The role of lycopene and its derivatives in the regulation of transcription systems: implications for cancer prevention1–4

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ABSTRACT
Evidence from epidemiologic studies has suggested that carotenoids, and lycopene in particular, decrease the risk of cancer: however, not all studies support this view. To gain insight into the molecular mechanisms whereby lycopene and other carotenoids may exert their chemoprotective effects, we and others performed a series of studies that used a large panel of cancer cell lines of different lineages and animal models of human cancer. In this review we address some of the mechanisms proposed for the cancer-preventive activity of tomato lycopene, focusing on the induction of the antioxidant response element transcription system and the inhibition of the transcriptional activity of sex hormones, such as estrogens and androgens, and the activity of growth factors, such as insulin-like growth factor. We also considered the modulation by lycopene of the transcription factors peroxisome proliferator–activated receptor, retinoid X receptor, liver X receptor, and activating protein-1. The ligands and the phytonutrient regulators of these transcription systems contain electrophilic active groups, whereas lycopene and nonxanthophyll carotenoids are devoid of them. Thus, we suggest that at least some of the cellular effects of carotenoids are mediated through their derivatives formed either by chemical oxidation or by enzymatic cleavage inside the cells. This review highlights findings that pertain to this exciting avenue of research, which is currently under intense scrutiny in several laboratories worldwide. Am J Clin Nutr doi: 10.3945/ajcn.112.034645.

INTRODUCTION
There is considerable epidemiologic evidence that suggests an association between the consumption of fruit and vegetables and reduced incidence of cancer (1, 2). In particular, carotenoids have been implicated as cancer-preventive agents (3). β-Carotene has received the most attention because of its prevalence in many foods. However, in more recent years other carotenoids such as lycopene, the main tomato carotenoid, have become the subject of intense investigation (4). In a comprehensive analysis of the epidemiologic literature related to tomato consumption and cancer prevention, Giovannucci (5, 6) found that most of the reviewed studies reported an inverse association between tomato intake or lycopene concentration in blood and the risk of various types of cancer. The anticancer effects of these and other carotenoids were also observed with the use of human cancer cell lines (7–10). For example, we have shown that lycopene inhibits mammary, endometrial, lung, and leukemic cancer cell growth in a dose-dependent manner (7, 8, 11).

The biochemical processes involved in the chemoprotective effects of fruit and vegetables are not completely understood. In this review we primarily address the mechanisms proposed for the cancer-preventive activity of carotenoids in general and tomato lycopene in particular, focusing on their modulation of various transcription systems, such as the electrophile/antioxidant response element (EpRE/ARE)5 and the estrogen and androgen receptors.

LYCOPENE INHIBITION OF ESTROGENIC ACTIVITY IN BREAST AND ENDOMETRIAL CANCERS
Evidence has accrued that lycopene may play a significant role in the prevention of sex hormone–dependent cancers. Among these are the major human malignancies, such as estrogen-dependent breast and endometrial cancers in women and androgen-dependent prostate cancer in men.

Breast cancer is the second leading cause of cancer deaths in women today and the most common cancer among women in Western societies (12). There is substantial evidence to suggest that endogenous or administered estrogens play an important role in both the development and progression of breast cancer (13). Therefore, anti-estrogens, which reduce estrogenic activity, represent an important adjuvant treatment of the prevention of breast cancer recurrence and for primary prevention in women who are at high risk of this malignancy. The awareness that estrogenic activity is essential for bone health led to the de-
development of specific estrogen receptor modulators (SERMs) for the treatment of breast cancer. SERMs were shown to selectively inhibit estrogenic activity in some tissues, such as the breast, but stimulate it in other tissues, such as bone, thus preventing bone loss. The recent success of breast cancer prevention trials that used SERMs such as tamoxifen (14) and raloxifene (15) suggests that chemoprevention of breast cancer is a valid therapeutic approach. However, for long-term prevention in the general population, changes in diet, when proven effective, rather than sustained administration of drugs has long been considered to be a more sensible approach.

Several studies examined whether fruit, vegetable, and antioxidant micronutrient consumption is associated with a reduction in breast cancer incidence. A large, population-based case-control study has shown that among postmenopausal women, reduced ORs were noted for the highest fifth compared with the lowest fifth of oxidant micronutrient consumption is associated with a reduction in breast cancer risk. In an additional animal model of human prostate cancer, they would appear to be too severe for preventive approaches. Thus, an alternative approach must be sought to reduce androgen activity in the prostate; in this context, the nutritional approach appears to be the most suitable for populations at large because of feasibility in application and the absence of toxicity.

