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Lycopene: modes of action to promote prostate health

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Abstract

Epidemiological evidence strongly suggests that lycopene consumption contributes to prostate cancer risk reduction. Preclinical studies show that lycopene acts via different mechanisms, which have the potential to cooperate in reducing the proliferation of normal and cancerous prostate epithelial cells, in reducing DNA damage, and in improving oxidative stress defense. The mechanisms include inhibition of prostatic IGF-I signaling, IL-6 expression, and androgen signaling. Moreover, lycopene improves gap-junctional communication and induces phase II drug metabolizing enzymes as well as oxidative defense genes. These findings provide plausible explanations for the epidemiological findings how lycopene can contribute to reduced prostate cancer risk. The novel finding that lycopene reduces local androgen signaling in the prostate suggests also efficacy in prevention of benign prostate hyperplasia. Intervention trials in humans are required to finally prove clinical efficacy of the lycopene molecule in prostate health.
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Prostate cancer incidence varies widely between different geographic regions [1,2]. Migrant studies have impressively demonstrated that the risk for prostate cancer is predominantly influenced by environmental factors. Genetic factors involved in hereditary prostate cancer development have been identified [3,4]. Only about 9% of all cases seem to be directly linked to a family history of prostate cancer, which contributes mainly to prostate risk in younger men. Here, the attributable risk of genetic factors may be as high as 43% [3]. Regarding the lifetime risk for prostate cancer, however, the role of these genetic loci is less pronounced compared to the impact of life style habits, including nutrition.

A considerable body of epidemiological evidence demonstrates an association of tomato consumption with reduced prostate cancer risk [5]. Recent data demonstrate that consumption of tomato products confers more potent protection against sporadic prostate cancer than against familial prostate cancer [6]. Tomato is the main dietary source of lycopene intake. Lycopene (ψ,ψ-carotene) is the main carotenoid in tomato and is responsible for its red color. In the Health Professionals Follow Up Study, Giovannucci et al. [7] showed that estimated intake of lycopene (in form of tomato based products), but not of other carotenoids, was linked to lower prostate cancer risk. Further investigations consolidated the epidemiological evidence for a role of lycopene in prostate cancer prevention by showing that in addition to lycopene intake [8] also lycopene blood levels [9] are inversely correlated with prostate cancer risk [10,11].

In most foods, lycopene is mainly present in the all-*trans* configuration, but significant amounts of various *cis* isomers, mainly 5-*cis*, can be produced during food processing. The stomach initiates the transfer of carotenoids from the vegetable matrix to the fat phase of the meal. Following absorption into enterocytes, carotenoids are incorporated into triglyceride-rich chylomicron particles for transport to the liver, the major storage site. Secondary organs are supplied with lycopene from the liver lycopene pool. Lycopene is not significantly isomerized in the human stomach during digestion [12]. Nevertheless in plasma, *cis*-lycopene isomers, mainly 5-*cis*, can contribute to more than 50% of

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the total lycopene [13–16]. Lycopene concentrations in plasma vary considerably between geographic regions due to different nutritional habits [17]. In Mediterranean countries, lycopene reaches plasma levels of approximately 1 μM. In Northern Europe and Japan, lycopene concentrations in plasma are 2- to 8-fold lower. For lycopene plasma levels in the US population, values as low as 0.01 μM, and as high as 1.8 μM were reported [17].

As expected, the highest lycopene concentrations in tissues are detected in liver. In secondary organs, the highest levels were detected in adrenals, testes, and prostate [14,18–22].

In prostate, the ratio of all-*trans* to *cis*-lycopene was even lower than in plasma, and *cis* isomers can contribute up to nearly 90% of the total lycopene contents [14].

Lycopene is not a precursor for retinol or retinoic acid (RA),[1] but it can be oxidized to acyclo retinoic acid [23]. Acyclo RA has weak retinoid activity in reporter gene assays [24], although it remains to be shown that acyclo RA is a physiologically active metabolite of lycopene in humans. Possibly, eccentric enzymatic cleavage of lycopene is a first step of acyclo RA formation, since lycopene is a substrate for the eccentric β-carotene cleavage enzyme β-carotene-9′,10′-oxygenase [25]. The resulting 10′-apo-lycopenal may, analogously to β-apocarotenals [26], be oxidized to the corresponding acid, and shortened to acyclo RA by β-oxidation. So far, no specific enzymes for lycopene metabolism have been identified. Khachik et al. [27,28] described two further oxidative metabolites of lycopene, 2,6-cyclolycopene-1,5-diol A and B, which occur only at low levels in prostate.

