ether anesthesia and necropsied after 18 months on study. Kidneys were removed and kept at -80 °C until they were cut for analysis. At a later date they were cut and fixed in 10% neutral buffered formalin at room temperature for 18–24 h, then transversely trimmed into several sections per kidney. These were embedded in paraffin and further sectioned to a thickness of 4 µm, then H&E stained for histologic evaluation for angiomyolipoma and adenocarcinoma lesions. Some tissue specimens were lost during transport from Detroit to Atlanta. Therefore only tissues from 56 of 99 animals were available for analysis.

Immunohistochemistry

For immunohistochemistry analysis, 4-µm sections were deparaffinized through graded ethanol series, rehydrated, and washed with Tris-buffered saline (TBS) containing 0.1% (w/v) Tween 20. Sections were then incubated overnight at 4 °C with the following primary antibodies at the indicated dilution: (1) phospho-mTOR (Ser2448) (49F9) rabbit mAb (1:50) (Cell Signaling Technology, Beverly, MA, USA), (2) phospho-S6 ribosomal protein (Ser235/ 236) (91B2) rabbit mAb (1:75) (Cell Signaling Technology), (3) EGFR rabbit mAb (1:400) (Cell Signaling Technology). Antibody binding was detected by the avidin-biotin complex (ABC) method of staining using a Vectastain Elite ABC kit (PK-6101; Vector Laboratories, Burlingame, CA, USA) for which the Dako EnVision Plus HRP system (K4006) was used following the manufacturer's protocol.

Statistical analyses

Presence of tumor and number of tumor were subjected to cross-tabulation with level of dietary lycopene for Chi-square analysis. The mean size and length of tumors were analyzed using oneway ANOVA. Contrast options were built to evaluate effect of lycopene effect (0 vs average of 100 and 200 mg/kg) and dose–response relationship (linear and quadratic effect of lycopene). Results were considered significant at $P < 0.05$.

Results

As shown in Table 1, supplementation with lycopene at low (100 mg/kg of diet) or high (200 mg/kg of diet) dosage could effectively inhibit the development and growth of renal tumors in the Eker rat model. Presence of tumors decreased from 94% in the control group to 65–75% in the experimental groups, but the differences were not statistically significant ($P < 0.12$). The incidence of tumor was 15/16 (94%) in the control, and 15/20 (75%) in the 100 group, and 13/20 (65%) mm in the 200 group. Mean numbers of renal carcinomas were significantly decreased in lycopene-treated rats ($P < 0.008$) when compared to untreated control group. Group 200 had a mean number of 2.85 tumors (± 2.99) per rat, compared to 3.65 (± 2.62) in group 100 and 6.71 (± 6.13) in the control group, with a significant linear effect of lycopene ($P < 0.003$). Moreover, tumor numbers significantly decreased ($P < 0.002$) and tumor number tended to decrease linearly ($P < 0.003$) as supplemental lycopene increased from 0 to 100 to 200. Average tumor size was 3.45 mm, 3.31 mm and 3.44 mm in groups 200, 100 and control, respectively. However, the total tumor size per rat was 11.29 mm (± 13.36 mm) in group 200, compared to 10.00 mm (± 10.04 mm) in group 100 and 26.14 mm (± 24.27 mm) in the control group, with a linear effect of lycopene ($P < 0.03$).

Immunohistochemical analyses

Immunohistochemical analyses were used to examine the status of the lycopene-sensitive mTOR pathway, which is negatively regulated by the Tsc2 complex. All tumors showed strong staining with antibodies against mTOR, phospho-S6 and EGFR (Fig. 1). We did imaging analysis of immunohistochemical markers which did not reveal significant differences in expression between the groups (data not shown).

Discussion

There were about 54,000 cases of kidney cancer and about 13,010 people died from this disease in the United States in 2008 [1]. RCC has been related to overweight and obesity, hypertension, tobacco smoking, and family history of the disease [29]. A case-control studies on RCC reported a significant inverse association with long-term supplementation with antioxidants [5,3].

Lycopene has been implicated as having a potentially beneficial impact in a number of chronic diseases including cancer. Lycopene is also a powerful antioxidant with a singlet-oxygen-quenching capacity 47 and 100 times greater than that of β -carotene and vitamin E, respectively [6]. Epidemiological and animal studies show a significant inverse association between lycopene intake and the risk for different cancer types, including prostate, lung, stomach, pancreas, colon, rectum, esophagus, oral cavity, breast, and cervix [18]. However,

to the authors' knowledge, this is the first study evaluating the effects of lycopene supplementation on the development of renal cell cancer in the Eker rat model. The present study demonstrated that consumption of lycopene could significantly inhibit the growth of renal cell cancer in this model.

There was no previous study investigating the effects of lycopene supplementation on the prevention of RCC in Eker rats. However, in our previous *in vivo* study in Japanese quail model we observed that lycopene could inhibit the formation of fibroid tumors in a dose-dependent manner [25]. Tang et al. [28] suggested that lycopene could act as a chemopreventive agent against the growth and progression of colorectal cancer in a mouse xenograft model.

The postulated mechanisms of action of lycopene include (1) inhibition of growth and induction of differentiation in cancer cells by modulating the expression of cell cycle regulatory proteins [2], (2) modulation of the IGF-1/IGFBP-3 system [9], (3) up-regulation of gap-junctional gene connexin 43 (Cx43) and increased gap junctional intercellular communication [32], (4) prevention of oxidative DNA damage [24], (5) inhibition of IL-6 and androgen [26], (6) inhibition of 5-lipoxygenase [13], (7) modulation of carcinogen metabolizing enzymes [17], and (8) modulation of immune function [4].

van Dijk et al. [12] reported that dietary intake of carotenoids was inversely associated with the risk of RCC in humans. However, another cohort study found that individual carotenoids, except lycopene, were significantly associated with a lower risk of RCC in men [19].

The results of this study indicate that dietary supplementation with lycopene reduces the number and size of renal carcinomas developing in the genetically susceptible TSC2 mutant Eker rats. These results may be generalizable and applicable to humans because TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling [15]. Two FDA approved drugs, everolimus and temsirolimus, targeting the mTOR pathway are currently used in the treatment of patients with metastatic renal cancer. Clinical trials should be conducted to investigate the efficacy of lycopene supplementation in the prevention and treatment of renal cancer in humans.

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Fig. 1. Immunohistochemical analysis of renal tumors. (A) H&E; (B) mTOR; (C) pS6; (D) EGFR.

Table 1

The effect of lycopene supplementation on kidney tumor development in Eker rats.

Item	Dietary lycopene, mg/kg diet				P
	0	100	200	SEM	
Presence of tumor in Eker rats (n/n)	15/16	15/20	13/20		$\chi^2 = 4.18, P < 0.12$
Number of tumors ^a	6.94	3.65	2.85	0.87	0.008
0	1	5	7		$\chi^2 = 38.21, P < 0.05$
1	1	1	0		
2	2	0	3		
3	1	3	3		
4	2	0	1		
5	0	6	1		
6	4	2	1		
7	0	3	1		
8	0	0	1		
10	1	0	1		
11	1	0	0		
14	1	0	0		
15	1	0	0		
21	1	0	0		
Size (mm)	3.41	3.31	3.45	1.05	0.60
Total length ^b (mm)	23.98	12.90	11.07	3.75	0.05

^a 0 vs average 100 and 200, $P < 0.002$; linear effect of lycopene, $P < 0.003$.

^b 0 vs average 100 and 200, $P < 0.02$; linear effect of lycopene, $P < 0.03$.