An increase in dietary consumption of lycopene was associated with decreased prostate cancer development in a case-control study in Iran (23). The question of whether tomato consumption may affect androgen activity in prostate cancer was examined in animal models of the human disease. Male rats treated with a carcinogen (N-methyl-N-nitrosourea) and testosterone to induce prostate cancer were fed diets containing whole-tomato powder, synthetic lycopene beadlets, or control beadlets (24). The risk of death from prostate cancer was lower for rats fed the tomato-powder diet than for those fed control beadlets; in contrast, lycopene beadlets did not have a significant effect. Thus, consumption of tomato powder but not lycopene alone inhibited prostate carcinogenesis, which suggests that in addition to lycopene, tomato products contain compounds that modify prostate carcinogenesis. Indeed, short-term consumption of phytofluene, lycopene, or tomato powder decreased serum testosterone in rats (25). These results indicating that short-term intake of tomato carotenoids alters androgen status provide a plausible mechanism for the beneficial effects of tomato intake in lowering prostate cancer risk. In an additional animal model of human prostate cancer, synthetic lycopene alone failed to reduce prostate tumor volume. However, combined treatment with lycopene and vitamin E, suppressed the growth of human PC-346C prostate tumor cells xenografted in nude mice by 73% and increased median survival time by 40% (26). These results suggest that the concerted action of several tomato ingredients and not lycopene alone is important for the reduction in prostate cancer development.

Another study evaluated the effect of a combination of tomatoes and broccoli in the Dunning R3327-H prostate adenocarcinoma rat model (27). Male rats injected subcutaneously with...
tumor implants were fed for 22 wk diets containing either tomatoes or broccoli or a combination of both. Synthetic lycopene alone did not reduce tumor weight significantly, whereas a significant reduction was obtained with tomato or broccoli powder. The combination of these dietary powders caused the more pronounced decrease in tumor weights. These results clearly show that the combination of tomatoes and broccoli was more effective at slowing tumor growth than either tomatoes or broccoli on their own.

The mechanism by which lycopene may contribute to a reduction in prostate cancer risk was tested in the Dunning prostate cancer model (28). Lycopene supplementation for 4 wk led to plasma lycopene concentrations comparable to those found in humans. Macroscopic evaluation of the tumors by MRI showed that lycopene interfered with local testosterone activation by downregulating 5α-reductase and consequently reduced the expression of androgen target genes (28). The enzyme 5α-reductase converts testosterone to the more active androgen, dihydrotestosterone, and therefore activity and expression of the reductase is crucial for androgen function in the prostate tissue. The activity of 5α-reductase was also inhibited by astaxanthin and by saw palmetto lipid extract in human prostate cancer cell lines in vitro (29). Inhibition of 5α-reductase by drugs has been reported to decrease the symptoms of benign prostate hyperplasia. It is used to treat existing prostate cancer and leads to prevention of prostate cancer (30). Cumulatively, these findings indicate that inhibition by lycopene of 5α-reductase expression (28) may provide a valuable nondrug alternative for the prevention of prostate cancer and for alleviation of existing prostate diseases. It is important to note that in another rat model of prostate cancer (24), synthetic lycopene had a small and insignificant effect on prostate carcinogenesis. Thus, the question of whether synthetic lycopene can replace the natural carotenoids in the tomato is still open, and more studies on this issue are warranted.

The interference by lycopene and other carotenoids with androgen cancer–promoting activity was examined in our laboratory in the human LNCaP prostate cancer cell line, which expresses androgen receptors and responds to dihydrotestosterone with enhanced proliferation. We found that lycopene, phytoene plus phytofluene, and astaxanthin inhibited dihydrotestosterone-stimulated cell growth in a dose-dependent manner with an IC_{50} of 2–5 μmol/L (G Zango, J Levy, and Y Sharoni, unpublished results, 2009). This inhibition was also evident in the presence of various apo-carotenals (31), which are carotenoid oxidation products that are discussed below for their effects on several cellular pathways. Interestingly, the carotenoids inhibited only marginally the proliferation of fibroblast cell lines, which suggests that the growth of normal tissues is not significantly affected by these dietary compounds. The carotenoid concentrations that are required for significant inhibition of prostate cancer cell proliferation when added alone (>2 μmol/L) are higher than those present in human plasma and tissues. To explain the efficacy of carotenoids in vivo, we surmised that low concentrations of different active nutritional ingredients act in combination to produce additive or synergistic effects. Indeed, in these preliminary studies we observed a strong synergistic interaction between the carotenoids mentioned above in the inhibition of prostate and mammary cancer cell growth (G Zango, J Levy, and Y Sharoni, unpublished results, 2009).