Mechanisms of lycopene action

Antioxidant function

Carotenoids are well known as highly efficient scavengers of singlet oxygen ($^1O_2$) and other excited species [29–31]. During $^1O_2$ quenching, energy is transferred from $^1O_2$ to the lycopene molecule, converting it to the energy-rich triplet state. Lycopene in the triplet state can return to the ground state by dissipating the energy as heat or by physical quenching, leaving the lycopene molecule intact and ready for further quenching events. Cantrell et al. [32] and Stahl et al. [33] showed that lycopene is also an excellent $^1O_2$ quencher in biological membrane models, such as liposomes. Trapping of other reactive oxygen species, like $OH^\bullet$, $NO_2^\bullet$ or peroxynitrite, in contrast, leads to oxidative breakdown of the lycopene molecule [34]. In organic solution, lycopene was the most rapidly destroyed carotenoid upon reaction with peroxyl radicals [35,36], indicating its presence in the first line of defense. Given this potent antioxidant function in vitro, lycopene may protect also in vivo against oxidation of lipids, proteins, and DNA. Oxidative DNA damage gives rise to DNA mutations and is therefore implicated in cancer initiation. Prevention of oxidative DNA damage accordingly is of interest for primary cancer prevention. Lycopene has been shown to reduce the amount of oxidative DNA damage in cell culture and in rats in vivo [37,38]. Moreover, several clinical studies demonstrated that tomato consumption protected human leukocytes against oxidative DNA damage in vitro [39–41]. Bowen et al. [42] provided the first in vivo evidence that a lycopene-rich diet reduced oxidative DNA damage also in the prostate.

In addition to its antioxidant function, lycopene also influences several biological processes important in regulating cell fates, such as cell cycle progression, cell communication, and signaling of cytokines, hormones, and growth factors. It is unclear, whether lycopene mediates these effects through its antioxidant function or through additional, so far unknown, mechanisms.

Inhibition of cell cycle progression

Lycopene was shown to inhibit cell growth of a variety of cancer cell lines, including prostate cancer cells [43–47], mammary cancer cells [48,49], endometrial cancer cells [50], and promyelocytic leukemia cells [51].

Pastori et al. [43] described the influence of lycopene on the growth of the androgen-insensitive human prostate carcinoma cell lines DU-145 and PC-3. Lycopene alone did not influence proliferation of these cell lines. However, co-treatment with physiological concentrations of lycopene (below 1 μM) and α-tocopherol (below 50 μM) inhibited prostate carcinoma cell proliferation nearly 10-fold. The effect of lycopene with α-tocopherol was synergistic and was not shared by β-tocopherol, ascorbic acid or probucol.

Other reports describe that lycopene alone does inhibit growth of prostate cancer cell lines DU-145 [45,47], PC-3 [45], and LNCaP [45,46]. Kim et al. [46] found that lycopene reduced proliferation of LNCaP cells at physiological concentrations (1 μM). Kotake-Nara et al. [45] demonstrated growth inhibition of all three broadly used prostate cancer cell lines PC-3, DU-145, and LNCaP, although supra-physiological concentrations (20 μM) were used. On the other hand, Hall [47] describe growth inhibition of DU-145 cells by lycopene at extremely low concentrations (10 nM).

[1] *Abbreviations used:* RA, retinoic acid; GJC, gap-junctional cell communication; IGF-I, insulin-like growth factor I; PIA, proliferative inflammatory atrophy; PIN, prostatic intraepithelial neoplasi; GSTP1, glutathione-*S*-transferase π1; GP, glutathione peroxidase; GR, glutathione reductase; ARE, antioxidant-response element; PSA, prostate-specific antigen; ROS, reactive oxygen species.

The mechanism, by which lycopene inhibits cell growth, involves downregulation of cyclin D1, but not cyclin E, at the protein level, and leads to cell cycle arrest at the G0/G1 phase of the cell cycle. G0/G1 arrest was also observed in lycopene-treated HL-60 promyelocytic leukemia cells [51]. In MCF-7 and T-47D breast cancer cells, as well as in ECC-1 endometrial cancer cells, lycopene delayed G1-S transition by downregulating cyclin D1 and D3 protein expression [50].

In addition to delaying growth of prostate cancer cells, lycopene also inhibited cell growth of normal prostate epithelial cells in a dose-dependent fashion [44]. Normal prostate epithelial cells were even more sensitive to growth inhibition by lycopene than cancer cells. Growth started to be inhibited by concentrations above 1 μM, and the degree of inhibition reached 80% at concentrations of 2 μM and higher. This finding is most relevant, since it relates to the activity of lycopene in primary prevention of prostate cancer, and potentially also of benign prostate hyperplasia.

Apoptosis induction

There are very few reports on apoptosis induction by lycopene in prostate cells. Hall [47] and Kotake-Nara et al. [45] did not find apoptosis induction by lycopene in PC-3, DU-145, and LNCaP prostate cancer cells even at very high lycopene concentrations. In contrast, the oxidative metabolite of lycopene, acyclo RA, induced apoptosis in the androgen-independent prostate cancer cell lines PC-3 and DU-145, but not in the androgen-responsive LNCaP cell line. Supra-physiological concentrations of the compound of up to 40 μM were, however, required [52]. Oxidized lycopene also induced apoptosis in HL-60 cells [53].

On the other hand, a 3-week tomato intervention caused an increased apoptotic index in hyperplastic and neoplastic cells in the resected prostate tissue in prostate cancer patients [42].

Increase of gap-junctional communication

Several studies suggest an effect of lycopene on gap-junctional cell communication (GJC), a key factor in tissue homeostasis. Gap junctions are channels connecting two neighboring cells, thus enabling the exchange of small molecules, such as nutrients or intracellular signaling molecules [54]. Gap junctions consist of two half-channels (connexons), each made up by hexameric complexes of different connexins. Forced expression of connexin 43 has been demonstrated to reduce the neoplastic potential of prostate carcinoma cells [55]. Relative to normal tissue, a decreased expression of connexins, including connexin 43, has been detected in different human tumors [56]. In healthy prostate, connexin 43 is expressed in basal epithelial cells and connexin 32 in luminal epithelial cells. The abundance of both connexins is increased in benign prostate hyperplasia. In contrast, connexins 32 and 43 are decreased in prostate cancer [57].

In cell culture, lycopene enhanced GJC in human fetal skin fibroblasts [24] and in KB-1 human oral cancer cells [58]. This was accompanied by upregulation of connexin 43 mRNA and protein levels. Lycopene has been shown to inhibit carcinogen-induced neoplastic transformation in cell culture [59]. This action involved upregulation of connexin 43 expression as well as improved GJC [60], and was independent of antioxidant activity or pro-vitamin A activity [61]. First data in humans indicate that lycopene may indeed increase connexin 43 expression in the prostate [62].

Inhibition of IGF-I signal transduction

Elevated serum concentrations of insulin-like growth factor I (IGF-I) are associated with an increased risk for several types of cancer, including prostate cancer [63,64]. Moreover, IGF-I upregulation accompanies tumor progression in the TRAMP mouse prostate cancer model [65]. Also, IGF-I overexpression in the prostate epithelium suffices to cause prostatic intraepithelial neoplasia in transgenic mice [66]. This shows that IGF-I is not only a marker for cancer risk, but is causally involved in tumorigenesis.

Lycopene markedly reduced IGF-I-stimulated growth in MCF-7 breast cancer cells. Growth inhibition was associated with a delayed G1-S cell cycle progression. This effect may have been mediated by upregulation of IGF-binding proteins by lycopene. As a consequence, IGF signaling was inhibited [48]. Upregulation of IGF-binding protein-3 by lycopene was also demonstrated in ferret lungs [67]. Recently, we demonstrated in the MatLyLu Dunning prostate cancer model that IGF-I expression locally in prostate tumors was decreased by lycopene supplementation at 200 ppm in feed [68]. Available clinical data indicate that in response to tomato consumption also changes of systemic IGF-I levels occur. Consumption of cooked tomatoes was inversely associated with IGF-I plasma levels [69]. In contrast, supplementation with tomato extract oleoresin for 3 weeks did not lower IGF-I plasma levels in a treatment-dependent manner [62]. Rather, IGF-I plasma levels decreased independent of the intervention over the course of the study. The reason for these conflicting results is not resolved. In any case, since the majority of circulating IGF-I is secreted from the liver, a downregulation of IGF-I in the prostate by lycopene is not necessarily reflected in the serum IGF-I concentration. This may explain the observations by Kucuk et al. [62], and argues that the lycopene effects should be addressed in prostate tissue in addition to serum.