**THE EFFECT OF TOMATO CAROTENOIDS ON THE INSULIN-LIKE GROWTH FACTOR SYSTEM**

Growth factor activity is a well-documented target for many anticancer drugs. These factors are crucial for the uncontrolled proliferation of cancer cells. Thus, a putative inhibitory action of phytonutrients in general and tomato carotenoids in particular on growth factor signaling has been an intense focus of interest for us and for other researchers.

Insulin-like growth factor (IGF) I is an important risk factor for several major cancers (32, 33). In addition, the activity of this growth factor system can be modulated by various dietary regimes, including tomato carotenoids (34). Two possible mechanisms can account for the lowering of IGF-related cancer risk by tomato lycopene. The carotenoid may decrease plasma concentrations of IGF-I, thereby diminishing the risk associated with its elevation, and/or it may interfere with IGF-I activity in the cancer cell. Evidence for the successful cancer-preventive intervention of tomato carotenoids in the IGF system using these 2 strategies in animal model systems and in humans is presented below.

The IGF system includes the IGF ligands (IGF-I and IGF-II) and the structurally related hormone insulin. Most of the biological effects of the IGFs are mediated by the IGF-I receptor (IGF-IR), which is a transmembrane protein tyrosine kinase (35). In addition, a family of IGF-binding proteins, IGFBP-1 to -6, is involved in determining the half-lives, availability, and activity of the IGFs. Thus, IGF action depends on the availability of IGF-I to interact with the IGF-IR, which is regulated by the relative concentrations of the IGFBPs (35). The binding of IGF-I to IGF-IR results in receptor autophosphorylation, subsequent phosphorylation of intracellular substrates, and activation of specific signaling processes, which ultimately promote growth (35).

Some epidemiologic studies addressed the association between tomato products or lycopene consumption and components of the IGF system (36, 37) and found positive associations between lycopene intake and IGFBP-3 (38) or an inverse association with IGF-I in blood or its molar ratio with IGFBP-3 (39, 40).

At the cellular level, we showed previously that lycopene inhibits the growth of human breast, endometrial, lung, and leukemic cancer cells (7, 8). Furthermore, growth stimulation of MCF-7 human breast cancer cells by IGF-I was markedly reduced by lycopene (41). Interestingly, these effects were associated with a marked inhibition of serum and IGF-I–stimulated cell-cycle progression from the G1 to the S phase (7, 11, 41, 42). Lycopene treatment markedly reduced the IGF-I stimulation of tyrosine phosphorylation of insulin receptor substrate 1 and binding capacity of the activating protein-1 transcription complex (41). The activation of this transcription system is a key event in the mitogenic signaling of IGF-I and other growth factors. The inhibition of these intracellular events, which are downstream to the IGF-I receptor, was not associated with changes in the number or affinity of these receptors but with an increase in membrane-associated IGFBPs.

IGF-I and other growth factors, either in the blood or as part of autocrine or paracrine loops, are important for cancer progression. The cumulative results therefore indicate that attenuation of IGF signaling by dietary tomato carotenoids may result in cancer prevention.
ACTIVATION BY CAROTENOIDS OF THE EpRE/ARE TRANSCRIPTION SYSTEM AND THE INDUCTION OF PHASE II ENZYMES

In recent years, evidence has accumulated indicating that the cancer-preventive action of dietary ingredients in general and tomato lycopene in particular is, at least in part, to the induction of phase II detoxifying and antioxidant enzymes (43). This is a major mechanism for chemical protection against carcinogenesis, mutagenesis, and other forms of toxicity by any of a diverse array of naturally occurring and synthetic chemical agents (44–47). The phase II cytoprotective enzymes include detoxifying and antioxidant enzymes such as glutathione S-transferase, NAD(P)H:quinone oxidoreductase 1, superoxide dismutase, and heme oxygenase 1. The promoter regions of the inducible genes encoding phase II enzymes contain the EpRE/ARE, which on binding of the transcription factor Nrf2 leads to increased expression of these genes. Nrf2 is a redox-sensitive transcription factor that under normal, unstimulated conditions is present in the cytosol bound to an inhibitory protein, Kelch-like ECH-associated protein 1 (Keap1). Chemopreventive agents disrupt the Nrf2-Keap1 association, thereby releasing “free” Nrf2, which then translocate to the nucleus and drive the expression of phase II enzymes (48).

Several studies have shown that dietary antioxidants, such as 1,2-dithiole-3-thiones (49), phenolic flavonoids (50, 51), curcuminoids (52), and isothiocyanates (53), may function as anticancer agents by activating the Nrf2/ARE transcription system. We hypothesized that carotenoids also activate this transcription system, because several carotenoids have been found to induce phase II enzymes (54, 55). Indeed, we have shown that different tomato carotenoids (lycopene, phytoene, phytofluene, and β-carotene) as well as lutein and the algal xanthophyll astaxanthin transactivate EpRE/ARE and induce the expression of phase II enzymes (56).