Inhibition of IL-6 expression

IL-6 is a pleiotropic cytokine, which promotes inflammation, and acts as a paracrine and autocrine growth factor in prostate epithelial cells [70]. There is evidence that a history of prostatitis is linked to a higher prostate cancer risk [71]. Moreover, focal atrophic lesions in the prostate epithelium are often associated with chronic inflammation and increased cell turnover (proliferative inflammatory atrophy, PIA). These foci are thought to represent precancerous lesions, which may progress to prostatic intraepithelial neoplasia (PIN) and/or adenocarcinoma [72,73].

Moreover, IL-6 can transactivate the androgen receptor [74]. Serum IL-6 levels have been found to correlate with the stage of prostate cancer. Accordingly, serum IL-6 levels are regarded as a prognostic factor for prostate cancer.

We recently found in the MatLyLu Dunning rat prostate tumor model that supplementation of pure lycopene reduced IL-6 expression locally in prostate tumors [68]. This effect should add to the efficacy of lycopene in prostate cancer risk reduction.

Induction of phase II enzymes

Induction of phase II enzymes plays a crucial role in providing a first barrier against toxic low molecular agents, including exogenous carcinogens [75]. Silencing of the phase II enzyme glutathione-*S*-transferase $\pi 1$ (GSTP1) by promoter methylation occurs very frequently in prostate cancer [76]. Moreover, GSTP1 silencing has been detected in single cells of proliferative inflammatory atrophy (PIA) lesions [77].

Lycopene increased the activity of the phase II enzymes glutathione peroxidase (GP), glutathione-*S*-transferase (GST), and glutathione reductase (GR), as well as GSH levels in several animal models (healthy rats [78]; rat model for gastric carcinogenesis [79], DMBA-induced hamster buccal pouch carcinogenesis model [80,81]; and T2-toxin-treated chicken [82]). Induction of phase II enzymes was paralleled by suppressed DMBA-induced oral carcinogenesis in hamsters [80]. At the same time, also enzymes of oxidative defense were induced [78], and lipid peroxidation was reduced [79,81,82]. Co-regulation of genes encoding phase II enzymes and the oxidative defense system is mediated via the antioxidant-response element (ARE) in the promoter of these genes [83]. Of the many transcription factors that bind to the ARE, Nrf-Jun heterodimers positively regulate ARE-mediated induction of genes in response to antioxidants and xenobiotics.

Although it has not been clarified how lycopene induces phase II enzymes, an antioxidant mechanism is conceivable. Often, the same set of redox reactions is responsible for activation or detoxification of carcinogens; and many inhibitors of cancer initiation target these reactions [84]. In line with this, upregulation of phase II enzymes indicates an improved protection against carcinogens. Since lycopene can also reduce oxidative DNA damage (as discussed above) in the prostate, lycopene should contribute to reduced prostate cancer initiation by two mechanisms.

Inhibition of androgen activation and signaling

Androgens are the steroid hormones responsible for the male phenotype (reviewed in [85]). There is evidence suggesting that androgenic influences over a period of time favor prostate cancer development [86].

Studies of prostate biology suggest that 5-α-dihydrotestosterone is the principal androgen responsible for both normal and hyperplastic growth of the prostate gland. 5-α-Dihydrotestosterone is produced from testosterone by steroid 5-α-reductase. In case of elevated expression of oxidative 3-α-hydroxysteroid dehydrogenase, it can also be formed from 3-α-androstanediol [87]. 5-α-Reductase occurs in two isoforms, I and II. In healthy prostate, both isoforms are expressed [88], with type II being the predominant isoform [89,90]. In prostate cancer cell lines DU-145 and PC3, on the other hand, 5-α-reductase I is strongly expressed [91], and 5-α-reductase II expression is lower in prostate cancer samples than in benign prostate hyperplasia samples [92]. The central role of 5-α reductase in prostate cancer is evident from the observation that the prostate in men deficient for this enzyme is hypoplastic and does not develop cancer [89,93,94].

We found that lycopene reduced expression of 5-α-reductase I in prostate tumors in the rat MatLyLu Dunning prostate cancer model [68] (5-α-reductase II was not detectably expressed in the tumors (Siler, unpublished observation)). As a consequence, several androgen target genes were drastically downregulated in these tumors [68]. Downregulated androgen targets comprise cystatin related protein 1 and 2, prostatic spermine-binding protein, prostatic steroid-binding protein C1, C2, and C3 chain, and probasin [68]. Thus, we showed for the first time that lycopene suppresses the key pathway in development of prostate cancer as well as of benign prostate hyperplasia. Suppression of androgen signaling was accompanied with increased necrosis rates (35.97%, compared to 19.98 and 23.27% in the untreated vs the placebo group) of the prostate tumors, as demonstrated by in vivo magnetic resonance imaging.

Androgen deprivation is the therapy of choice against prostate cancer. Moreover, anti-androgens are among the promising chemopreventive agents [95], since epidemiological studies strongly suggest that surgical castration prior to age 40 prevents both benign prostatic hypertrophy (BPH) and prostate cancer (reviewed in

[94]). Thus, our findings provide a promising mechanistic explanation for the beneficial effect of lycopene in prostate cancer prevention.

Boileau et al. [96] used the Bosland rat prostate cancer model, in which tumor formation is initiated by the carcinogen NMU and promoted by exogenous testosterone application [97]. In contrast to our results in the MatLyLu Dunning model, pure lycopene was not significantly effective in this model, as opposed to freeze-dried tomatoes. Since prostate tumor formation in the Bosland model relies on exogenous testosterone treatment, the pathway, by which lycopene acts, is constantly activated. In a system that is flooded with supra-physiological testosterone levels, we expect the local anti-androgen effect of lycopene to become overwhelmed. Lycopene not only affects the androgen metabolism in prostate, but androgen, conversely, also influences lycopene uptake: Boileau et al. [98,99] showed that castrated male rats accumulate more lycopene in their livers than do intact male rats. In addition to the androgen aspect, lycopene exposure—as judged by plasma levels—was 10-fold lower in the Boileau study compared to our study (0.1 μM vs 1 μM), despite similar lycopene dosage (161 vs 200 ppm) for a shorter period of time (67 weeks vs 4 weeks).

In conclusion, our animal data argue that lycopene has an anti-androgen effect in prostate, thus targeting the key pathway in prostate cancer development. Tomatoes probably contain compounds that in addition to lycopene could contribute to reduced prostate cancer risk.

Besides the epidemiological data on primary prevention, there are some reports on short-term clinical intervention trials with lycopene, which indicate an efficacy of lycopene in secondary prevention. The data consistently demonstrate that lycopene or tomato intervention reduces serum levels of prostate-specific antigen (PSA) [42,62,100]. The gene encoding PSA is one of the well-known androgen target genes [101]. Decrease of serum PSA levels is therefore in line with an anti-androgen effect of lycopene also in the human prostate.

Mechanistic integration of lycopene effects and outlook

A variety of lycopene-induced events have been described on the cellular level, which result in reduced cell proliferation and increased oxidative defense (see Fig. 1). The cascade of molecular events, however, is not known. Lycopene, due to its lipophilic character, is expected to reside in cellular membranes. The first