Various electrophilic phytonutrients have been shown to induce the EpRE/ARE system via interaction with Keap1 (48). However, hydrophobic carotenoids such as lycopene are devoid of electrophilic groups and thus are unlikely to directly interact with Keap1. We have previously suggested that carotenoid oxidation products and not the intact carotenoids stimulate the EpRE/ARE system. Indeed, our recent study (31) proved this hypothesis to be valid, and findings indicate that apo-carotenals and diapo-carotenals potentially can be produced by in vivo metabolism of carotenoids and interact with the inhibitory protein Keap1, leading to the activation of EpRE/ARE.

In addition to EpRE/ARE, carotenoid modulation of other transcription systems contributes to their intracellular effect. For example, lycopene decreased the invasive ability of hepatoma cells by suppressing the activity of nuclear transcription factor κB (NF-κB), the key inflammation-related transcription system (57). This inhibitory effect of lycopene may be related to down-regulation of IGF-IR. Another study (58) suggested that lycopene via the inhibition of NF-κB might be useful in the treatment of benign prostate hyperplasia, a nonmalignant condition associated with inflammation. Consistent with these findings, our preliminary studies indicate that putative carotenoid cleavage products such as apo-carotenals and diapo-carotenals inhibit NF-κB activity (K Linweill-Hermoni, J Levy, and Y Sharoni, unpublished results, 2011).

Earlier studies have shown that β-carotene excentric cleavage derivatives, such as β-apo-14′-carotenal (59) and β-apo-13-carotenone (60, 61), inhibit the activity of 2 nuclear receptor transcription systems, the peroxisome proliferator–activated receptor (PPAR) and retinoid X receptor. The inhibition of PPARα and PPARγ activity resulted in suppression of their target gene expression and in adipogenesis in 3T3-L1 preadipocytes (59). In contrast to the inhibition of PPAR or retinoid X receptor by the β-carotene derivatives, a recent article suggested that lycopene inhibition of LNCaP prostate cancer cell growth is mediated by stimulation of the PPARγ and the liver X receptor α transcription systems (62). Another proliferation-related transcription factor, activating protein-1, was found to be inhibited in MCF-7 mammary cancer cells by lycopene (33). This inhibition was associated with interference in IGF-I receptor signaling and with inhibition of cell-cycle progression.

It is important to note that most of the findings cited above were based on studies that used cancer cell lines and their relevance to human physiology and pathology awaits confirmation. However, it is becoming increasingly evident that the current available information is only the tip of the iceberg, and we can confidently predict a wealth of exciting observations pertaining to the modulation of the activity of additional transcription systems by carotenoids and their derivatives.

CONCLUSIONS

The prevention of cancer by diet-derived compounds has obvious advantages over drug-based interventions. In this review we addressed the modulation by tomato lycopene and other carotenoids of several transcription systems including the EpRE/ARE, estrogen and androgen receptors, PPAR, and NF-κB. These transcription systems are known to affect carcinogenesis and tumor progression, and thus their modulation by carotenoids is consistent with epidemiologic studies that show an association between carotenoid consumption and reduced incidence of cancer.

There is convincing evidence that neither synthetic lycopene nor natural tomato lycopene can act alone to prevent cancer. We suggest that combinations of carotenoids alone or with other phytonutrients, at concentrations that can be achieved in the body, will result in significant beneficial health effects, most probably elicited by synergistic modulation of various transcription systems. Thus, health benefits can be gained from the addition of tomato-based foods to the diet (particularly cooked tomato products containing oil) or from supplements of tomato extract suspended in oil. These tomato-derived products are devoid of any significant toxicity and thereby warrant additional testing for putative preventive effects on cancers in humans.

The authors’ responsibilities were as follows—KL-H, GZ, MK, HS, and AV: performed the studies done in the authors’ laboratory as cited in the relevant references in the review and were involved in writing the paragraphs relevant to their studies; and YS, JL, and MD: supervised the studies and were responsible for most of the review writing and editing. LycoRed Ltd did not have any role in the performance of the experiments or in the analysis and interpretation of the data presented in this review. JL and YS are consultants for LycoRed Ltd, Beer Sheva, Israel. None of the other authors had any conflicts of interest. LycoRed Ltd is a supplier to the dietary supplement and functional food industries worldwide.

REFERENCES

LYCOPENE AND TRANSCRIPTION