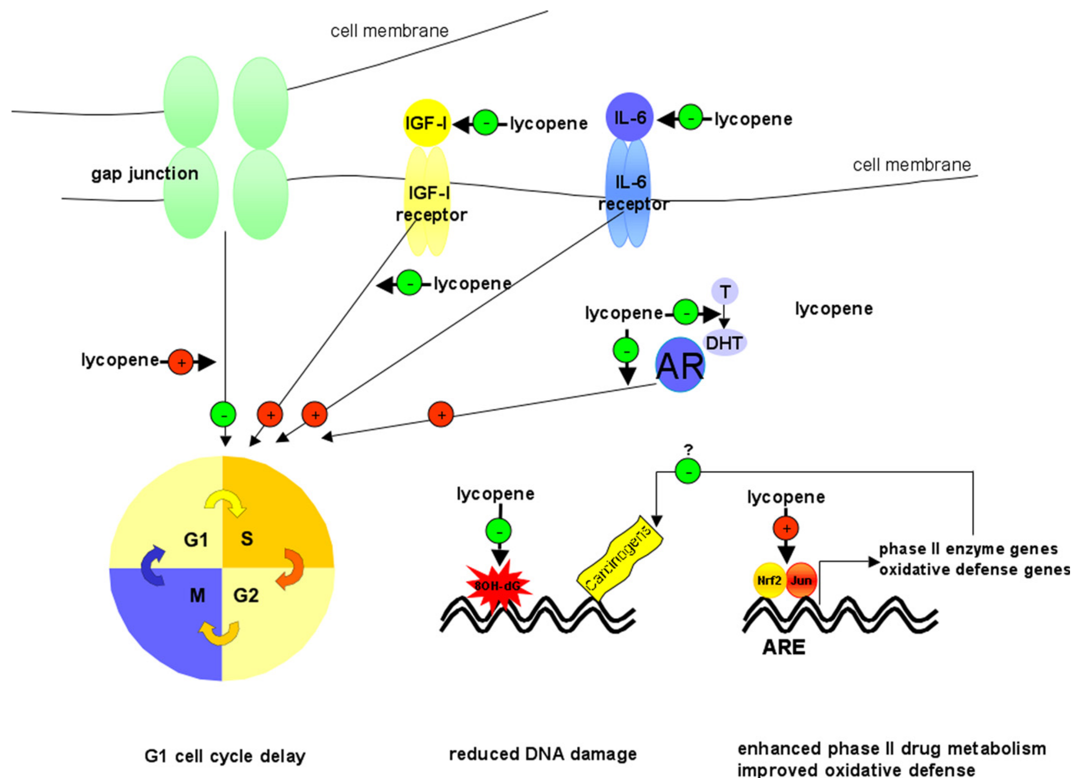

Fig. 1. Modes of action of lycopene to contribute to reduced prostate cancer risk. Lycopene delays cell cycle progression at the G1 phase by different mechanisms. Furthermore, lycopene reduces oxidative DNA damage, as shown by reduced prevalence of 8OH-dG. Lycopene also induces oxidative defense genes and promotes phase II metabolism, which is involved in detoxification of carcinogens. Since carcinogen action often involves the formation of mutagenic DNA adducts, lycopene may also reduce DNA damage via increased phase II metabolism. IGF-I, insulin-like growth factor I; IL-6, interleukin 6; AR, androgen receptor; T, testosterone; DHT, 5-α-dihydrotestosterone; 8OH-dG, 8-hydroxy-2′-deoxyguanosine; and ARE, antioxidant-response element.

molecular effect of lycopene, which initiates the series of events leading to the observed cellular changes, has not been defined. It is also unresolved, whether lycopene mediates these effects by its antioxidant function, or via so far unknown mechanisms.

Induction of phase II enzymes, as well as of oxidative defense genes via transactivation of the antioxidant-response element, may well be mediated by an antioxidant mechanism. Lycopene may also contribute to reduced growth factor signaling by its antioxidant function. Signaling through receptor tyrosine kinases, e.g., IGF-I and EGF R, involves formation of reactive oxygen species (ROS) (reviewed in [102]). Hence, ROS generation during activation of transmembrane receptor tyrosine kinases could well be reduced by lycopene molecules in the vicinity. Such an effect would be expected to inhibit growth factor signaling and subsequent cell proliferation. Androgen signaling was also reported to cause ROS generation [103]. In this case, ROS are not generated during signaling, but are likely the indirect consequence of stimulated cellular metabolism and oxygen consumption. Nevertheless, antioxidants can inhibit late androgen-induced signaling events, which are associated with oxidative stress and which regulate proliferation and inflammatory events [104].

Lycopene could also act by mechanisms independent of its antioxidant function. Possible modes of action include changes in membrane fluidity, e.g., in lipid rafts, and interaction of lycopene with transmembrane proteins, such as transport proteins or signal transducers. Lycopene could also act through metabolites, although none of them have been detected at higher concentrations in the prostate in vivo.

Several genes are regulated by lycopene. It is not known whether the reported changes are induced individually and converge to induce delayed cell growth. Alternatively, the genes/proteins regulated by lycopene could be organized in a pathway, where the observed molecular changes influence each other, and result in the cellular changes observed. Data in the published literature indeed indicate mechanistic links between IGF-I, IL-6, gap-junctional communication, and androgen signaling [105–110].

Conclusion

Taken together, lycopene acts via different mechanisms that have the potential to cooperatively delay cell cycle progression in prostate epithelial cells, to reduce DNA damage, and to improve oxidative stress defense. These findings provide plausible explanations for the epidemiological findings that lycopene can contribute to a reduced prostate cancer risk. The order of molecular events, which are initiated by lycopene located in the cellular membranes and which lead to gene regulation and cellular changes, will have to be determined in future studies.

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